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BHAS 42 CELL TRANSFORMATION ASSAY VALIDATION STUDY REPORT

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Series on Testing and Assessment

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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FOREWORD

The project for developing a validation report and a Test Guideline for the Bhas 42 Cell Transformation Assay (CTA) was proposed by Japan and included in the Test Guideline work plan in 2010, after completion of international validation studies. The validation report was subsequently developed and peer-reviewed by the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) Scientific Advisory Committee (ESAC). The ESAC peer review served as a basis for the development of the EURL ECVAM Recommendation on the Cell Transformation Assay based on the Bhas 42 cell line, publicly available on the EURL ECVAM website.

The validation report of the Bhas CTA was circulated for comments to the Working group of the National Coordinators for the Test Guidelines Programme (WNT) in October 2013. It was discussed by the expert group on cell transformation assays in a meeting held in Paris in January 2014 and subsequently endorsed by the WNT at its 26th meeting in April 2014. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 7th July, 2014.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

30 July, 2012

Bhas 42 Cell Transformation Assay

Validation Study Report

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Contents

List of Abbreviations

BSRC	Biosafety Research Center, Foods, Drugs and Pesticides
CAS no.	Chemical Abstract Service registry number
CTA	Cell Transformation Assay
CV	Crystal violet
DF5F	Dulbecco's modified Eagle's medium/Ham's F12 supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin and 5% FBS
DMSO	Dimethyl Sulfoxide
DRP	Detailed review paper
DRP 31	Detailed Review Paper on Cell Transformation Assays for Detection of Chemical Carcinogens, OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 31 [OECD, 2007]
ECVAM	European Centre for the Validation of Alternative Methods
FBS	Fetal Bovine Serum
FDSC	Food and Drug Safety Center
Harlan	Harlan Cytotest Cell Research GmbH
HRI	Hadano Research Institute
IARC	International Agency for Research on Cancer
IC_{50}	50% inhibitory concentration
IC_{90}	90% inhibitory concentration
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ID	Identification
JaCVAM	Japanese Centre for the Validation of Alternative Methods
JBRC	Japan Bioassay Research Center
M10F	Eagle's minimum essential medium supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin and 10% fetal bovine serum
MCA	3-Methylcholanthrene
Mitsubishi	Mitsubishi Chemical Medience
MNNG	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
NEDO	New Energy and Industrial Technology Development Organization
NIHS	National Institute of Health Sciences
NOEL	No-effect level
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
SHE	Syrian Hamster Embryo
TPA	12-*O*-tetradecanoylphorbol-13-acetate

Modular approach

The European Centre for the Validation of Alternative Methods (ECVAM) developed a modular approach to the validation of alternative methods, where the various information requirements for peer-review and as generated during the validation process are broken down into 7 independent modules. In this report, module 1 (test definition) is described in section 1, Rationale for the proposed test, section 2, Organization of the study, sections 3.1.1 and 4.1.1, Study design (on the 6-well method and on the 96-well method, respectively) and sections 3.2 and 4.2, Protocol, and briefly mentioned in section 5, General discussion. After the presentation of assay results in sections 3.3 and 4.3, modules 2-5 (within-laboratory reproducibility, transferability, between-laboratory reproducibility and predictive capacity) are assessed in sections 3.4 and 4.4, Discussion and section 5, General discussion. Modules 6 and 7 (applicability domain and performance standards) are not addressed in this report.

Preface

The Organisation for Economic Co-operation and Development (OECD) has published a detailed review paper (DRP) on the cell transformation assays (CTAs) [OECD, 2007], where Syrian Hamster Embryo (SHE) and BALB/c 3T3 CTAs are dealt with as assays having potential to detect non-genotoxic as well as genotoxic carcinogens. Meanwhile, a new CTA using the Bhas 42 cells which were clonally derived from the BALB/c 3T3 cells transfected with v-Ha-*ras* gene was developed at about the same time as the compilation of the review. Hadano Research Institute (HRI), Food and Drug Safety Center (FDSC) undertook a research project financed by the New Energy and Industrial Technology Development Organization (NEDO, Japan) to refine, evaluate and validate the Bhas 42 CTA. HRI applied the assay to various chemicals under the project, and the accumulated data showed that the assay was promising to predict chemical carcinogenicity. The study presented in this report was executed to validate the Bhas 42 CTA in a formal inter-laboratory validation study. Two protocol variants of the Bhas 42 CTA were considered in this study: the 6-well plate and the 96-well plate protocols. The ultimate goal of this study is to develop an OECD Test Guideline for the Bhas 42 CTA. The study was supervised by the Validation Management Team (VMT) established by the Japanese Centre for the Validation of Alternative Methods (JaCVAM). This report includes a short introduction on the context and background of the study, the presentation of the results generated in the validation study and the conclusions by the VMT.

Summary

Carcinogenicity is a key element in the hazard and risk assessment of chemicals for human health and has traditionally been assessed using the chronic carcinogenicity bioassay in rodents. Rodent carcinogenicity tests are costly in terms of time, money, and the large number of animals sacrificed. With the aims of reducing the number of rodent bioassays conducted, as well as the associated time and costs, while maintaining efficient discrimination of carcinogens from non-carcinogens, several *in vitro* alternatives have been developed. Among them, *in vitro* genotoxicity tests have been routinely employed to provide adjunct data with which to screen chemicals for carcinogenicity. However, direct DNA damage is but one of mechanisms involved in chemical carcinogenesis, since some carcinogens are non-genotoxic. *In vitro* cell transformation mimics multistage carcinogenesis in experimental animals, and cell transformation assays (CTAs) can detect both non-genotoxic as well as genotoxic carcinogens. The conventional CTAs have been carried out using primarily Syrian hamster embryo (SHE) cells, BALB/c 3T3 cells and C3H/10T1/2 cells. The Bhas 42 cells were established from the BALB/c 3T3 cells by the transfection of v-Ha-*ras* gene more than twenty years ago and postulated to be an initiated cell population in the two-stage carcinogenesis theory. Using the Bhas 42 cells, a sensitive short-term CTA was developed which is capable of detecting both tumor-initiating and tumor-promoting activities of chemical carcinogens.

The evolution of the Bhas 42 CTA has spawned two methods. One is a 6-well method where the assay is carried out using 6-well micro-plates and the other is a 96-well method where 96-well micro-plates are used. The Bhas 42 CTA was developed, refined and applied to various chemicals using 6-well micro-plates. Later the 96-well method was added as a modification that has the potential to be utilized for high throughput automated applications. The protocol of each of the 6-well and 96-well methods consists of two assay components, the initiation assay and the promotion assay, to detect the tumor-initiating activity and the tumor-promoting activity of chemicals, respectively. The assay procedures are fundamentally the same between the 6-well and 96-well methods. The cells are apportioned relative to each well-size (6-well micro-plate or 96-well micro-plates), cultured and treated with a test chemical for the same duration and the production of transformed foci is observed.

Hadano Research Institute (HRI), Food and Drug Safety Center (FDSC) undertook a New Energy and Industrial Technology Development Organization (NEDO) Project in Japan. Among the purposes of this large project was to refine, evaluate and validate the Bhas 42 CTA. HRI finished three validation studies, pre-validation and validation studies on the 6-well method and a validation study on the 96-well method. The report of the pre-validation study on the 6-well method (available elsewhere) is excluded from this report since a validation management team (VMT) was not organized for that study. Nevertheless those study results are compared with those of other validation studies in section 5, General discussion. This validation study report provides details on the latter two validation studies (6-well method and 96-well method) that were supervised by the CTA Advisory Committee and VMT.

The validation study performed on the 6-well method involved six laboratories from three countries. Twelve coded chemicals were examined in total and each chemical was tested by three laboratories. In the initiation assay, concordant results were obtained by three laboratories for eight out of ten chemicals and in the promotion assay, concordant

results were achieved for ten of twelve chemicals. The 6-well method discriminated all six carcinogens and two tumor-promoters from four non-carcinogens. Thus, the present study demonstrated that the Bhas 42 CTA using 6-well micro-plates is transferable between laboratories, reproducible between laboratories and applicable to the prediction of chemical carcinogenicity. In addition, by comparison of the present results with intra-laboratory data previously published, within-laboratory reproducibility in the 6-well method was also confirmed.

The validation study performed on the 96-well method involved four participating laboratories. The study was conducted in a stepwise manner (pre-validation phase, validation phase I and validation phase II). 3-Methylcholanthrene (MCA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were examined in the pre-validation phase, and coded chemicals were tested in the validation phases I and II. A total of 25 chemicals were tested including duplicate chemicals between phases. In the pre-validation phase, the respective results of MCA and TPA were quite similar among all four laboratories. In validation phase I, seven coded chemicals were examined and each chemical was tested by the four laboratories. In the initiation assay, concordant results were obtained by four laboratories for six chemicals. In the promotion assay, concordant results among all four laboratories were achieved for four chemicals and for the remaining three chemicals concordant results were obtained by three laboratories. In validation phase II, 16 coded chemicals were examined and each chemical was tested by two laboratories. The results were concordant between the two respective laboratories for all 16 chemicals. MCA and TPA were tested under coded conditions in phase I, and the results were quite similar to those in the pre-validation phase. Benzo[*a*]pyrene was examined in phases I and II and the judgments were identical between both phases in every laboratory. The transformation frequency of each positive control was statistically significantly different from that of corresponding negative control and the cluster of transformation frequency in positive controls was quite separate from that in negative controls. Overall, 10 out of 12 carcinogens and tumor-promoters were positive and 8 out of 9 non-carcinogens were negative in the 96-well method of Bhas 42 CTA. These results proved that the 96-well method is robust, transferable between laboratories, reproducible both within and between laboratories, and applicable to the prediction of chemical carcinogenicity.

The 6-well method and the 96-well method produced the same results for 15 out of the 17 chemicals duplicated between the validation study on the 6-well method and that of the 96-well method.

The VMT reviewed the results of the validation studies on the 6-well method and the 96-well method.

1 Rationale for the proposed test

1.1 Introduction

The assessment of carcinogenicity is one of the most important issues in the regulation of chemicals. Carcinogenicity is traditionally assessed using chronic carcinogenicity bioassays performed in rodents. Standard carcinogenicity tests are performed using hundreds of mice and rats and incur large expenditures in terms of cost and time, usually 2 years. The new European chemical legislation (REACH, Registration, Evaluation, Authorization and Restriction of Chemicals), which limits animal use for safety assessment, has recently been enacted. Situations such as this, other regulatory global requirements, and public pressures necessitate the use of validated cost-effective *in vitro* alternative test methods that can reduce/replace the use of animals for the assessment of chemical carcinogenicity. Genotoxicity tests (*in vivo* and *in vitro*) have come to serve as adjuncts to routine *in vivo* carcinogenicity tests, both for screening purposes and prediction purposes and to help resolve mechanism(s) of action. However, a significant number of carcinogens are considered to be non-genotoxic [Hernández *et al.*, 2009]. In order to avoid this limitation by using standard genotoxicity tests, it is desirable that a more encompassing reliable *in vitro* test other than the genotoxicity assays be included in the battery of short-term assays for the prediction of chemical carcinogenicity. An *in vitro* cell transformation assay (CTA) is such a unique system. The CTA, of which there are several, is an assay measuring the phenotypic conversion from normal to malignant characteristics in cultured mammalian cells exposed to test chemicals. Among the available CTAs, focus formation assays, generally using BALB/c 3T3 cells or C3H 10T1/2 cells, and colony forming assays, most often using Syrian hamster embryo (SHE) cells, have been acknowledged as the principal *in vitro* screening transformation methods for chemical carcinogens [Kakunaga and Yamasaki, 1985; Montesano *et al.*, 1986]. However, these CTAs have not yet been fully embraced by regulatory authorities for routine use in the regulation of potential chemical carcinogens [IARC/NCI/EPA Working Group, 1985; OECD, 2007].

Although a two-stage theory of chemical carcinogenesis has been hypothesized [Berenblum I, 1941b], at present, it is considered to involve multiple stages. However, the two-stage model of carcinogenesis can serve as a suitable, though abridged, representation of multi-stage carcinogenesis. The two-stage carcinogenesis model been described as being comprised of an initiation stage and a promotion stage. In the initiation stage, a target cell is damaged with a genotoxic carcinogen (tumor-initiator), the damage is fixed as a mutation in the genome, and the fixed mutation is never reversed and is retained in the genome. Some of those mutations render the target cells predisposed for carcinogenesis although they are not yet cancerous. The process to this point is the initiation stage and such cells are termed initiated cells. In the promotion stage, the initiated cell is stimulated with a non-genotoxic carcinogen such as TPA to proliferate and proceed to tumor formation. The carcinogen that promotes the initiated cell toward tumor formation is termed a tumor-promoter. Thus, the tumor-promoter is non-genotoxic and does not produce tumors by itself, but promotes carcinogenesis in initiated cells. This two-stage carcinogenesis theory serves to explain the phenomenon in mouse skin that neither treatment with a sub-threshold dose of a genotoxic carcinogen, such as benzo[a]pyrene, alone, nor treatment with a tumor-promoter, such as croton oil, alone produced tumors but that a sequential treatment with a sub-threshold dose of a genotoxic carcinogen and followed by treatment with a tumor-promoter produced tumors [Berenblum, 1941a]. The two-stage carcinogenesis

progression has been reported in other organs and in other experimental animals as well as in mouse skin [Beebe *et al.*, 1995; Nishiwaki-Matsushima *et al.*, 1991; Reddy *et al.*, 1977; Sakai, 2008]. Many non-genotoxic carcinogens are considered to be tumor-promoters, since somatic cells can be initiated by a small amount of environmental and/or endogenous genotoxic carcinogens. Meanwhile, tumor-promoters are different from receptor-mediated non-genotoxic carcinogens which are able to cause tumors without initiation by a genotoxic carcinogen.

Cell transformation *in vitro* is regarded as a model system for carcinogenesis *in vivo* [Smets, 1980; Barrett *et al.*, 1986; Landolph, 1985]. Initiation and promotion stages have been described in cell transformation just like the two-stages in mouse skin carcinogenesis [Mondal *et al.*, 1976; Hirakawa *et al.*, 1982]. A sub-threshold dose of a genotoxic carcinogen (tumor-initiator) alone does not cause cell transformation in the cultures nor does any tumor-promoter alone. When the cells are treated with a sub-threshold dose of a genotoxic carcinogen and then with a tumor-promoter, however, transformation is induced in the target cells. Therefore, two-stage CTAs that mimic a two-stage carcinogenesis test *in vivo* can be performed using BALB/c 3T3 and C3H 10T1/2 cells. In the two-stage CTAs, the target cells are initiated with a sub-threshold dose of a known genotoxic carcinogen prior to treatment with a test chemical. In this way, many non-genotoxic carcinogens can be detected as promoters of cell transformation [Kuroki and Sasaki, 1985; Sakai, 2008; Sakai *et al.*, 2002]. Generally, genotoxic carcinogens can be detected as transforming agents by conventional (one-stage) CTAs and as initiators by two-stage CTAs [IARC/NCI/EPA Working Group, 1985; Sakai and Sato, 1989; Tsuchiya *et al.*, 1999].

The Bhas 42 CTA is a sensitive short-term system that has reduced associated cost and labor compared with those associated with the conventional BALB/c 3T3 and C3H10T1/2 CTAs [Sasaki et al., 2012]. Bhas 42 cells were developed from BALB/c 3T3 cells through the transfection with a plasmid pBR322 containing Ha-MuSV-DNA, clone H1 (v-Ha-*ras*) [Dhar *et al.*, 1982; Ellis *et al.*, 1980; Sasaki, K. *et al,* 1988]. It has been confirmed that the cell population still retains v-Ha-*ras* gene (presently at passage 18) and m-RNA of the viral *ras* gene is expressed in the cultures as well [Tanabe *et al.*, in preparation]. The Bhas 42 cells are transformed by known tumor-promoters, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA), okadaic acid and lithocholic acid, without initiating treatment with a known tumor-initiator such as 3-methylcholanthrene (MCA) [Omori et al., 2004], and are presumed to be initiated toward transformation by the introduced *ras* sequence [Sasaki *et al.*, 1990]. Using the Bhas 42 cells, Omori *et al.* developed a short-term CTA to identify tumor-promoters [Omori *et al.*, 2004]. The Bhas 42 CTA does not require pretreatment with an initiator to detect tumor-promoting activity, and the assay period is 3 weeks instead of 6 weeks, as is the case with the BALB/c 3T3 two-stage CTA [Sakai, 2008]. Because of the increased sensitivity of cells to transformation, the assay scales such as the dish size, the dish number, the medium volume, the serum concentration in the cultures and the number of medium exchanges have been diminished, resulting in a reduction in the cost and labor expended. Asada *et al.* [2005] modified the Bhas 42 CTA such that it was capable of detecting tumor-initiating activity as well as tumor-promoting activity of chemicals. The current protocol consists of two assay components, the initiation assay and the promotion assay, to detect the tumor-initiating activity and the tumor-promoting activity of chemicals, respectively. In the initiation assay, the cells are inoculated at a low density and treated with a test chemical in the beginning of the assay period so that the target cells can undergo cell division several times before reaching contact inhibition of growth at confluence, and thus DNA damage is fixed as mutations in the genes. In the promotion

assay, the cells are seeded more densely than in the initiation assay, and the treatment with a test chemical is started at sub-confluence and continued beyond confluence, and thus longer than in the initiation assay.

HRI undertook a research project financed by NEDO to characterize, evaluate, refine and validate the Bhas 42 CTA. Under the project, the Bhas 42 CTA has been applied to 98 chemicals including carcinogens and non-carcinogens. The results have been published in Mutation Research [Sakai et al., 2010], the PDF file of the article is presented as Annex 16. The results confirm that the performance of the assay for the prediction of chemical carcinogenicity is superior or equivalent to that of conventional genotoxicity assays and that the assay is capable of detecting Ames-negative and Ames-discordant carcinogens in addition to Ames-positive carcinogens.

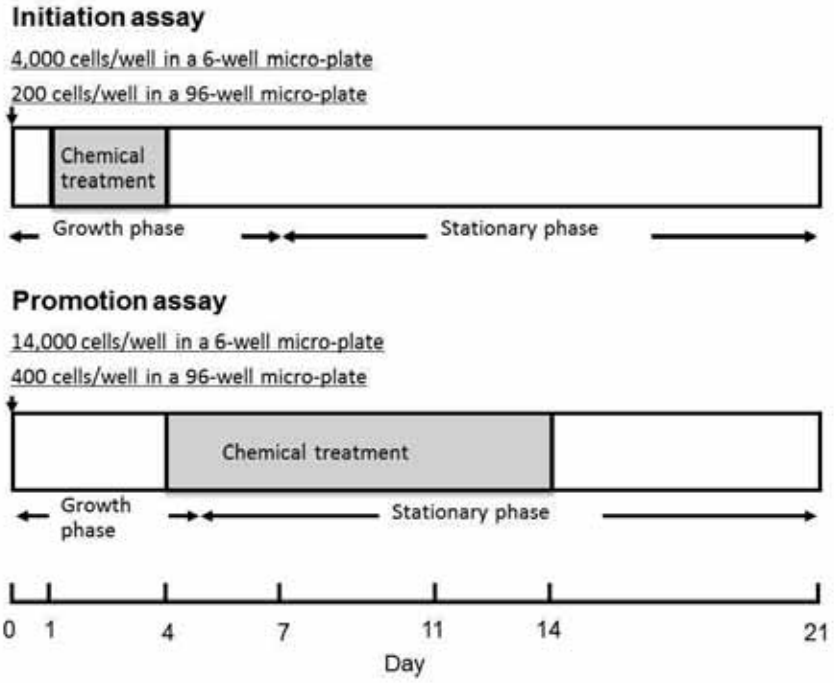

Fig. 1. Illustration of the Bhas 42 cell transformation assay methodology.

The above-mentioned studies on the Bhas 42 CTA, i.e. development, improvement and application to 98 chemicals, were performed using 6-well micro-plates (6-well method). Meanwhile, the Bhas 42 CTA using 96-well micro-plates (96-well method) has been developed in anticipation of its potential utility for high throughput automated applications. The assay procedures are fundamentally the same between the 6-well and 96-well methods as shown in Fig. 1. The cells are seeded in proportion to the respective well-sizes, 6-well and 96-well micro-plates, cultured and treated with a test chemical on the very same schedule and the induction of transformed foci is observed. Quantification of transformation frequency, however, is different, ie., in the 6-well method, the number of transformed foci in every well of a given plate is recorded, and in the 96-well method, the number of wells in a given plate having transformed foci is

recorded. Accordingly, the statistical analysis of each is different between the two methods. An analysis by multiple comparison using the Dunnett test is performed in the 6-well method and the chi-square test with Bonferroni adjustment is carried out in the 96-well method.

Three validation studies on the Bhas 42 CTA, a pre-validation study of the 6-well method, a validation study of the 6-well method and a validation study of the 96-well method, were carried out under the NEDO project.

The pre-validation study of 6-well method involved six laboratories and nine coded chemicals were examined. Each chemical was tested by three laboratories. The pre-validation study results have been published previously [Tanaka *et al.*, 2009], and are excluded from this report since a VMT was not organized for the pre-validation study. But its results are compared with those of other validation studies in section 5, Discussion.

The validation study of the 6-well method involved six laboratories from three countries, USA, Germany and Japan, and twelve coded chemicals were tested. Each chemical was assayed by three laboratories. This study is included in this validation report. The summary of results has been published by the Executive Team and the participating laboratories [Sakai *et al.*, 2011].

The validation study of 96-well method was proceeded in a stepwise manner (pre-validation phase, validation phase I and validation phase II) and included four laboratories in Japan. MCA and TPA were examined in the pre-validation phase, and coded chemicals were tested in validation phases I and II. A total of 25 chemicals were tested including duplicate chemicals between phases. Each chemical was assayed by four laboratories in the pre-validation phase and validation phase I studies, and by two laboratories in the validation phase II studies.

The Bhas 42 cells were established by Sasaki *et al.* in the National Institute of Health Sciences, Japan [Sasaki, *et al,* 1988]. The cells were propagated and at present are stored at HRI, FDSC (Hadano, Japan) and Japanese Collection of Research Bioresources (JCRB) Cell Bank, National Institute of Biomedical Innovation (Osaka, Japan). Those cells have been confirmed to be free from mycoplasma and adequate for transformation assays. They are distributed to worldwide laboratories through Health Science Research Resources Bank (HSRRB, Osaka, Japan) [http://cellbank.nibio.go.jp/cellbank_e.html].

The current report, which was prepared by HRI with the support of the VMT, presents the outcome of the validation studies on the 6-well method and the 96-well method in the Bhas 42 CTA under the NEDO Project.

1.2 Objective and goals

The aim of these studies was to scientifically validate the Bhas 42 CTA in formal inter-laboratory studies in order to assess the within- and between-laboratory reproducibility of the assay, its transferability and its predictive capacity. The ultimate goal is to demonstrate its utility for adoption as an OECD Test Guideline that could be

used as a component of a test battery for the prediction of chemical carcinogenicity. In this respect, the Bhas 42 CTA should supplement conventional genotoxicity tests with the ability of detecting non-genotoxic carcinogens including tumor-promoters. The present objective was to assess the transferability, reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of this test system with a set of coded chemicals whose carcinogenicities are known *in vivo*. It is recommended that both of the 6-well method and the 96-well method should be integrated in the OECD test guideline so as to call attention to the availability of both variations and their different potential applications (i.e. manual procedures or automated procedures).

1.3 Intended use

The Bhas 42 CTA is expected to have its greatest impact as a component of a battery with genotoxicity assays for the prediction of chemical carcinogenicity. No single genotoxicity assay is capable of predicting or assessing all chemicals for their carcinogenic potential. DNA damage is not the only mechanism involved in carcinogenesis. Some carcinogens are not genotoxic [Hernández *et al.*, 2009]. Non-genotoxic events, which are at least initially independent of direct DNA damage, can play a critical role in chemical carcinogenesis. The strength of the Bhas 42 CTA is that it (a) can detect both genotoxic and non-genotoxic carcinogens [Asada *et al.*, 2005]、(b) is sensitive to tumor-promoters [Omori *et al.*, 2004, 2005], (c) discriminates tumor-initiating activity and tumor-promoting activity of carcinogens [Sakai, 2008]. When applied to mycotoxins derived from *Fusarium* and related substances, it was expected that the Bhas 42 CTA would show a good correlation with an *in vivo* two-stage carcinogenesis test using experimental animals for estimation of the tumor-promoting activity [Sakai et al., 2007]. In the in-house assay of 98 chemicals, the Bhas 42 CTA could identify a considerable number of carcinogens which are negative or discordant in the Ames assay [Sakai *et al.*, 2010].

1.4 Current use

The Bhas 42 cells have been used for academic studies in Japan. The role of cytokines in tumor-promotion and the mechanisms of stomach cancer caused by *Helicobacter pylori* have been investigated utilizing the Bhas 42 cells as well as BALB/c 3T3 cells, though the protocols are different from those proposed in the present validation report [Komori *et al.*, 1993; Suganuma *et al.*, 1999, 2001,2005]. The Bhas 42 CTA has been applied by researchers in Kanagawa Prefectural Institute of Public Health (Japan) and National Institute of Health Sciences (Japan) to abietic acid and dehydroabietic acid, which are the components of resin from a needle–leaf tree and possible contaminants in paper and paperboard for food contact use, and those resin components have been shown to have positive activity in the promotion assay [Ohmori and Kawamura, 2009]. In-house use of the Bhas 42 CTA by industry for safety assessment of chemicals, pharmaceuticals, cosmetics, pesticides and biocides is limited, since, at present, such companies tend to employ the conventional genotoxicity assays that are accepted by regulatory authorities. It is expected that the utilization of Bhas 42 CTA will be expanded as a consequence of the development of an OECD Test Guideline.

1.5 Recent research

The Bhas 42 CTA was assessed in-house against 98 chemicals in order to characterize it and evaluate its performance for the detection of chemical carcinogens [Sakai *et al.*, 2010]. When the assay results were compared with the existing genotoxicity data, the Bhas 42 CTA could detect a considerable number of the carcinogens that are negative or discordant in the Ames test, and most of those Ames-negative and Ames-discordant carcinogens were detected by the promotion assay in the Bhas 42 CTA. This fact suggested that the Bhas 42 cells behaved as initiated cells in CTA. The performance indices were calculated from the assay results of 52 carcinogens and 37 non-carcinogens (The other nine chemicals were of unknown carcinogenicity). The concordance was 78%, sensitivity 73%, specificity 84%, positive predictivity 86%, negative predictivity 69%, false negatives 27% and false positives 16%. Of these values, the concordance, specificity, negative predictivity and false positives were superior to those of conventional genotoxicity tests and the other performance indices were equivalent to those of conventional genotoxicity tests. From these results, the authors concluded that the overall accuracy of prediction of chemical carcinogenicity would be improved by introducing the Bhas 42 CTA into the standard battery of *in vitro* assays.

1.6 Published data on inter-laboratory reproducibility

No formal between-laboratory trial had been previously conducted to assess the transferability and reproducibility of the Bhas 42 CTA and its applicability to the prediction of chemical carcinogenicity. However, there were two inter-laboratory collaborative studies conducted prior to the validation studies presented in the current report. The first study, which involved 14 participating laboratories, was conducted on the promotion assay by the Non-genotoxic Carcinogen Study (NGCS) Group in the Environmental Mutagen Society of Japan [Omori *et al.*, 2005]. After confirmation that these laboratories could obtain positive results with two tumor-promoters, TPA and lithocholic acid, 12 coded chemicals were assayed. Each chemical was tested in four laboratories. For eight chemicals, all four laboratories obtained consistent results, and for two of the other four chemicals, one of the four laboratories showed inconsistent results. It should be noted that only the promotion assay of Bhas 42 CTA had been developed at this stage. The second study was carried out by HRI as the lead laboratory, after the Bhas 42 CTA protocol was modified so as to detect tumor-initiating activity in addition to tumor-promoting activity. This second study was conducted as a part of the NEDO Project and is referred to as “a pre-validation study of the 6-well method” in this report. The pre-validation study has been published [Tanaka *et al.*, 2009], and the results are compared with those of the validation studies on the 6-well method and on the 96-well method in section 5 General discussion.

1.7 Patents

The test method and the cells employed in this study are in the public domain and have not been patented.

2. Organization of the study

2.1 Executive team

The current validation study was carried out by HRI as a part of the project entrusted from NEDO.

Executive Team
Noriho Tanaka (Director), HRI, FDSC (Kanagawa, Japan)
Makoto Umeda, HRI, FDSC
Ayako Sakai, HRI, FDSC
Assistant
Shoko Arai, HRI, FDSC (until December 31, 2010)

2.2 Validation management team

Validation management team

Chairman	Makoto Hayashi (Biosafety Research Center, Foods, Drugs and Pesticides (BSRC), Shizuoka, Japan)
Representative of JaCVAM	Hajime Kojima (Japanese Center for the Validation of Alternative Methods (JaCVAM), National Institute of Health Sciences (NIHS), Tokyo, Japan)
Representative of ECVAM	Raffaella Corvi (European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy)
Representative of ICCVAM	William Strokes (Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, National Institute of Environmental Health Sciences, North Carolina, USA)
Representative of ICCVAM	(Provisional, 2008) Abigail Jacobs (Food and Drug Administration, Maryland, USA)
Expert	Takeshi Morita (NIHS)
Expert	Leonard Schechtman (Innovative Toxicology Consulting LLC, Florida, USA)
Statistician	Masaya Suzuki (BSRC) until July 31, 2010

*Advisory Committee**

Chairman	Makoto Hayashi (NIHS; currently BSRC)
Expert	Raffaella Corvi (ECVAM)
Representative JaCVAM	Hajime Kojima (JaCVAM, NIHS)
Expert	Takeshi Morita, (NIHS)
Statistician	Sebastian Hoffmann, (ECVAM; currently seh consulting + services, Germany)

* This Committee was active in the beginning of the validation study on Bhas 42 CTA using 6-well micro-plates and reorganized to eventually become the formal VMT on 2008.10.29.

2.3 Participating laboratories

The validation study of the 6-well method included six laboratories, four in Japan, one from the USA and one from Germany; the validation study of the 96-well method included four Japanese laboratories. Those laboratories are listed below, and their data are shown in Tables and Figures under lab identification (ID) codes presented in Table 1. HRI, Mitsubishi Chemical Medience (Mitsubishi), BSRC and Japan Bioassay Research Center (JBRC) had considerable expertise in the Bhas 42 CTA, since they had participated in the pre-validation study on the Bhas 42 CTA (6-well method) [Tanaka et al., 2009]. Harlan Cytotest Cell Research (Harlan) had a little experience and BioReliance had limited knowledge of the Bhas 42 system, although they had taken part in the ECVAM validation study on the BALB/c 3T3 and SHE CTAs. The latter two laboratories were included in order to involve "naive" laboratories experienced in CTAs but not specifically in the Bhas 42 CTA.

6-well method validation study

Laboratory I: HRI (Kiyoshi Sasaki, Dai Muramatsu, Kumiko Hayashi, Nobuko Endou, Sachiko Kuroda; Kanagawa, Japan)
Laboratory II: Mitsubishi (Hideki Hirose, Nana Ishii; Tokyo, Japan)
Laboratory III: BSRC (Fukutaro Mizuhashi, Sawako Kasamoto, Miho Nagai; Shizuoka, Japan)
Laboratory IV: JBRC (Masumi Asakura; Kanagawa, Japan)
Laboratory V: BioReliance Corporation (Kamala Pant, Shannon W. Bruce, Jamie E. Sly; Rockville, Maryland, Japan)
Laboratory VI: Harlan (Albrecht Poth, Susanne Bohnenberger, Thorsten Kunkelmann; Rossdorf, Germany)

96-well method validation study

Laboratory 1: BSRC (Fukutaro Mizuhashi, Sawako Kasamoto, Miho Nagai, Maiko Takai; Shizuoka, Japan)
Laboratory 2: JBRC (Masumi Asakura; Kanagawa, Japan)
Laboratory 3: HRI (Kiyoshi Sasaki, Dai Muramatsu, Nobuko Endou; Kanagawa, Japan)
Laboratory 4: Mitsubishi (Nobuhiko Tashiro, Nana Ishii; Tokyo, Japan)

Table 1
Participating laboratories, the validation studies to which they contributed, and their identification (ID) codes in respective studies

Laboratory	6-Well method	96-Well method		
		Pre-validation	Phase I	Phase II
HRI	Lab I	Lab 3	Lab 3	Lab 3
Mitsubishi	Lab II	Lab 4	Lab 4	--*
BSRC	Lab III	Lab 1	Lab 1	Lab 1
JBRC	Lab IV	Lab 2	Lab 2	Lab 2
BioReliance	Lab V	--	--	--
Harlan	Lab VI	--	--	--

* Not participated

2.4 Quality assurance schemes employed by the testing laboratories

The present study was conducted under conditions that closely approximated Good Laboratory Practice (GLP) by all laboratories.

2.5 Meetings held

27/08/2007 (HRI)
Workshop for technical training of the Bhas 42 CTA using 6-well micro-plates (6-well method)*
Albrecht Poth: RCC (Harlan)
Kamala Pant: BioReliance
Kiyoshi Sasaki: HRI.
Subjects: explanation of the Bhas 42 CTA and its protocol, technical training and judgment of transformed foci.
* Since the other participating laboratories had experience in the 6-well method, they did not take part in this workshop.

10-12/01/2008 (HRI)
Meeting of Advisory Committee
Makoto Hayashi: NIHS
Raffaella Corvi: ECVAM, Italy
Hajime Kojima: JaCVAM, NIHS
Takeshi Morita: NIHS
Sebastian Hoffmann: ECVAM, Italy
Plus participating laboratories
Subjects: protocol for the 6-well method and consultation on statistical analysis of the data from the 6-well method validation study.

14/03/2008 (HRI)
Meeting on appropriate statistics for the Bhas 42 CTAs using 6-well micro-plates and 96-well micro-plates
Sebastian Hoffmann (ECVAM)
Kiyoshi Sasaki (HRI)
Ayako Sakai (HRI)
Kumiko Hayashi (HRI)
Subject: consultation on statistical methods suitable for data analysis in the 6-well method and the 96-well method.

18/04/2008 (HRI)
Meeting on the validation study of Bhas 42 CTA using 96-well micro-plates (96-well method)
Hajime Kojima (JaCVAM)
Noriho Tanaka (HRI)
Ayako Sakai (HRI)
Subject: providing a framework for the validation study on the 96-well method

15/10/2008 (Kyoto Univ.)
Meeting on appropriate statistics for the 96-well method
Takashi Omori (Kyoto Univ.)
Shoko Arai (HRI)
Subjects: preparation of data sheet for the 96-well method validation study and construction of a database for the study

29-30/10/2008 (HRI)
1st VMT Meeting
VMT members: Makoto Hayashi, Raffaella Corvi, Hajime Kojima, Takeshi Morita, Abby Jacob
Adviser: Takashi Omori (Kyoto Univ. 29 October)
Participating laboratories: HRI, Mitsubishi, BSRC, JBRC
Subjects: study plan, protocol and test chemicals for the validation study of 96-well method.

18/11/2008 (HRI)
Workshop for technical inter-laboratory transfer of 96-well method protocol
Participating laboratories: HRI, Mitsubishi, BSRC, JBRC
Subjects: explanation of protocol and technical training

24/12/2008 (BSRC)
Meeting on statistics
Takashi Omori (Kyoto Univ.)
Masaya Suzuki (BSRC)
Shoko Arai (HRI)
Subject: decision on the method of statistical analysis for the data in the validation study of 96-well method

17/01/2009 (Kyoto Univ.)
Meeting on statistics
Takashi Omori (Kyoto Univ.)
Masaya Suzuki (BSRC)
Shoko Arai (HRI)
Subject: analysis of the results in pre-validation phase of the 96-well method validation study

26/01/2009 (HRI)
Joint meeting of Japanese VMT and participating laboratories about the results of pre-validation phase
VMT members: Makoto Hayashi and Hajime Kojima
Statistics team: Masaya Suzuki and Shoko Arai
Participating Laboratories: HRI, Mitsubishi, BSRC, JBRC
Subject: evaluation of results in the pre-validation phase, harmonization of focus identification and counting among laboratories, adoption of assay acceptance criteria* and planning of phase I in the validation study of 96-well method
* In accordance with the discussion at this joint meeting, the protocol for the 96-well method was revised (Ver. 3, 2009.1.26)

15/10/2009 (HRI)
Joint meeting of Japanese VMT and participating laboratories regarding the results of phase I

VMT members: Makoto Hayashi and Takeshi Morita
Statistics team: Masaya Suzuki and Shoko Arai
Participating Laboratories: HRI, Mitsubishi, BSRC, JBRC
Subject: preliminary evaluation of the results in the phase I validation study of 96-well method

9-10/11/2009 (NEDO Shiroganedai Training Center)
2nd VMT Meeting
VMT members: Makoto Hayashi, Hajime Kojima, Raffaella Corvi, William Stokes, Leonard Schechtman, Takeshi Morita:
Participating laboratories: HRI, Mitsubishi, BSRC, JBRC
Subjects: evaluation of the phase I results and planning of phase II in the 96-well method validation study.

6-7/10/2010 (NEDO Kawasaki Head Office)
3rd VMT Meeting
VMT members: Makoto Hayashi, William Stokes, Raffaella Corvi, Hajime Kojima, Leonard Schechtman, Takeshi Morita
Participating laboratories: HRI, BSRC, JBRC
Subjects: evaluation of the results in the 96-well method phase II validation study and approval of the 96-well method; evaluation of the results in the 6-well method validation study and approval of the 6-well method

15/9/2011 (Keisuikan, Sinmachi-Campus, Doshisha Univ., Kyoto)
Meeting for international Bhas 42 CTA validation study
VMT member: Makoto Hayashi, Hajime Kojima, Raffaella Corvi, Takeshi Morita and Leonard Schechtman
The other participants: Noriho Tanaka, Makoto Umeda, Ayako Sakai, Kiyoshi Sasaki and Koji Yamakage
Subjects: Future schedule on the Bhas 42 CTA to develop OECD Test Guideline: VMT members and the other participants agreed that the Bhas 42 CTA validation study report should undergo peer review by the ESAC through the ECVAM process.

3 Validation Study on the 6-well method

The summary of this validation study has been published by the executive team and the participating laboratories [Sakai *et al.*, 2011].

3.1 Study design and chemicals tested

3.1.1 Study design

In the validation study of 6-well method, six laboratories participated. Twelve coded chemicals were tested. Each chemical was assayed by three laboratories so that the inter-laboratory reproducibility could be assessed. The intra-laboratory reproducibility was retrospectively assessed by comparing data between this validation study and pre-validation study reported by Tanaka *et al.* [2009] and also between this validation study and in-house HRI studies [Sakai et al., 2010]. The transferability of the test protocol was determined by comparing the data from HRI, which had developed the current protocol, with those from the other laboratories in this validation study.

A workshop on the technical training necessary for the proper conduct of the Bhas 42 CTA using 6-well micro-plates was held by HRI on 27 August, 2007 before starting the validation study of the 6-well method. The study directors of Harlan and BioReliance participated in the workshop to learn the protocol, the assay procedures and judgment criteria for identifying transformed foci. Since the other participating laboratories had experience in the pre-validation study of 6-well method [Tanaka *et al.*, 2009], they did not take part in this workshop.

3.1.2 Chemicals tested and negative and positive controls

The chemicals were selected so that 2/3 were carcinogens including non-genotoxic ones and 1/3 were non-carcinogens (Tables 2 and 3). Ideally, it would have been good to include chemicals with low potency. However, it is difficult to assess precise potency based on available data. It could be argued that the process of dilution of test chemicals resulting in reduced concentrations may approximate potency reduction so as to mimic the testing of low potency chemicals. The chemical properties and classes of 12 test chemicals and two positive controls are presented in Annex 14. The test chemicals, their Chemical Abstract Service registry numbers (CAS no.), their code names for the validation study and the laboratories that took charge of each chemical are shown in Table 2. Each of the participating laboratories independently determined the appropriate solvent (either water or DMSO) for the test chemicals with the exception of dibenz[*a,h*]anthracene and mezerein. Solvent determination was based upon the results of preliminary solubility testing. The test chemical dose ranges for the transformation assays were selected according to dose setting procedures using the cell growth assays. For the two chemical exceptions, the solvent, dimethyl sulfoxide (DMSO) was pre-selected and the maximum concentrations in culture medium of each were likewise pre-assigned (≤10 μg/mL and ≤0.01 μg/mL, respectively). These amounts were predetermined due to the expense and limited availability of each chemical.

MCA and TPA were chosen as positive controls for the initiation assay and the

promotion assay, respectively, because they induce a strong positive response in the Bhas 42 CTA, have been used as positive controls since the development of Bhas 42 CTA [Omori *et al.*, 2004; Asada *et al.*, 2005], and are common positive controls in the BALB/c 3T3 and C3H10T1/2 CTA two-stage systems. The historical data of these chemicals as positive controls were available at the lead laboratory, HRI. The doses of MCA and TPA (1 μg/mL and 0.05 μg/mL, respectively) to be used as positive controls were determined based on the HRI historical data.

Table 2
Test chemicals, code names and laboratory allocation in the 6-well method validation study.

Chemical	CAS no.[a]	Carcinogenicity[b]	Code	Allocation to lab					
				I	II	III	IV	V	VI
2-Acetylaminofluorene	53-96-3	+	A	○			○		○
Cadmium chloride	10108-64-2	+	F	○	○	○			
Dibenz[*a,h*]anthracene*	53-70-3	+	C	○	○			○	
Lithocholic acid	434-13-9	TP[c]	E	○			○		○
Methapyrilene HCl	135-23-9	+,TP	H	○	○		○		
Mezerein*	34807-41-5	TP	G	○		○	○		
Sodium arsenite	7784-46-5	+	D	○		○			○
o-Toluidine HCl	636-21-5	+	B	○				○	○
Anthracene	120-12-7	–	I	○	○				○
L-Ascorbic acid	50-81-7	–	L	○	○		○		
Caffeine	58-08-2	–	K	○				○	○
D-Mannitol	69-65-8	–	J	○		○	○		
3-Methylcholanthrene	56-49-5	+	Positive control for initiation assay						
TPA[d]	16561-29-8	+	Positive control for promotion assay						

[a] Chemical abstract service registry number.
[b] +, carcinogen; –, non-carcinogen; TP, tumor-promoter
[c] Tumor-promoter
[d] 12-*O*-tetradecanoylphorbol-13-acetate.
* The solvent (DMSO) and the highest concentrations in the culture medium (dibenz[*a,h*]anthracene, 10 μg/mL; mezerein, 0.01 μg/mL) were designated in advance.

Data regarding genotoxic and carcinogenic activity, including tumor-promoting activity, of the test chemicals were garnered from (a) the Detailed Review Paper on Cell Transformation Assays for Detection of Chemical Carcinogens, OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 31 (DRP 31) [OECD, 2007], (b) a review by Kirkland *et al.* [2005], and (c) other original publications [Reddy *et al.*, 1975; Lijinsky *et al.*, 1992]. In DRP 31, the reference data to *in vivo* carcinogenicity are from well-regarded sources, such as IARC, Gold & Zeiger [1997], NTP databases. The carcinogenicity and genotoxicity of the test chemicals are presented in Table 3.

The chemicals tested were purchased and coded by the HRI chemical repository supervisor, who was independent of the participating laboratories and the executive team, and distributed by him to the chemical repository officer of each individual participating laboratory. The suppliers, catalog numbers and lot numbers of the test chemicals are presented in Annex 1 (Table 54).

Table 3
Genotoxicity and carcinogenicity of the test chemicals in the 6-well method validation study.

Chemicals	Genotoxicity[a]				IARC class	Carcinogenicity[b]
	In vitro			*In vivo*		
	Ames[c]	ML[d]	CA[e]	MN[f]		
2-Acetylaminofluorene	+	+	+	+		+
Cadmium chloride	–	+	+/–	–	1	+
Dibenz[a,h]anthracene	+	+	+		2A	+
Lithocholic acid	–	+/–	–			TP[g]
Methapyrilene HCl	–	+/–	+			+, TP[h]
Mezerein	–					TP
Sodium arsenite	–	+		+	1	+
o-Toluidine HCl	+/–					+
Anthracene	+/–	+	–		3	–
L-Ascorbic acid	+w/–		–	+		–
Caffeine	–		+	+	3	–
D-Mannitol	–	–	–	–		–

[a] Source: DRP 31 [OECD, 2007] and a review by Kirkland *et al.* [2005]: +, positive; –, negative; +/–, positive and negative results in DRP 31; +w, weakly positive.
[b] Source: DRP 31 [OECD, 2007] and original papers: +, carcinogen; –, non-carcinogen; TP, tumor-promoter
[c] Ames test
[d] Mouse lymphoma test
[e] Chromosomal aberration test
[f] Micronucleus test
[g] Reddy *et al.*, [1975].
[h] Lijinsky *et al.*, [1992].

3.1.3 Timeline

27/08/2007	Workshop for technical transfer of Bhas 42 CTA using 6-well micro-plates.
1/10/2007	Start of the 6-well method validation study
1-26/10/2007	Discussion, questions and answers about the protocol exchanged between HRI and participating laboratories (via e-mail)
26/10/2007	Determination of the protocol for the 6-well method validation study
1/11/2007	Shipment of test chemicals to Japanese laboratories
12/11/2007	Shipment of test chemicals to overseas laboratories
10-12/02/2008	Meeting of Advisory Committee
13/10/2009	Last submission of study results: Data submission from Lab 6 was delayed. The other laboratories reported all the data including re-test by October, 2008.
28/10/2009	Decoding of the test chemicals.
6-7/10/2010	Evaluation and approval of the study results by VMT (in the 3rd VMT Meeting)

15/9/2011 Meeting for the international Bhas 42 CTA validation study: Future schedule on the Bhas 42 CTA to develop OECD/TG: VMT members and the other participants agreed that the Bhas 42 CTA validation study report should undergo peer review by the ESAC through the ECVAM process.

3.2 Protocol

The transformation assay was performed according to the procedures as described previously [Sakai *et al.*, 2010]. The detailed test protocol used in the 6-well method validation study is presented in Annex 2. Fig. 2 shows a schematic representation of the Bhas 42 CTA protocol. The protocol consists of two assay components, the initiation assay to detect tumor-initiating activity and the promotion assay to detect tumor-promoting activity. The cell growth assays were carried out prior to the transformation assays to determine the doses applicable to the cell transformation tests. Cell growth assays were also performed concurrently with every transformation assay (every initiation and promotion assay) to estimate the effect of each treatment on the cell growth and survival.

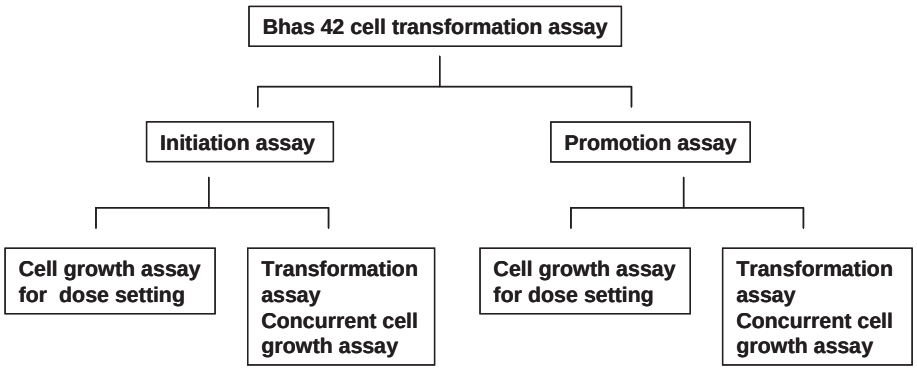

Fig. 2. Schematic of the Bhas 42 cell transformation assay protocol.

3.2.1 Preparation

3.2.1.1 Cells

The Bhas 42 cells from the same cell pool at passage 17 were distributed by HRI to the participating laboratories. We confirmed that Bhas 42 cells at passage 18 retained the transfected v-Ha-*ras* gene and expressed the introduced mRNA at a level similar to that of the c-Ha-*ras* gene.

3.2.1.2 Materials

Eagle's minimum essential medium supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin and 10% fetal bovine serum (FBS) (M10F) was used for cell expansion (for mother cultures). Dulbecco's modified Eagle's medium/Ham's F12 supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin and 5% FBS (DF5F) was used for the transformation assays and cell growth assays.

The batch of FBS used throughout this validation study by all participating laboratories was selected by HRI, having been prescreened to produce a minimal number of transformed foci in the Bhas 42 cells treated with the solvent control, DMSO, and a substantially elevated number of transformed foci in the cells treated with the positive control, MCA (1 µg/mL) or TPA (0.05 µg/mL).

The all materials and reagents used are shown in the test protocol presented in Annex 2, and their suppliers, catalog numbers and lot numbers are also listed.

3.2.1.3 Cell stocks

The distributed Bhas 42 cells were thawed, expanded and cryopreserved in aliquots so as to generate a large batch of that cell pool in the respective laboratories for use in the transformation assays. The cells were cultivated in M10F in a humidified 5% CO_2 incubator at 37˚C and when the cultures reached about 70% confluence they were subcultured using 0.25% trypsin in order to maintain a sub-confluent state. Within two passages after thawing, the cultured cells were suspended at 5 x 10^5 cells/mL in fresh M10F containing 5% DMSO, frozen in 0.5 mL volumes at -80 °C and stored in liquid nitrogen. The transformation assays were always started from this frozen cell stock. The cell growth assay were not necessary performed using cells from this frozen stock, however, properly passaged cells were always used in such the cell growth assays, which were conducted for the determination of test chemical concentrations.

3.2.1.4 Chemicals

Test chemicals were dissolved or suspended in DMSO or sterile distilled water. The vehicle of choice was decided by each laboratory depending on its respective solubility test. The vehicles used and their final concentrations in the medium are presented in the tables of assay results (Tables 4-15, below).

MCA and TPA were dissolved in DMSO and served as positive controls for the initiation assay and the promotion assay, respectively. The same lots of each were purchased by each of the participating laboratories.

3.2.2 Initiation assay

3.2.2.1 Cell growth assay

A cell suspension at 2,000 cells/mL in DF5F was distributed into each well of 6-well micro-plates in a 2 mL volume (4,000 cells/well, day 0). Three wells were prepared for each treatment group. At 24 h after seeding, the culture medium was replaced with medium containing a test chemical at various concentrations or vehicle alone, or a test

chemical dissolved in DMSO or sterile distilled water was added directly to the culture medium in the well without medium exchange. On day 4, the medium containing the test chemical was replaced with the fresh DF5F. On day 7, the cells were fixed with 10% formalin or methanol and stained with a 0.1% crystal violet (CV) solution in 5% ethanol. CV was extracted from the stained cells with a solution containing 0.02 mol/L hydrochloric acid in 50% ethanol. The optical density of the extract was measured at a wavelength between 540 and 570 nm. The relative cell growth of cultures treated with a chemical was calculated as follows:

relative cell growth = [(At – Ab)/(Ac – Ab)] x 100

where At was the absorbance of CV extract from a well with the chemically-treated cells, Ac was the absorbance of CV extract from a well with the solvent-treated cells and Ab was the absorbance of CV extract from a well with medium only.

3.2.2.2 Dose-setting for the transformation assay

Five or more concentrations were selected based on the results of cell growth assays, so that the concentrations used covered a range from little or no toxicity to a toxicity level that resulted in less than 20% survival compared to the control cultures. In practice, the concentrations generally employed included one dose below the no-effect level (NOEL), two doses between the NOEL and the 50% inhibitory concentration (IC_{50}) and two doses between the IC_{50} and the 90% inhibitory concentration (IC_{90}).

The highest concentration of test chemical in the medium was 5 mg/mL in this study.

3.2.2.3 Transformation assay to examine initiating activity

One tube of the frozen Bhas 42 cells was rapidly thawed and grown in M10F up to about 70% confluence and then subcultured in DF5F to reach about 70% confluence again. Thereafter, cells were cultured in DF5F. The cells were trypsinized and suspended at a density of 2,000 cells/mL and seeded into each well of 6-well micro-plates in 2 mL volumes (4,000 cells/well, day 0). Nine wells were prepared per concentration, of which six wells were reserved for the transformation assay and three were reserved for the concurrent cell growth assay. At 24 h after seeding, the cells were treated by the addition to the cultures of a test chemical solution or the vehicle alone, or by complete replacement of the medium containing either the test chemical or the solvent vehicle. The treatment in the initiation phase was continued for 72 h. Following the exposure period, all treatment media were removed and the cells were refed with medium without the test chemical (day 4) and subsequently cultured in the normal medium until day 21, receiving medium exchanges on day 7, day 11 and day 14. The cells were then fixed with methanol and stained with Giemsa's solution. Each assay also included MCA (1 μg/mL) as the positive control.

3.2.3 Promotion assay

3.2.3.1 Cell growth assay

Two milliliter of a cell suspension at 7,000 cells/mL was seeded into each well (14,000 cells/well, day 0), and on day 4, the culture medium was replaced with a medium containing a test chemical. On day 7, the cells were fixed and stained with CV, and the

relative cell growth of cultures treated with a chemical was calculated according to the procedures as described in 3.2.2.1.

3.2.3.2 Dose-setting for the transformation assay

Five or more concentrations were selected based on the results of cell growth assays.

For the chemicals that exhibited marked growth enhancement, the test concentrations were selected to cover a range from little effect on cell growth to growth enhancement. In practice, the concentrations generally employed included one dose below the NOEL, three doses resulting in growth enhancement, and one more higher dose that produces weak growth inhibition. For the chemicals that did not induce marked growth enhancement, the test concentrations were selected to range from a dose two or three levels lower than the no-effect concentration to that showing a survival less than 50%. In practice, the concentrations generally employed included two doses below NOEL, two doses between NOEL and IC_{50}, and one dose above IC_{50}.

For a chemical that caused a sharp decline of cell growth within a narrow concentration range, one or two additional doses outside the established concentration range were set up as a precaution against the fluctuation of cell response among experiments.

The highest concentration of test chemical in the medium was 5 mg/mL in this study.

3.2.3.3 Transformation assay to examine promoting activity

The promotion assay was carried out in the same manner as the initiation assay (3.2.2.3) except for the following steps. The cells were seeded at a density of 7,000 cells/mL (14,000 cells/well, day 0) and cultured for 4 days without a medium exchange. On day 4, day 7, and day 11, the culture medium was replaced with fresh medium containing a test chemical or vehicle alone and the treatment in the promotion phase was continued until day 14 (for a total of 10 days). The cells were then cultured in the normal medium without the test chemical for one week until day 21. Each assay also included TPA (0.05 μg/mL) as the positive control.

3.2.4 Focus count and statistical analysis

The transformed foci were judged on the basis of the morphological characteristics: (a) more than 100 cells, (b) spindle-shaped cells whose morphology was distinctly different from the contact-inhibited monolayer cells, (c) deep basophilic staining, (d) random orientation of cells, especially at the edge of foci, (e) dense multilayering of cells, and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. The number of transformed foci in every well was recorded and a statistical analysis for the increase in the number of the transformed foci produced by a test chemical was performed by multiple comparison using the one-sided Dunnett test ($p<0.05$). The statistical significance of positive controls was evaluated by the t-test or Aspin-Welch test depending on the result of the F-test for homoscedasticity (homogeneity of variance). "Toxic" was recorded for wells in which the cells were not confluent at the end of the transformation assay because of cytotoxicity resulting from chemical treatment.

A Photo Catalog used for judging the morphology of foci in the Bhas 42 CTA is attached (Annex 3).

3.2.5 Judgment

The results in the Bhas 42 CTAs were judged positive when there existed two or more sequential doses that induced statistically significant increases in the number of transformed foci, and negative when there was no dose showing a statistically significant increase of foci. When the statistically significant increase was at only one dose, the assay result was regarded as equivocal, and then the initiation or promotion assay together with the concomitant cell growth assay was repeated including the positive dose in the first assay. The chemical was judged to be positive if a statistically significant increase in the number of transformed foci resulted at one or more concentrations in the second assay.

3.2.6 Assay acceptance criteria

In the 6-well method validation study, assay acceptance criteria were not described in the protocol. Instead of acceptance criteria, the following determining factors, which are described in protocol section D. Judgment, were observed: “When low transformation frequency in the positive control or high transformation frequency in the negative control is obtained, the experiment should be repeated using optimized test conditions. When biologically suspicious results are obtained, a re-experiment may be requested after discussion.”

3.3 Results

This international validation study of the Bhas 42 CTA using 6-well micro-plates was carried out on twelve coded chemicals in total for the initiation and promotion assays. Each chemical was examined by three laboratories. The data for the negative and positive controls obtained in individual laboratories are collected and illustrated in Figures 3 and 4. The results of each individual test chemical in the Bhas 42 CTAs, including the respective concurrent cell growth assays are presented in Tables 4 - 15 and in Figures 5 - 16. The raw data submitted by individual laboratories are presented in Annex 4.

3.3.1 Quality assurance aspects

All the data were sent to Noriho Tanaka and Ayako Sakai. Since at that stage there was no acceptance criteria in the protocol, all the data submitted from the participating laboratories were accepted, and are presented and discussed in this validation study report. In certain instances, specific experiments were repeated by a particular laboratory. The data from both the first and repeated experiments are presented for each chemical, including the reason why the experiment was repeated. The statistical analysis was carried out by A. Sakai.

3.3.2 Negative and positive controls

The numbers of transformed foci produced in the negative (DMSO) and positive controls are presented in Tables 4-15 as part of the assay results derived for each test chemical. In Fig. 3, those control values are plotted pairwise, assay by assay, and the pairs are clustered by a given laboratory. The number of transformed foci was statistically analyzed by t-test or Aspin-Welch test and there were significant differences between positive controls and the corresponding negative controls in all the initiation and promotion assays ($p<0.05$), although the absolute number of foci induced by the positive control varied between laboratories and sometimes within laboratories. In Fig 4, the numbers of transformed foci in the negative and positive controls are averaged laboratory by laboratory.

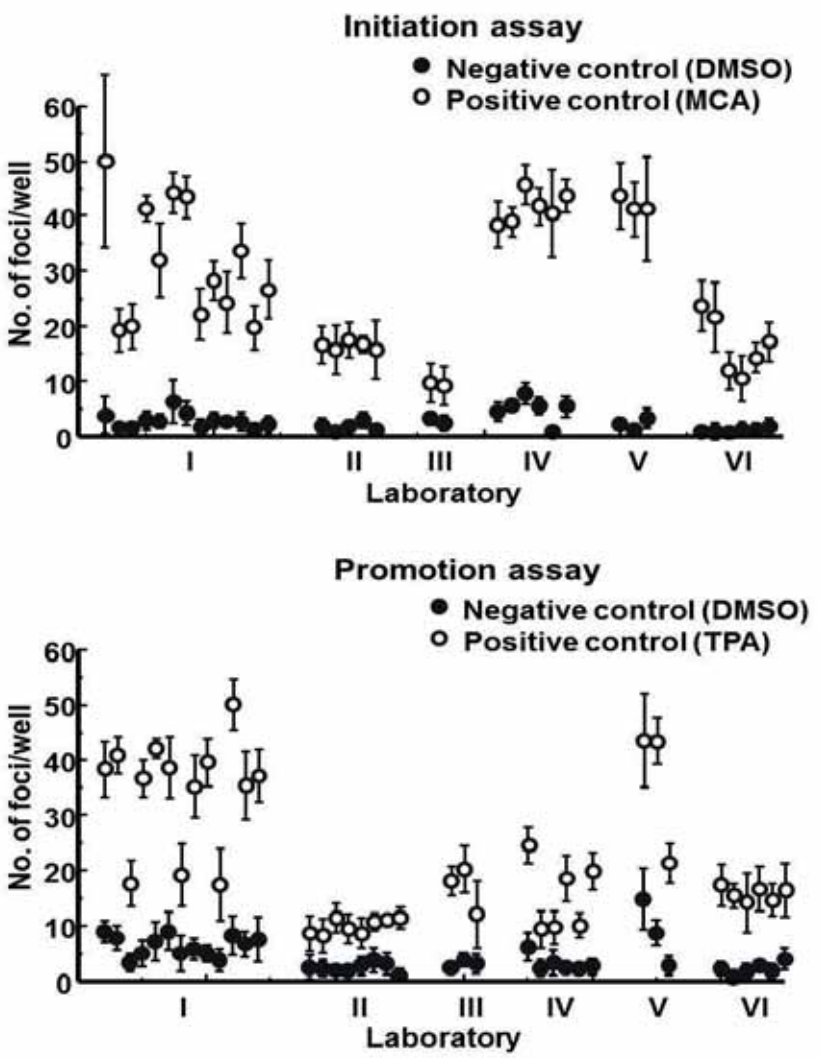

Fig. 3. Number of transformed foci per well in the negative and positive controls of the Bhas 42 cell transformation assay in the 6-well method validation study.
Negative and positive controls are paired assay by assay and the pairs are clustered by a given laboratory. Bars indicate standard deviation.

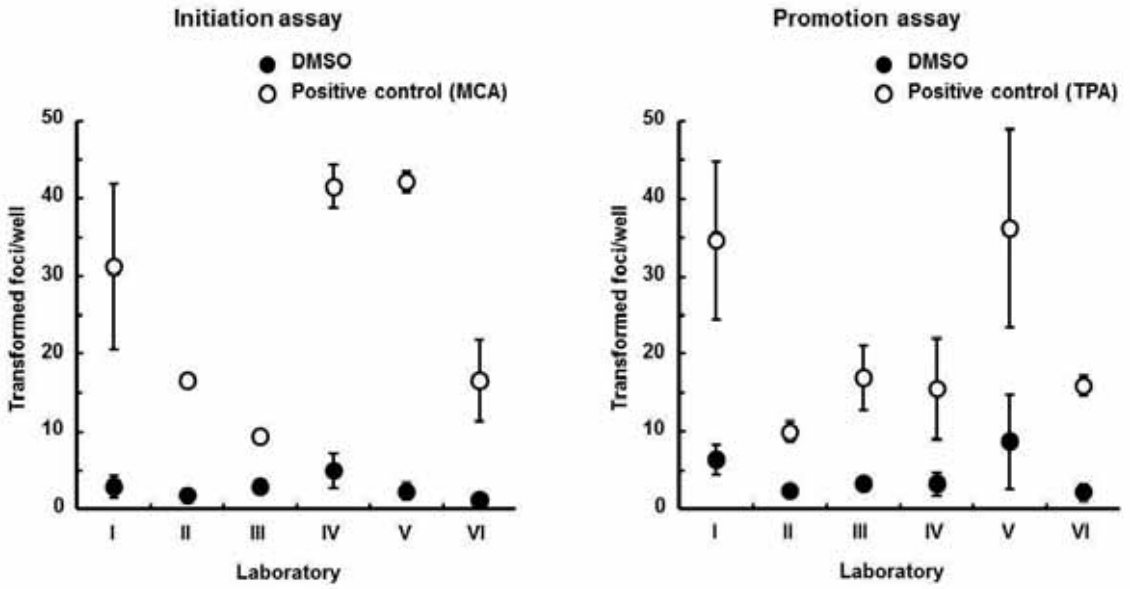

Fig. 4 Average number of the transformed foci per well produced in negative and positive controls of the Bhas 42 cell transformation assay in individual laboratories in the 6-well method validation study. Bars indicate standard deviation.

3.3.3 2-Acetylaminofluorene

The results for 2-acetylaminofluorene are shown in Table 4 and Fig. 5. All three laboratories judged 2-acetylaminofluorene as positive both in the initiation assay and in the promotion assay since it induced statistically significant increases in the number of transformed foci at two or more sequential concentrations in both assays in all laboratories.

Table 4.
Results of transformation assay on 2-acetylaminofluorene in the 6-well method validation study

a) Initiation assay

Concentration (μg/mL)		Lab I CG[a]	Lab I Foci/well[b]	Lab IV CG	Lab IV Foci/well	Lab VI CG	Lab VI Foci/well
0[c] (0.1% DMSO)		100	3.8 ± 3.4	100	4.5 ± 1.6		
0 (0.5% DMSO)						100	1.0 ± 0.6
10		118	4.5 ± 2.3	101	5.5 ± 2.3		
12						103	2.7 ± 2.1
14						86	5.7 ± 2.5 *
15		130	7.0 ± 1.7	104	5.8 ± 3.1		
16						87	9.2 ± 3.5 *
18						60	6.3 ± 4.1 *
20		135	10.0 ± 3.7 *	94	8.7 ± 2.6 *	45	4.2 ± 2.3
25		91	14.5 ± 1.9 *	41	12.5 ± 1.9 *	20	1.8 ± 1.5
30		46	14.3 ± 1.6 *	26	12.3 ± 2.2 *	20	Toxic
35				18	14.2 ± 2.9 *		
40				17	13.5 ± 3.7 *	9	Toxic
50		22	8.8 ± 5.7 *				
100		19	5.2 ± 2.4				
300		14	7.8 ± 3.5				
MCA	1[d] (0.1% DMSO)	82	50.0 ± 15.6 †	72	38.5 ± 4.1 †		
MCA	1 (0.5% DMSO)					53	23.7 ± 4.6 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab IV		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.1% DMSO)	100	9.0 ± 2.0	100	6.2 ± 2.6		
0 (0.5% DMSO)					100	2.2 ± 1.3
0.03	103	5.7 ± 1.0				
0.1	103	4.2 ± 1.9				
0.3	104	6.3 ± 2.3				
1	101	9.2 ± 3.1				
2.5					98	6.0 ± 3.6
3	102	6.8 ± 2.6				
5			100	9.5 ± 1.9 *	79	10.2 ± 2.9 *
10	85	13.0 ± 3.6 *	98	10.8 ± 3.2 *	88	15.5 ± 2.1 *
15	83	19.5 ± 3.9 *	89	12.0 ± 2.1 *	58	11.7 ± 4.5
20	71	19.3 ± 3.4 *	85	9.7 ± 1.6 *	61	5.3 ± 3.8
25			78	10.0 ± 2.5 *	49	Toxic
30			72	12.7 ± 1.6 *		
35			78	18.2 ± 1.7 *		
40			76	1.3 ± 1.0		
50					47	Toxic
TPA 0.05[d] (0.1% DMSO)	125	38.5 ± 5.0 †	219	24.7 ± 3.3 †		
TPA 0.05 (0.5% DMSO)					104	17.5 ± 3.7 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

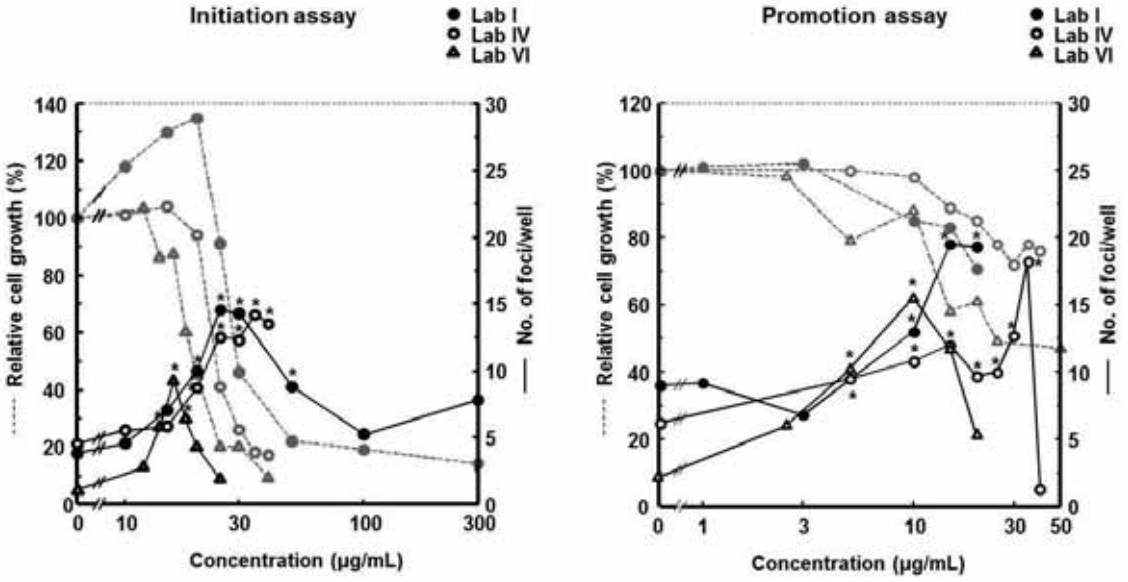

Fig. 5. Graphic view of the results of transformation assay and concurrent cell growth assay on 2-acetylaminofluorene in the 6-well method validation study.
* $p<0.05$; one-sided Dunnett test.

3.3.4 Cadmium chloride

The results for cadmium chloride are presented in Table 5 and Fig. 6. The chemical gave negative results in the initiation assays as determined by all three laboratories.

The promotion assay was repeated by all three laboratories. The reason for the repetition was the cell death caused by cytotoxicity at high concentrations of the test chemical. All three laboratories performed the second assay in a lower concentration range. In the first run of the promotion assay, Lab I obtained statistically significant increases in the number of transformed foci at the lowest two concentrations, 0.4 and 0.6 μg/mL, but the chemical was toxic at the higher five doses. In the second run of the assay, the positive results were confirmed at concentrations ≤0.6 μg/mL. In Lab II, the seven highest of eight doses tested were completely cytotoxic in the first run of the assay. However, in the second run of the assay, which employed lower doses, the number of transformed foci at one dose (the highest dose tested, which was also the lowest dose tested in the first run) was statistically significantly increased. Lab II again repeated the assay for a third time and obtained a significant increase at a single dose again. Thus, based upon the response criteria, cadmium chloride was judged to be positive in the Lab II. Since Lab III set the test concentrations over a wide range, cell killing was caused at only two doses in the first promotion assay. Meanwhile, Lab III obtained a statistically significant increase in transformed foci at a single dose in that first assay. In the second assay that was carried out using a lower and narrower concentration range, there were the significant increases of foci at 3 consecutive doses. Therefore, cadmium chloride was judged positive by Lab III.

Table 5.
Results of transformation assay on cadmium chloride in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab II		Lab III	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
	0[c] (5% Water)	100	4.8 ± 2.5	100	1.5 ± 1.4	100	3.0 ± 1.7
	0.1			111	1.7 ± 1.4		
	0.2			104	0.8 ± 1.2		
	0.4			96	2.0 ± 1.4		
	0.402					110	1.7 ± 1.0
	0.482					105	1.3 ± 1.4
	0.579					106	1.7 ± 1.6
	0.6			93	1.0 ± 1.1		
	0.694					95	1.3 ± 1.5
	0.8	88	2.7 ± 0.8	72	0.7 ± 1.2		
	0.833					72	1.3 ± 1.2
	1	82	3.3 ± 0.5	41	0.7 ± 0.8	55	1.3 ± 1.0
	1.2	80	2.3 ± 1.8	13	0.5 ± 1.2	7	0.3 ± 0.5
	1.4	44	1.3 ± 1.2				
	1.44					5	1.0 ± 0.6
	1.5			4	0.2 ± 0.4		
	1.6	21	0.7 ± 0.5				
	1.73					-1	0.3 ± 0.8
	1.8	6	0.8 ± 1.2				
	2	3	0.3 ± 0.5				
	0[d] (0.1% DMSO)	100	4.3 ± 2.1		1.0 ± 0.9		
MCA	1[e] (0.1% DMSO)	74	43.5 ± 3.8 †		15.8 ± 4.4 †	14[f]	9.2 ± 2.0 ‡

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

b) Promotion assay

Concentration		Lab I, 1st run		Lab I, 2nd run		Lab II, 1st run		Lab II, 2nd run		Lab II, 3rd run		Lab III, 1st run		Lab III, 2nd run	
	(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well	CG	Foci/well	CG	Foci/well	CG	Foci/well	CG	Foci/well
	0[c] (5% Water)	100	5.3 ± 2.3	100	2.5 ± 0.8	100	2.6 ± 1.1	100	1.3 ± 0.8	100	1.3 ± 1.5	100	2.0 ± 0.6	100	1.2 ± 0.8
	0.001							92	1.0 ± 0.6						
	0.01							100	0.3 ± 0.5						
	0.05							97	1.7 ± 1.4					98	1.2 ± 1.6
	0.0625											97	2.2 ± 0.8		
	0.1			101	2.0 ± 0.9			102	0.8 ± 1.3					102	1.3 ± 0.5
	0.125											99	2.3 ± 0.8		
	0.2			104	4.2 ± 1.0			116	2.8 ± 1.9	110	1.3 ± 1.4			108	3.7 ± 1.6
	0.25									124	1.8 ± 1.7	108	4.0 ± 1.8 *		
	0.275									109	2.7 ± 1.8				
	0.3			110	11.0 ± 4.7 *	106	4.0 ± 2.2	119	4.7 ± 1.9 *	120	3.5 ± 1.2			109	4.2 ± 2.7 *
	0.35			108	13.5 ± 4.0 *					123	5.3 ± 2.7 *				
	0.4	114	21.8 ± 4.4 *	114	13.3 ± 5.4 *					131	3.3 ± 2.3			114	4.0 ± 2.0 *
	0.45			117	14.5 ± 2.2 *					126	4.0 ± 3.2				
	0.5			118	14.2 ± 3.1 *	121	Toxic			138	2.5 ± 1.4	109	3.2 ± 1.8	112	5.3 ± 2.7 *
	0.6	126	14.7 ± 5.6 *	123	14.2 ± 5.6 *									110	2.3 ± 1.2
	0.8	122	Toxic			117	Toxic								
	1	113	Toxic			114	Toxic					68	Toxic		
	1.2	96	Toxic			90	Toxic								
	1.4	70	Toxic			67	Toxic								
	1.6	54	Toxic			52	Toxic								
	2					31	Toxic					25	Toxic		
	0[d] (0.1% DMSO)	100	9.0 ± 3.4	100	5.0 ± 3.3		2.3 ± 1.6	100	2.0 ± 0.9	100	1.8 ± 0.4			100	3.8 ± 1.3
TPA	0.05[e] (0.1% DMSO)	125	38.7 ± 5.6 †	132	19.3 ± 5.6 †		8.3 ± 3.1 †	133	11.5 ± 2.7 †	134	9.5 ± 2.7 †	155[f]	15.7 ± 4.5 ‡	166	20.3 ± 4.2 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

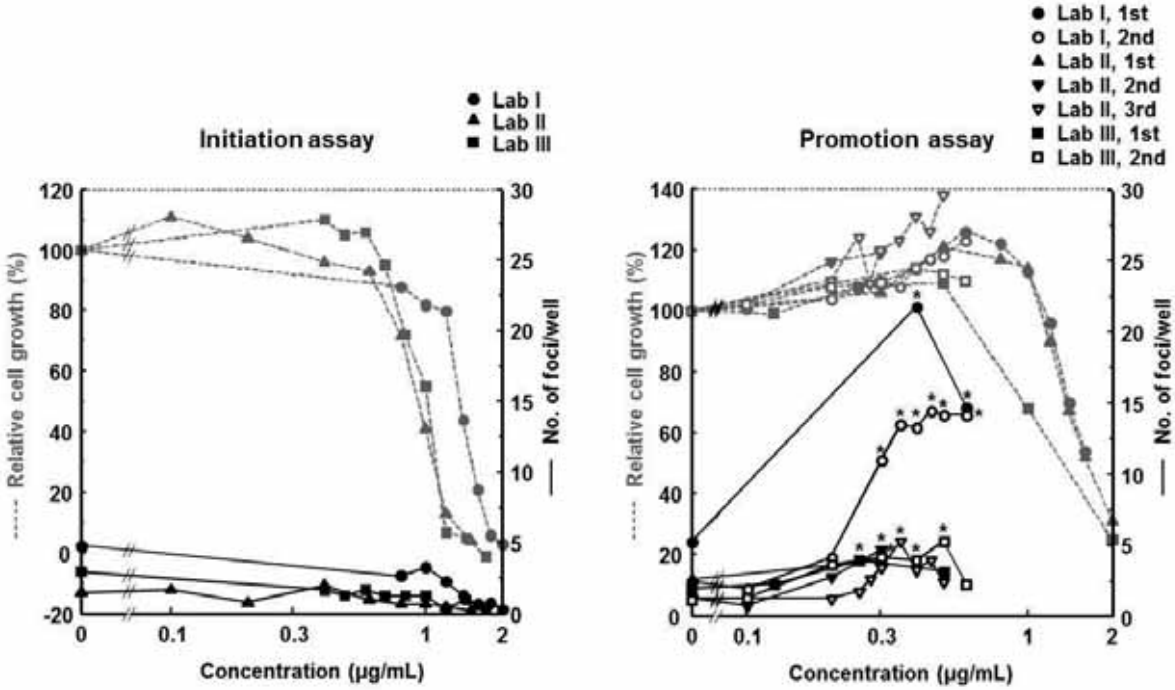

Fig. 6. Graphic view of the results of transformation assay and concurrent cell growth assay on cadmium chloride in the 6-well method validation study.
* $p<0.05$; one-sided Dunnett test.

3.3.5 Dibenz[*a,h*]anthracene

The results for dibenz[*a,h*]anthracene are presented in Table 6 and Fig. 7. All three laboratories reported that dibenz[*a,h*]anthracene was clearly positive in the initiation assay, but negative in the promotion assay.

Table 6.
Results of transformation assay on dibenz[*a,h*]anthracene in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab II		Lab V	
	(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
	0[c] (0.1% DMSO)	100	1.5 ± 1.2			100	2.8 ± 1.9
	0 (0.2% DMSO)			100	0.8 ± 0.8		
	0.0033					89	8.0 ± 1.4
	0.01	113	6.0 ± 2.5	99	2.5 ± 1.5	73	13.3 ± 3.1 *
	0.03	110	8.7 ± 2.9 *	80	6.2 ± 2.4 *		
	0.033					37	22.5 ± 3.0 *
	0.04			71	7.3 ± 3.3 *		
	0.05			65	8.3 ± 2.9 *		
	0.1	84	15.0 ± 4.2 *	53	11.2 ± 3.9 *	11	18.5 ± 4.1 *
	0.3	63	24.2 ± 3.9 *				
	0.33					3	22.7 ± 7.1 *
	1	58	24.2 ± 3.3 *	30	12.7 ± 2.9 *	4	16.3 ± 4.2 *
	3	54	17.8 ± 4.5 *				
	3.3					7	17.5 ± 4.8 *
	7	57	17.2 ± 3.5 *				
	10	58	17.5 ± 3.5 *	38	9.3 ± 2.4 *	1	17.3 ± 7.8 *
	0[d] (0.1% DMSO)			100	2.0 ± 1.3	100	1.2 ± 0.4
MCA	1[e] (0.1% DMSO)	79	20.0 ± 4.1 †	53	16.7 ± 3.4 †	23	41.3 ± 4.9 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab II		Lab V	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.1% DMSO)	100	3.3 ± 1.6			100	8.7 ± 2.3
0 (0.2% DMSO)			100	2.8 ± 1.5		
0.001			81	1.5 ± 0.5	83	9.0 ± 2.1
0.0033					94	11.8 ± 3.3
0.005			82	2.5 ± 1.5		
0.01	83	2.2 ± 2.1			70	11.2 ± 3.1
0.03	80	2.3 ± 2.0				
0.033					70	11.5 ± 4.4
0.05			76	2.7 ± 1.9		
0.1	74	2.7 ± 1.2			67	10.5 ± 3.0
0.2			69	1.5 ± 1.0		
0.3	65	0.8 ± 1.6				
0.33					59	5.0 ± 4.1
1	66	1.0 ± 1.3	71	0.2 ± 0.4	71	2.3 ± 2.4
3	66	1.7 ± 0.8				
3.3					68	3.2 ± 1.2
7	71	0.7 ± 1.0				
10	66	1.0 ± 0.9	74	1.0 ± 1.3		
0[d] (0.1% DMSO)			100	2.5 ± 2.1		
TPA 0.05[e] (0.1% DMSO)	118	17.7 ± 4.2 †	128	8.8 ± 3.2 †	136	43.5 ± 4.1 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

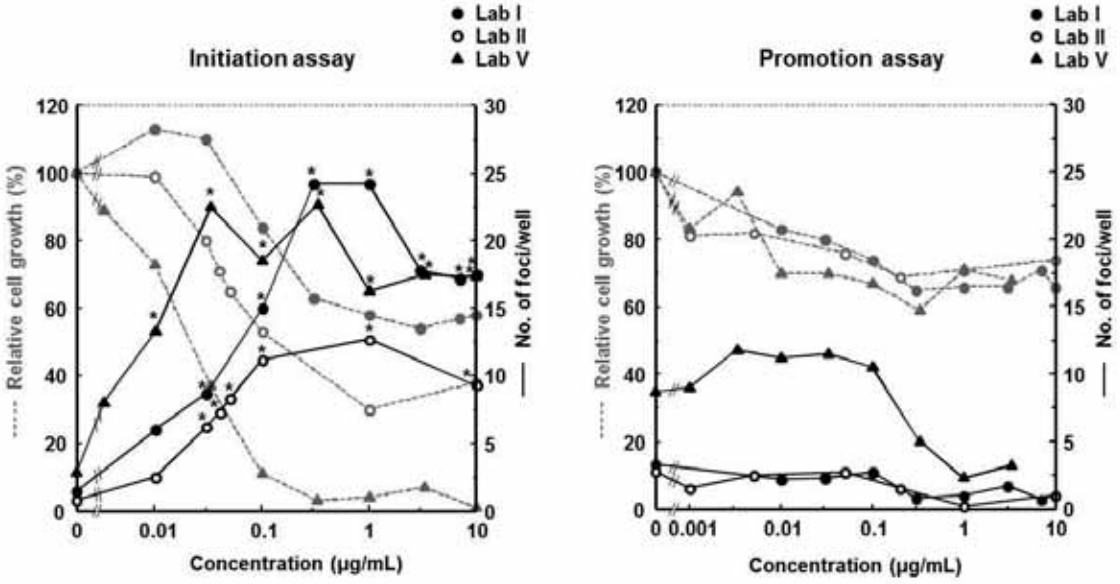

Fig. 7. Graphic view of the results of transformation assay and concurrent cell growth assay on dibenz[*a,h*]anthracene in the 6-well method validation study.
***** $p<0.05$; one-sided Dunnett test.

3.3.6 Lithocholic acid

The results obtained with lithocholic acid are shown in Table 7 and Fig. 8. The data from all laboratories revealed that lithocholic acid was negative in the initiation assay and positive in the promotion assay.

Table 7.
Results of transformation assay on lithocholic acid in the 6-well method validation study

a) Initiation assay

Concentration	Lab I		Lab IV		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (DMSO 0.1%)	100	6.3 ± 3.9	100	5.7 ± 1.0		
0 (DMSO 0.5%)					100	1.3 ± 1.4
7			92	5.7 ± 2.3		
10	105	5.5 ± 1.9	94	4.5 ± 3.3		
14			96	5.7 ± 2.4		
15	106	4.5 ± 2.3				
17.5	108	2.0 ± 1.4				
20	104	3.7 ± 2.3	85	3.7 ± 1.9		
22					75	1.8 ± 1.5
22.5	107	2.7 ± 0.5				
25	89	4.3 ± 2.5				
27.5	56	5.5 ± 3.7				
28			112	3.2 ± 1.2	10	0.2 ± 0.4
28.25					9	0.5 ± 0.5
28.5					7	0.8 ± 0.8
28.75					8	0.5 ± 0.8
29					11	0.0 ± 0.0
29.25					9	Toxic
29.5					8	Toxic
29.75					8	Toxic
30	11	3.0 ± 1.4			9	Toxic
40			3	4.2 ± 0.8		
56			0	Toxic		
80			1	Toxic		
MCA 1[d] (0.1% DMSO)	73	44.2 ± 3.7 †	57	39.0 ± 2.6 †		
MCA 1 (0.5% DMSO)					56	10.5 ± 4.2 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab IV		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (DMSO 0.1%)	100	7.2 ± 3.5	100	2.3 ± 1.6		
0 (DMSO 0.5%)					100	2.7 ± 0.8
2.5	93	9.3 ± 2.6				
5	89	15.7 ± 5.1 *				
10	80	27.3 ± 4.2 *			83	19.7 ± 5.2 *
12			86	6.3 ± 4.1 *		
15	72	44.8 ± 9.8 *				
18			86	13.5 ± 2.1 *		
20	75	45.7 ± 5.8 *			83	33.2 ± 4.9 *
25	86	12.2 ± 2.4	89	14.0 ± 1.8 *		
30	16	Toxic			106	Toxic
35			84	Toxic	50	Toxic
40					38	Toxic
42.5					19	Toxic
45					14	Toxic
50			5	Toxic	11	Toxic
TPA 0.05[d] (0.1% DMSO)	124	42.3 ± 1.8 †	136	9.5 ± 3.4 †		
TPA 0.05 (0.5% DMSO)					145	16.8 ± 4.1 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

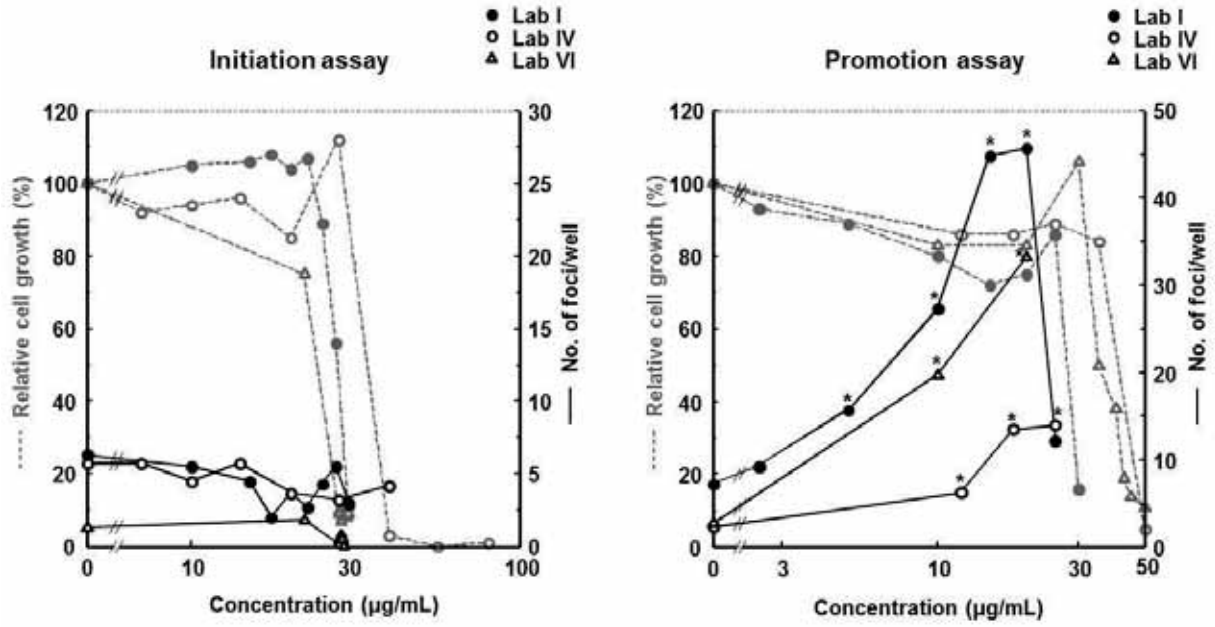

Fig. 8. Graphic view of the results of transformation assay and concurrent cell growth assay on lithocholic acid in the 6-well method validation study.
* $p<0.05$; one-sided Dunnett test.

3.3.7 Methapyrilene hydrochloride

The results for methapyrilene hydrochloride are shown in Table 8 and Fig. 9. All three laboratories demonstrated methapyrilene hydrochloride to be negative in the initiation assay and positive in the promotion assay.

Table 8.
Results of transformation assay on methapyrilene hydrochloride in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab II		Lab IV	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (5% water)		100	2.7 ± 1.6	100	2.0 ± 1.4	100	6.8 ± 1.8
53						120	6.2 ± 1.2
75						149	7.2 ± 3.1
100		109	3.3 ± 1.5				
110						124	6.2 ± 1.5
150		86	2.8 ± 1.0			129	6.5 ± 2.6
200		71	3.2 ± 2.1	45	1.8 ± 0.8		
210						132	5.8 ± 1.5
220				23	1.0 ± 0.6		
240				13	1.7 ± 1.2		
250		56	3.0 ± 1.9				
260				5	0.8 ± 0.8		
280				3	1.3 ± 0.8		
300		32	2.0 ± 1.4	1	0.5 ± 0.8	64	4.8 ± 1.3
350		16	1.0 ± 0.9				
400		6	1.7 ± 1.0				
450		4	1.3 ± 1.5				
	0[d] (0.1% DMSO)	100	3.0 ± 1.4	100	1.8 ± 1.2	100	5.5 ± 1.5
MCA	1[e] (0.1% DMSO)	45	28.3 ± 3.6 †	54	17.5 ± 3.1 †	70	41.8 ± 3.4 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab II		Lab IV	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (5% Water)	100	5.7 ± 2.3	100	2.3 ± 1.8	100	2.7 ± 1.2
5			104	3.0 ± 1.5	111	6.7 ± 1.5
10	121	10.5 ± 2.3	116	4.5 ± 2.6	116	9.2 ± 5.2 *
20					126	16.0 ± 1.4 *
25	130	16.2 ± 2.6 *				
40					140	29.7 ± 4.8 *
50	151	35.7 ± 5.6 *	159	18.0 ± 4.8 *		
75	168	46.0 ± 8.0 *				
80					199	52.3 ± 7.3 *
100	184	33.8 ± 4.5 *	190	24.7 ± 6.2 *		
160					217	34.5 ± 2.3 *
200	164	5.3 ± 1.9	161	1.0 ± 1.3		
300	139	0.7 ± 0.8				
400	114	Toxic				
500			72	Toxic		
0[d] (0.1% DMSO)	100	5.0 ± 1.5		2.7 ± 1.6		2.5 ± 0.8
TPA 0.05[e] (0.1% DMSO)	126	39.7 ± 4.3 †		8.7 ± 2.8 †		18.7 ± 4.2 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

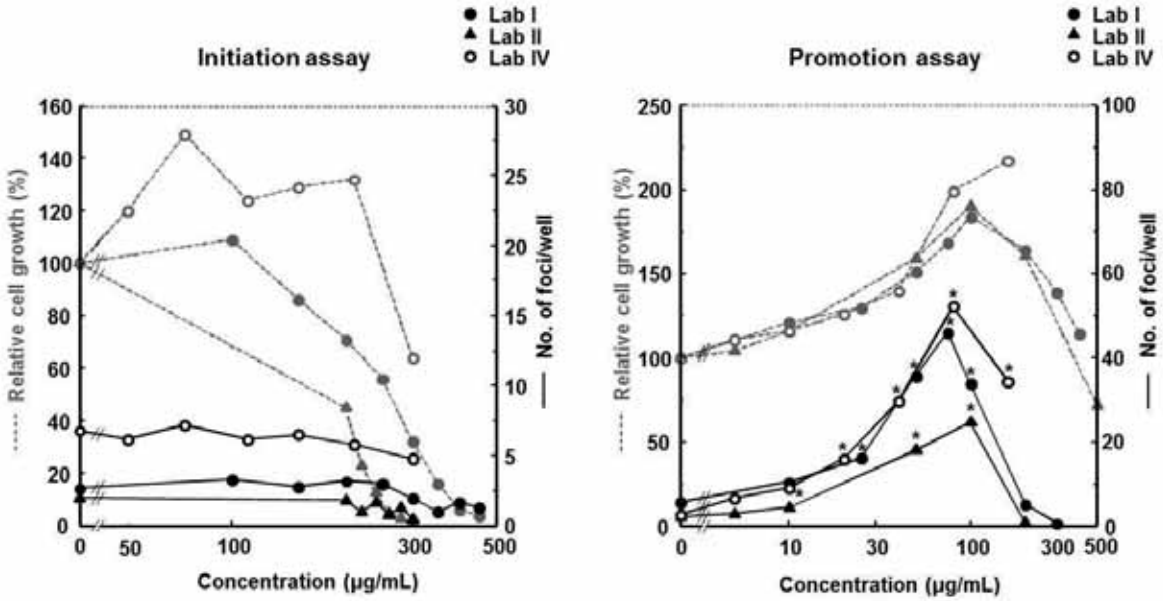

Fig. 9. Graphic view of the results of transformation assay and concurrent cell growth assay on methapyrilene hydrochloride in the 6-well method validation study.
* $p<0.05$; one-sided Dunnett test.

3.3.8 Mezerein

Table 9 and Fig. 10 show the results for mezerein. All three laboratories reported clearly positive results in the promotion assay. The chemical induced marked and dose-dependent increases in the number of transformed foci relative to the solvent control in all cases.

In the initiation assay performed by Lab I, mezerein did not induce statistically significantly increases in the number of transformed foci at any concentration up to 0.01 µg/mL, which was the maximum concentration used in the present study. In the initiation assays performed by Lab III and Lab IV, a statistically significant increase in the number of transformed foci was observed only at the maximum (preset) concentration used. The results of the initiation assay by both laboratories remained inconclusive since neither laboratory repeated the assay at higher concentrations (due to limited availability) in an attempt to verify the initial results. Rather, they considered that the promotion assay had established that the chemical was clearly positive in the Bhas 42 CTA and that it was not necessary to further differentiate the initiation vs. promotion properties of the compound for the purpose of this exercise.

Table 9.
Results of transformation assay on mezerein in the 6-well method validation study

a) Initiation assay

Concentration	Lab I		Lab III		Lab IV	
(µg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.1% DMSO)	100	1.7 ± 1.5	100	2.5 ± 1.4	100	7.8 ± 2.0
0.000010			104	1.8 ± 1.0		
0.000039			102	2.3 ± 0.8		
0.0001	94	1.7 ± 1.6				
0.000156			110	1.5 ± 1.5		
0.0003	94	1.5 ± 1.0				
0.0005	93	2.2 ± 0.8				
0.000625			113	1.7 ± 1.2		
0.00063					94	7.7 ± 1.5
0.001	94	1.5 ± 1.4				
0.0013					109	7.8 ± 3.2
0.0025			140	3.0 ± 0.6	130	7.3 ± 2.3
0.003	111	1.7 ± 0.8				
0.005	141	1.3 ± 1.2			195	10.2 ± 2.6
0.01	150	3.2 ± 1.9	202	13.3 ± 2.9 *	208	13.2 ± 1.7 *
MCA 1[d] (0.1% DMSO)	56	22.2 ± 4.7 †	28	9.2 ± 3.5 †	71	45.7 ± 3.6 †

[a] % of cell growth compared to that of that solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab III		Lab IV	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.1% DMSO)	100	5.7 ±1.9	100	3.2 ± 1.8	100	3.3 ± 2.3
0.000005			100	2.2 ± 2.1		
0.000020			104	4.5 ± 1.5		
0.000026					100	5.3 ± 2.1
0.000064					102	4.3 ± 2.0
0.000078			107	9.2 ± 2.6		
0.0001	103	17.5 ± 5.4 *				
0.00016					113	8.2 ± 1.6 *
0.0003	121	37.2 ± 4.2 *				
0.000313			143	31.8 ± 6.0 *		
0.0004					139	18.3 ± 2.2 *
0.0005	142	46.5 ± 4.4 *				
0.001	184	62.7 ± 2.1 *			208	40.2 ± 4.1 *
0.00125			257	46.5 ± 7.4 *		
0.0025					236	75.2 ± 5.4 *
0.003	181	Toxic				
0.005	183	Toxic	206	37.2 ± 7.9 *		
0.01	193	Toxic				
TPA 0.05[d] (0.1% DMSO)	124	35.3 ± 5.6 †	143	12.2 ± 6.1 †	142	9.8 ± 3.1 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

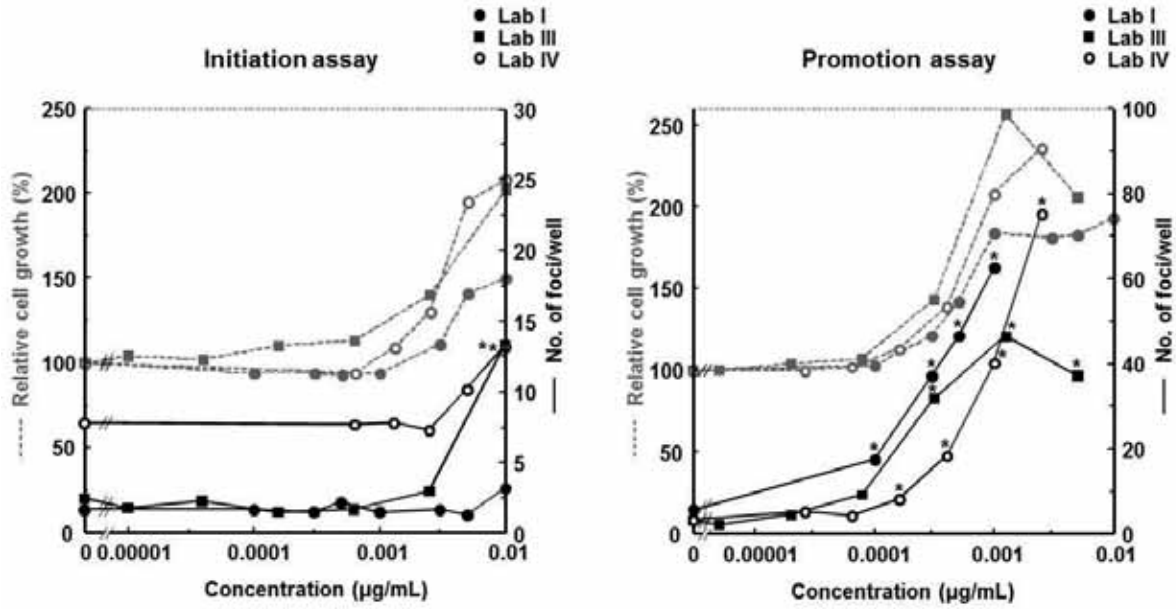

Fig. 10. Graphic view of the results of transformation assay and concurrent cell growth assay on mezerein in the 6-well method validation study.
* $p<0.05$; one-sided Dunnett test.

3.3.9 Sodium arsenite

Table 10 and Fig. 11 show the assay results for sodium arsenite. In the first initiation assay of sodium arsenite by Lab I, there was a statistically significant increase in the number of transformed foci at a single dose of 0.8 μg/mL. In accordance with the protocol, Lab I repeated the initiation assay, but there was no significant increase of transformed foci in the second assay. Thus, sodium arsenite was judged as negative in the initiation assay in Lab I. Since Lab III first obtained statistically significant increases of transformed foci at two non-sequential doses, 0.455 and 0.769 μg/mL, they performed a second initiation assay and again obtained a significant increase at 0.769 μg/mL. Consequently, sodium arsenite was judged to be positive in the initiation assay by Lab III. Lab VI carried out initiation assay only once and observed no increase of transformed foci. However, sodium arsenite did not produce any cytotoxicity at the tested concentrations in the concurrent cell growth assay. In view of its high solubility in the solvent (water) and its lack of cytotoxicity at the lower concentration initially used, sodium arsenite should have been retested by Lab VI at higher concentrations in order to achieve cytotoxicity. Therefore, the initiation assay conducted on sodium arsenite by Lab VI was judged to be incomplete because of inadequate dosing.

In the promotion assay on sodium arsenite, all three laboratories reported positive results. Despite having obtained statistically significant increases of transformed foci at two sequential doses in the first assay, however, Lab III repeated the promotion assay. The compound was considered positive and the repeat was not necessary. Lab III's decision was the result of having lost cultures at the two highest concentrations in the transformation assay because of cytotoxicity, although the cell growth rates at respective concentrations were 77% and 26% of control in the concurrent cell growth assay. The second assay was voluntarily carried out in a lower and narrower concentration range and confirmed the positive results obtained in the first assay.

Table 10.
Results of transformation assay on sodium arsenite in the 6-well method validation study
a) Initiation assay

Concentration		Lab I, 1st run		Lab I, 2nd run		Lab III, 1st run		Lab III, 2nd run		Lab VI	
	(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well	CG	Foci/well	CG	Foci/well
	0[c] (0.5% DMSO)									100	0.8 ± 1.0
	0 (5% Water)	100	2.0 ± 2.0	100	2.8 ± 1.3	100	1.5 ± 0.5	100	1.5 ± 1.8		
	0.01									99	2.3 ± 1.6
	0.025									99	1.2 ± 1.2
	0.05									96	2.2 ± 1.9
	0.075									108	1.0 ± 1.3
	0.1	113	1.7 ± 1.2							101	1.8 ± 1.7
	0.11									100	0.0 ± 0.0
	0.12									96	2.2 ± 2.5
	0.123					103	0.7 ± 0.5				
	0.13									104	2.2 ± 1.7
	0.14									100	0.5 ± 0.8
	0.15									98	1.5 ± 1.4
	0.159					96	1.5 ± 0.8				
	0.2	114	2.3 ± 1.8								
	0.207					107	1.3 ± 0.5	103	2.7 ± 2.0		
	0.269					101	2.2 ± 1.6	108	1.8 ± 1.5		
	0.3			99	2.3 ± 1.5						
	0.35					110	2.8 ± 1.3	102	3.0 ± 2.5		
	0.4	120	3.3 ± 1.6	104	2.3 ± 1.4						
	0.455					103	3.3 ± 1.6 *	84	2.5 ± 2.2		
	0.5			103	1.7 ± 2.3						
	0.592					91	1.3 ± 1.0	61	2.5 ± 2.3		
	0.6	97	3.0 ± 1.3	103	1.0 ± 1.1						
	0.7			106	1.7 ± 1.4						
	0.769					74	3.5 ± 1.0 *	35	6.3 ± 2.7 *		
	0.8	49	5.0 ± 3.0 *	96	1.2 ± 1.5						
	0.9			96	1.2 ± 1.2						
	1	16	2.5 ± 1.9			35	2.2 ± 1.3	6	4.7 ± 3.8		
	2	3	0.8 ± 1.0								
	0[d] (0.1% DMSO)	100	3.0 ± 1.7	100	2.8 ± 1.3			100	3.3 ± 1.2		
MCA	1[e] (0.1% DMSO)	60	41.3 ± 2.3 †	47	32.0 ± 6.7 †	22[f]	15.0 ± 3.3 ‡	21	9.7 ± 3.6 †		
MCA	1 (0.5% DMSO)									79	12.0 ± 3.5 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

b) Promotion assay

Concentration		Lab I		Lab III, 1st run		Lab III, 2nd run		Lab VI	
	(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well	CG	Foci/well
	0[c] (0.5% DMSO)							100	1.7 ± 1.5
	0 (5% Water)	100	4.5 ± 1.8	100	2.5 ± 1.4	100	0.7 ± 0.8		
	0.0158			107	2.5 ± 2.1				
	0.025					104	2.5 ± 1.4	109	6.5 ± 1.9 *
	0.05	94	6.3 ± 2.6	106	5.2 ± 1.2 *	118	4.7 ± 1.6 *	103	7.3 ± 1.8 *
	0.1	101	13.0 ± 2.2 *			104	2.8 ± 1.5 *	105	11.8 ± 2.9 *
	0.15					102	3.7 ± 1.2 *		
	0.158			109	6.2 ± 2.6 *				
	0.2					112	3.5 ± 2.3 *	101	13.7 ± 2.7 *
	0.25					125	1.7 ± 1.0		
	0.3	99	9.0 ± 0.9 *			127	0.2 ± 0.4	96	1.5 ± 0.8
	0.4							96	0.7 ± 0.8
	0.5			97	0.3 ± 0.8			95	0.0 ± 0.0
	0.7	86	1.7 ± 0.8						
	0.75							80	Toxic
	1	83	Toxic						
	1.3	85	Toxic						
	1.58			77	Toxic				
	1.7	78	Toxic						
	5			26	Toxic				
	0[d] (0.1% DMSO)	100	5.0 ± 2.4			100	2.5 ± 1.0		
TPA	0.05[e] (0.1% DMSO)	128	36.7 ± 3.3 †	133[f]	13.7 ± 6.8 ‡	198	18.2 ± 2.7 †		
TPA	0.05 (0.5% DMSO)							109	14.3 ± 5.4 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

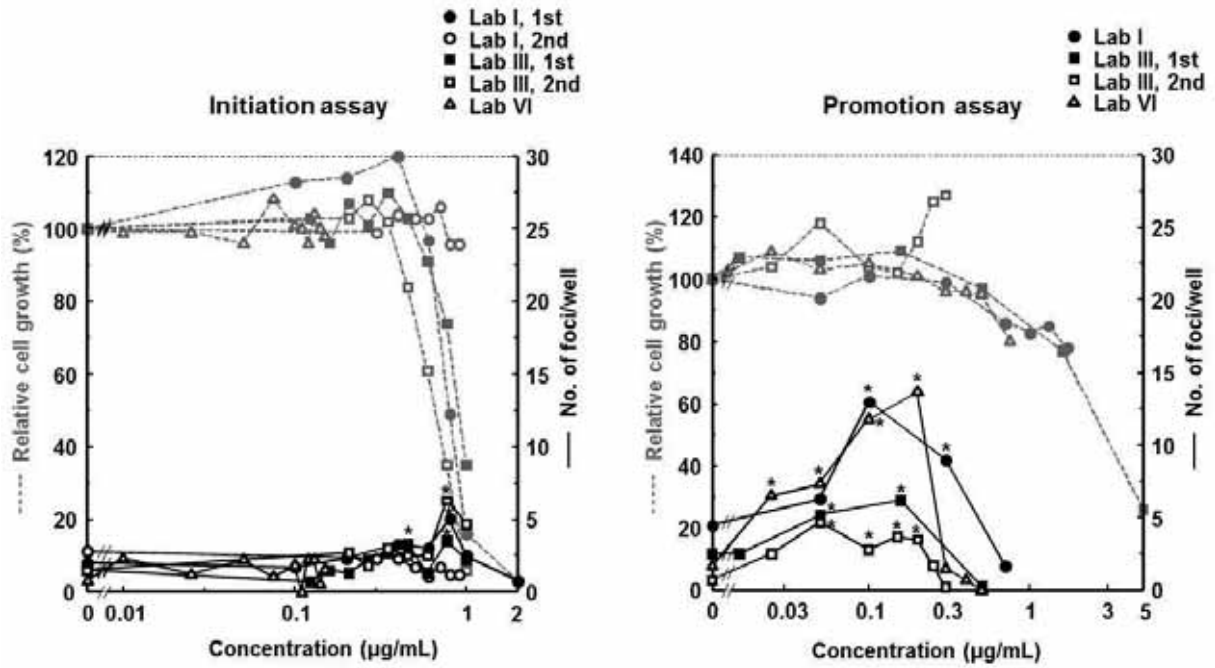

Fig. 11. Graphic view of the results of transformation assay and concurrent cell growth assay on sodium arsenite in the 6-well method validation study.
***** $p<0.05$; one-sided Dunnett test.

3.3.10 *o*-Toluidine hydrochloride

Table 11 and Fig. 12 represent the results of *o*-toluidine hydrochloride. Two laboratories, Lab V and Lab VI, gave positive results both in the initiation assay and in the promotion assay. The other laboratory, Lab I, gave negative results in both assays, although acceptable responses were obtained with the positive controls.

Table 11.
Results of transformation assay on *o*-toluidine hydrochloride in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab V		Lab VI	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)						100	1.0 ± 1.5
0 (5% Water)		100	1.8 ± 1.3	100	2.0 ± 2.4		
100		98	2.8 ± 1.8	92	2.5 ± 2.5		
150						123	2.0 ± 1.8
250				79	6.8 ± 3.2 *		
300		77	2.5 ± 1.5			89	1.7 ± 1.2
450						109	1.8 ± 1.3
500		61	3.2 ± 2.5	50	7.0 ± 2.0 *		
600						73	4.5 ± 2.7 *
700		29	2.8 ± 1.8				
750						64	3.7 ± 1.0 *
1000		18	1.8 ± 1.6	0	9.5 ± 4.0 *	29	2.0 ± 1.1
1250						13	0.3 ± 0.5
1500						9	0.3 ± 0.8
2000				-6	Toxic		
3000		3	Toxic	-4	Toxic		
5000		3	Toxic				
0[d] (0.1% DMSO)		100	1.7 ± 0.8	100	2.3 ± 0.8		
MCA	1[e] (0.1% DMSO)	53	19.3 ± 3.9 †	33	43.7 ± 6.0 †		
MCA	1 (0.5% DMSO)					40	21.7 ± 6.3 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab V		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)					100	0.7 ± 0.8
0 (5% Water)	100	4.5 ± 1.6	100	16.3 ± 4.1		
10	94	5.5 ± 2.0	94	27.0 ± 4.6 *		
12.5					128	0.5 ± 0.5
25					65	2.5 ± 2.6
30	87	4.3 ± 1.5	92	31.0 ± 1.7 *		
100	83	3.7 ± 1.6	75	26.7 ± 7.4 *		
250			55	27.8 ± 4.6 *	91	6.2 ± 2.8 *
300	65	4.8 ± 1.7				
500			42	28.0 ± 6.4 *	48	8.0 ± 3.5 *
700	50	Toxic				
1000	13	Toxic	33	6.0 ± 4.3	68	0.5 ± 0.8
1500					28	Toxic
2000					21	Toxic
0[d] (0.1% DMSO)	100	7.8 ± 2.2	100	14.8 ± 5.5		
TPA 0.05[e] (0.1% DMSO)	127	41.0 ± 3.3 †	134	43.7 ± 8.4 †		
TPA 0.05 (0.5% DMSO)					104	15.5 ± 2.2 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
***** $p<0.05$; one-sided Dunnett test, vs. corresponding solvent control.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

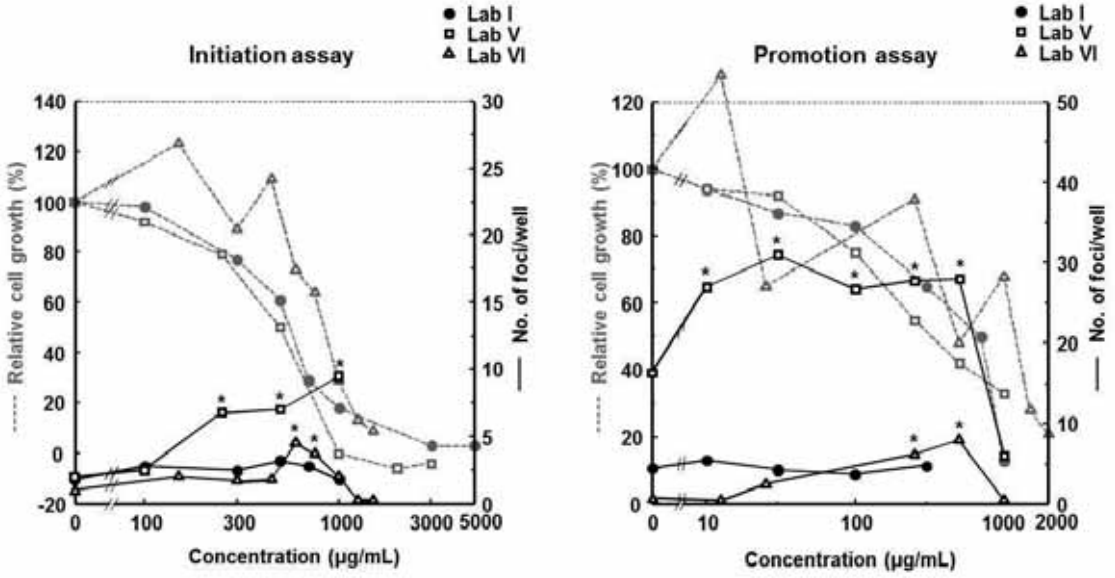

Fig. 12. Graphic view of the results of transformation assay and concurrent cell growth assay on *o*-toluidine hydrochloride in the 6-well method validation study.
***** $p<0.05$; one-sided Dunnett test.

3.3.11 Anthracene

The results for anthracene are exhibited in Table 12 and Fig. 13. All three laboratories obtained negative results in the initiation assay. In the promotion assay, the results were negative in two laboratories, Lab I and Lab II, but positive in Lab VI.

Inhibition of cell growth or focus formation by cytotoxicity was not observed at the concentrations tested in both initiation and promotion assays in all three laboratories. However, anthracene is poorly soluble in DMSO and other usable organic solvents and insoluble in water. The assays by three laboratories were conducted up to the limit of solubility in DMSO or beyond by using a chemical suspension in the vehicle. Despite this, the dosing employed in these assays was considered to be sufficient.

Table 12.
Results of transformation assay on anthracene in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab II		Lab VI	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)		100	4.2 ± 2.6	100	2.5 ± 0.8	100	1.3 ± 1.2
1.25						95	1.5 ± 1.0
1.56		107	5.8 ± 2.7	99	2.0 ± 0.6		
2.5						86	2.2 ± 1.2
3.13		102	5.5 ± 2.9	116	2.3 ± 1.5		
5						95	2.5 ± 1.6
6.25		109	4.0 ± 1.9	112	3.5 ± 2.6		
10						93	1.3 ± 0.8
12.5		106	2.8 ± 2.2	105	2.7 ± 1.6		
20						100	2.5 ± 1.0
25		110	5.7 ± 2.1	110	1.8 ± 1.3		
50		107	6.3 ± 2.2				
0[d] (0.1% DMSO)		100	2.7 ± 0.8	100	3.0 ± 1.5		
MCA	1[e] (0.1% DMSO)	48	24.3 ± 5.6 †	62	16.8 ± 1.5 †		
MCA	1 (0.5% DMSO)					59	14.3 ± 2.6 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab II		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)	100	2.3 ± 1.2	100	2.3 ± 1.0	100	1.8 ± 1.3
1.25					104	5.5 ± 2.3
1.56	96	2.7 ± 2.0	106	2.7 ± 0.8		
2.5					97	6.7 ± 2.3 *
3.13	94	2.0 ± 0.9	102	3.8 ± 2.8		
5					105	6.7 ± 4.4 *
6.25	97	2.7 ± 0.8	108	4.7 ± 1.5		
10					96	9.8 ± 4.4 *
12.5	98	2.8 ± 0.8	93	3.5 ± 2.5		
20					97	10.2 ± 3.5 *
25	98	3.0 ± 1.4	96	2.7 ± 1.2		
50	96	1.3 ± 0.8				
0[d] (0.1% DMSO)	100	3.7 ± 2.0	100	3.7 ± 2.2		
TPA 0.05[e] (0.1% DMSO)	131	17.5 ± 6.5 †	137	10.8 ± 1.6 †		
TPA 0.05 (0.5% DMSO)					136	14.7 ± 2.9 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
***** p<0.05; one-sided Dunnett test, vs. corresponding solvent control.
† p<0.05; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

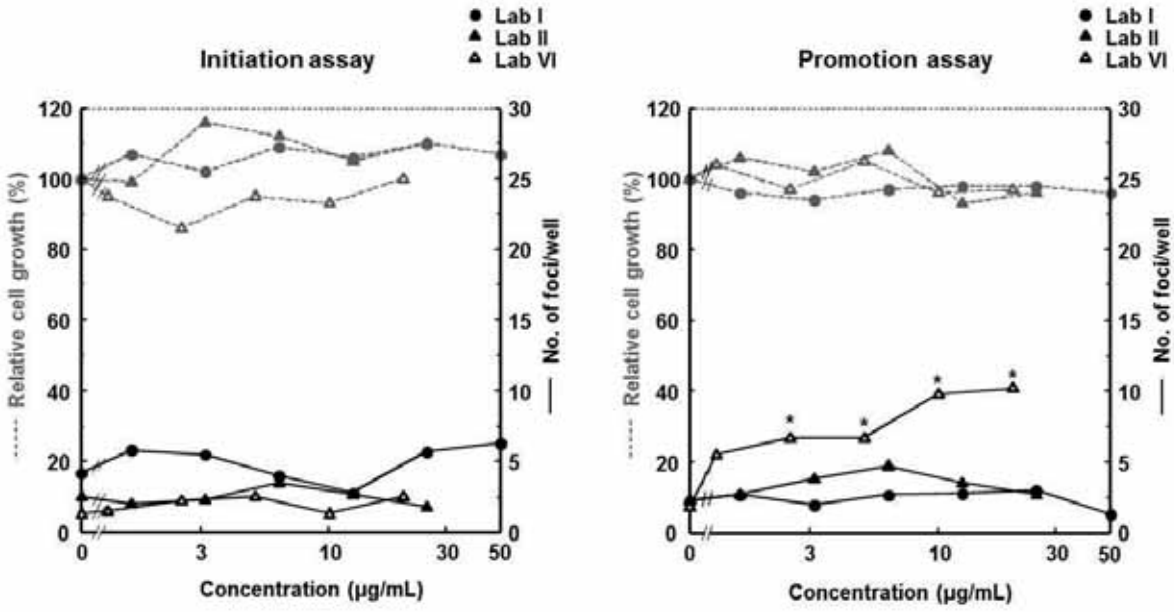

Fig. 13. Graphic view of the results of transformation assay and concurrent cell growth assay on anthracene in the 6-well method validation study.
* p<0.05; one-sided Dunnett test.

3.3.12 L-Ascorbic acid

Table 13 and Fig. 14 show the results for L-ascorbic acid. All three laboratories reported L-ascorbic acid to be negative in both the initiation and promotion assays. Lab II voluntarily repeated the promotion assay. In the first promotion assay, Lab II observed that the transformed focus formation was reduced in the cultures treated with ascorbic acid and the number of foci diminished to 0 at the highest three concentrations. Despite this reduction in transformed foci, obvious general cytotoxicity was not observed. In the second promotion assay using a lower concentration range, Lab II confirmed that L-ascorbic acid decreased the number of transformed foci as compared to the negative control. Ascorbic acid also inhibited transformed focus formation in the promotion assays performed by the other two laboratories. Sodium ascorbate has been reported to inhibit (a) spontaneous focus formation, (b) MCA-induced focus formation, and (c) MCA-initiated and phorbol ester-promoted focus formation in the two-stage BALB/c 3T3 CTA [Sivak and Tu, 1980; Tsuchiya *et al.*, 2000].

Table 13. Results of transformation assay on L-ascorbic acid in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab II		Lab IV	
	(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
	0[c] (5% Water)	100	2.7 ± 2.2	100	2.0 ± 1.9	100	4.2 ± 1.5
	10			108	2.2 ± 1.0		
	25	91	1.7 ± 1.2				
	50	87	1.7 ± 1.5				
	75					96	4.7 ± 2.0
	100	78	2.7 ± 1.0				
	110					101	4.8 ± 1.8
	150	63	2.0 ± 1.1	85	2.2 ± 1.5	95	4.8 ± 1.0
	200	46	2.5 ± 1.4				
	210					91	3.7 ± 1.6
	250	25	2.8 ± 0.8				
	300	14	2.3 ± 1.9	73	1.0 ± 0.6	73	3.2 ± 1.2
	340			52	1.3 ± 1.0		
	380			32	0.2 ± 0.4		
	400	4	2.5 ± 1.4	6	0.5 ± 0.8		
	420			0	0.2 ± 0.4	5	2.8 ± 2.0
	600					1	Toxic
	0[d] (0.1% DMSO)	100	2.3 ± 1.5	100	1.3 ± 1.0		5.5 ± 1.9
MCA	1[e] (0.1% DMSO)	48	26.7 ± 5.3 †	55	15.8 ± 5.3 †		43.7 ± 3.0 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab II, 1st run		Lab II, 2nd run		Lab IV	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well	CG	Foci/well
0[c] (5% Water)	100	4.5 ± 1.6	100	1.8 ± 1.2	100	1.0 ± 1.1	100	2.2 ± 0.8
0.1					99	0.5 ± 0.5		
1					103	0.3 ± 0.5		
10					95	0.2 ± 0.4		
50	92	1.2 ± 1.2			102	0.0 ± 0.0		
100	96	1.5 ± 1.0			82	0.0 ± 0.0		
110							104	0.5 ± 0.5
150			101	0.2 ± 0.4	92	0.0 ± 0.0	100	0.7 ± 0.5
200	92	1.5 ± 0.8						
210							101	1.5 ± 1.0
300	91	0.8 ± 0.4	95	0.2 ± 0.4			107	0.8 ± 1.0
400	90	2.2 ± 2.0						
420							111	1.7 ± 1.2
500	89	Toxic						
600	86	Toxic	103	0.5 ± 0.8			39	Toxic
700			102	0.3 ± 0.5				
800	41	Toxic	92	0.0 ± 0.0				
900			78	0.0 ± 0.0				
1500			36	0.0 ± 0.0				
0[d] (0.1% DMSO)	100	7.5 ± 4.0	100	3.2 ± 1.9	100	1.0 ± 1.3		2.7 ± 1.4
TPA 0.05[e] (0.1% DMSO)	123	37.2 ± 4.7 †	144	11.2 ± 0.8 †	156	11.5 ± 1.9 †		20.0 ± 3.3 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

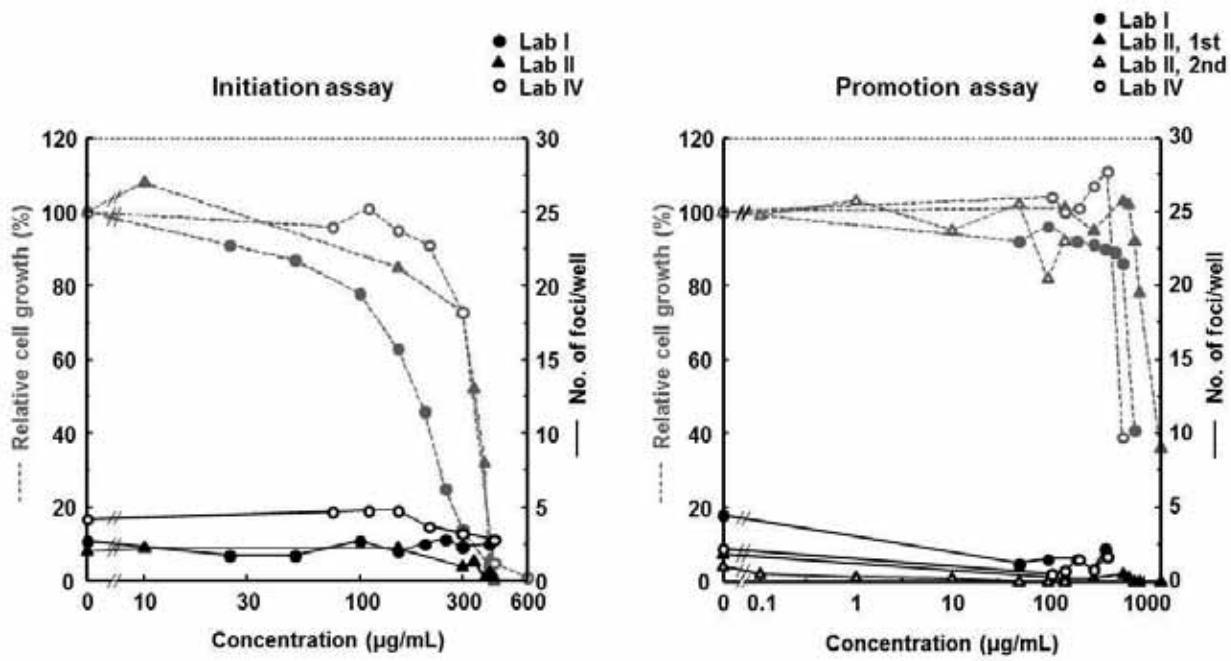

Fig. 14. Graphic view of the results of transformation assay and concurrent cell growth assay on L-ascorbic acid in the 6-well method validation study.

3.3.13 Caffeine

The results for caffeine are shown in Table 14 and Fig. 15. Two laboratories (Lab I and Lab V) presented convincing results that caffeine was negative in the initiation assay. Likewise, in the initiation assay reported by the other laboratory (Lab VI), no statistically significant increase in the number of transformed foci was reported, although cytotoxicity was also not induced at any of the concentrations of caffeine in the concurrent cell growth assay. Therefore, it was concluded that the initiation assay by Lab VI was incomplete because of inadequate dosing. Caffeine was judged negative by all three laboratories in the promotion assay.

Table 14.
Results of transformation assay on caffeine in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab V		Lab VI	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)						100	1.8 ± 1.5
0 (5% Water)		100	2.3 ± 1.6	100	2.7 ± 1.2		
6.25						87	2.2 ± 1.2
12.5						89	1.7 ± 0.8
25						97	0.5 ± 0.8
50						86	2.5 ± 1.8
100		114	1.8 ± 1.2	104	3.0 ± 2.5	90	4.0 ± 3.0
200				100	4.7 ± 2.1		
300		92	1.7 ± 1.0	76	5.2 ± 3.3		
400		51	2.0 ± 1.3	38	5.8 ± 2.3		
500		23	1.7 ± 1.0	18	3.7 ± 2.3		
600		13	2.5 ± 1.9				
700		11	1.0 ± 1.3				
800		11	1.8 ± 1.5				
0[d] (0.1% DMSO)		100	1.3 ± 1.2	100	3.3 ± 1.8		
MCA	1[e] (0.1% DMSO)	47	19.7 ± 4.0 †	54	41.3 ± 9.4 †		
MCA	1 (0.5% DMSO)					56	17.2 ± 3.5 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

b) Promotion assay

Concentration	Lab I		Lab V		Lab VI	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (0.5% DMSO)					100	4.0 ± 1.8
0 (5% Water)	100	5.0 ± 1.9	100	4.2 ± 2.3		
6.25					88	3.3 ± 1.2
10	93	2.0 ± 1.4	80	1.5 ± 1.0		
12.5					94	2.5 ± 1.4
25	87	4.0 ± 2.4			85	4.3 ± 1.4
30			73	2.2 ± 2.1		
50	82	3.5 ± 1.0			88	1.5 ± 1.0
100	70	1.5 ± 0.8	35	2.5 ± 1.6	78	1.8 ± 1.5
200	57	0.2 ± 0.4	33	2.3 ± 1.0		
300	49	0.0 ± 0.0	26	1.7 ± 1.6		
500	39	0.2 ± 0.4	27	0.5 ± 0.5		
0[d] (0.1% DMSO)	100	6.7 ± 2.3	100	2.7 ± 1.8		
TPA 0.05[e] (0.1% DMSO)	121	35.5 ± 6.1 †	106	21.5 ± 3.5 †		
TPA 0.05 (0.5% DMSO)					141	16.5 ± 4.8 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.

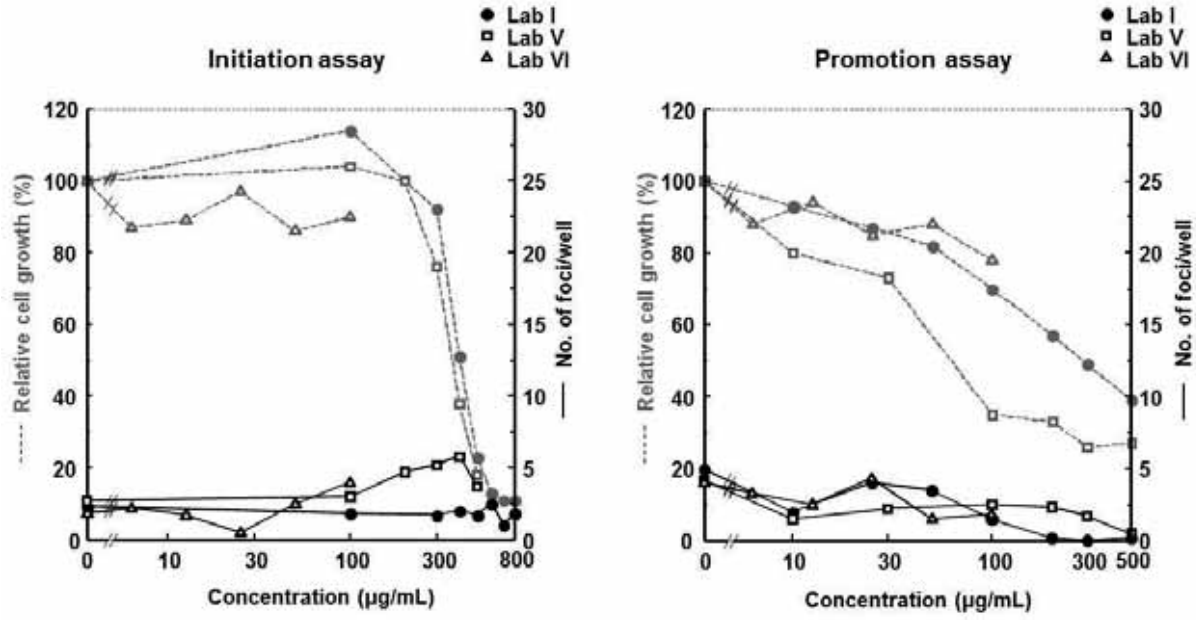

Fig. 15. Graphic view of the results of transformation assay and concurrent cell growth assay on caffeine in the 6-well method validation study.

3.3.14 D-Mannitol

Table 15 and Fig. 16 represent the results for D-mannitol. Both in the initiation assay and in the promotion assay, D-mannitol was consistently negative in the three laboratories up to the maximum concentration (5 mg/mL) tested.

Table 15.
Results of transformation assay on D-mannitol in the 6-well method validation study

a) Initiation assay

Concentration		Lab I		Lab III		Lab IV	
(μg/mL)		CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (5% Water)		100	2.7 ± 1.0	100	2.7 ± 1.4	100	1.2 ± 1.3
15.8				103	1.0 ± 0.9		
50				98	1.5 ± 0.8		
100		107	1.5 ± 1.0				
158				102	2.7 ± 1.6		
300		105	2.0 ± 2.4				
500				99	1.3 ± 1.2		
1000		111	0.8 ± 1.2				
1300						108	0.8 ± 1.0
1580				110	0.8 ± 0.8		
1800						111	1.8 ± 0.8
2500		106	3.2 ± 0.8			114	1.2 ± 0.8
3500						109	1.8 ± 1.2
5000		109	2.5 ± 1.0	102	1.8 ± 1.8	107	1.5 ± 1.6
	0[d] (0.1% DMSO)	100	2.7 ± 1.6				1.0 ± 0.9
MCA	1[e] (0.1% DMSO)	64	33.7 ± 5.0 †	33[f]	14.0 ± 4.4 ‡		40.5 ± 7.9 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

b) Promotion assay

Concentration	Lab I		Lab III		Lab IV	
(μg/mL)	CG[a]	Foci/well[b]	CG	Foci/well	CG	Foci/well
0[c] (5% Water)	100	5.8 ± 2.1	100	2.8 ± 1.5	100	1.5 ± 1.0
15.8			101	3.5 ± 1.6		
50			93	3.0 ± 1.4		
100	101	6.3 ± 4.5				
158			102	2.2 ± 1.9		
300	101	7.2 ± 2.0				
500			101	2.8 ± 2.3		
880					103	1.7 ± 0.5
1000	102	9.2 ± 3.2				
1300					107	1.5 ± 0.8
1580			104	1.7 ± 0.5		
1800					99	1.8 ± 1.7
2500	103	5.2 ± 2.6			110	1.7 ± 1.9
3500					101	1.3 ± 0.8
5000	103	3.7 ± 2.0	106	4.3 ± 1.5	99	1.2 ± 1.2
0[d] (0.1% DMSO)	100	8.2 ± 3.6				2.3 ± 1.0
TPA 0.05[e] (0.1% DMSO)	134	50.2 ± 4.6 †	157[f]	8.7 ± 3.6 ‡		10.2 ± 2.1 †

[a] % of cell growth compared to that of the solvent control.
[b] Average number of transformed foci/well ± SD.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
[f] % of cell growth compared to that of 5% water.
† $p<0.05$; one-sided t-test or Aspin-Welch test, vs. corresponding solvent control.
‡ $p<0.05$; one-sided t-test or Aspin-Welch test, vs. 5% water.

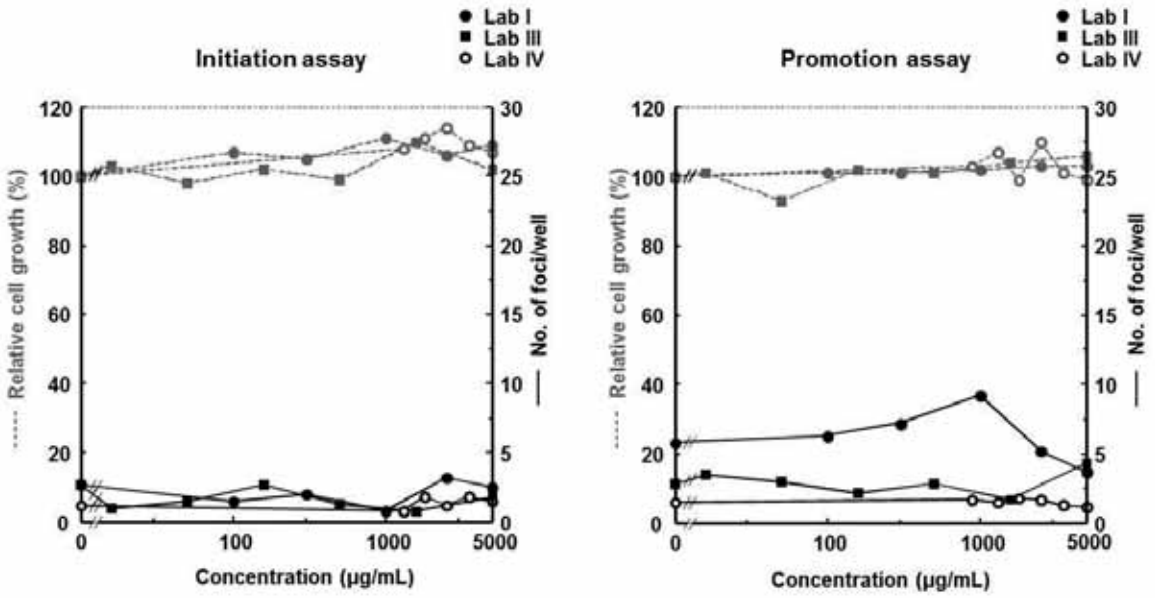

Fig. 16. Graphic view of the results of transformation assay and concurrent cell growth assay on D-mannitol in the 6-well method validation study.

3.4 Conclusion

The positive and negative calls for the test chemicals based on the results in the 6-well method validation study are summarized in Table 16. The positive or negative call for each chemical was individually determined based upon the Bhas 42 CTA results derived by each laboratory, into which the judgments in the initiation and promotion assays are integrated. Thus, a test chemical is positive in the Bhas 42 CTA, if it is positive in either of initiation and promotion assays. The overall judgment is determined by majority rule for the combined results obtained by all the participating laboratories.

3.4.1 Module 2 — Within-laboratory reproducibility

In this validation study, every test chemical was assayed including the negative control (DMSO) and each positive control (MCA in the initiation assay and TPA in the promotion assay). The numbers of transformed foci per well produced in the negative and positive controls are collected from all assays in the 6-well method validation study, clustered by individual laboratories and plotted in Fig. 3 for the initiation assay and the promotion assay. The average number of transformed foci per well in each laboratory is presented in Fig. 4. The ranges of the number of transformed foci per well produced in the negative control were narrow except for Lab V, which was one of two naive laboratories. The numbers of transformed foci in all the positive controls were statistically significantly increased in all the laboratories compared with those in the corresponding negative controls, although the number was considerably varied in Lab I (initiation and promotion assays) and Lab V (promotion assay). Thus, the VMT considered that the within-laboratory reproducibility for the negative and positive controls was satisfactory in all laboratories.

To estimate the within-laboratory reproducibility, the results obtained by Lab I were compared with those from a previously conducted Lab I in-house study (Table 17) [Sakai *et al.*, 2010]. With the exception of *o*-toluidine, all assay results between the two studies were concordant. Therefore, the within-laboratory reproducibility for the Bhas 42 CTA was 92% (11/12) in Lab I.

Lithocholic acid, methapyrilene hydrochloride, mezerein and anthracene had been examined in the 6-well method pre-validation study [Tanaka *et al.*, 2009] prior to this validation program. We then compared the results obtained by the same laboratories in the pre-validation study and the validation study. In Table 18, Lab A and Lab IV, Lab F and Lab I, and Lab C and Lab II were the same laboratories. Their results between pre-validation and validation studies were determined to be highly comparable. Their calls in the transformation assay likewise agreed. The chemicals methapyrilene and mezerein, which showed potent promoting activity in the Bhas 42 CTA, caused weak but statistically significant increases in the number of transformed foci at their high concentrations in single instances in several initiation assays.

3.4.2 Module 3 — Transferability

In general, the Bhas 42 CTA can be performed in a laboratory that has experience in routine cell culture techniques. General cell culture laboratory equipment and instruments are sufficient to perform the proposed test method. All supplies and

reagents are readily available commercially. Like the other CTAs, however, the Bhas 42 CTA requires staff training beyond that of general cell culture techniques in order to distinguish transformed foci from non-transformed foci. A training day was held in January, 2007 at HRI for the pre-validation study of the 6-well method, which preceded the validation study of the 6-well method (this validation study) and all the Japanese laboratories that participated in the pre-validation study of the 6-well method, the validation study of the 6-well method and the validation study of the 96-well method were involved. Thus, Japanese laboratories had been trained in the procedures used for the 6-well method Bhas 42 CTA and in the identification of Bhas 42 transformed foci. Consequently, those laboratories were able to gain experience in the Bhas 42 CTA prior to the conduct of the validation study of the 6-well method (this validation study). On the other hand, American and German laboratories, Lab V and Lab VI, respectively, which had been naive to the Bhas 42 CTA, received one day training at HRI for the validation study of the 6-well method (this validation study) in August, 2007. There, they were trained for the procedures of 6-well method Bhas 42 CTA and the identification of Bhas 42 transformed foci. They started the validation study of the 6-well method (this validation study) after preliminary efforts using MCA, TPA and DMSO (solvent control) in their own laboratories.

In the Bhas 42 CTA, foci are differentiated into two types, i.e., transformed foci and non-transformed foci, instead of type III foci, type II foci and type I foci, which have been used as descriptors in the BALB/c 3T3 CTA and the C3H101/2 CTA. A photo catalog of non-transformed foci and different morphological variants of transformed foci has been compiled to assist in the discrimination of transformed and non-transformed foci. The photo catalog is attached to this report as Annex 3.

In Table 16, Lab I is the lead laboratory and Lab V and Lab VI are naïve laboratories. Lab II, Lab III and Lab IV had some previous experience with the Bhas 42 CTA before this validation study. To evaluate its inter-laboratory transferability, Bhas 42 CTA results were compared between the lead laboratory and the naïve laboratories and between the lead laboratory and the laboratories having Bhas 42 CTA experience. The latter laboratories obtained results similar to those of the lead laboratory; furthermore, the judgment calls for transformation were completely concordant. The naïve laboratories, however, reported different results for *o*-toluidine hydrochloride and anthracene. This experience suggests that the proper conduct of the Bhas 42 CTA requires adequate training and practice to accurately discriminate and quantify transformed foci and non-transformed foci.

3.4.3 Module 4 – Between-laboratory reproducibility

As shown in Figures 3 and 4, the numbers of transformed foci per well in the negative controls for the initiation assay and the promotion assay were consistently low and less than 10 in all the laboratories with an exception of negative control for the promotion assay in Lab V, which was a naive laboratory. Although the values in the positive controls varied between laboratories, they were always statistically significantly different from those in the corresponding negative controls in all the laboratories. Thus, the VMT considered that the between-laboratory reproducibility was satisfactory for the negative and positive controls.

In addition to the reproducibility findings resulting from the studies reported for Module 2 and Module 3, above, the inter-laboratory reproducibility of the Bhas 42 CTA

was determined for the test chemicals listed in Table 16, each of which was distributed to three laboratories. In the initiation assay, concurring conclusions were reached by the three laboratories testing the same respective compounds with 8 out of 10 chemicals. Thus, the between-laboratory reproducibility for the initiation assay was 80% in this validation study. Since the tests of mezerein and caffeine were considered incomplete in two of three laboratories for mezerein and one of three laboratories for caffeine, those two chemicals were excluded from this calculation. In the promotion assay, concurrent conclusions were reached with 10 of 12 chemicals, and therefore between-laboratory reproducibility for the promotion assay was 83%. The judgments in the transformation assay were concordant between laboratories except for *o*-toluidine hydrochloride and anthracene, and consequently the between-laboratory reproducibility of Bhas 42 CTA was 83% (10/12). It is possible that the differences in results obtained were a consequence of those chemicals being examined by the naive laboratories, Lab V and Lab VI. Although mezerein was designated as equivocal (based upon the statistically significant increase in the number of transformed foci at only one dose) in the initiation assays conducted in two of the laboratories, the positive results obtained in the promotion assay were considered to have established mezerein as clearly positive in the Bhas 42 CTA. Likewise, despite the results for sodium arsenite in the initiation assay, which were split between negative and weakly positive, sodium arsenite was clearly positive in the promotion assay and thus considered positive in the Bhas 42 CTA in the three laboratories.

Between-laboratory reproducibility can be summarized as follows:

Between-laboratory reproducibility	
Assay	%
Initiation assay	80 (8/10)
Promotion assay	83 (10/12)
Transformation assay (Bhas 42 CTA)	83 (10/12)

3.4.4 Module 5 — Predictive capacity

The results demonstrated that the predictive capacity of Bhas 42 CTA is exceedingly high. When we compared *in vivo* carcinogenicity results and the overall judgments (judgment by majority rule among laboratories), all carcinogens including tumor-promoters were positive and all non-carcinogens were negative in the Bhas 42 CTA (Table 16). A 2x2 contingency table of the results obtained from 12 tested chemicals is presented below:

2x2 Contingency table of the results in the 6-well method Bhas 42 CTA validation study

		In vivo carcinogenicity		
		Carcinogen	Non-carcinogen	Total
Bhas 42 cell transformation assay	+	8	0	8
	–	0	4	4
Total		8	4	12

Table 16.

Judgments of the result for 12 test chemicals in the validation study of 6-well method.

Compound	Assay	Laboratory						Overall	Carcinogenicity
		I	II	III	IV	V	VI	Judgment[a]	*in vivo*
2-Acetylaminofluorene	Initiation	+			+		+	+	+
	Promotion	+			+		+	+	
	Transformation[b]	+			+		+	+	
Cadmium chloride	Initiation	-	-	-				-	+
	Promotion	+	+	+				+	
	Transformation	+	+	+				+	
Dibenz[*a,h*]anthracene	Initiation	+	+			+		+	+
	Promotion	-	-			-		-	
	Transformation	+	+			+		+	
Lithocholic acid	Initiation	-			-		-	-	TP[c]
	Promotion	+			+		+	+	
	Transformation	+			+		+	+	
Methapyrilene HCl	Initiation	-	-		-			-	+, TP
	Promotion	+	+		+			+	
	Transformation	+	+		+			+	
Mezerein	Initiation	-		±[d]	±				TP
	Promotion	+		+	+			+	
	Transformation	+		+	+			+	
Sodium arsenite	Initiation	-		+			i[e]		+
	Promotion	+		+			+	+	
	Transformation	+		+			+	+	
o-Toluidine HCl	Initiation	-				+	+	+	+
	Promotion	-				+	+	+	
	Transformation	-				+	+	+	
Anthracene	Initiation	-	-				-	-	-
	Promotion	-	-				+	-	
	Transformation	-	-				+	-	
L-Ascorbic acid	Initiation	-	-		-			-	-
	Promotion	-	-		-			-	
	Transformation	-	-		-			-	
Caffeine	Initiation	-				-	i	-	-
	Promotion	-				-	-	-	
	Transformation	-				-		-	
D-Mannitol	Initiation	-		-	-			-	-
	Promotion	-		-	-			-	
	Transformation	-		-	-			-	

[a] Judgment by majority rule.
[b] Judgment in the Bhas 42 CTA, into which judgments in the initiation and promotion assays are integrated.
[c] Tumor-promoter.
[d] Equivocal: there was a statistically significant increase in transformation frequency at only one concentration.
[e] Incomplete: unable to judge the results due to inadequate dosing.

Table 17.
Within-laboratory reproducibility of the 6-well method: Comparison of Lab I results for the 6-well method validation study and the in-house study.

Compound	Assay	Validation study	In-house study[a]
2-Acetylaminofluorene	Initiation	+	+
	Promotion	+	+
	Transformation	+	+
Cadmium chloride	Initiation	-	-
	Promotion	+	+
	Transformation	+	+
Dibenz[*a,h*]anthracene	Initiation	+	+
	Promotion	-	-
	Transformation	+	+
Lithocholic acid	Initiation	-	-
	Promotion	+	+
	Transformation	+	+
Methapyrilene HCl	Initiation	-	-
	Promotion	+	+
	Transformation	+	+
Mezerein	Initiation	-	-
	Promotion	+	+
	Transformation	+	+
Sodium arsenite	Initiation	-	±[b]
	Promotion	+	+
	Transformation	+	+
o-Toluidine (HCl)[c]	Initiation	-	-
	Promotion	-	+
	Transformation	-	+
Anthracene	Initiation	-	-
	Promotion	-	-
	Transformation	-	-
L-Ascorbic acid	Initiation	-	-
	Promotion	-	-
	Transformation	-	-
Caffeine	Initiation	-	-
	Promotion	-	-
	Transformation	-	-
D-Mannitol	Initiation	-	-
	Promotion	-	-
	Transformation	-	-

[a] Results of Lab I in-house study [Sakai *et al.*, 2010].
[b] Equivocal: there was a statistically significant increase in transformation frequency at only one concentration.
[c] Hydrochloride was tested in the 6-well method validation study, but free *o*-toluidine was used in the in-house study.

Table 18
Within-laboratory reproducibility of the 6-well method: Comparison of results of 4 chemicals that were duplicated between the pre-validation and validation studies of the 6-well method

Compound	Assay	Pre-validation[a] Laboratory[c]			Validation[b] Laboratory			Carcinogenicity *in vivo*
		A	C	F	I (F)	II (C)	IV (A)	
Lithocholic acid	Initiation	-		-	-		-	TP[d]
	Promotion	+		+	+		+	
	Transformation[e]	+		+	+		+	
Methapyrilene HCl	Initiation			+	-			+, TP
	Promotion			+	+			
	Transformation			+	+			
Mezerein	Initiation	-		-	-		±[f]	TP
	Promotion	+		+	+		+	
	Transformation	+		+	+		+	
Anthracene	Initiation		-	-	-	-		-
	Promotion		-	-	-	-		
	Transformation		-	-	-	-		

[a] The results in Lab A, Lab C and Lab F were obtained from the report on the pre-validation study of the 6-well method [Tanaka et al., 2009].
[b] The results of Lab I, Lab II and Lab IV were obtained from Table 16 in the present validation report.
[c] Lab A and Lab IV are the same laboratory; Lab C and Lab II are the same laboratory; Lab F and Lab I are the same laboratory.
[d] Tumor-promoter.
[e] Judgment in the Bhas 42 CTA, into which judgments in the initiation and promotion assays are integrated.
[f] Equivocal: there was a statistically significant increase in transformation frequency at only one concentration.

3.4.5 Negative and positive controls

Although the number of foci induced by the positive control varied between laboratories and sometimes within a given laboratory (Fig. 3), there was a statistically significant difference between the positive control and the corresponding negative control in every initiation and promotion assay. Taken together with the results reported for Modules 2-5, these data indicate that the Bhas 42 CTA 6-well method is both feasible and reliable.

3.4.6 Vehicle

In this validation study, the choice of solvent/vehicle (DMSO or distilled water) was left to each laboratory, considering the conditions of actual assay practice. Consequently, different solvents were chosen among the laboratories for 3 chemicals, sodium arsenite, *o*-toluidine hydrochloride and caffeine. Despite this difference, the judgment of transforming activity did not appear to be affected for these chemicals (Tables 10, 11 and 14). Furthermore, there is no information in literature that these chemicals are

unstable in either solvent.

3.4.7 Chemical treatment procedures in the initiation assay

In the initiation assay the cells were treated with test chemical by the direct addition of chemical solution to the culture medium or by the replacement of existing medium with fresh medium containing the chemical, although in the promotion assay the chemical treatment was always carried out by periodic exchanges of medium containing test chemical, as described in section 3.2.2, Initiation assay in the text and in the protocol for the 6-well method validation study in Annex 2. The choice of treatment procedure in the initiation assay was left to each laboratory. Only Lab IV out of 6 participating laboratories treated the cells by the medium exchange method. There was no difference in the judgment of results obtained with the two treatment procedures in the initiation assay for the particular 6 chemicals, 2-acetylaminofluorene, lithocholic acid, methapyrilene hydrochloride, mezerein, L-ascorbic acid and D-mannitol (Table 16).

4 Validation Study on the 96-well method

4.1 Study design and chemicals tested

4.1.1 Study design

Four laboratories participated and the study proceeded stepwise (pre-validation phase, validation phase I and validation phase II).A total of 25 chemicals were tested including duplication between phases, i.e., chemicals tested in (a) both the pre-validation phase and validation phase I, or (b) both validation phase I and validation phase II. Such chemicals were recorded as having been tested twice. Before starting the validation study, a workshop for the technical transfer of the 96-well method was held by HRI on 18 November, 2008. All participating laboratories took part in the workshop to learn the protocol, the assay procedures and judgment criteria for identifying transformed foci (i.e., how to count wells with transformed foci).

In the pre-validation study, MCA and TPA were examined to confirm the transferability of the test protocol. The chemical names were accessible and the concentrations tested were given in this phase: 0.03, 0.1, 0.3 and 1 μg/mL of MCA, and 0.025, 0.05, 0.1 and 0.2 μg/mL of TPA. Based on the transformation results obtained for the negative and positive controls in the pre-validation study, assay acceptance criteria were established.

In the validation phase I study, seven coded chemicals were examined and all of the chemicals were tested by all four laboratories to assess the inter-laboratory reproducibility of the 96-well method (Tables 19 and 21). MCA and TPA were included among the coded chemicals to evaluate intra-laboratory reproducibility by comparing the results obtained between the pre-validation phase and the validation phase I. The selected chemicals except TPA were the same as those tested in the ECVAM-initiative pre-validation study of BALB/c 3T3 CTA so that the results could be compared between the two CTAs.

In the validation phase II study, 16 coded chemicals were examined. Lab 4 did not participate in the phase II study. Each chemical was tested by two laboratories to confirm the inter-laboratory reproducibility of the 96-well method (Table 20). In order to assess the predictivity of the Bhas 42 CTA for chemical carcinogenicity, the chemicals selected included both tumor-promoters and non-carcinogens, with the latter comprising half of the total chemicals (Table 22). Ideally, it would have been good to include chemicals with low potency. However, it is difficult to assess precise potency based on available data. It could be argued that the process of dilution of test chemicals resulting in reduced concentrations may approximate potency reduction so as to mimic the testing of low potency chemicals. Benzo[*a*]pyrene was included among the coded test chemicals to ensure within-laboratory reproducibility between phases I and II. The chemicals which were used in the 6-well method validation study were also included among the coded test chemicals to confirm that the 6-well method and the 96-well method were equivalent in the transformation response.

The chemical properties and classes of test chemicals for the 96-well method validation study are presented Annex 15.

4.1.2 Chemicals tested and negative and positive controls

The chemicals selected, their CAS number, their distribution among laboratories and chemical codes for the validation study are listed for phase I and II studies in Tables 19 and 20, respectively. In the phase I study, the solvent (DMSO) for MCA, 2-acetylaminofluorene, benzo[*a*]pyrene and TPA was pre-selected by the VMT and their highest concentrations were also pre-assigned to be ≤100 μg/mL, ≤ 100 μg/mL, ≤100 μg/mL and ≤1 μg/mL, respectively, in the culture medium. In the phase II study, the approximate water solubility of each coded chemical was suggested and the top doses of benzo[*a*]pyrene, dibenz[*a,h*]anthracene, mezerein and phorbol were pre-assigned to be ≤10 μg/mL, ≤ 10 μg/mL, ≤0.1 μg/mL and ≤5 μg/mL, respectively, in the medium. These amounts were predetermined because these chemicals were expensive and their availability was limited. For the other chemicals, each individual participating laboratory determined the dose ranges for the transformation assays according to dose setting procedures using the cell growth assays.

MCA and TPA were chosen as positive controls for the initiation assay and the promotion assay, respectively.

Table 19
Test chemicals, CAS numbers and code names with allocation to the laboratories in phase I of 96 well method validation study.

Chemical	CAS no[a]	Carcinogenicity[b]	Code			
			Allocation to lab			
			1	2	3	4
3-Methylcholanthrene*	56-49-5	+	A-01	C-03	B-06	D-02
Benzo[*a*]pyrene*	50-32-8	+	A-02	C-04	B-07	D-01
2-Acetylaminofluorene*	53-96-3	+	A-03	C-07	B-01	D-04
TPA[c]*	16561-29-8	+,TP	A-04	C-05	B-03	D-07
o-Toluidine	95-53-4	+	A-05	C-06	B-02	D-03
Anthracene	120-12-7	–	A-06	C-01	B-04	D-05
Phenanthrene	85-01-8	–	A-07	C-02	B-05	D-06
3-Methylcholanthrene	56-49-5	+	Positive control for Initiation assay			
TPA[c]	16561-29-8	+	Positive control for promotionassay			

[a] Chemical Abstract Service registry number.
[b] +, carcinogen; –, non-carcinogen; TP, tumor-promoter
[c] 12-*O*-tetradecanoylphorbol-13-acetate.
* The solvent (DMSO) and the highest concentrations (3-methylcholanthrene, 100 μg/mL; benzo[*a*]pyrene, 100 μg/mL; 2-acetylaminofluorene, 100 μg/mL; TPA, 1 μg/mL) were designated by VMT in advance.

Table 20
Test chemicals, CAS numbers and code names with allocation to the laboratories in phase II of 96-well method validation study.

Chemical	CAS no.[a]	Carcinogenicity[b]	Code		
			Allocation to lab		
			1	2	3
N-Methyl-*N*'-nitro-*N*-nitrosoguanidine	70-25-7	+	B121		A101
Benzo[*a*]pyrene*	50-32-8	+	B122		A102
Dibenz[*a,h*]anthracene*	53-70-3	+		C131	A103
Sodium arsenite	7784-46-5	+	B123		A104
Cadmium chloride	10108-64-2	+	B124		A105
Methapyrilene HCl	135-23-9	+, TP		C132	A106
Mezerein*	34807-41-5	TP		C133	A107
Lithocholic acid	434-13-9	TP		C134	A108
Pyrene	129-00-0	–		C135	A109
Caprolactam	105-60-2	–	B125		A110
Ampicillin sodium	69-52-3	–	B126		A111
L-Ascorbic acid	50-81-7	–	B127		A112
D-Mannitol	69-65-8	–		C136	A113
Caffeine	58-08-2	–		C137	A114
Phorbol*	17673-25-5		B128		A115
Eugenol	97-53-0	–		C138	A116
3-Methylcholanthrene	56-49-5	+	Positive control		
TPA[c]	16561-29-8	+	Positive control		

[a] Chemical Abstract Service registry number.
[b] +, carcinogen; –, non-carcinogen; TP, tumor-promoter
[c] 12-*O*-tetradecanoylphorbol-13-acetate.
* The highest concentrations in the final culture medium (benzo[*a*]pyrene, 10 µg/mL; dibenz[*a,h*]anthracene, 10 µg/mL; mezerein, 0.1 µg/mL; phorbol, 5 µg/mL) were suggested by VMT in advance.

The carcinogenicity, including tumor-promoting activity, and genotoxicity of test chemicals in phases I and II are presented in Tables 21 and 22, respectively. Those activities of test chemicals were based on the review paper, DRP 31[OECD, 2007] and a review by Kirkland *et al.*, [2005], except for the carcinogenicity of methapyrilene hydrochloride, lithocholic acid and phorbol. Methapyrilene was included in the list of carcinogens in DRP 31 and reported to be a tumor-promoter by Lijinsky et al. [1992]. The tumor-promoting activity of lithocholic acid was reported by Reddy *et al.* [1975]. Carcinogenicity (leukaemogenic action) of phorbol has been reported to be positive in SWR mice [Berenblum and Lonai, 1970], and positive or negative depending on the mouse strains [Armuth, 1976], and its tumor-promoting activity in mouse skin is also positive in a sensitive stain, STS mice, but negative in other strains, Charles River CD1 mice and Sencar mice [Baird and Boutwell, 1971; Slaga et al., 1976 and 1980].

Table 21
Genotoxicity and carcinogenicity of the test chemicals for phase I of 96-well method validation study.

Chemicals	Genotoxicity[a]				IARC class	Carcinogenicity[b]
	In vitro			*In vivo*		
	Ames[c]	ML[d]	CA[e]	MN[f]		
3-Methylcholanthrene	+	+	+			+
Benzo[*a*]pyrene	+	+	+	+	2A	+
2-Acetylaminofluorene	+	+	+	+		+
TPA[g]	–		+			+, TP
o-Toluidine	+/–	+/–	+	–	2A	+
Anthracene	+w/–	+	–		3	–
Phenanthrene	+w/–		–		3	–

[a] Source: DRP 31 [OECD, 2007] and a review by Kirkland *et al.* [2005]: +, positive; –, negative; +/–, positive and negative results in DRP 31; +w, weakly positive.
[b] Source: DRP 31 [OECD, 2007]: +, carcinogen; –, non-carcinogen; TP, tumor-promoter.
[c] Ames test.
[d] Mouse lymphoma test.
[e] Chromosomal aberration test.
[f] Micronucleus test.
[g] 12-*O*-Tetradecanoylphorbol-13-acetate

Table 22
Genotoxicity and carcinogenicity of the test chemicals for phase II of 96-well method validation study.

Chemicals	Genotoxicity[a]				IARC class	Carcinogenicity[b]
	In vitro			*In vivo*		
	Ames[c]	ML[d]	CA[e]	MN[f]		
MNNG[g]	+	+	+	+		+
Benzo[*a*]pyrene	+	+	+	+	2A	+
Dibenz[*a,h*]anthracene	+	+	+		2A	+
Sodium arsenite	–	+		+	1	+
Cadmium chloride	–	+	+/–	–	1	+
Methapyrilene HCl	–	+/–	+			+,TP[h]
Mezerein	–					TP
Lithocholic acid	–	+/–	–			TP[i]
Pyrene	–	+	–		3	–
Caprolactam	–	–	–	–	4	–
Ampicillin sodium	–	–	–		3	–
L-Ascorbic acid	+w/–		–	+		–
D-Mannitol	–	–	–	–		–
Caffeine	–		+	+	3	–
Phorbol						*
Eugenol	–	+	+	+/–	3	–

[a] Source: DRP 31 [OECD, 2007] and a review by Kirkland *et al.* [2005]: +, positive; –, negative; +/–, positive and negative results in DRP 31; +w, weakly positive.
[b] Source: DRP 31 [OECD, 2007] except for lithocholic acid and phorbol: +, carcinogen; –, non-carcinogen; TP, tumor-promoter
[c] Ames test
[d] Mouse lymphoma test
[e] Chromosomal aberration test
[f] Micronucleus test
[g] *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine
[h] Lijinsky *et al.*, [1992]
[i] Reddy *et al.*, [1975]
* Carcinogenicity in mice (leukaemogenic action) is positive or negative depending on the strains [Berenblum and Lonai, 1970; Armuth, 1976]. Tumor-promoting activity in mouse skin is positive in a sensitive strain but negative in other strains [Baird and Boutwell, 1971; Slaga et al., 1976 and 1980]. These strains are mentioned in the text.

In the pre-validation study, the chemicals tested (MCA and TPA) were selected by VMT and distributed by the lead laboratory, HRI, to the other participating laboratories. In phases I and II, the test chemicals were selected by VMT, and purchased, coded and distributed by JaCVAM, which is institutionally and geographically independent of the participating laboratories. The chemicals were distributed to the chemical repository officer of each individual participating laboratory. The participating laboratories were not informed of the chemical names or the chemical selection criteria used in phases I and II. The suppliers, catalog numbers and lot numbers of the test chemicals are presented in Tables 55 and 56 of Annex 5.

4.1.3 Time line

18/04/2008 Preparation of a framework for the validation study on the 96-well method.

29-30/10/2008	1st VMT meeting: explanation of study plan, discussion on and determination of the protocol for pre-validation study (Ver. 2), test chemical selection for the pre-validation study to optimize the protocol (MCA and TPA), schedule of the pre-validation phase; chemical selection by VMT for the phase I validation study.
12/11/2008	Start of the pre-validation study. (MCA, TPA, Bhas 42 cells and FBS were shipped by HRI).
18/11/2008	Workshop for technical transfer of the 96-well method according to the protocol Ver. 2.
24/12/2008	Decision on the method of statistical analysis for the data.
13/1/2009	Deadline for data submission in the pre-validation study.
17/1/2009	Statistical analysis of the results in the pre-validation study by the statistical team.
26/1/2009	Joint meeting of Japanese VMT and participating laboratories: evaluation of the results in the pre-validation study, exchange of information on the technical issues, harmonization of focus counting among laboratories, decision regarding assay acceptance criteria*, and planning of phase I study. * As a result of the discussion in this joint meeting the protocol of 96-well method was amended for the phase I validation study (Ver. 3).
29/1/2009	Start of the phase I validation study. (The test chemicals were shipped by JaCVAM to participating laboratories.)
16/9/2009	Deadline for data submission in the phase I validation study
15/10/2009	Joint meeting of Japanese VMT and participating laboratories: report of the results in the phase I validation study, decoding of test chemicals.
9-10/11/2009	2nd VMT Meeting: evaluation of the results in the phase I validation study, and planning of the phase II study.
6/1/2010	Start of the phase II validation study. (Test chemicals were shipped by JaCVAM to participating laboratories.)
14/7/2010	Deadline for data submission in the phase II study.
6-7/10/2010	3rd VMT Meeting: report and evaluation of the results in the phase II validation study, approval of the 96-well method, decoding test chemicals.
15/9/2011	Meeting for the international Bhas 42 CTA validation study: Future schedule on the Bhas 42 CTA to develop OECD/TG: VMT members and the other participants agreed that the Bhas 42 CTA validation study report should undergo peer review by the ESAC through the ECVAM process.

4.2 Protocol

The schematic of 96-well method is the same as that of 6-well method (Fig. 2). The procedures of 96-well method is fundamentally the same as those of 6-well method as shown in Fig. 1. The differences from the 6-well method are as follows:

- The cell number inoculated per well are proportional relative to the well-size.
- The number of wells having transformed foci is quantified relative to the total number of observed wells, instead of the number of transformed foci in every

well, as is the case in the 6- well method. The wells having transformed foci in the bottom are counted, but the wells having transformed foci only on the wall are not counted. This is because the narrow shape of the well in a 96-well micro-plate is not conducive to observing and distinguishing transformed foci from non-transformed foci on the well wall. (In the 6-well micro-plate, transformed foci are counted both on the bottom and on the wall since technical problems of this nature are not encountered.)

- The statistical analysis for the 96-well method is carried out using the chi-square test with Bonferroni adjustment, instead of the multiple comparison using the Dunnett test which is applied for the 6-well method.

The detailed test protocols used in the pre-validation phase, validation phase I and validation phase II of the 96-well method validation study are presented in Annex 6,7 and 8, respectively. The protocol, Ver. 2 was used for the pre-validation phase, and amended to Ver. 3 for the phase I studies and Ver. 4 for the phase II studies to reflect the results, discussion and decisions made from the previous phases.

4.2.1 Preparation

Cells, materials and chemicals were prepared in the same manner as described in 3.2.1 of the 6-well method validation study. All materials, reagents, and FBS used are shown in the test protocols annexed (Annex 6, 7 and 8). The vehicles used to dissolve test chemicals and their final concentrations in the media are presented in the tables of assay results (Tables 23-47).

Concerning the preparation and storage of test-chemical solutions, a section was added in " I.1.3) Chemicals" in the protocol Ver. 3 (Page 1 in Annex 7). This addition was not an amendment but a correction, because the procedures included were explained in the workshops for technical transfer of the protocol and were performed in the 6-well method validation study and the pre-validation phase of 96-well method validation study. The insertion was as follows:

- Preparation and storage of test-chemical solutions: Test-chemical solutions are prepared before use as a general rule. Working solutions may be preserved in aliquots at -20°C for less than 10 days and thawed before use, but must not be re-frozen. When the amount of test chemical supplied is too little to be weighed for every assay, a stock solution is prepared at a concentration as high as possible or at some other appropriate concentration and stored in aliquots at -20℃ for up to a year and thawed before use. The stock solution is never re-frozen. When suspensions of test chemicals are used, they must be prepared before use and cannot be stored for the future use. The stock solutions of MCA and TPA in DMSO can be stored at -20 ℃ for at least two years, if they are not thawed. [Page 1 in Annex 7 and 8]

4.2.2 Initiation assay

4.2.2.1 Cell growth assay

A cell suspension at 4000 cells/mL in DF5F was distributed into each well of 96-well

micro-plates in a 0.05 mL volume (200 cells/well, day 0). Eight wells were prepared for each treatment group. At 24 h after seeding, medium containing a test chemical at two times the final concentration or containing vehicle alone was prepared, and 0.05 mL of the preparation was added to each well without medium exchange so that the volume of medium was 0.1 mL/well in total. On day 4, the medium containing the test chemical was replaced with 0.1 mL/well of fresh DF5F. On day 7, the cells were fixed and stained with CV, and the relative cell growth of cultures treated with a chemical was calculated according to the procedures as described in 3.2.2.1.

4.2.2.2 Dose setting for the transformation assay

Five or more concentrations were selected based on the results of cell growth assays as described in 3.2.2.2.

4.2.2.3 Transformation assay to examine initiating activity

One tube of the frozen Bhas 42 cells was thawed and grown in M10F up to about 70% confluence and then subcultured in DF5F to reach about 70% confluence again as described in 3.2.2.3. Thereafter, cells were cultured in DF5F. The cells were trypsinized and suspended at a density of 4,000 cells/mL and seeded into each well of 96-well micro-plates in 0.05 mL volumes (200 cells/well, day 0). One plate and 8 wells were prepared per concentration, of which one plate was reserved for the transformation assay and eight wells were reserved for the concurrent cell growth assay. At 24 h after seeding, medium containing a test chemical at two times the final concentration or containing vehicle alone was prepared, and 0.05 mL of the preparation was added to each well without medium exchange so that the volume of medium was 0.1 mL/well in total. The treatment in the initiation phase was continued for 72 h. Following the exposure period, all treatment media were removed and the cells were refed with 0.1 mL/well of medium without the test chemical (day 4) and subsequently cultured in the normal medium until day 21, receiving medium exchanges on day 7, day 11 and day 14. The cells were then fixed with methanol and stained with Giemsa's solution. Each assay also included MCA (1 μg/mL) as the positive control.

4.2.3 Promotion assay

4.2.3.1 Cell growth assay

A cell suspension of 4000 cells/mL in DF5F was distributed into each well in 0.1 mL of medium (400 cells/well, day 0), and on day 4, the culture medium was replaced with a medium containing a given test chemical. On day 7, the cells were fixed and stained with CV, and the relative cell growth of cultures treated with a chemical (chemical vs. vehicle) was calculated, as described in 3.2.2.1.

4.2.3.2 Dose setting for the transformation assay

Five or more concentrations were selected based on the results of cell growth assays as described in 3.2.3.2.

4.2.3.3 Transformation assay to examine promoting activity

The promotion assay was carried out in the same manner as the initiation assay

(4.2.2.3) except for the following steps. The cells were seeded at a density of 400 cells/well in 0.1 mL of medium (day 0) and cultured for 4 days without a medium exchange. On day 4, day 7, and day 11, the culture medium was replaced with fresh medium containing a given test chemical or vehicle alone and the treatment in the promotion phase was continued until day 14 (for a total of 10 days). The cells were then cultured in normal medium without the test chemical for one week until day 21. Each assay also included TPA (0.05 μg/mL) as the positive control.

4.2.4 Focus count and statistical analysis

The transformed foci were judged using the same criteria as those used in the 6-well method (Annex 3). The number of wells having transformed foci versus the total number of observed wells was recorded. The bottom area of each well was observed under a stereoscopic microscope, but the wall of each well was excluded from consideration for the reasons discussed under 4.2 above. The proportion of wells having transformed foci was statistically analyzed by the chi-square test with Bonferroni adjustment. "Toxic" was recorded for plates or wells that were not confluent at the end of transformation assay because of cytotoxicity resulting from chemical treatment.

- The p-value of the chi-square test was tentatively set at <0.05 or <0.025 (upper-sided) in protocol Ver. 2 and Ver. 3. The transformation frequency was analyzed with two p-values, <0.025 and <0.05 (upper-sided), in the phase I study, and the p-value was finally fixed at <0.5 (upper-sided) in protocol Ver. 4, based on the results in the phase I study (Page 8 in Annex 7-8).

4.2.5 Judgment

The assay results were judged positive when there existed two or more sequential doses that induced statistically significant increases in the proportion of wells having transformed foci, and negative when there was no dose showing a statistically significant increase in the proportion of wells having transformed foci. When the statistically significant increase was at only one dose, the assay result was regarded as equivocal. In such cases, the initiation or promotion assay together with the concomitant cell growth assay was repeated and included the positive dose in the first assay. The chemical was judged to be positive if a statistically significant increase in the proportion of wells having transformed foci resulted at one or more concentrations in the second assay.

- In the pre-validation phase and the validation phase I study, the repeat of assay to make a final judgment was compulsory for the participating laboratory when the statistically significant increase was at only one dose. In the validation phase II study, that requirement was amended so that the decision to re-test was left up to each laboratory. However, in the absence of the repeat test, the judgment remained equivocal. This amendment was introduced in the interest of time and cost savings. (Page 8 in Annex 7-8)).

4.2.6 Acceptance criteria

The assay acceptance criteria were established in protocol Ver. 2 for the pre-validation

study, as follows (citation from Annex 6, protocol Ver. 2):

The initiation or promotion assay is repeated independently, as needed, to satisfy the assay acceptance criteria.

1. Concurrent cell growth assay
   - At least one concentration should be included near the NOEL which gives 80-120% cell growth compared to solvent/vehicle control.
   - When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration.
2. Transformation assay

- Initiation assay and promotion assay
  - If the cells are killed and/or not confluent at the end of transformation assay, the concentration is not valid and excluded from focus-counting, statistical analysis and judgment. In such cases, "toxicity" is entered in the data sheet.
  - When contamination or technical problems are observed, if, for a given concentration, the number of damaged wells is ≥7, the concentration is not considered valid and is excluded from focus-counting, statistical analysis and judgment. In such cases, "contamination", "accident", "technical error",etc. are entered in the data sheet.
- Initiation assay
  - Negative control: The number of wells having transformed foci should be 10 wells/plate or less.
  - Positive control: The number of wells having transformed foci should be 30 wells/plate or more.
  - Test chemical concentrations: Four valid concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and three concentrations in the range between the NOEL and IC_{90}.
- Promotion assay
  - Negative control: The number of wells having transformed foci should be 20 wells/plate or less.
  - Positive control: The number of wells having transformed foci should be 30 wells/plate or more. Furthermore, the proportion of wells having transformed foci should be significantly increased in the positive control plate compared to the solvent control plate (chi-square test, $p<0.05$).
  - Test chemical concentrations: Four valid concentrations are necessary, at the minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increases cell density). The durations of chemical exposure to the cells are different between the cell growth assay and the transformation assay. Consequently, chemical toxicity is sometimes accumulated over the 10 day duration of the promotion assay and plates may be lost because of chemical toxicity. In such cases, four valid plates are also necessary in the concentration range where cells are not killed and are confluent at the end of the transformation assay. If excessive toxicity is encountered, it may be necessary to repeat the assay in a lower concentration range.

The transformation frequency in the CTAs is known to be influenced by the batch of

FBS [Sakai *et al.*, 2002]. The parts of the assay acceptance criteria that are underlined (Annex 6, section F.), which concern the transformation frequency of negative and positive controls, were tentatively set based on the HRI historical data, that was produced using FBS obtained from Moregate (Lot no. 7825120). The 96-well method validation study, including the pre-validation phase, was carried out using FBS obtained from GIBCO (Lot no. 1391481). Hence, three out of four of the underlined parts of the acceptance criteria were modified in protocol Ver. 3 for phase I based on the results obtained from MCA, TPA and negative controls in the pre-validation study: these amendments were incorporated into protocol Ver. 4 for phase II.

The amendments of acceptance criteria concerning the transformation frequency of negative and positive controls in the protocol Ver. 3 were as follows (Page 6-7 in Annexes 7 and 8):

2. Transformation assay
- Initiation assay
  - Negative control: The number of wells having transformed foci should be 15 wells/plate or less.
  - Positive control: The number of wells having transformed foci should be 40 wells/plate or more.
- Promotion assay
  - Negative control: The number of wells having transformed foci should be 20 wells/plate or less.
  - Positive control: The number of wells having transformed foci should be 40 wells/plate or more.

- An overlapping item of acceptance criteria in the protocol Ver. 2 was deleted in the protocol Ver. 3. That is:

  The item "・ At least should exist one concentration near NOEL which gives 80-120% cell growth compared to solvent/vehicle control." was deleted from "II.F.1 Concurrent cell growth assay" in Ver. 2 (Page 6), and instead was included in the item "・ Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: at least one concentration near the NOEL (80 – 120 %) and three concentrations in the range between the NOEL and IC_{90} in the concurrent cell growth assay." of "II F. 2.2) Initiation assay" in Ver. 3 (Page. 7 in Annex 6-8).

4.3 Results

The validation study of the Bhas 42 CTA 96-well method was carried out on a total of 25 coded chemicals for the initiation and promotion assays. Each chemical was examined by four laboratories in the pre-validation phase and validation phase I and by two laboratories in validation phase II. The data for the negative and positive controls obtained in individual laboratories are illustrated in Figures 17 and 18. The transformation results of each test chemical in the 96-well method, including the respective concurrent cell growth assay, are presented in Tables 23 to 47 and in Figures 19 to 43. The raw data submitted by individual laboratories for the pre-validation phase,

and for validation phase I and phase II are presented in Annex 9, 10 and 11, respectively.

4.3.1 Quality assurance aspects

All of the data from all laboratories were sent at the same time to Masaya Suzuki and Shoko Arai, who belong to geographically and administratively different laboratories, and they assured the quality of all the data and records, by evaluating their compliance with the acceptance criteria. Subsequently, M. Suzuki statistically analyzed the data. In some instances, certain experiments were repeated by a particular laboratory. For such cases, the data from both the first and repeated experiments are presented in the detail result section for each chemical, including the reason(s) as to why the experiment was repeated.

4.3.2 Negative and positive controls

The transformation frequencies for the positive and negative (DMSO) controls are presented in Tables 25-47 as part of the assay results derived for each test chemicals. All data of negative and positive controls satisfied assay acceptance criteria except for one case: i.e., the first promotion assay of L-ascorbic acid by Lab 3 in validation phase II. In Fig. 17, those control values are plotted pairwise, assay by assay, and the pairs are clustered by laboratory. The transformation frequency of each positive control was statistically significantly different from that of the corresponding negative control. The cluster of transformation frequencies of positive controls was quite distinct from that of negative controls. In Fig. 18, the transformation frequencies for the negative and positive controls are averaged per individual laboratory.

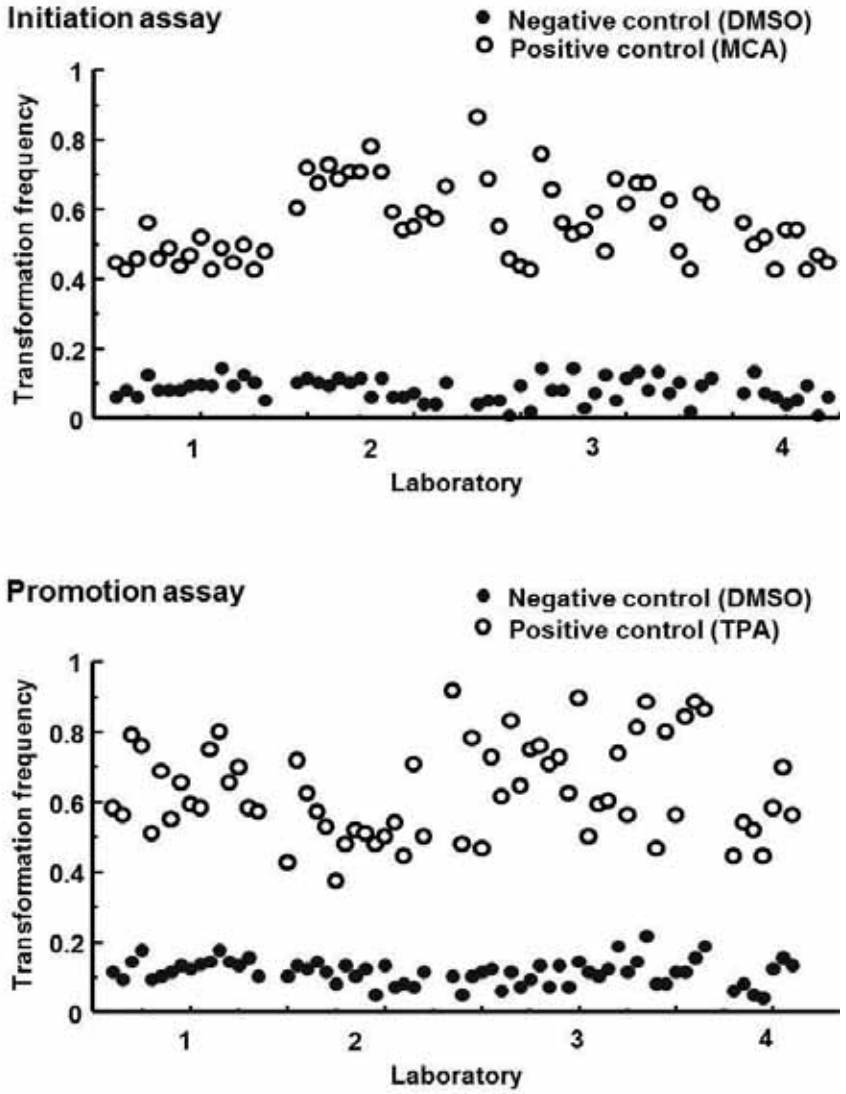

Fig. 17. Transformation frequency of the negative and positive controls in the 96-well method validation study. The negative and positive controls are paired assay by assay and the pairs are clustered laboratory by laboratory.

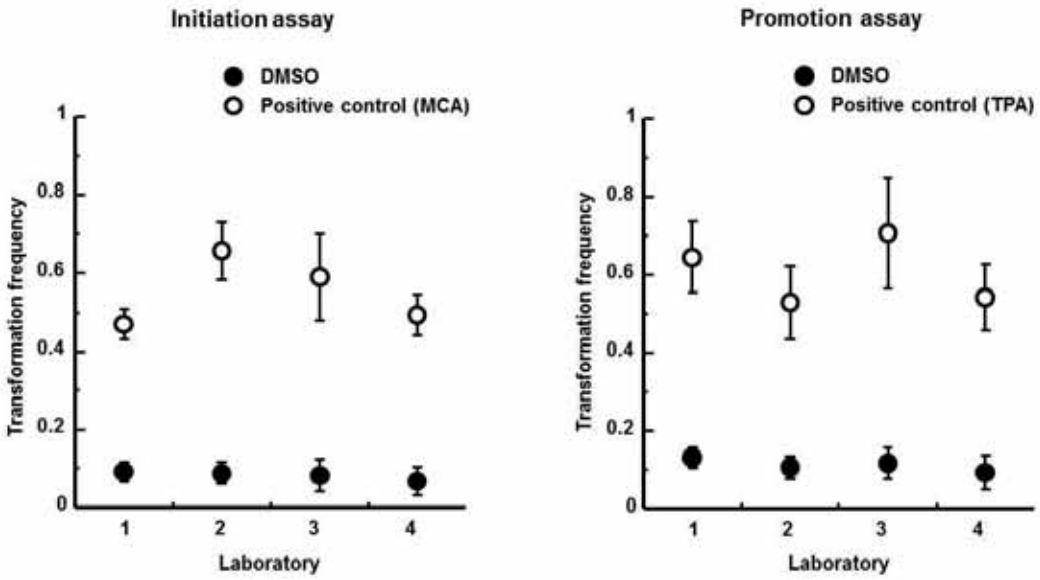

Fig. 18. Average transformation frequency of negative and positive controls per individual laboratory in the 96-well method validation study including phase I and phase II. Bars indicate standard deviation.

4.3.3 Pre-validation (transferability phase)

With the aim of assessing the transferability of the assay to the participating laboratories, MCA and TPA were examined without having been coded at the concentrations designated by the VMT.

4.3.3.1 3-Methylcholanthrene

As shown in Table 23 and Fig. 19, MCA was positive in the initiation assay in all the participating laboratories and the dose response curves were concordant between the laboratories.

Table 23.
Results of transformation assay on 3-methylcholanthrene in the pre-validation phase of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	7/96	100	11/96	100	8/96	100	5/96
0.03	95	17/96	115	20/96	87	16/96	89	14/96
0.1	90	31/96 *	140	35/96 *	80	27/96 *	84	19/96 *
0.3	75	46/96 *	130	51/96 *	70	44/96 *	72	30/96 *
1	53	71/91 *	83	66/96 *	50	59/96 *	47	33/96 *

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. solvent control.

b) Promotion assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	14/94	100	15/96	100	6/96	100	16/96
0.03	91	10/96	86	13/96	78	2/96	78	13/96
0.1	84	19/96	86	22/96	71	2/96	65	15/96
0.3	75	15/96	73	38/96 *	64	5/96	52	15/96
1	86	9/96	89	15/96	65	3/96	45	6/96

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. solvent control.

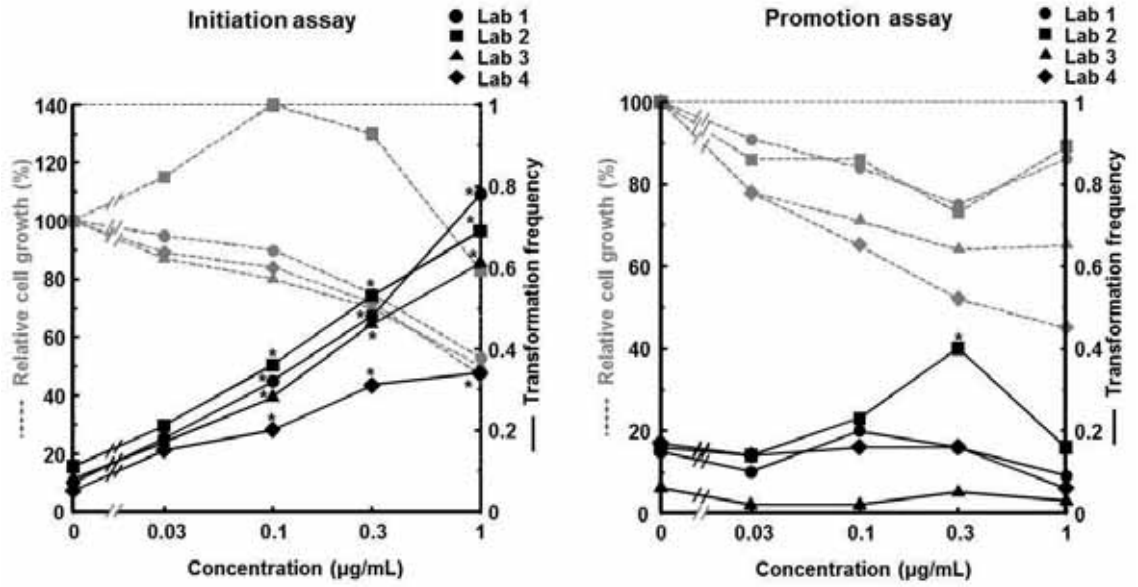

Fig. 19. Graphic view of the results of transformation assay and concurrent cell growth assay on 3-methylcholanthrene in the pre-validation phase of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.3.2 TPA

Table 24 and Fig. 20 represent the results for TPA. TPA was positive in the promotion assay in all the four laboratories and induced similar responses between laboratories.

Table 24.
Results of transformation assay on TPA in the pre-validation phase of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	8/96	100	10/96	100	8/96	100	7/96
0.025	95	4/96	99	4/96	91	0/96	86	2/96
0.05	97	4/96	115	4/96	94	2/96	87	0/96
0.1	100	1/96	115	5/96	97	4/96	89	3/96
0.2	111	5/96	122	6/96	107	5/96	98	2/96

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control.

b) Promotion assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	14/96	100	14/96	100	7/96	100	15/96
0.025	127	78/96 *	146	52/96 *	110	54/96 *	145	50/96 *
0.05	153	71/96 *	157	71/96 *	117	66/96 *	161	46/96 *
0.1	194	69/96 *	231	65/96 *	129	56/96 *	194	41/96 *
0.2	255	77/96 *	301	59/96 *	146	55/96 *	238	25/96

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. solvent control.

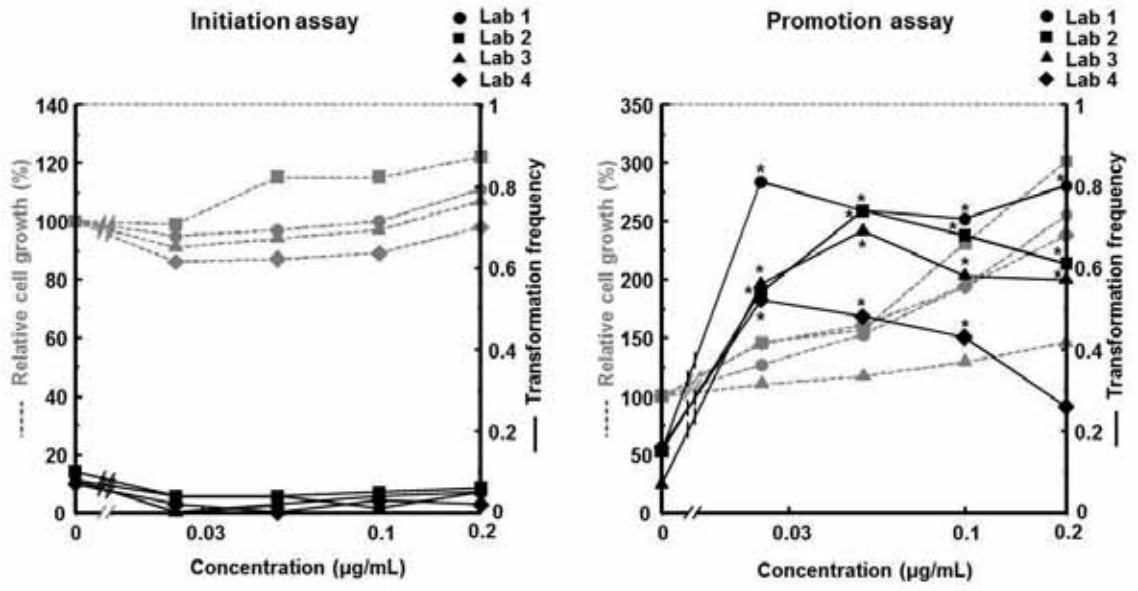

Fig. 20. Graphic view of the results of transformation assay and concurrent cell growth assay on TPA in the pre-validation phase of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

Thus, all laboratories obtained comparable results from MCA and TPA and proceeded to the validation phase I study. The acceptance criteria for the transformation frequency in the negative and positive controls were amended for phase I based on the results of MCA and TPA in the pre-validation phase. The criteria applied in protocol Ver. 2 had been tentatively established based on the assays that had been carried out using a different batch of FBS prior to the 96-well validation study. Use of a new batch of FBS throughout the 96-well method validation study necessitated the revision of the acceptance criteria. These amended criteria were also employed in the validation phase II studies.

4.3.4 Validation phase I

Seven coded chemicals were tested in each laboratory.

4.3.4.1 2-Acetylaminofluorene

The results for 2-acetylaminofluorene are shown in Table 25 and Fig. 21. In the initiation assay, the chemical was negative in the Lab 1 but positive in Lab 2, Lab 3 and Lab 4. In Lab 4, there was a statistically significant increase in transformation frequency at only one concentration, 21 μg/mL, in the first run of the initiation assay. In accordance with the protocol, the assay was repeated and significant increases in transformation frequency at two serial concentrations were obtained. Hence the chemical was positive in the initiation assay in Lab 4. In the promotion assay, this chemical was positive in Lab 1, Lab 3 and Lab 4 but negative in Lab 2.

Table 25.
Results of transformation assay on 2-acetylaminofluorene in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4, 1st run		Lab 4, 2nd run	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	6/96	100	11/96	100	4/96	100	6/96	100	4/96
2.5	100	6/96								
5	97	10/96								
6.25			98	5/96						
8.84			102	16/96						
10	98	7/96					113	8/91	103	-/- [d]
12					104	14/96				
12.5			101	20/96						
15							90	10/93	82	6/96
16					97	17/96 *				
16.5							72	16/96	72	10/96
17.7			101	25/96 *						
18							62	15/96	45	9/96
19					75	19/96 *				
19.5							39	16/96	42	20/95 *
20	79	12/96								
21							28	25/96 *	23	18/96 *
22					53	23/96 *				
22.5							21	13/96	20	9/96
24							12	7/96	17	2/96
25			47	34/96 *	33	20/96 *				
29					20	16/96 *				
30	13	12/96					5	5/96	7	4/94
34					13	17/96 *				
35.4			14	25/96 *						
40					11	8/96				
50	6	Toxic	8	14/96	9	15/96 *				
100	4	Toxic								
MCA 1[e] (0.1% DMSO)	26	44/96 †	48	68/96 †	49	83/96 †	56	41/96 †	47	52/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)	100	14/96	100	13/96	100	10/96	100	4/96
	0.01							111	6/96
	0.1							112	11/96
	0.4							117	8/96
	0.5					105	28/96 *		
	0.78			108	8/96				
	1					99	15/96		
	1.56	90	24/96	114	16/96			119	3/96
	2					103	11/96		
	3					99	15/96		
	3.13	84	16/96	121	18/96				
	5					92	22/96		
	6.25	72	43/96 *	102	19/96			104	18/96 *
	9					81	29/96 *		
	12.5	60	44/96 *	83	22/96			82	21/94 *
	15					63	35/96 *		
	25	44	16/96	57	10/96	50	22/96	53	9/96
	40					47	Toxic		
	50	33	0/96	37	0/96			38	0/96
	100	33	Toxic					33	Toxic
TPA	0.05[d] (0.1% DMSO)	111	76/96 †	137	46/96 †	145	88/96 †	155	43/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

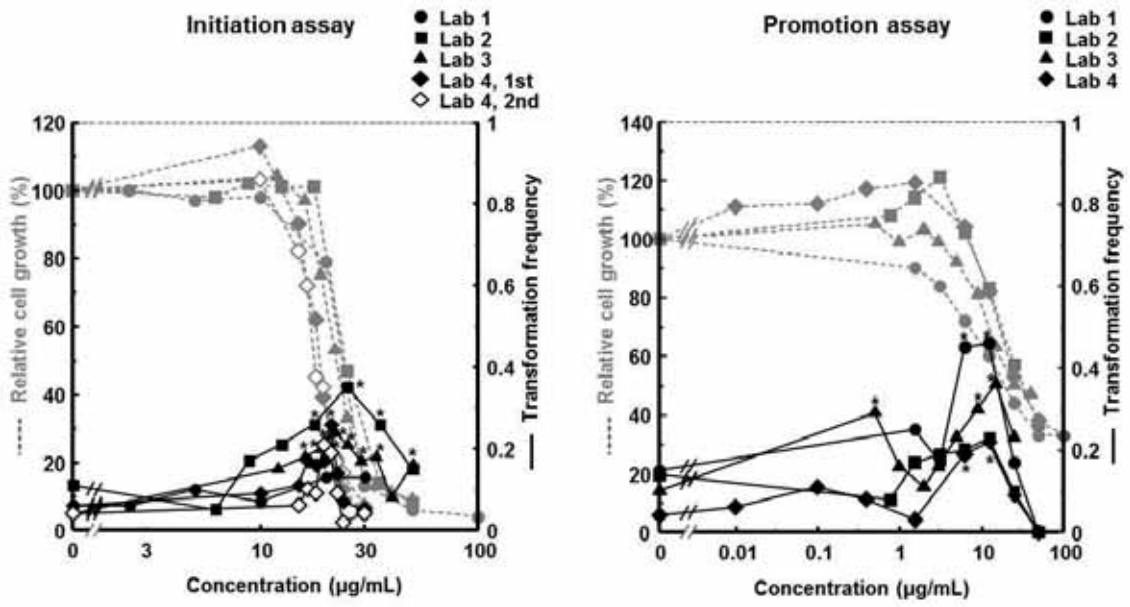

Fig. 21. Graphic view of the results of transformation assay and concurrent cell growth assay on 2-acetylaminofluorene in phase I of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.4.2 Benzo[*a*]pyrene

In all the laboratories, benz[a]pyrene was strongly positive in the initiation assay and negative in the promotion assay as shown in Table 26 and Fig. 22.

Table 26.
Results of transformation assay on benzo[*a*]pyrene in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0 [c] (0.1% DMSO)	100	8/96	100	9/96	100	14/96	100	7/96
0.0016							109	11/96
0.003					93	5/96		
0.0031			92	13/96			113	8/96
0.00316	91	13/96						
0.0063			101	12/96			114	13/96
0.01	96	8/96			90	14/96		
0.0125							109	9/96
0.013			95	22/96 *				
0.025			88	29/96 *			113	18/96
0.03					80	15/96		
0.0316	67	21/96 *						
0.05			78	34/96 *	65	31/96 *	91	16/96
0.1	24	20/96 *	60	39/96 *	43	35/96 *	65	29/96 *
0.2			36	48/96 *	24	36/96 *	31	25/95 *
0.3					19	36/96 *		
0.316	9	Toxic						
0.4			24	45/96 *			12	25/96 *
0.5					12	32/96 *		
0.8			14	41/96 *				
1	7	Toxic						
3.16	3	Toxic						
MCA 1[d] (0.1% DMSO)	28	41/96 †	35	70/96 †	43	73/96 †	51	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)	100	9/96	100	14/96	100	11/96	100	6/96
	0.0001	95	10/96						
	0.0003					93	11/96		
	0.001	91	7/96			91	8/96		
	0.0016			88	2/96				
	0.002							107	4/96
	0.003					89	5/96		
	0.0031			93	6/96				
	0.0045							110	7/96
	0.0063			83	7/96				
	0.009							109	4/96
	0.01	88	7/96			81	8/96		
	0.013			87	2/96				
	0.02					84	4/96	105	5/96
	0.025			75	5/96				
	0.03					75	5/96		
	0.0316	81	7/96						
	0.045							91	4/96
	0.05			64	6/96	67	2/96		
	0.09							79	7/96
	0.1	63	6/96	51	7/96	51	1/96		
	0.2			47	11/96			59	7/96
	0.4			44	6/96				
	0.45							46	Toxic
	1	45	Toxic					42	Toxic
	10	50	Toxic						
TPA	0.05[d] (0.1% DMSO)	164	54/96 †	107	55/96 †	136	80/96 †	161	43/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

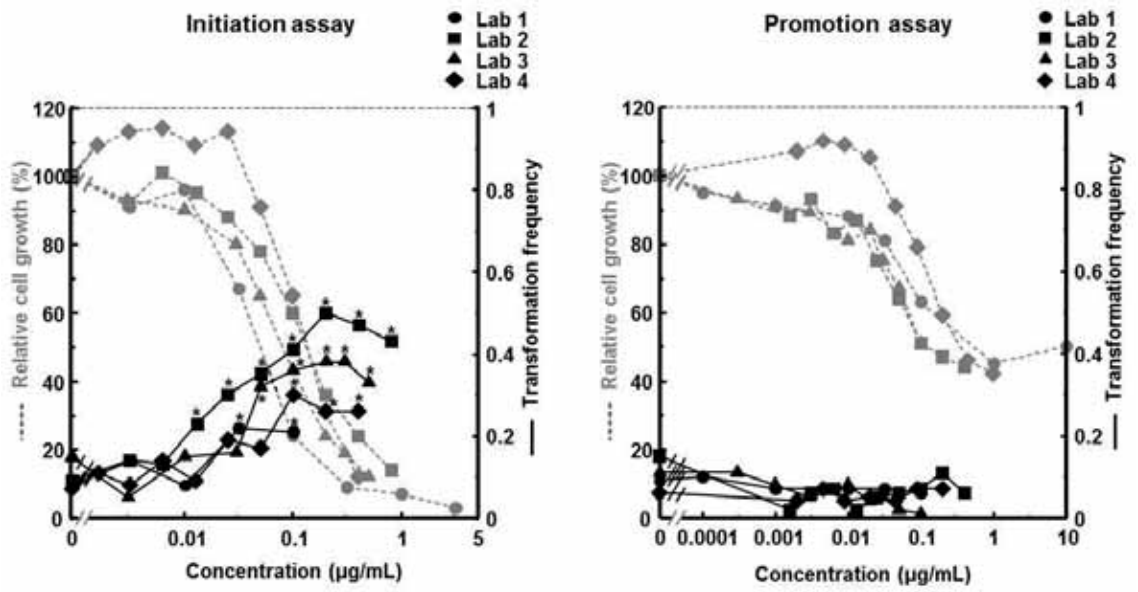

Fig. 22. Graphic view of the results of transformation assay and concurrent cell growth assay on benzo[*a*]pyrene in phase I of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.4.3 3-Methylcholanthrene

The results for MCA are presented in Table 27 and Fig. 23. This chemical was clearly positive in the initiation assay in all the laboratories. In the promotion assay, it was negative in Lab 1, Lab 2 and Lab 3. In Lab 4, there was a statistically significant increase at the highest usable doses (0.2 μg/mL) before the onset of overwhelming toxicity. However, Lab 4 did not repeat the promotion assay. They considered that the initiation assay had established that the chemical was clearly positive in the Bhas 42 CTA and that it was not necessary to further differentiate the initiation vs. promotion properties of the compound.

Table 27.
Results of transformation assay on 3-methylcholanthrene in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1%DMSO)			100	10/96				
0 (0.5% DMSO)	100	10/96			100	4/96	100	6/90
0.005							122	12/96
0.01	105	27/96 *						
0.025			81	20/96				
0.03					95	13/96		
0.05			80	28/96 *				
0.07							106	21/96 *
0.1	93	36/96 *	68	43/96 *	84	11/96		
0.2			61	60/96 *				
0.21							96	27/96 *
0.3					66	33/96 *		
0.316	62	44/96 *						
0.4			52	63/96 *				
0.42							76	41/96 *
0.7							65	51/96 *
0.8			46	65/96 *				
1	44	39/96 *			46	47/96 *	65	56/96 *
1.6			39	72/96 *				
2.5							53	61/96 *
3					38	31/96 *		
3.16	34	36/96 *						
3.2			41	75/96 *				
5							50	61/96 *
6.4			43	59/96 *				
10	28	Toxic			35	15/96 *	47	50/96 *
30					34	12/96		
100	25	Toxic			35	Toxic		
0[d] (0.1%DMSO)	100	6/96			100	2/96	100	13/96
MCA 1[e] (0.1% DMSO)	34	43/96 †	49	65/96 †	42	41/96 †	51	48/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)			100	12/96				
	0 (0.5% DMSO)	100	12/96			100	8/96	100	9/95
	0.00001	91	9/96						
	0.0001	89	13/96						
	0.001	86	4/93			96	7/96	95	13/96
	0.0016			96	11/96				
	0.003					93	5/96		
	0.0031			90	8/96				
	0.004							95	7/96
	0.0063			86	12/96				
	0.01	73	6/96			85	3/96		
	0.013			84	10/96				
	0.015							88	11/96
	0.025			85	13/96				
	0.03					82	1/96		
	0.05			82	10/96				
	0.06							86	8/96
	0.1	56	10/95	70	13/96	74	2/96		
	0.2			65	16/96			69	25/96 *
	0.3					59	0/96		
	0.4			55	12/96			66	Toxic
	1	58	Toxic			61	0/96	64	Toxic
	2.2							66	Toxic
	3					62	0/96		
	5							64	Toxic
	10	58	Toxic						
	100	60	Toxic						
	0[d] (0.1%DMSO)	100	11/96			100	6/96	100	8/96
TPA	0.05[e] (0.1% DMSO)	160	56/96 †	126	60/96 †	147	59/96 †	166	52/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

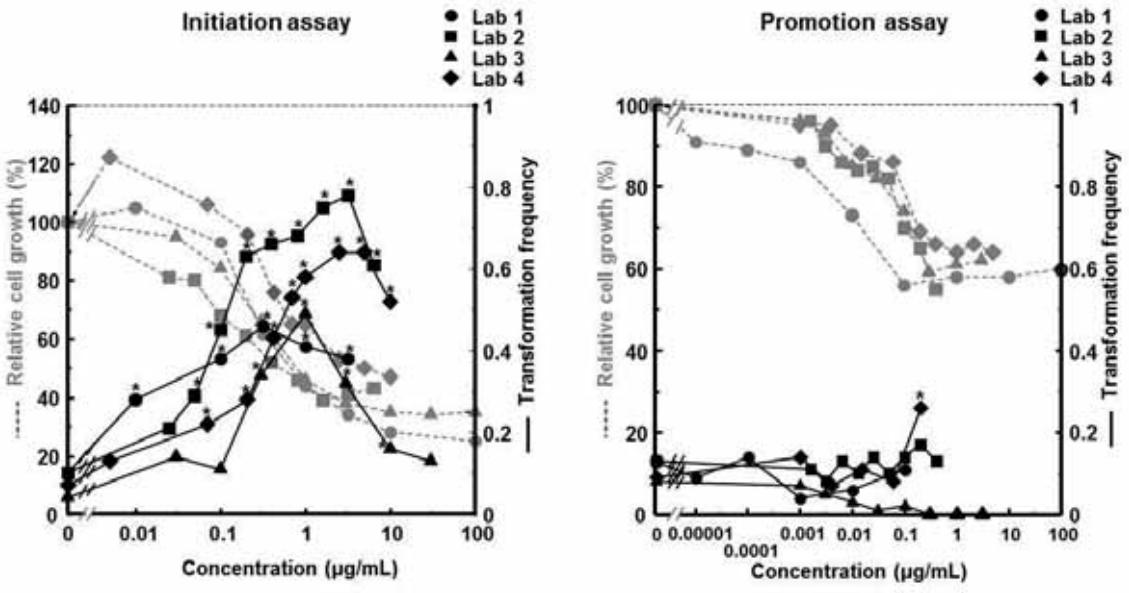

Fig. 23. Graphic view of the results of transformation assay and concurrent cell growth assay on 3-methylcholanthrene in phase I of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.4.4 *o*-Toluidine

Table 28 and Fig. 24 represent the results of *o*-toluidine. This hydrochloride was negative in both initiation and promotion assays.

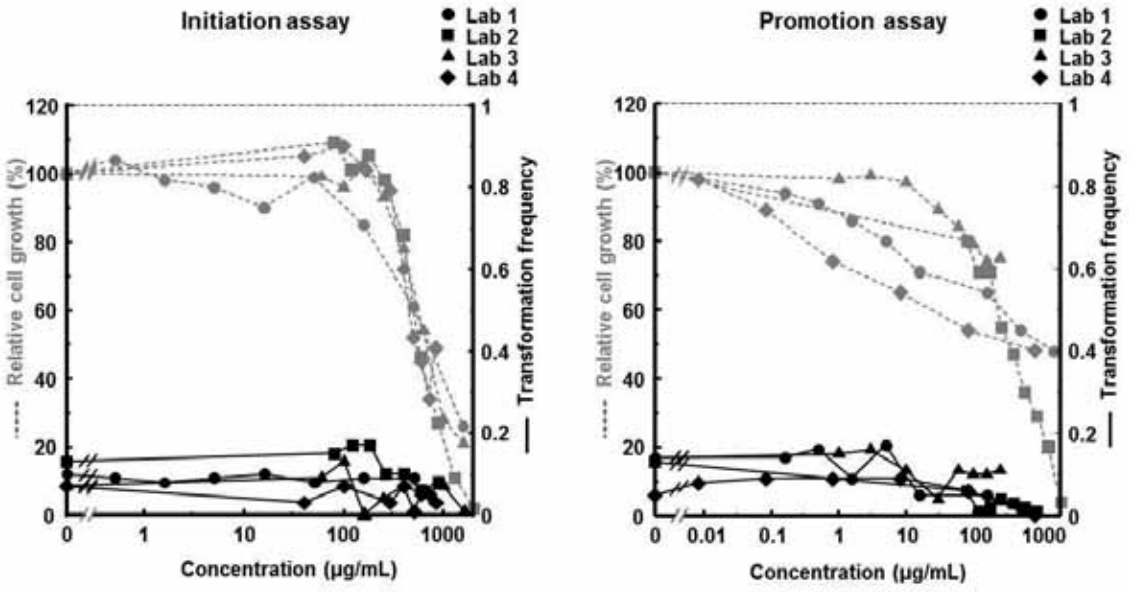

Fig. 24. Graphic view of the results of transformation assay and concurrent cell growth assay on *o*-toluidine in phase I of the 96-well method validation study.

Table 28.
Results of transformation assay on *o*-toluidine in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)							100	7/96
	0 (0.5% DMSO)	100	10/96	100	12/96	100	7/96		
	0.5	104	9/96						
	1.58	98	8/96						
	5	96	9/96						
	15.8	90	10/96						
	40							105	3/96
	50	99	8/96						
	60					99	9/96		
	80			109	14/96				
	100					96	12/96	108	7/96
	120			101	16/96				
	158	85	9/96						
	160					102	0/96		
	167							101	-/- [d]
	180			105	16/96				
	250					93	4/96		
	260			98	10/96				
	292							95	3/96
	400			82	10/96	78	7/96	72	6/92
	500	61	9/96					52	1/96
	590			46	6/96				
	600							45	5/93
	630					54	5/96		
	720							34	5/96
	833							49	3/96
	890			27	8/96				
	1000					28	7/96		
	1300			11	Toxic				
	1580	26	Toxic						
	1600					21	1/96		
	2000			2	Toxic				
	0[e] (0.1% DMSO)	100	8/96	100	10/96	100	5/96		
MCA	1[f] (0.1% DMSO)	30	44/96 †	57	68/96 †	38	66/96 †	27	50/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[f] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)							100	5/96
	0 (0.5% DMSO)	100	13/96	100	12/96	100	13/96		
	0.00000833							112	6/96
	0.0000833							115	8/96
	0.000833							107	7/96
	0.00833							98	8/96
	0.0833							89	9/96
	0.158	94	13/96						
	0.5	91	15/96						
	0.833							74	9/96
	1					98	14/96		
	1.58	86	9/96						
	3					99	15/96		
	5	80	16/96						
	8.33							65	9/96
	10					97	11/96		
	15.8	71	5/96						
	30					89	4/96		
	60					84	11/96		
	80			80	6/96				
	83.3							54	6/96
	100					79	10/96		
	120			71	1/96				
	158	65	5/96						
	160					74	10/96		
	180			71	2/96				
	250					75	11/96		
	260			55	4/96				
	400			47	3/96				
	500	54	2/96						
	590			36	2/96				
	833							48	0/96
	890			29	1/96				
	1300			20	Toxic				
	1580	48	Toxic						
	2000			4	Toxic				
	0[d] (0.1% DMSO)	100	9/96	100	8/96	100	5/96		
TPA	0.05[e] (0.1% DMSO)	110	49/96 †	137	36/96 †	171	46/96 †	89	50/96 †

[a] % of cell growth compared to that of solvent control.

[b] Transformation frequency: number of wells having transformed foci/number of wells counted.

[c] Solvent control: final solvent concentration in the working culture media in parentheses.

[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.

[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.

† $p<0.05$; one-sided chi-square test, vs corresponding solvent control.

4.3.4.5 TPA

The results of TPA are exhibited in Table 29 and Fig. 25. All four laboratories demonstrated TPA to be clearly positive in the promotion assay. In the initiation assay, TPA was negative in the concentration range pre-assigned by the VMT (≤1 μg/mL), where cytotoxicity was not induced in the concurrent cell growth assay. To fulfill the assay acceptance criteria of the initiation assay, the chemical needed to be examined up to the concentrations that show cytotoxicity. However, it was considered that the promotion assay had established that TPA was cleary positive in the Bhas 42 CTA and that it was not necessary to further differentiate the initiation vs. promotion properties of the comound for the purpose of this exercise. The results do, however, indicate that for TPA, initiating activity was not detected in the concentration range at which potent promoting activity was detected.

Table 29.
Results of transformation assay on TPA in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	12/96	100	11/96	100	5/96	100	6/96
0.0001					95	3/96	98	3/96
0.0003					97	5/96		
0.000977	90	4/96						
0.001					92	1/96	89	4/96
0.003					86	2/96		
0.0032							87	2/96
0.00391	92	0/96						
0.0078			78	1/96				
0.01					89	2/96	91	1/96
0.0156	98	4/96						
0.016			85	1/96				
0.03					94	1/96		
0.031			84	1/96				
0.032							96	1/96
0.0625	103	0/96						
0.063			87	1/96				
0.1					104	1/96	104	4/96
0.13			94	4/96				
0.25	119	6/96	107	7/96				
0.3					115	4/96		
0.32							116	3/96
0.5			132	5/96				
0.64							130	-/- [d]
1	134	12/96	147	5/96	139	9/96	131	-/-
MCA 1[e] (0.1% DMSO)	47	54/96 †	50	66/96 †	49	53/96 †	41	43/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)	100	17/96	100	11/95	100	10/96	100	13/96
	0.00001							112	14/96
	0.0001					99	15/96	117	-/- [d]
	0.00014			109	10/95				
	0.0003					101	10/96		
	0.00041			110	12/96				
	0.000977	107	30/96						
	0.001					107	11/96	117	17/91
	0.0012			114	12/95				
	0.003					111	23/96		
	0.0037			112	19/96				
	0.00391	109	47/96 *						
	0.01					120	43/96 *	132	47/96 *
	0.011			122	31/95 *				
	0.0156	118	69/96 *						
	0.03					136	68/96 *		
	0.032							142	59/96 *
	0.033			128	34/96 *				
	0.0625	134	74/96 *						
	0.1			151	54/94 *	158	66/96 *	174	47/96 *
	0.25	164	68/96 *					237	45/96 *
	0.3			166	46/96 *	190	50/96 *		
	0.5							254	-/-
	1	165	90/96 *			200	81/96 *	254	80/96 *
TPA	0.05[e] (0.1% DMSO)	129	73/96 †	111	51/96 †	145	75/96 †	143	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

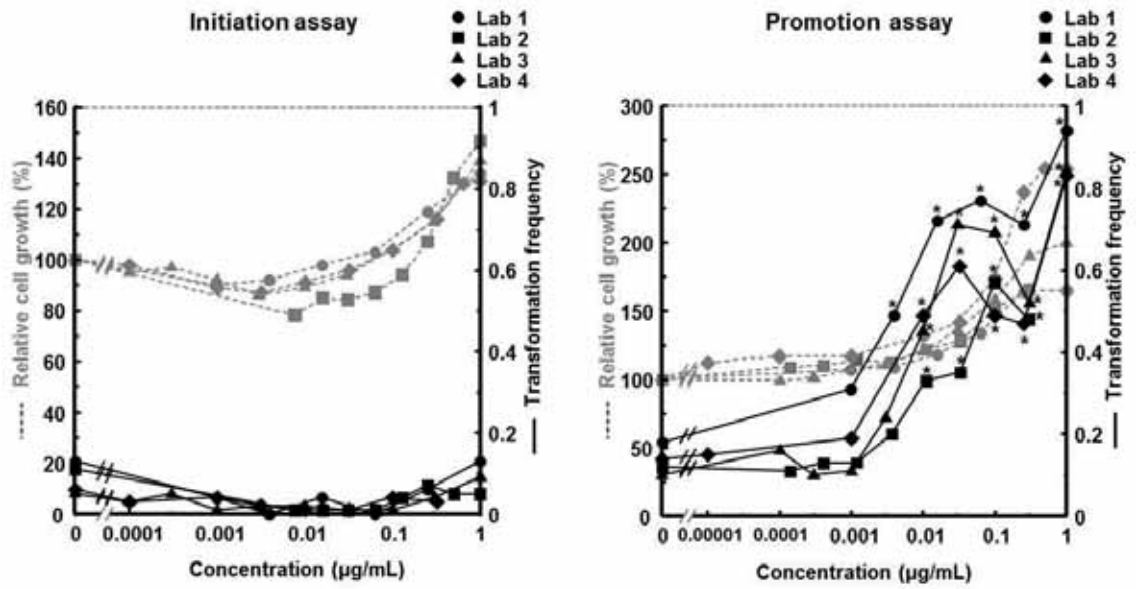

Fig. 25. Graphic view of the results of transformation assay and concurrent cell growth assay on TPA in phase I of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.4.6 Anthracene

The results obtained with anthracene are shown in Table 30 and Fig. 26. Anthracene was negative both in the initiation assay and in the promotion assay. There was a large difference in the maximum concentrations applied between the laboratories. Lab 1, Lab 2 and Lab 3 treated cells with the chemical up to the highest concentration soluble in DMSO, but Lab 4 applied the chemical in suspension to achieve inhibition in the cell growth assay. Despite the differences in treatment concentrations, anthracene failed to induce transformation, either as an initiator or as a promoter, in the Bhas 42 CTA.

Table 30.
Results of transformation assay on anthracene in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
0[c] (0.5% DMSO)	100	12/96	100	12/96	100	6/96	100	8/96
3					95	10/96		
4.7			101	18/96				
5					99	14/96		
7					99	1/96		
9.4			106	17/96				
10					97	6/96		
15.6	107	12/93						
19			102	18/96				
30					94	7/96		
31.3	101	16/96						
38			104	14/96				
50					104	6/96		
62.5	105	18/96						
70					97	9/96		
75			104	13/95				
100					101	9/96	118	5/96
125	132	15/96						
150			108	10/96				
250	112	14/96						
300			110	17/94				
600			111	15/95				
1000							117	12/96
1500							112	-/- [d]
2000							105	10/96
2500							84	7/96
3000							56	10/96
3500							33	1/96
4000							58	8/96
4500							17	6/96
0[e] (0.1% DMSO)	100	8/96	100	10/96	100	1/96	100	5/93
MCA 1[f] (0.1% DMSO)	33	47/96 †	58	58/96 †	40	44/96 †	42	52/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[f] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF
	0[c] (0.5% DMSO)	100	12/96	100	5/96	100	12/96	100	18/96
	1					103	7/96		
	1.56			103	12/96				
	3					103	11/96		
	3.13			98	9/96				
	5					103	12/96		
	6.25			100	15/96				
	7					102	13/96		
	10					102	8/96		
	12.5			100	10/96				
	15.6	103	13/96						
	25			101	15/96				
	30					99	9/96		
	31.3	106	10/96						
	50			103	10/96	99	10/96		
	62.5	96	14/96						
	70					93	14/96		
	100			93	8/96	94	7/96	116	18/96
	125	103	15/96						
	250	101	20/96					114	19/96
	500							119	28/96
	700							106	14/96
	875							107	10/96
	1000							102	26/96
	2000							78	21/96
	3500							54	Toxic
	5000							47	Toxic
	0[d] (0.1% DMSO)	100	10/96	100	10/96	100	11/96	100	12/96
TPA	0.05[e] (0.1% DMSO)	158	66/96 †	128	41/96 †	120	45/96 †	121	56/96 †

[a] % of cell growth compared to that of solvent control.

[b] Transformation frequency: number of wells having transformed foci/number of wells counted.

[c] Solvent control: final solvent concentration in the working culture media in parentheses.

[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.

[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.

† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

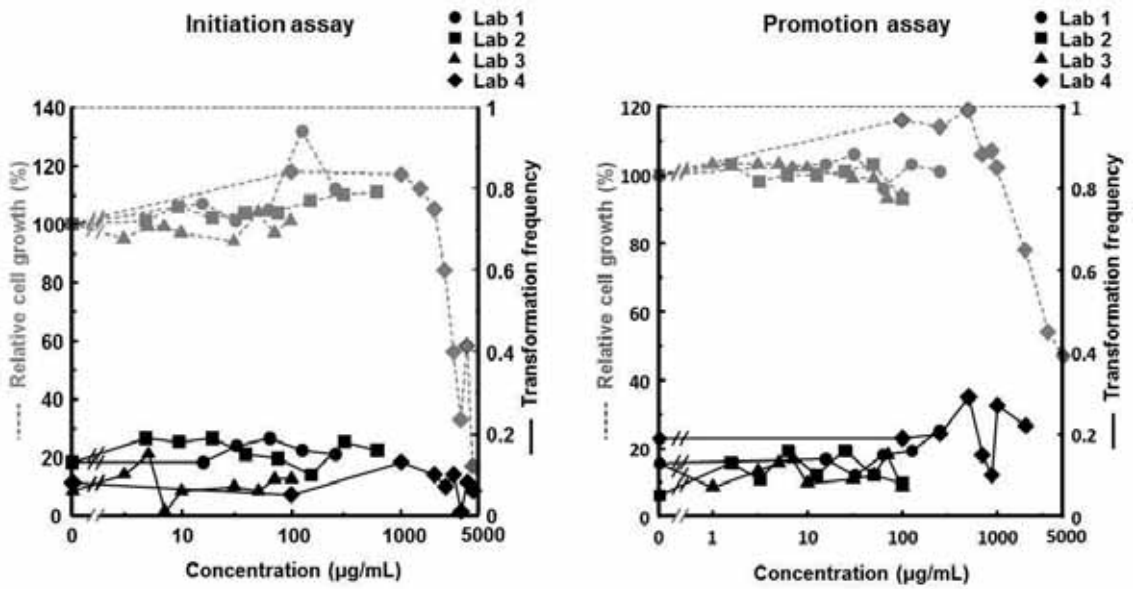

Fig. 26. Graphic view of the results of transformation assay and concurrent cell growth assay on anthracene in phase I of the 96-well method validation study.

4.3.4.7 Phenanthrene

Table 31 and Fig. 27 present the results of phenanthrene. In all four laboratories, the chemical was negative in the initiation assay. It did induce a statistically significant increase in transformation frequency at a single dose in the first run of the assay conducted by Lab 4. In accordance with the protocol, Lab 4 then repeated the initiation assay at concentrations that bracketed the positive dose, but there was no significant increase of transformation frequency at any dose in the second run. In the promotion assay, phenanthrene was negative in Lab 1, Lab 3 and Lab 4, but positive in Lab 2. Lab 1 repeated the promotion assay to verify that the chemical was negative, since it induced a statistically significant increase in the transformation frequency at one dose in the first run of promotion assay. In Lab 3 and Lab 4, the chemical did not induce a significant increase in the transformation frequency at any dose. In Lab 2, phenanthrene induced a statistically significant increase in transformation frequency at two successive concentrations.

Table 31.
Results of transformation assay on phenanthrene in phase I of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 2		Lab 3		Lab 4, 1st run		Lab 4, 2nd run	
	CG[a]	TF[b]	CG	TF	CG	TF	CG	TF	CG	TF
0[c] (0.1% DMSO)	100	8/96			100	9/96				
0 (0.5% DMSO)			100	12/96			100	4/96	100	7/96
0.5									106	-/-[d]
1							107	14/96	104	5/96
4									112	7/96
6	109	6/96							106	8/96
8							111	17/96 *	113	5/96
10	120	5/96							111	5/96
12									108	4/96
12.5			102	14/96						
13					103	10/96				
15									111	6/96
16							117	8/96		
17.7			102	10/96						
20	129	10/96								
24							123	7/96		
25			120	8/96	114	12/96				
30									118	5/96
35.4			77	8/96						
38					110	9/96				
40	124	9/96								
48							84	12/96		
50			34	6/96	94	3/96				
60	91	7/96								
63					76	17/96				
70.7			1	9/96						
75					59	15/96				
80							36	5/96		
88					49	5/96				
100	59	5/96	-1	7/96	45	5/96				
130					50	8/96				
160							20	10/96		
200	68	11/96								
240							17	2/96		
500							17	3/96		
0[e] (0.1%DMSO)			100	11/96			100	9/94	100	1/96
MCA 1[f] (0.1% DMSO)	40	42/96 †	55	69/96 †	34	42/96 †	31	41/96 †	48	45/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] No data.
[e] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[f] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1, 1st run		Lab 1, 2nd run		Lab 2		Lab 3		Lab 4	
		CG[a]	TF[b]	CG	TF	CG	TF	CG	TF	CG	TF
	0[c] (0.1% DMSO)	100	11/96	100	13/96			100	12/96		
	0 (0.5% DMSO)					100	10/96			100	13/96
	0.0001									110	12/96
	0.001									118	10/96
	0.01									123	6/96
	0.1									108	11/96
	1	102	15/96	93	10/96					108	11/96
	1.56					98	10/96				
	2	108	16/96								
	2.5							105	10/96		
	3.13					94	15/95				
	5	111	16/96					102	14/96		
	6.25					90	24/96 *				
	10	115	18/96	87	13/96			99	3/96	101	10/96
	12.5					79	30/96 *				
	15							97	9/96		
	20	112	23/96	77	14/96						
	22							83	13/96		
	25					62	13/96				
	30			65	8/96			68	9/96		
	40			57	6/96			57	9/96		
	50	86	26/96 *	54	8/96	48	0/96				
	60			55	6/96			49	0/96		
	90							46	0/96		
	100	86	Toxic			32	0/96			67	6/96
	1000									63	Toxic
	5000									62	Toxic
	0[d] (0.1% DMSO)					100	13/96			100	15/96
TPA	0.05[e] (0.1% DMSO)	185	53/96 †	134	63/96 †	112	69/96 †	144	70/96 †	114	67/96 †

[a] % of cell growth compared to that of solvent control.

[b] Transformation frequency: number of wells having transformed foci/number of wells counted.

[c] Solvent control: final solvent concentration in the working culture media in parentheses.

[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.

[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.

* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.

† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

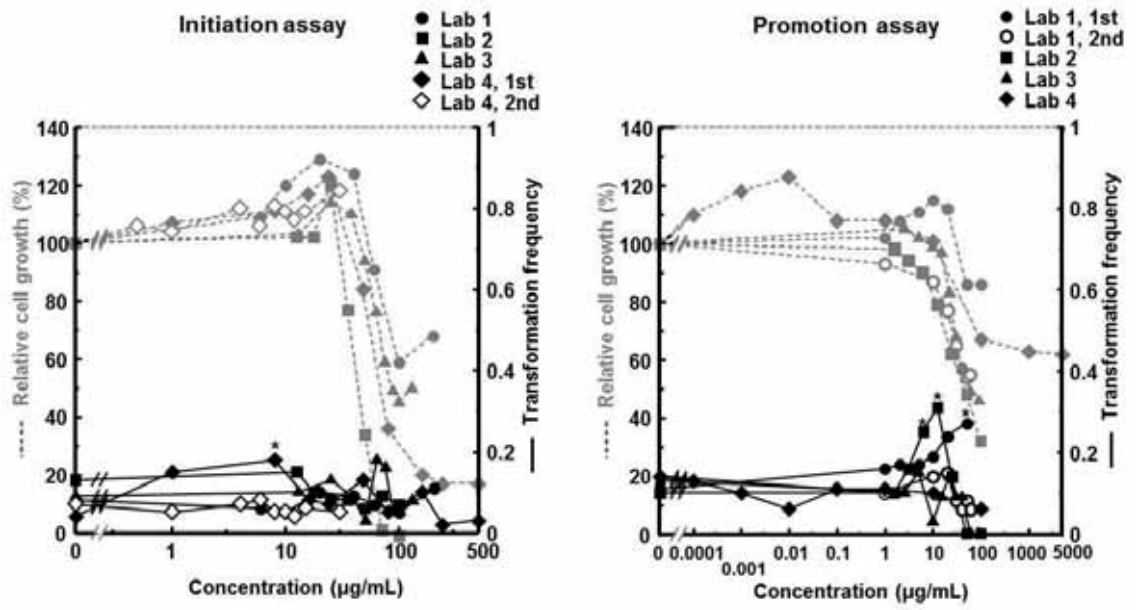

Fig. 27. Graphic view of the results of transformation assay and concurrent cell growth assay on phenanthrene in phase I of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.4.8 Conclusion of phase I for the phase II study plan

As stated above, the phase I study demonstrated good reproducibility between laboratories. When we compare the results in the initiation assay or in the promotion assay between laboratories, positive and negative calls were concordant except for one call in the initiation assays and one call in the promotion assays for 2-acetylaminofluorene and one call in the promotion assays for phenanthrene (see summary data presented in Table 49). That is, the discrepancy in calls were 3 out of 55 assays (including one equivocal call in the promotion assays for MCA, which was excluded from this calculation to avoid unintended ambiguity in the absence of a confirmatory/repeat test [see 4.3.4.3]). Considering the high degree of inter-laboratory reproducibility associated with the 96 well method, it was decided that for phase II of the 96-well method the validation study for each chemical would be tested by only two laboratories.

4.3.5 Validation phase II

For the validation phase II study, three laboratories were involved; Lab 4 did not take part in this phase of study. A total of sixteen chemicals were examined; each chemical was tested by two of the three participating laboratories.

4.3.5.1 Benzo[*a*]pyrene

As shown in Table 32 and Fig. 28, benzo[*a*]pyrene was positive in the initiation assay and negative in the promotion assay in both laboratories.

Table 32
Results of transformation assay on benzo[*a*]pyrene in phase II of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (0.1% DMSO)	100	9/93	100	8/96
	0.001			97	8/96
	0.003			99	14/96
	0.01			97	14/96
	0.0156	96	19/95		
	0.03			89	16/96
	0.0313	76	33/94 *		
	0.045	61	30/95 *		
	0.05			77	38/96 *
	0.0625	45	28/95 *		
	0.1			62	40/96 *
	0.125	29	21/95		
	0.15			44	26/96 *
	0.25	13	21/94		
	0.3			25	31/96 *
	0.5	9	Toxic		
	1			11	20/96
MCA	1[d] (0.1% DMSO)	45	50/96 †	55	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (0.1% DMSO)	100	13/95	100	9/96
	0.0001			101	12/96
	0.0003			101	4/96
	0.000316	91	8/94		
	0.001	91	9/96	100	10/96
	0.003			98	6/96
	0.00316	88	9/95		
	0.01	79	2/95	90	0/96
	0.03			81	3/96
	0.0316	69	5/93		
	0.1	55	Toxic	68	2/96
	0.3			55	2/96
	0.316	46	Toxic		
	1	45	Toxic	54	Toxic
TPA	0.05[d] (0.1% DMSO)	137	56/96 †	132	72/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

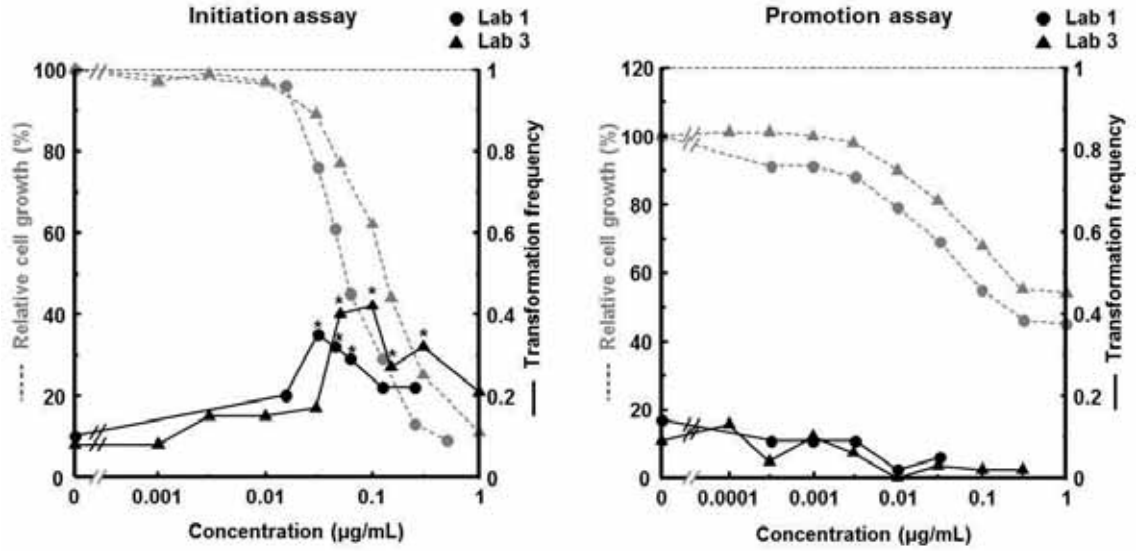

Fig. 28. Graphic view of the results of transformation assay and concurrent cell growth assay on benzo[*a*]pyrene in phase II of the 96-well method validation study.
***** p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.2 Cadmium chloride

The results of cadmium chloride are represented in Table 33 and Fig. 29. Cadmium chloride was negative in the initiation assay but positive in the promotion assay in both laboratories. All but one dose of cadmium chloride used by Lab 3 were found to be toxic in the first run of promotion assay. The second run of the assay performed by Lab 3 at lower concentrations yielded positive results.

Table 33
Results of transformation assay on cadmium chloride in phase II of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	9/96	100	5/96
	0.335	86	8/96		
	0.402	92	9/96		
	0.482	90	5/96		
	0.579	91	4/96		
	0.6			91	7/96
	0.694	85	6/96		
	0.8			78	8/96
	0.833	74	10/95		
	1	44	5/94	43	6/96
	1.2			10	3/96
	1.3			4	2/94
	1.4			1	Toxic
	1.5			0	Toxic
	1.6			0	Toxic
	0[d] (0.1% DMSO)	100	14/96	100	7/96
MCA	1[e] (0.1% DMSO)	31	47/96 †	42	57/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3, 1st run		Lab 3, 2nd run	
		CG[a]	TF[b]	CG	TF	CG	TF
	0[c] (5% Water)	100	18/96	100	10/96	100	14/96
	0.0625	97	17/96				
	0.1					98	17/96
	0.125	98	19/96				
	0.15					99	13/96
	0.2			86	19/96	101	24/96
	0.25	96	37/96 *			106	26/96
	0.3					106	43/96 *
	0.35					105	53/96 *
	0.4			106	Toxic	105	38/95 *
	0.45					108	47/96 *
	0.5	104	44/91 *				
	0.6			115	Toxic		
	0.75	110	Toxic				
	0.8			114	Toxic		
	1	103	Toxic	111	Toxic		
	1.2			102	Toxic		
	1.5			77	Toxic		
	1.8			38	Toxic		
	2	11	Toxic				
	0[d] (0.1% DMSO)	100	17/96	100	13/96	100	7/96
TPA	0.05[e] (0.1% DMSO)	177	77/96 †	161	70/96 †	138	60/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

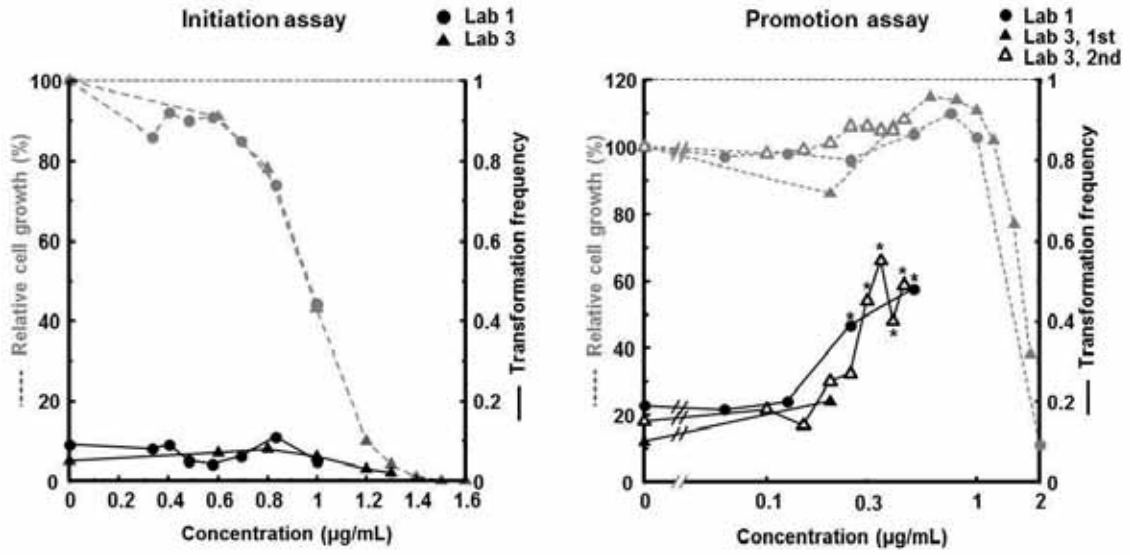

Fig. 29. Graphic view of the results of transformation assay and concurrent cell growth assay on cadmium chloride in phase II of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.3 Dibenz[*a,h*]anthracene

Both laboratories reported dibenz[*a,h*]anthracene to be clearly positive in the initiation assay and negative in the promotion assay, as shown in Table 34 and Fig. 30.

Table 34.
Results of transformation assay on dibenz[*a,h*]anthracene in phase II of the 96-well method validation study

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (0.1% DMSO)	100	6/96	100	14/96
0.01			87	25/96
0.02	96	29/96 *		
0.03			76	34/96 *
0.04	84	42/96 *		
0.08	64	48/96 *		
0.1			59	53/96 *
0.15			52	50/96 *
0.16	50	56/96 *		
0.3			45	48/96 *
0.31	35	60/96 *		
0.5			39	51/96 *
0.63	25	63/96 *		
1			34	33/96 *
1.25	28	38/96 *		
2.5	36	34/96 *		
3			35	Toxic
5	38	29/96 *		
10			43	Toxic
MCA 1[d] (0.1% DMSO)	49	75/96 †	60	51/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (0.1% DMSO)	100	10/96	100	13/96
	0.001			87	6/96
	0.003			81	7/96
	0.01			79	5/96
	0.02	66	7/96		
	0.03			73	1/87
	0.04	67	5/96		
	0.08	63	5/96		
	0.1			67	6/96
	0.16	60	3/96		
	0.3			62	6/96
	0.31	58	8/96		
	0.63	56	5/96		
	1			65	3/96
	1.25	61	2/96		
	2.5	60	3/96		
	3			66	0/96
	5	63	2/96		
	10			68	0/96
TPA	0.05[d] (0.1% DMSO)	135	50/96 †	127	73/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

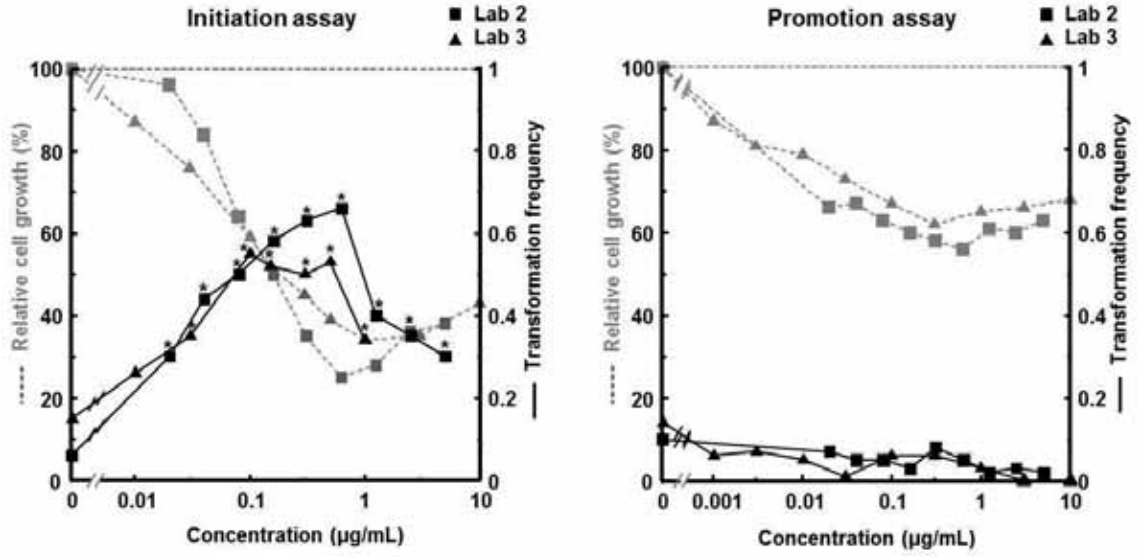

Fig. 30. Graphic view of the results of transformation assay and concurrent cell growth assay on dibenz[*a,h*]anthracene in phase II of the 96-well method validation study.
***** p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.4 Lithocholic acid

Table 35 and Fig. 31 present the results of lithocholic acid. The chemical was negative in the initiation assay but clearly positive in the promotion assay in the two laboratories.

Table 35
Results of transformation assay on lithocholic acid in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (0.1% DMSO)	100	6/96	100	11/96
2.5	107	10/96		
3.5	111	6/96		
5	113	6/96		
7	113	4/96		
10	119	6/96	104	10/96
14	114	6/96		
15			110	5/96
20	121	1/96	114	1/96
22			112	3/96
24			89	4/96
26			45	4/96
28	15	2/96	19	2/95
30			2	1/91
32			1	2/89
40	2	Toxic		
MCA 1[d] (0.1% DMSO)	63	52/96 †	55	59/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (0.1% DMSO)	100	13/96	100	10/96
	0.3125			106	21/96
	0.625			102	19/96
	1.25			84	15/96
	2.5	111	12/96	96	13/96
	3.5	107	16/96		
	5	105	13/96	90	24/96 *
	7	104	31/96 *		
	10	104	33/96 *	74	40/96 *
	14	93	42/96 *		
	20	85	46/96 *	68	46/96 *
	28	90	0/96		
	30			70	0/96
	40	14	Toxic	7	Toxic
TPA	0.05[d] (0.1% DMSO)	155	48/96 †	125	57/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

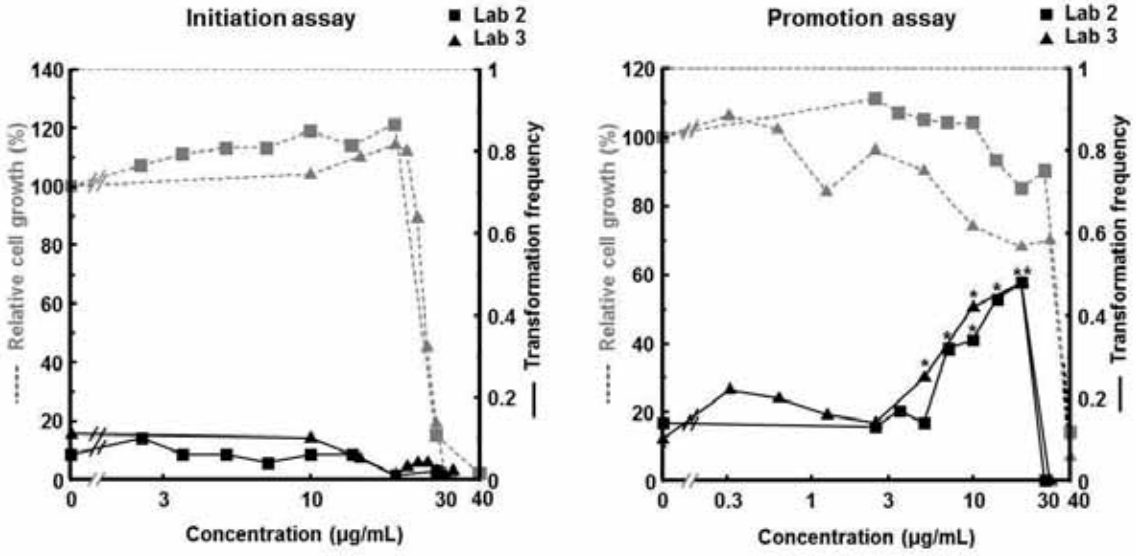

Fig. 31. Graphic view of the results of transformation assay and concurrent cell growth assay on lithocholic acid in phase II of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.5 Methapyrilene hydrochloride

Both two laboratories demonstrated methapyrilene hydrochloride to be negative in the initiation assay but clearly positive in the promotion assay, as shown in Table 36 and Fig. 32.

Table 36
Results of transformation assay on methapyrilene hydrochloride in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (5% Water)	100	12/96	100	9/96
150	113	9/96		
180	103	5/96	110	6/96
210	80	10/96	84	6/96
240	38	7/96	38	3/96
250			44	4/96
260			37	3/96
270	38	4/96		
300	15	4/96	7	3/96
320			3	0/95
330	6	4/96		
360	5	2/96		
400	2	3/96		
500			0	Toxic
0[d] (0.1% DMSO)	100	11/96	100	12/96
MCA 1[e] (0.1% DMSO)	64	68/96 †	53	46/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	11/96	100	11/96
	1			100	12/96
	5			111	27/96 *
	5.9	113	21/96		
	8.8	108	20/96		
	10			115	25/96 *
	13	107	27/96 *		
	20	125	30/96 *		
	25			125	39/96 *
	30	121	26/96 *		
	44	127	44/96 *		
	50			162	83/96 *
	67	138	55/96 *		
	100	144	46/96 *	155	78/96 *
	150	126	17/96	140	24/96
	280			103	Toxic
	0[d] (0.1% DMSO)	100	12/96	100	14/96
TPA	0.05[e] (0.1% DMSO)	139	49/96 †	126	86/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

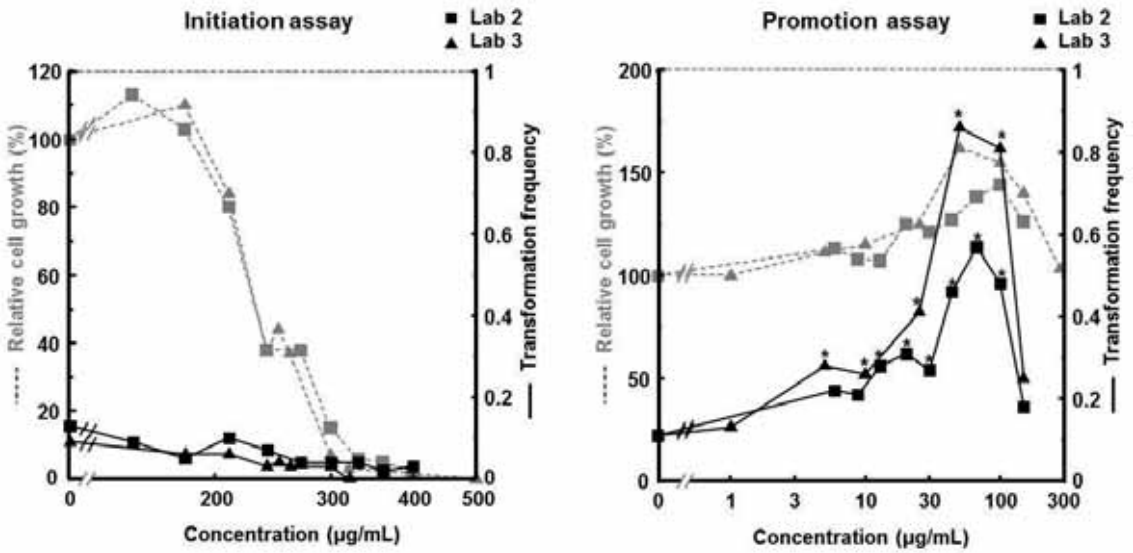

Fig. 32. Graphic view of the results of transformation assay and concurrent cell growth assay on methapyrilene hydrochloride in phase II of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.6 Mezerein

The results of mezerein are represented in Table 37 and Fig. 33. Mezerein proved to be positive in both the initiation assay and the promotion assay. In the promotion assay, mezerein was strongly positive in both laboratories, inducing the highest transformation response among all the chemicals tested in this validation study. The chemical caused statistically significant increase of transformation frequency at a concentration as low as 0.000156 μg/mL or 0.0003 g/mL, and produced transformed foci in almost all the wells at the highest 4 doses in either laboratory. In the initiation assay, mezerein was also positive, inducing statistically significant increases in transformation frequency in both laboratories at the highest two or four concentrations, respectively, although those concentrations were 100 times higher than the minimum dose at which promoting activity of mezerein was detected. It is noteworthy that despite the positive transformation response to mezerein in the initiation assay, the chemical was non-cytotoxic up to the maximum concentration used, which had been pre-assigned by the VMT (0.1 μg/mL).

Table 37
Results of transformation assay on mezerein in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (0.1% DMSO)	100	6/96	100	5/96
0.00001			104	7/96
0.00003			106	7/96
0.0001			98	6/96
0.0003			99	5/96
0.00039	96	5/96		
0.00078	97	6/96		
0.001			104	4/96
0.00156	124	4/96		
0.003			100	3/96
0.00313	110	3/96		
0.00625	144	6/96		
0.01			120	2/96
0.0125	166	18/96 *		
0.025	138	33/96 *		
0.03			94	20/96 *
0.05	137	70/96 *		
0.1	141	72/96 *	87	41/96 *
MCA 1[d] (0.1% DMSO)	37	57/96 †	54	66/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (0.1% DMSO)	100	5/96	100	11/96
	0.00001			100	17/96
	0.00003			101	26/96
	0.000078	116	13/96		
	0.0001			104	15/96
	0.000156	126	18/96 *		
	0.0003			111	37/96 *
	0.000313	161	25/96 *		
	0.000625	190	61/96 *		
	0.001			139	57/96 *
	0.00125	200	82/96 *		
	0.0025	173	92/96 *		
	0.003			180	96/96 *
	0.005	158	91/96 *		
	0.01	165	92/96 *	147	94/96 *
	0.02	190	93/96 *		
	0.03			166	94/96 *
	0.1			174	96/96 *
TPA	0.05[d] (0.1% DMSO)	175	46/96 †	135	48/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

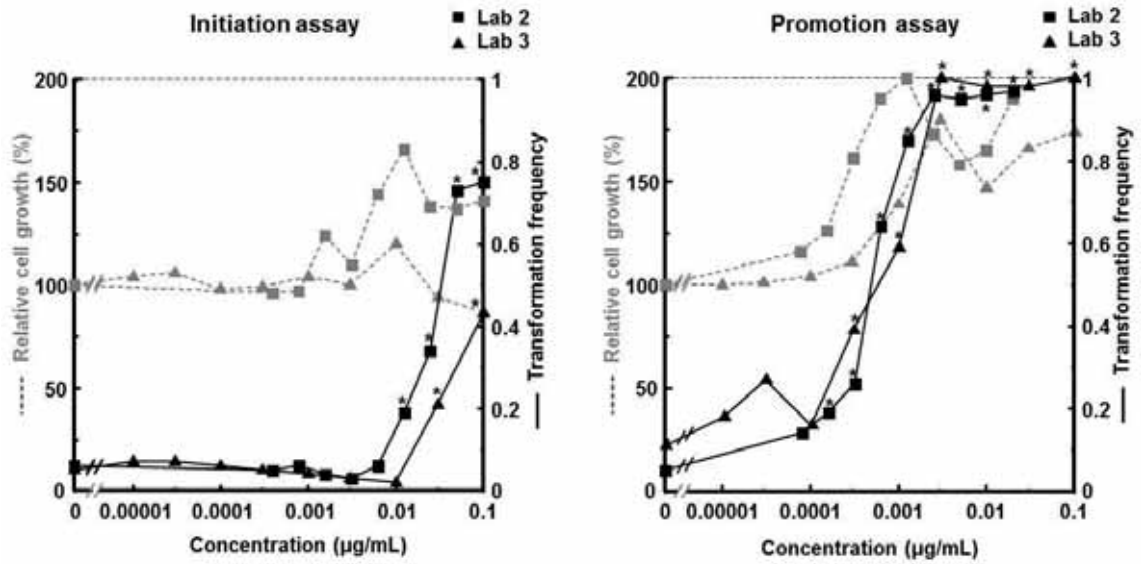

Fig. 33. Graphic view of the results of transformation assay and concurrent cell growth assay on mezerein in phase II of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.7 *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine

The results of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) are exhibited in Table 38 and Fig. 34. MNNG was positive in the initiation assay and negative in the promotion assay in the two laboratories.

Table 38.
Results of transformation assay on *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	3/96	100	9/96
	0.25			97	7/96
	0.5			104	16/96
	0.75			99	14/96
	1			96	20/96
	1.2	109	6/96		
	1.25			87	23/96 *
	1.5			78	31/96 *
	1.55	113	10/95		
	2			52	32/96 *
	2.02	107	13/96 *		
	2.5			22	24/96 *
	2.62	101	26/95 *		
	3.41	97	23/94 *		
	4.43	73	18/95 *		
	5.75	41	23/96 *		
	7.5	13	14/94 *		
	0[d] (0.1% DMSO)	100	9/96	100	8/96
MCA	1[e] (0.1% DMSO)	37	44/94 †	50	63/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	10/96	100	6/96
	0.1			97	10/96
	0.2			102	7/96
	0.5			101	9/96
	0.75			99	7/96
	0.78	100	12/96		
	1			98	4/96
	1.5			93	5/96
	1.56	94	11/96		
	2.5			86	3/96
	3.13	93	9/96		
	5			68	0/96
	6.26	82	2/95		
	12.5	63	0/96		
	25	57	0/96		
	50	13	Toxic		
	0[d] (0.1% DMSO)	100	12/95	100	7/96
TPA	0.05[e] (0.1% DMSO)	143	57/96 †	136	62/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

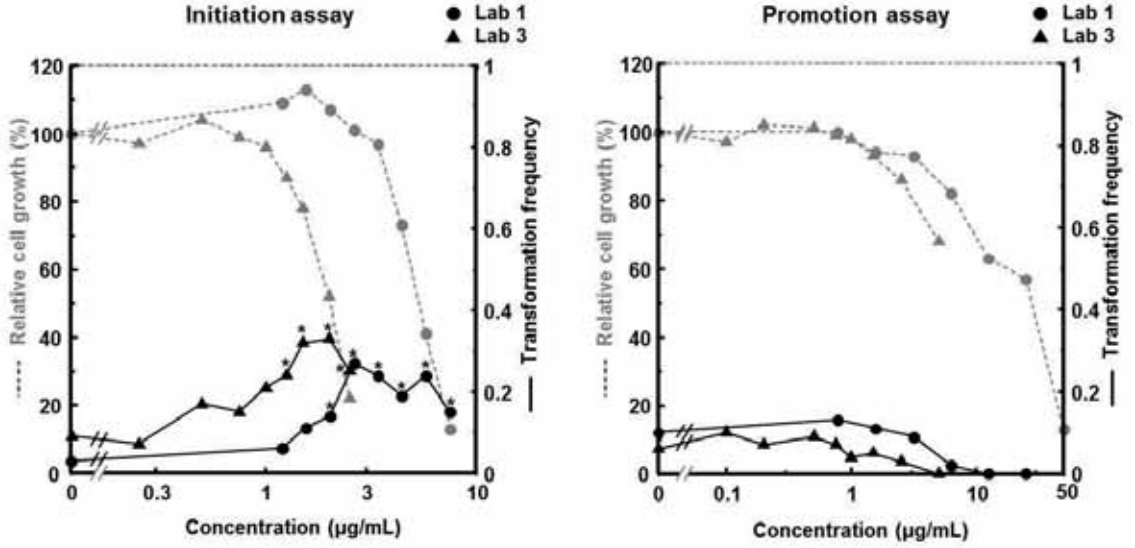

Fig. 34. Graphic view of the results of transformation assay and concurrent cell growth assay on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in phase II of the 96-well method validation study.
***** p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.8 Sodium arsenite

Both of the laboratories reported negative results in the initiation and promotion assays, as shown in Table 39 and Fig. 35.

Table 39.
Results of transformation assay on sodium arsenite in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	7/96	100	1/96
	0.05			107	0/96
	0.1			106	2/96
	0.135	87	7/96		
	0.175	80	1/96		
	0.2			99	4/96
	0.228	82	8/96		
	0.296	57	3/96		
	0.3			68	4/96
	0.385	33	8/96		
	0.4			35	5/96
	0.5	12	3/96	16	3/96
	0.6	9	8/96	4	4/96
	0.7			3	2/96
	0.72	3	6/95		
	0[d] (0.1% DMSO)	100	9/96	100	3/96
MCA	1[e] (0.1% DMSO)	29	41/96 †	52	52/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	15/96	100	12/96
	0.025			101	10/96
	0.0469	100	21/96		
	0.05			95	11/96
	0.0938	93	26/96		
	0.1			99	8/96
	0.188	96	25/96		
	0.25			100	5/96
	0.375	86	7/96		
	0.5			81	0/96
	0.725	72	6/96		
	1			63	Toxic
	1.5	42	Toxic	51	Toxic
	2			13	Toxic
	3	6	Toxic		
	0[d] (0.1% DMSO)	100	14/96	100	7/96
TPA	0.05[e] (0.1% DMSO)	167	72/96 †	168	68/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

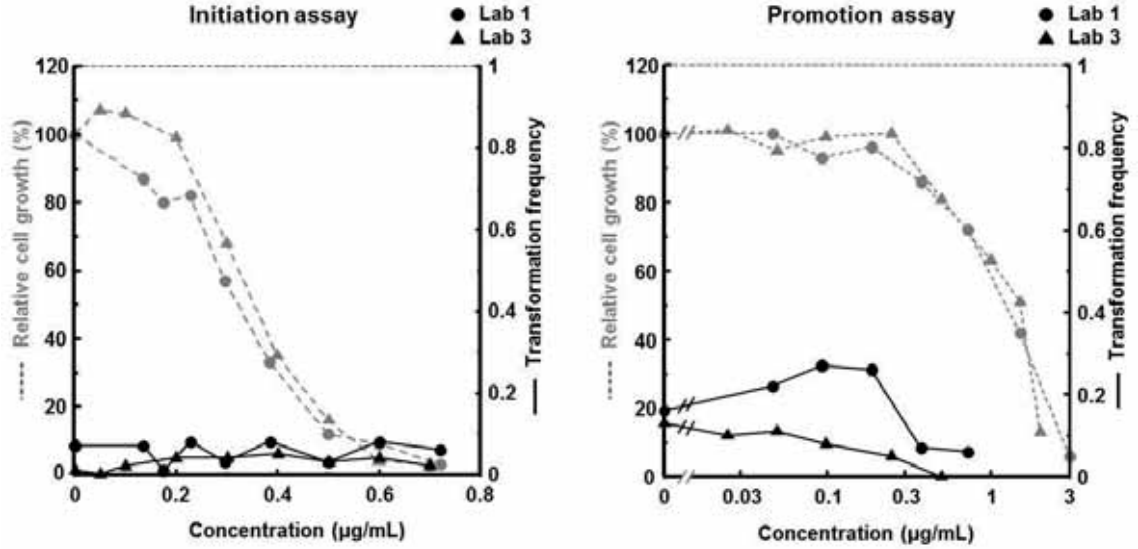

Fig. 35. Graphic view of the results of transformation assay and concurrent cell growth assay on sodium arsenite in phase II of the 96-well method validation study.

4.3.5.9 Ampicillin sodium

The results of Ampicillin sodium were presented in Table 40 and Fig. 36. The chemical was negative in the initiation and promotion assays in both laboratories.

Table 40.
Results of transformation assay on ampicillin sodium in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	13/96	100	13/96
	502	100	12/96		
	603	98	10/96		
	724	95	4/96		
	800			98	6/96
	868	95	7/96		
	1000			103	6/96
	1040	86	7/96		
	1200			103	2/96
	1250	59	7/96		
	1400			86	2/96
	1500	11	6/94	80	0/96
	1600			55	1/96
	1700			55	4/96
	1800			27	5/96
	0[d] (0.1% DMSO)	100	12/96	100	13/96
MCA	1[e] (0.1% DMSO)	39	48/96 †	52	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	18/96	100	9/96
	250	97	9/96		
	500	97	9/96	91	15/96
	800			93	11/96
	1000	97	12/95	95	10/96
	1500			91	19/96
	2000	84	8/96	85	11/96
	3000	72	2/96	80	12/96
	4000	66	1/96	72	0/96
	5000	56	3/96	64	1/96
	0[d] (0.1% DMSO)	100	13/96	100	14/96
TPA	0.05[e] (0.1% DMSO)	138	67/96 †	144	78/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

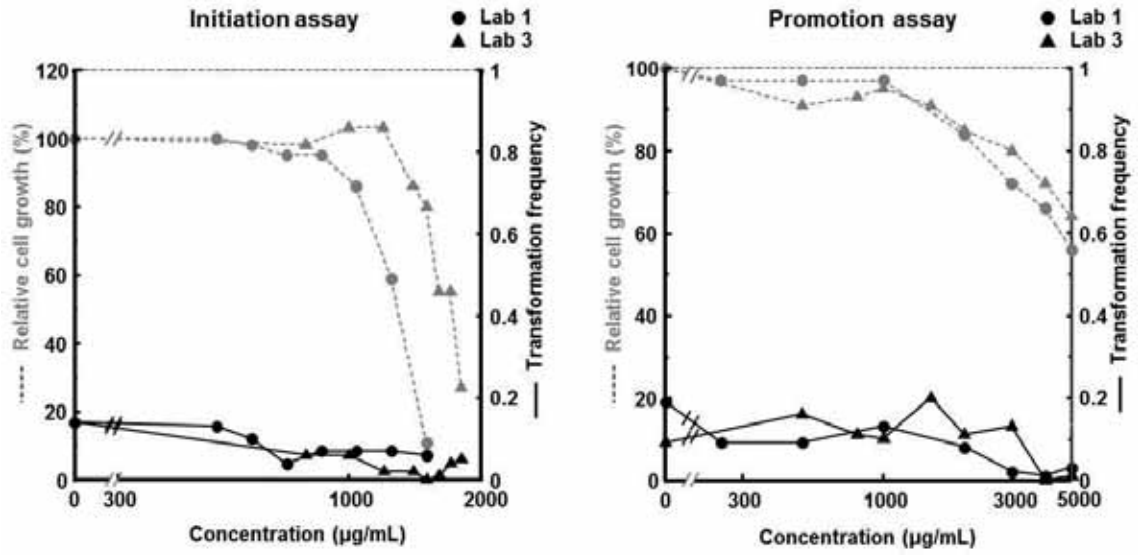

Fig. 36. Graphic view of the results of transformation assay and concurrent cell growth assay on ampicillin sodium in phase II of the 96-well method validation study.

4.3.5.10 L-Ascorbic acid

The results for L-ascorbic acid are shown in the Table 41 and Fig. 37. Ascorbic acid was negative in both assays in both laboratories. In the first run of promotion assay in Lab 3, only three out of eight plates treated with the chemical were observable for the transformed foci, because the cells were killed by the toxic effect of chemical treatment at the highest five concentrations. In addition, the incidence of transformed foci in the negative control for TPA (21 wells/96 wells) exceeded the acceptance criteria (≤20 wells/96 well). Since this first run did not satisfy the assay acceptance criteria, Lab 3 repeated the promotion assay in a lower concentration range and confirmed L-ascorbic acid to be negative.

Table 41.
Results of transformation assay on L-ascorbic acid in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (5% Water)	100	9/96	100	7/96
50			89	5/96
100			83	8/96
112	90	9/96		
134	89	7/96		
161	81	10/96		
193	74	4/96		
200			67	8/96
231	62	8/96		
278	43	5/96		
300			53	3/96
333	8	6/96		
350			51	1/96
380			42	6/96
400	-1	Toxic	37	2/96
450			13	1/96
0[d] (0.1% DMSO)	100	10/96	100	7/96
MCA 1[e] (0.1% DMSO)	40	41/96 †	56	60/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3, 1st run		Lab 3, 2nd run	
		CG[a]	TF[b]	CG	TF	CG	TF
	0[c] (5% Water)	100	13/96	100	7/96	100	5/96
	12.5					97	4/96
	25					99	0/96
	50					101	2/96
	96.6	90	2/96				
	100					105	0/96
	145	89	0/96				
	200					101	0/96
	217	91	1/96				
	280			99	0/96		
	326	86	1/96				
	350			95	1/96	96	1/96
	489	83	7/95				
	500			92	2/96	92	Toxic
	650			87	Toxic	62	Toxic
	733	72	Toxic				
	800			76	Toxic		
	900			65	Toxic		
	1000			52	Toxic		
	1100	13	Toxic	40	Toxic		
	0[d] (0.1% DMSO)	100	15/96	100	21/96	100	8/96
TPA	0.05[e] (0.1% DMSO)	137	56/96 †	128	85/96 †	120	45/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

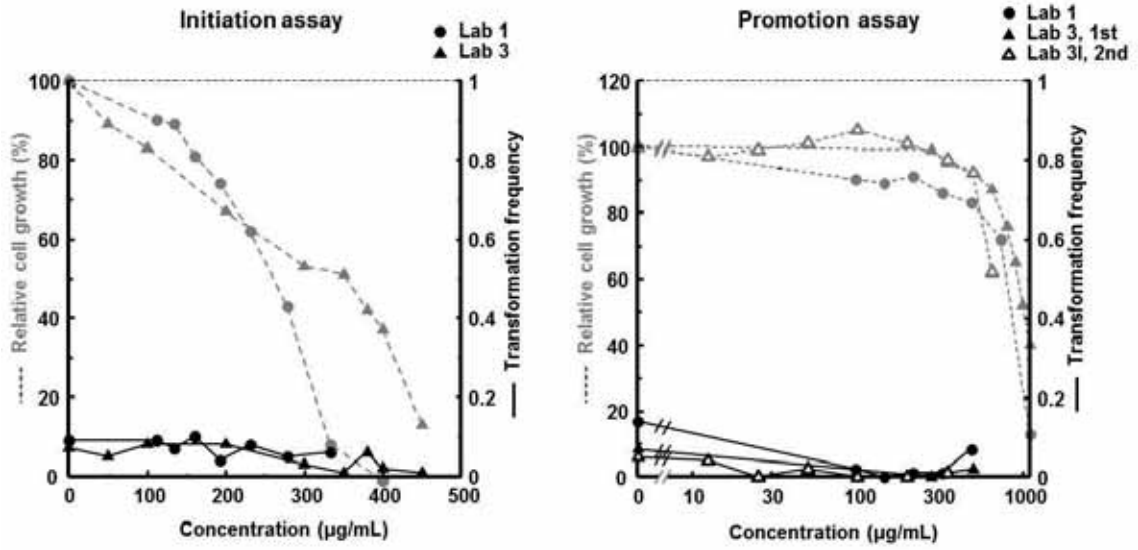

Fig. 37. Graphic view of the results of transformation assay and concurrent cell growth assay on L-ascorbic acid in phase II of the 96-well method validation study.

4.3.5.11 Caffeine

The results of caffeine are presented in Table 42 and Fig. 38. Caffeine was negative in both assays in both laboratories.

Table 42.
Results of transformation assay on caffeine in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (5% Water)	100	5/96	100	3/96
200			107	7/96
226	107	8/96		
250			102	4/96
259	105	9/96		
298	105	4/96		
300			81	4/96
340			83	11/96
343	90	4/96		
370			58	12/96
395	57	4/96		
400			51	4/96
450			20	8/96
454	29	3/96		
500			7	6/96
522	13	3/96		
600	5	4/96		
0[d] (0.1% DMSO)	100	4/96	100	2/96
MCA 1[e] (0.1% DMSO)	56	55/96 †	44	41/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	6/96	100	4/96
	2.5			112	6/96
	5			110	7/96
	10			108	7/96
	18	99	5/96		
	25			104	8/96
	26	97	4/96		
	40	94	2/96		
	50			99	5/96
	59	87	6/96		
	89	76	3/96		
	100			83	5/96
	130	71	4/96		
	200	57	3/96		
	250			60	2/96
	300	43	3/96		
	500			43	0/96
	0[d] (0.1% DMSO)	100	7/96	100	11/96
TPA	0.05[e] (0.1% DMSO)	149	68/96 †	138	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

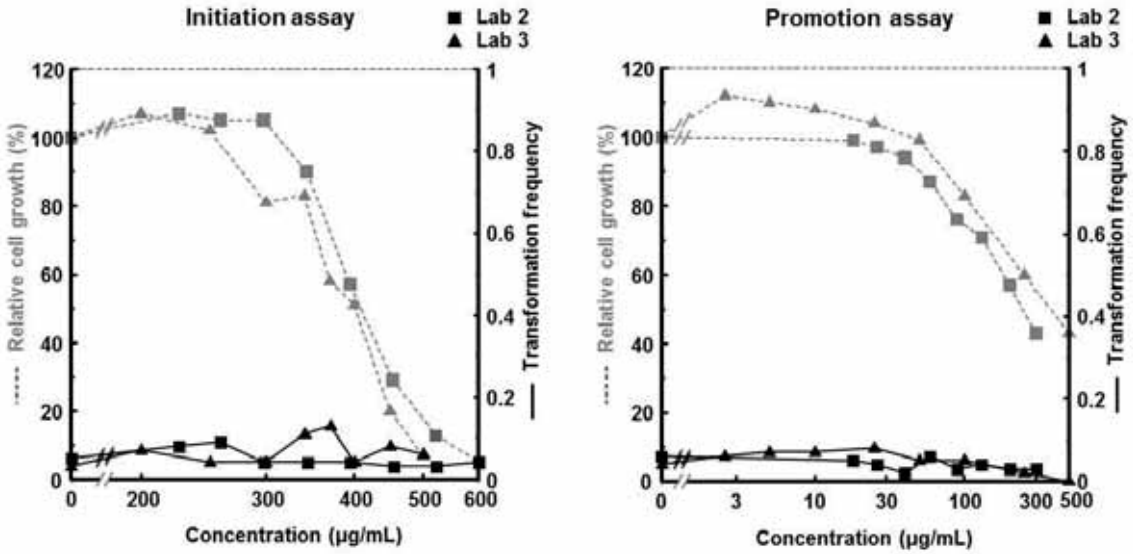

Fig. 38. Graphic view of the results of transformation assay and concurrent cell growth assay on caffeine in phase II of the 96-well validation study.

4.3.5.12 Caprolactam

Table 43 and Fig. 39 present the results of caprolactam. The chemical was determined to be negative in both the initiation and promotion assays despite the unusual outcome of the initiation assay. In the initiation assay, caprolactam induced statistically significant increases in transformation frequency at the extremely high concentrations of 2890 and 3470 μg/mL (25.54 and 30.66 mM) in Lab 1 and 3700 and 4000 μg/mL (32.70 and 35.35 mM) in Lab 3. However, in accordance with the Bhas 42 CTA protocol, those concentrations exceed the maximum allowable concentration that can be tested, i.e. 10 mM. Since the test chemical was coded for assessment in the validation study, the molecular weight was unknown to the laboratories, and so the studies were performed using these unknowingly improper chemical concentrations. The VMT concluded that the increase in transformation frequency induced by caprolactam at these excessive concentrations was, therefore, not biologically relevant and that, in fact, all test concentrations were in excess of the 10 mM maximum allowable concentration that can be tested. Thus, under the test conditions employed, caprolactam was considered to be negative in the initiation assay.

Table 43.
Results of transformation assay on caprolactam in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 1		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (5% Water)	100	7/96	100	9/96
1670 (14.76 mM)	113	12/96		
2010 (17.76 mM)	112	14/96		
2410 (21.30 mM)	113	18/96		
2500 (22.09 mM)			125	11/96
2890 (25.54 mM)	107	25/96 *		
3000 (26.51 mM)			117	11/96
3300 (29.16 mM)			96	16/96
3470 (30.66 mM)	87	20/96 *		
3700 (32.70 mM)			79	25/96 *
4000 (35.35 mM)			68	32/96 *
4170 (36.85 mM)	49	17/96		
4300 (38.00 mM)			56	16/96
4700 (41.53 mM)			42	11/96
5000 (44.19 mM)	15	7/96	25	19/96
0[d] (0.1% DMSO)	100	9/96	100	8/96
MCA 1[e] (0.1% DMSO)	36	43/96 †	40	65/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	12/96	100	10/96
	10			97	9/96
	30			100	12/96
	78.1	100	15/96		
	100			96	12/96
	156	103	23/96		
	300			91	14/96
	313	97	20/96		
	625	94	17/96		
	1000 (8.84 mM)			79	13/96
	1250 (11.05 mM)	84	12/96		
	2000 (17.67 mM)			66	13/96
	2500 (22.09 mM)	58	14/96		
	3000 (26.51 mM)			52	19/96
	4000 (35.35 mM)			47	10/96
	5000 (44.19 mM)	47	12/96		
	0[d] (0.1% DMSO)	100	14/96	100	11/96
TPA	0.05[e] (0.1% DMSO)	136	63/96 †	137	54/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

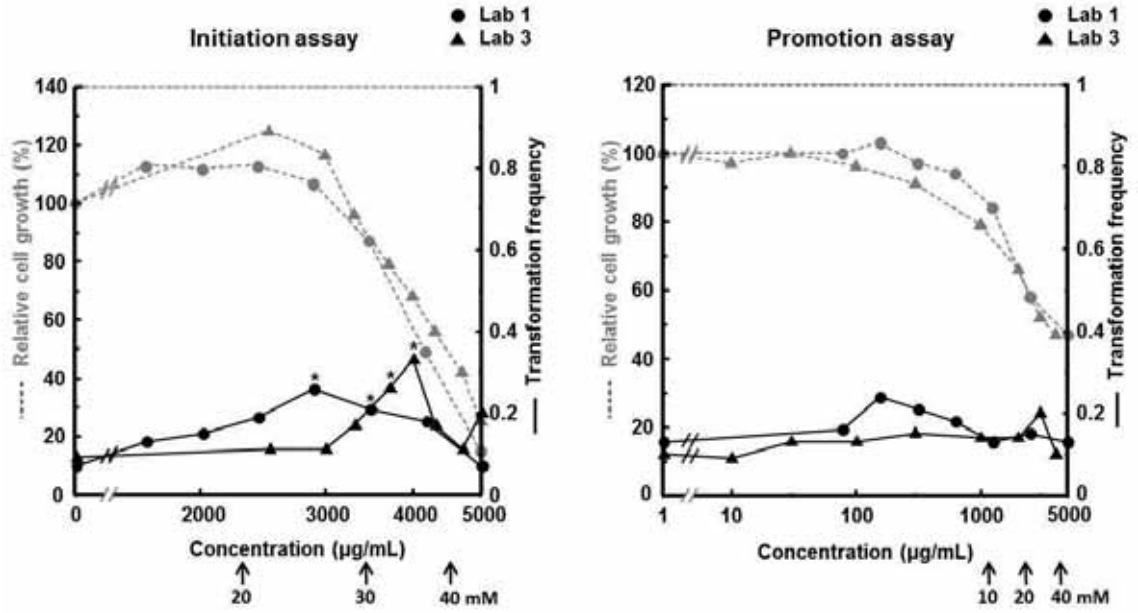

Fig. 39. Graphic view of the results of transformation assay and concurrent cell growth assay on caprolactam in phase II of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.13 Eugenol

The results of eugenol are shown in Table 44 and Fig. 40. Eugenol was negative in both assays and in both laboratories. The chemical induced a statistically significant increase in transformation frequency at a single concentration (2.5 μg/mL) in the first run of promotion assay in Lab 3. In accordance with the protocol, Lab 3 repeated the promotion assay, and in so doing, applied a narrower range of test concentrations that bracketed the 2.5 μg/mL concentration. The results of the second run confirmed the negative judgment.

Table 44.
Results of transformation assay on eugenol in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (0.1% DMSO)	100	10/96	100	11/96
18	111	10/96		
25	113	12/96		
30			104	7/96
35	112	10/96		
40			116	6/96
50	106	10/96	118	3/96
60			108	9/96
70	63	7/96	92	10/96
80			72	5/96
90			41	10/96
100	28	6/96	32	4/96
120			29	6/96
140	11	4/96		
200	10	3/96		
MCA 1[d] (0.1% DMSO)	21	64/96 †	18	59/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3, 1st run		Lab 3, 2nd run	
		CG[a]	TF[b]	CG	TF	CG	TF
	0[c] (0.1% DMSO)	100	11/96	100	15/96	100	18/96
	0.3125			105	22/96		
	0.625			108	25/96		
	1					105	17/96
	1.25			105	20/96		
	1.5					113	21/96
	2					112	11/96
	2.5			102	31/96 *	108	9/96
	3					115	16/96
	3.5					112	9/96
	4					112	16/96
	5			101	13/96	115	11/96
	6.3	104	12/96				
	7.5			99	14/96		
	8.8	105	4/96				
	10			92	10/96	115	4/96
	13	99	7/96				
	18	95	5/96				
	20			89	6/96		
	25	90	7/96				
	35	78	4/96				
	40			79	1/96		
	50	65	5/96				
	70	56	4/96				
	100	50	6/96				
TPA	0.05[d] (0.1% DMSO)	86	48/96 †	116	85/96 †	140	83/96 †

[a] % of cell growth compared to that of solvent control.

[b] Transformation frequency: number of wells having transformed foci/number of wells counted.

[c] Solvent control: final solvent concentration in the working culture media in parentheses.

[d] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.

* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.

† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

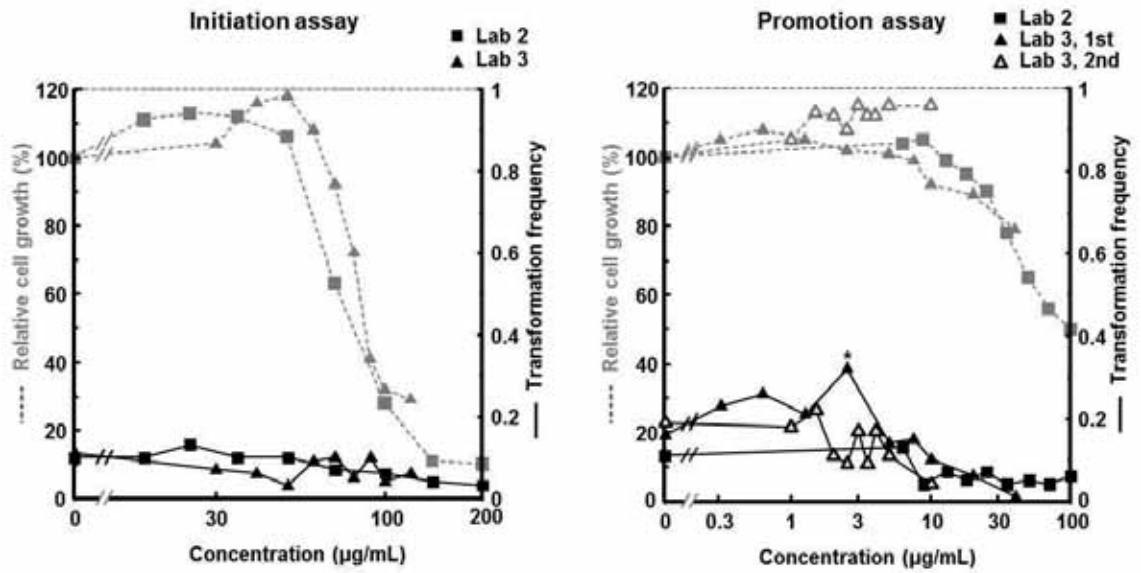

Fig. 40. Graphic view of the results of transformation assay and concurrent cell growth assay on eugenol in phase II of the 96-well method validation study.
***** $p<0.05$; one-sided chi-square test with Bonferroni's adjustment.

4.3.5.14 D-Mannitol

Both two laboratories found D-mannitol to be negative in both assays, as shown in Table 45 and Fig. 41.

Table 45.
Results of transformation assay on D-mannitol in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	3/96	100	6/96
	290	115	3/96		
	440	111	2/94		
	500			96	6/96
	660	122	2/96		
	990	117	1/96		
	1000			100	6/96
	1500	118	1/96		
	2000			103	1/96
	2200	114	2/95		
	3000			96	8/96
	3300	114	3/96		
	4000			98	7/96
	5000	121	3/96	101	7/96
	0[d] (0.1% DMSO)	100	4/96	100	10/96
MCA	1[e] (0.1% DMSO)	56	57/96 †	38	46/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	5/96	100	9/96
	290	105	5/96		
	440	112	4/96		
	500			99	7/96
	660	112	11/96		
	990	109	7/96		
	1000			99	6/96
	1500	106	9/96		
	2000			102	7/96
	2200	110	6/96		
	3000			104	5/96
	3300	101	6/96		
	4000			100	3/96
	5000	109	4/96	100	4/96
	0[d] (0.1% DMSO)	100	8/96	100	8/96
TPA	0.05[e] (0.1% DMSO)	156	43/96 †	153	77/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

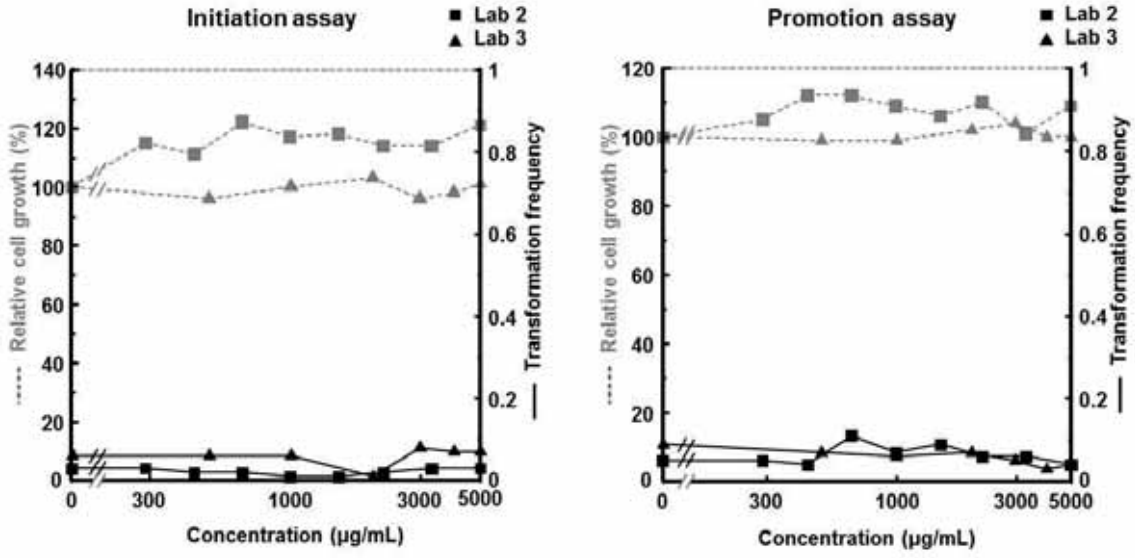

Fig. 41. Graphic view of the results of transformation assay and concurrent cell growth assay on D-mannitol in phase II of the 96-well method validation study.

4.3.5.15 Phorbol

The results of phorbol are represented in Table 46 and Fig. 42. The highest concentration of phorbol to be tested was pre-assigned by the VMT to be ≤ 5 μg/mL in the medium, because this chemical is expensive and its availability was limited. In the concentration range pre-assigned by VMT, phorbol was negative in both assays in both laboratories. However, cytotoxicity was also not induced at any of the concentrations in the concurrent cell growth assay. Phorbol is quite soluble in polar solvents, including water. In order to draw any conclusion regarding the transformation activity of phorbol, this chemical should have been tested at higher concentrations that it achieved cytotoxicity or induced a positive result in at least one of component assays (initiation assay or promotion assay). Therefore, it was concluded that these transformation assays for phorbol were incomplete because of inadequate dosing.

Table 46.
Results of transformation assay on phorbol in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	10/96	100	13/96
	0.5	110	12/96	98	8/96
	1	112	13/96	100	7/96
	2	105	10/96	95	5/96
	3	111	4/96	104	4/96
	4	103	6/96	97	6/96
	5	102	3/96	103	6/96
	0[d] (0.1% DMSO)	100	5/96	100	9/96
MCA	1[e] (0.1% DMSO)	43	46/96 †	50	62/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 1		Lab 3	
		CG[a]	TF[b]	CG	TF
	0[c] (5% Water)	100	9/96	100	10/96
	0.5	99	12/96	100	13/96
	1	98	6/96	101	12/96
	2	96	8/96	100	17/96
	3	96	13/96	103	13/96
	4	96	7/96	103	8/96
	5	97	11/96	99	12/96
	0[d] (0.1% DMSO)	100	10/96	100	11/96
TPA	0.05[e] (0.1% DMSO)	138	55/96 †	131	81/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

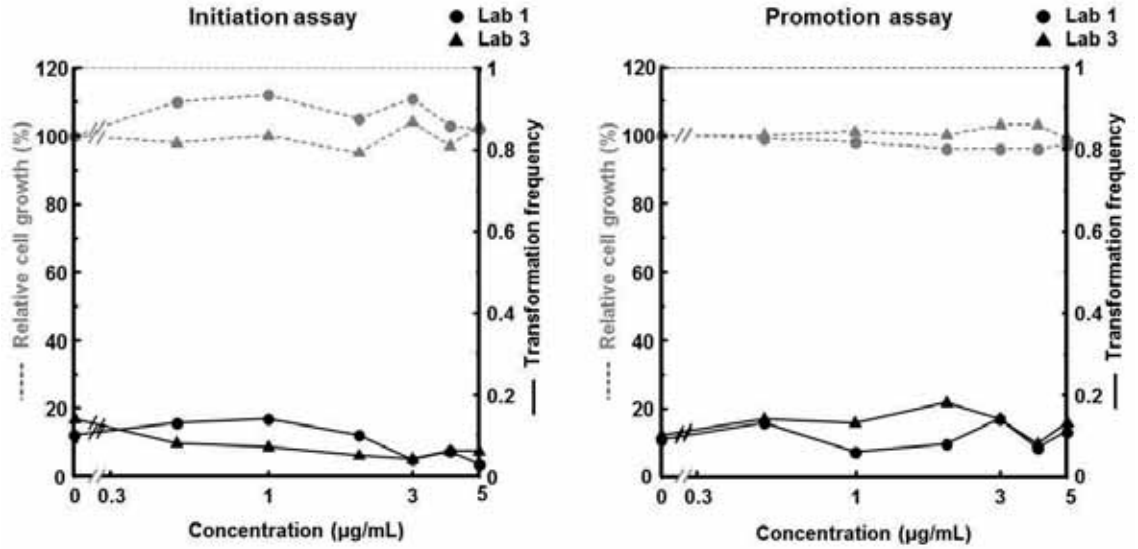

Fig. 42. Graphic view of the results of transformation assay and concurrent cell growth assay on phorbol in phase II of the 96-well method validation study.

4.3.5.16 Pyrene

Table 47 and Fig. 43 show the results for pyrene, which were positive in both the initiation and promotion assays in both laboratories. In the initiation assay, although the concentrations examined in Lab 2 were too low for the chemical to induce cytotoxicity in the concurrent cell growth assay, the fact that pyrene was clearly positive for transformation in that concentration range suggested that Lab 2 need not repeat the assay, as determined by the VMT. In the promotion assay, pyrene was clearly positive in Lab 2 as well. In contrast, Lab 3 observed a statistically significant increase in transformation frequency at only a single concentration (10 μg/mL) in the first run of promotion assay. Lab 3 repeated the assay at a slightly lower concentration range and once again obtained the statistically significant increase in transformation frequency at the same concentration. Thus, pyrene was judged to be positive in the promotion assay in Lab 3.

Table 47
Results of transformation assay on pyrene in phase II of the 96-well method validation study.

a) Initiation assay

Concentration (μg/mL)	Lab 2		Lab 3	
	CG[a]	TF[b]	CG	TF
0[c] (0.5% DMSO)	100	5/96	100	6/96
2.8	101	8/96		
5.6	111	6/96		
11	114	8/96		
23	100	12/96		
25			106	6/96
45	103	19/96 *		
50			106	17/96
90	111	20/96 *		
100			109	21/96 *
150			96	22/96 *
180	117	25/96 *		
200			106	23/96 *
250			94	25/96 *
300			84	14/96
400			68	16/96
0[d] (0.1% DMSO)	100	7/96	100	13/96
MCA 1[e] (0.1% DMSO)	59	53/96 †	54	65/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, MCA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the initiation assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

b) Promotion assay

Concentration (μg/mL)		Lab 2		Lab 3, 1st run		Lab 3, 2nd run	
		CG[a]	TF[b]	CG	TF	CG	TF
	0[c] (0.5% DMSO)	100	13/96	100	9/96	100	14/96
	2.8	101	10/96				
	5			104	13/96	100	14/96
	5.6	98	20/96				
	10			92	25/96 *	94	31/96 *
	11	92	30/96 *				
	15					90	27/96
	23	90	36/96 *				
	25			88	17/96	89	23/96
	45	97	40/96 *				
	50			94	15/96	93	27/96
	75					90	21/96
	90	91	47/96 *				
	100			96	16/96	88	26/96
	150			92	7/96	85	9/96
	180	95	28/96 *				
	200			74	Toxic		
	400			55	Toxic		
	0[d] (0.1% DMSO)	100	7/96	100	12/96	100	18/96
TPA	0.05[e] (0.1% DMSO)	159	52/96 †	134	58/96 †	169	71/96 †

[a] % of cell growth compared to that of solvent control.
[b] Transformation frequency: number of wells having transformed foci/number of wells counted.
[c] Solvent control: final solvent concentration in the working culture media in parentheses.
[d] Solvent control for the positive control, TPA: final solvent concentration in the working culture media in parentheses.
[e] Positive control in the promotion assay: final solvent concentration in the working culture media in parentheses.
* $p<0.05$; one-sided chi-square test with Bonferroni's adjustment, vs. corresponding solvent control.
† $p<0.05$; one-sided chi-square test, vs. corresponding solvent control.

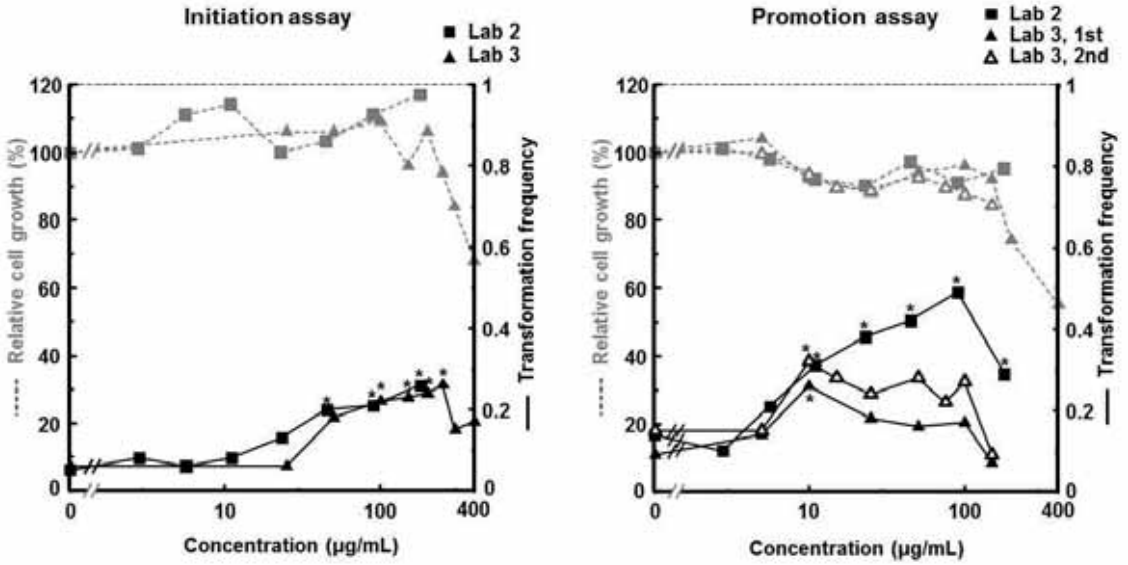

Fig. 43. Graphic view of the results of transformation assay and concurrent cell growth assay on pyrene in phase II of the 96-well method validation study.
* p<0.05; one-sided chi-square test with Bonferroni's adjustment.

4.3.6 Fulfillment of acceptance criteria

The success or failure to fulfill the assay acceptance criteria for each assay conducted in each lab is listed in Table 48.

Table 48.
Fulfillment of the assay acceptance criteria

Phase I					
Chemical	Lab	Initiation assay		Promotion assay	
		Run	All assay acceptance criteria	Run	All assay acceptance criteria
2-Acetylamino-fluorene	Lab 1		Fulfilled		Fulfilled
	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
	Lab 4	1st	Fulfilled. However, there was a statistically significant increase in transformation frequency at one concentration. The assay was repeated.		Fulfilled
		2nd	Fulfilled		
Benzo[*a*]pyrene	Lab 1		Fulfilled		Fulfilled
	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
	Lab 4		Fulfilled		Fulfilled
3-Methyl-cholanthrene	Lab 1		Fulfilled		Fulfilled
	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
	Lab 4		Fulfilled		Fulfilled
o-Toluidine	Lab 1		Fulfilled		Fulfilled
	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
	Lab 4		Fulfilled		Fulfilled
TPA	Lab 1		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (<1 µg/mL), has not achieved cytotoxicity.		Fulfilled
	Lab 2		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤1 µg/mL), had not achieved cytotoxicity		Fulfilled
	Lab 3		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤1 µg/mL), had not achieved cytotoxicity.		Fulfilled

Table 48.
(Continued)

Chemical	Lab	Initiation assay		Promotion assay	
		Run	All assay acceptance criteria	Run	All assay acceptance criteria
	Lab 4		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤1 μg/mL), had not achieved cytotoxicity.		Fulfilled
Anthracene	Lab 1		Fulfilled. The chemical was examined up to the maximum concentration soluble in DMSO, although it did not induced cytotoxicity.		Fulfilled
	Lab 2		Fulfilled. The chemical was examined up to the maximum concentration soluble in DMSO, although it did not induced cytotoxicity.		Fulfilled
	Lab 3		Fulfilled. The chemical was examined up to the maximum concentration soluble in DMSO, although it did not induced cytotoxicity.		Fulfilled
	Lab 4		Fulfilled. The chemical was applied as suspension to the cultures and induced cytotoxicity.		Fulfilled
Phenanthrene	Lab 1		Fulfilled	1st	Fulfilled. However, there was a statistically significant increase in transformation frequency at one concentration. The assay was repeated.
				2nd	Fulfilled
	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
	Lab 4	1st	Fulfilled. However, there was a statistically significant increase in transformation frequency at one concentration. The assay was repeated.		Fulfilled
		2nd	Fulfilled except for the concentration criterion, which was considered inapplicable for the following reason: to conclude positive or negative call, this assay was carried out in a narrower range around the concentration at which there had been a statistically significant increase of transformation frequency in the 1st run.		

Table 48.
(Continued)

Phase II					
Chemical	Lab	Initiation assay		Promotion assay	
		Run	All assay acceptance criteria	Run	All assay acceptance criteria
Benzo[*a*]pyrene	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Cadmium chloride	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled	1st	Not fulfilled. The criterion for the number of valid concentrations was not met, i.e., there was only one valid concentration.
				2nd	Fulfilled
Dibenz[*a,h*]-anthracene	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Lithocholic acid	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Methapyrilene HCl	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Mezerein	Lab 2		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤0.1 μg/mL), had not achieved cytotoxicity.		Fulfilled
	Lab 3		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤0.1 μg/mL), had not achieved cytotoxicity.		Fulfilled
MNNG	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Sodium arsenite	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Ampicillin sodium	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
L-Ascorbic acid	Lab 1		Fulfilled		Fulfilled
	Lab 3		Fulfilled	1st	Not fulfilled. The criterion for the number of valid concentrations was not met, i.e., there were only three valid concentrations. Also not fulfilled was the criterion for the negative control.
				2nd	Fulfilled
Caffeine	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled

Table 48.
(Continued)

Chemical	Lab	Initiation assay		Promotion assay	
		Run	All assay acceptance criteria	Run	All assay acceptance criteria
Caprolactam	Lab 1		Fulfilled		Fulfilled
	Lab 2		Fulfilled		Fulfilled
Eugenol	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled	1st	Fulfilled. However, there was a statistically significant increase in transformation frequency at only one concentration. The assay was repeated.
				2nd	Fulfilled
D-Mannitol	Lab 2		Fulfilled		Fulfilled
	Lab 3		Fulfilled		Fulfilled
Phorbol	Lab 1		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤5 μg/mL), had not achieved cytotoxicity.		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤5 μg/mL), had not achieved cytotoxicity.
	Lab 2		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤5 μg/mL), had not achieved cytotoxicity.		Not fulfilled. The concentrations used, the maximum concentration of which had been pre-assigned by the VMT (≤5 μg/mL), had not achieved cytotoxicity.
Pyrene	Lab 2		Not fulfilled. The concentrations used had not achieved cytotoxicity. The data were considered acceptable, however, since there were three sequential concentrations that induced statistically significant increases in transformation frequency and consequently the chemical was called positive.		Fulfilled
	Lab 3		Fulfilled	1st	Fulfilled. However, there was a statistically significant increase in transformation frequency at one concentration. The assay was repeated.
				2nd	Fulfilled

A total of 62 experiments of the initiation assay were performed in the validation phase I and phase II studies on the 96-well method. Out of 62 experiments, nine did not fulfill the assay acceptance criteria. The failure to satisfy those acceptance criteria by eight out of nine of those experiments were the result of the maximum concentrations employed, which were pre-assigned by the VMT (TPA, mezerein and phorbol) being too low. Therefore, only one out of 54 experiments was judged not to fulfill the assay acceptance criteria with respect to the conditions (including dose selection) for which the participating laboratories were responsible. The data of this particular experiment were considered acceptable, however, since there were three sequential concentrations that induced

statistically significant increases in transformation frequency and consequently the chemical was called positive although the concentrations used had not achieved cytotoxicity (pyrene in Lab 2).

A total of 65 experiments of promotion assay were carried out. Out of 65 experiments, four did not fulfill the assay acceptance criteria. The failure to satisfy those acceptance criteria by two out of four of those experiments were the result of the maximum concentrations employed, which were pre-assigned by the VMT (phorbol). Therefore, two out of 63 experiments were judged not to fulfill the assay acceptance criteria with respect to the conditions (including dose selection) for which the participating laboratories were responsible. These two particular experiments were repeated and the second runs succeeded to fulfill the assay acceptance criteria (cadmium chloride in Lab 3 and L-ascorbic acid in Lab 3).

4.4 Conclusion

The positive and negative calls for the test chemicals based on the results in the Bhas 42 CTA 96-well method validation study are summarized in Table 49. In the table, the positive and negative calls by each laboratory for each chemical are made based upon the integrated judgments from the initiation and promotion assay results. That is, a test chemical is positive in a given laboratory if it is positive in either the initiation or promotion assay. The overall judgment (AO) recorded for each chemical is determined by majority rule based on the results obtained by each of the participating laboratories.

4.4.1 Module 2 — Within-laboratory reproducibility

The transformation frequencies of the negative (DMSO) and positive controls obtained in all assays in phase I and phase II of the 96-well method validation study are clustered by individual laboratories and plotted for the respective initiation and promotion assays, as shown in Fig. 17 (section 4.3.2), The average transformation frequency of the negative and positive controls per individual laboratory in phase I and phase II of the 96-well method validation study is shown in Fig. 18 (section 4.3.2). The results depicted in these figures demonstrated that within-laboratory reproducibility was satisfactory in all the laboratories for the negative and positive controls in both the initiation assay and the promotion assay.

To estimate the within-laboratory reproducibility, results of the same test chemical in the same laboratory were compared between different phases.

MCA and TPA were examined in pre-validation phase and in the validation phase I by each laboratory. Benzo[a]pyrene was tested in validation phases I and II. When the results of these chemicals in each laboratory were compared for positive and negative calls between the phases, these three chemicals are all positive in the transformation assay in every laboratory in both phases (Table 49). MCA was judged to be equivocal in one of the promotion assays in the pre-validation phase and in one of the promotion assays in the validation phase I having induced a statistically significant increase of transformation frequency at a single high concentration. Nevertheless, the other judgments for all the test chemicals were concordant in both initiation and promotion assays within each laboratory between phases.

A comparison of Figures 19 and 23 for MCA, Figures 20 and 25 for TPA and Figures 22 and 28 for benzo[a]pyrene, showed that the dose-response relationships for each test chemical were similar between phases in a given laboratory. This held true even though the concentration scales were different between the figures for each respective chemical in the different phases.

These results demonstrated good intra-laboratory reproducibility of the 96-well method of Bhas 42 CTA.

Table 49.

Results of validation studies on the 96-well method of Bhas 42 cell transformation assay

Compound	Pre-validation study					Phase I study					Phase II study				Carcinogenicity
	Laboratory				OA[a]	Laboratory				OA	Laboratory			OA	*in vivo*
	I	II	III	IV		I	II	III	IV		I	II	III		
2-Acetylaminofluorene															+
Initiation[b]						-	+	+	+	+					
Promotion[c]						+	-	+	+	+					
Transformation[d]						+	+	+	+	**+**					
Benzo[*a*]pyrene															+
Initiation						+	+	+	+	+	+		+	+	
Promotion						-	-	-	-	-	-		-	-	
Transformation						+	+	+	+	**+**	+		+	**+**	
Cadmium chloride															+
Initiation											-		-	-	
Promotion											+		+	+	
Transformation											+		+	**+**	
Dibenz[*a,h*]anthracene															+
Initiation												+	+	+	
Promotion												-	-	-	
Transformation												+	+	**+**	
Lithocholic acid															TP[e]
Initiation												-	-	-	
Promotion												+	+	+	
Transformation												+	+	**+**	
Methapyrilene HCl															+, TP
Initiation												-	-	-	
Promotion												+	+	+	
Transformation												+	+	**+**	
3-Methylcholanthrene															+
Initiation	+	+	+	+	+	+	+	+	+	+					
Promotion	-	±[f]	-	-	-	-	-	-	±	-					
Transformation	+	+	+	+	**+**	+	+	+	+	**+**					
Mezerein															TP
Initiation												+	+	+	
Promotion												+	+	+	
Transformation												+	+	**+**	
MNNG															+
Initiation											+		+	+	
Promotion											-		-	-	
Transformation											+		+	**+**	
Sodium arsenite															+
Initiation											-		-	-	
Promotion											-		-	-	
Transformation											-		-	**-**	
o-Toluidine															+
Initiation						-	-	-	-	-					
Promotion						-	-	-	-	-					
Transformation						-	-	-	-	**-**					
TPA															TP
Initiation	-	-	-	-	-	-	-	-	-	-					
Promotion	+	+	+	+	+	+	+	+	+	+					
Transformation	+	+	+	+	**+**	+	+	+	+	**+**					
Ampicillin sodium															-
Initiation											-		-	-	
Promotion											-		-	-	
Transformation											-		-	**-**	
Anthracene															-
Initiation						-	-	-	-	-					
Promotion						-	-	-	-	-					
Transformation						-	-	-	-	**-**					

Table 49.
(Continued)

Compound	Pre-validation study					Phase I study					Phase II study				Carcinogenicity
	Laboratory				OA[a]	Laboratory				OA	Laboratory			OA	*in vivo*
	I	II	III	IV		I	II	III	IV		I	II	III		
L-Ascorbic acid															-
Initiation											-		-	-	
Promotion											-		-	-	
Transformation											-		-	**-**	
Caffeine															-
Initiation												-	-	-	
Promotion												-	-	-	
Transformation												-	-	**-**	
Caprolactam															-
Initiation											-[g]		-[g]	-	
Promotion											-		-	-	
Transformation											-		-	**-**	
Eugenol															-
Initiation												-	-	-	
Promotion												-	-	-	
Transformation												-	-	**-**	
D-Mannitol															-
Initiation												-	-	-	
Promotion												-	-	-	
Transformation												-	-	**-**	
Phenanthrene															-
Initiation						-	-	-	-	-					
Promotion						-	+	-	-	-					
Transformation						-	+	-	-	**-**					
Phorbol															*
Initiation											i[h]		i		
Promotion											i		i		
Transformation											i		i		
Pyrene															-
Initiation												+	+	+	
Promotion												+	+	+	
Transformation												+	+	**+**	

[a] Overall judgment: Judgment by majority rule among laboratories.
[b] Judgment in the initiation assay.
[c] Judgment in the promotion assay.
[d] Judgment in the Bhas 42 CTA into which judgments in initiation and promotion assays were integrated.
[e] Tumor-promoter.
[f] Equivocal: there was a statistically significant increase in transformation frequency at only one concentration.
[g] <21.3 mM and <29.2 mM, negative in Lab I and III, respectively; 25.5 mM< and 32.7 mM<, positive in Lab I and Lab III (see section 4.3.5.12 above).
[h] Incomplete: unable to judge the results due to inadequate dosing, which was the result of the VMT pre-assignment of too low a dose as the highest concentration to be tested (see section 4.3.5.15).
* Carcinogenicity in mice (leukaemogenic action) is positive or negative depending on the strains [Berenblum and Vlasta, 1970; Armuth, 1976]. Tumor-promoting activity in mouse skin is positive or negative depending on the strains [Baird and Boutwell, 1971; Slaga et al., 1976, 1980].

4.4.2 Module 3 — Transferability

Basically, the transferability of the 96-well method Bhas 42 CTA is the same as that of the 6-well method (section 3.4.2). The Bhas 42 CTA can be performed in a laboratory that has experience in routine cell culture techniques. General cell culture laboratory equipment and instruments are sufficient to perform the proposed test method. All supplies and reagents are readily available commercially. Like the 6-well method Bhas 42 CTA, however, the 96-well method requires staff training beyond that of general cell culture techniques in order to distinguish transformed foci from non-transformed foci. Training acquired in mastering focus discrimination using the 6-well method Bhas 42 CTA is directly applicable to that for the 96-well method. The identification of transformed foci is carried out using the same photo catalog that is used for the 6-well method Bhas 42 CTA (Annex 3), since the criteria of transformed foci are the same between the 6-well method and the 96-well method.

Although all the participating laboratories had experience in the 6-well method Bhas 42 CTA, a one-day workshop in November, 2008 was held at HRI for the technical transfer of the 96-well method. All the participating laboratories joined the workshop which was designed to reinforce their previous technical training and to apply it to the 96-well method Bhas 42 CTA, thereby ensuring proper identification of Bhas 42 transformed foci produced in a 96-well plate.

After the workshop, to estimate transferability, MCA and TPA at the designated concentrations were examined in the pre-validation phase (Tables 23 and 24, and Figures 19 and 20). MCA was positive in the initiation assay and negative in the promotion assay, showing concordant dose-response curves between the laboratories. TPA was positive in the promotion assay and negative in the initiation assay, inducing similar responses between the laboratories. These results demonstrated that with adequate training, the Bhas 42 CTA 96-well method is readily transferable between laboratories and because of its portability, the assay is one that can be efficiently and successfully performed by laboratories outside of that of the test method developer.

Although transferability had been demonstrated in the pre-validation phase study, both the VMT and the participating laboratories convened a joint meeting in January, 2009 in preparation for the subsequent validation phase I and phase II studies. At that meeting, experiential information was exchanged on various technical issues and the harmonization of focus counting (identification and discrimination) in order to ensure methodological consistency and pre-empt possible unanticipated issues that might arise.

4.4.3 Module 4 — Between-laboratory reproducibility

As shown in Figures 17 and 18, the transformation frequencies of the negative controls for the initiation assay and the promotion assay were similar between laboratories and those of the positive controls were also similar in range between laboratories. These data indicate that the between-laboratory reproducibility was satisfactory for negative and positive controls.

Table 49, which presents the summary results of 96-well method validation study, shows the good reproducibility obtained between laboratories. Positive or negative calls based upon the integrated judgments from the initiation and promotion assay results

per laboratory were concordant between laboratories, except for phenanthrene. Therefore, the between-laboratory reproducibility of the Bhas 42 CTA was 95% (21/22) with respect to a total of 22 chemicals which were examined in the validation phase I and phase II studies. Phorbol, of which the initiation and promotion assays were incomplete, was excluded from this calculation. Even if we compare the results of each of the component assays, i.e., initiation assay or promotion assay, the judgments were comparable between laboratories except for one of the initiation assays and one of the promotion assays for 2-acetylaminofluorene and one of the promotion assays for phenanthrene. Thus, the between-laboratory reproducibility of initiation assay was 95% (21/22) and that of promotion assay was 91% (20/22).

Between-laboratory reproducibility

Assay	%	
Initiation assay	95	(21/22)
Promotion assay	91	(20/22)
Transformation assay (Bhas 42 CTA)	95	(21/22)

Furthermore, the dose-response curves produced by positive chemicals were similar between laboratories in many instances, e.g., MCA, TPA, benzo[*a*]pyrene, cadmium chloride, dibenz[*a,h*]anthracene, lithocholic acid, methapyrilene hydrochloride, mezerein and pyrene.

These results demonstrated a high degree of inter-laboratory reproducibility of Bhas 42 CTA 96-well method.

4.4.4 Module 5 — Predictive capacity

As shown in Table 49, overall judgments (i.e., the judgment by majority rule) in the transformation assays were consistent with the reported *in vivo* carcinogenicity results except for sodium arsenite, *o*-toluidine and pyrene. The concordance for the prediction of chemical carcinogenicity was 86 % (18/21) in the 96-well method validation study: the sensitivity was 83% (10/12) and the specificity was 89% (8/9). A 2x2 contingency table of 21 tested chemicals and the performance indices of 96-well method Bhas 42 CTA for the prediction of chemical carcinogenicity are presented below (phorbol, of which the initiation and promotion assays were incomplete, was excluded from this calculation):

2x2 Contingency table of the results in the 96-well method Bhas 42 CTA validation study

		In vivo carcinogenicity		
		Carcinogen	Non-carcinogen	Total
Bhas 42 cell transformation assay	+	10	1	11
	–	2	8	10
Total		12	9	21

The performance of 96-well method Bhas 42 CTA for the prediction of chemical carcinogenicity

Performance index	%
Concordance	86 (18/21)
Sensitivity	83 (10/12)
Specificity	89 (8/9)
Positive predictivity	91 (10/11)
Negative predictivity	80 (8/10)
False negative	17 (2/12)
False positive	11 (1/9)

4.4.5 Positive and negative controls

The transformation frequency of each positive control was statistically significantly different from that of corresponding negative control in phase I and phase II of the validation study, and the cluster of transformation frequencies for the positive controls was quite distinct from that of the negative controls (Fig. 17). Therefore, the positive control responses are considered to be robust, and the assay acceptance criteria established for transformation frequency in the negative and positive controls were considered to be appropriate.

5 Discussion

5.1 Module 1— Test definition

As mentioned above, the Bhas 42 CTA is a short-term system to predict chemical carcinogenicity. The assay can detect genotoxic and non-genotoxic carcinogens. To detect genotoxic activity, cells are treated with a test chemical in the beginning of growth phase and then allowed to undergo several rounds of cell division until further growth is inhibited as a result of confluence (initiation assay). To detect tumor-promoting activity, cells are repetitively treated with a test chemical beginning at sub-confluence and continuing beyond confluence achievement of cell growth (promotion assay). The assay endpoint is the formation of transformed foci. The judgments in the initiation assay and the promotion assay for a test chemical are integrated into the positive or negative call in the Bhas 42 CTA. That is, the chemical is positive in the Bhas 42 CTA if it is positive in either the initiation or promotion assay. The two Bhas 42 CTA methods, i.e., the 6-well method and the 96-well method, are fundamentally the same. The 6-well method follows the conventional procedures developed for focus transformation assays and the 96-well method has been developed to be utilized for high throughout automated applications in addition to manual procedures. The data obtained in the 6-well method are statistically analyzed by multiple comparison using the Dunnett test and those in the 96-well method are statistically analyzed by the chi-square test with Bonferroni adjustment. The respective protocols for the 6-well method and the 96-well method have undergone refinement and validation and are both available for use by other laboratories.

5.2 Module 5 — Predictive capacity

The extent of chemical carcinogenicity predictivity of the Bhas 42 CTA was demonstrated by validation studies of the 6-well and 96-well methods as described in sections 3.4.4 and 4.4.4 (Tables 16 and 49). The strong evidence for the predictive capacity of the Bhas 42 CTA was derived using 23 test chemicals in this validation effort. Those results are further supported by an in-house study conducted by HRI in which 98 chemicals were assessed with the Bhas 42 CTA. The study showed performance indices superior or similar to those of conventional genotoxicity assays [Sakai *et al.*, 2010]. As shown in Table 50, HRI applied the Bhas 42 CTA to 52 *in vivo* carcinogens and obtained positive calls from 38 carcinogens and negative calls from 14. In addition, 37 *in vivo* non-carcinogens were tested, of which 6 were positive and 31 were negative in the Bhas 42 CTA. (The other nine out of 98 chemicals were of unknown carcinogenicity). Table 50 presents a 2 x 2 contingency table of the 89 reported chemicals from which the performance indices of Bhas 42 CTA for predicting the carcinogenicity of chemicals were calculated (Table 51).

Table 50.
2x2 Contingency table of the results of Bhas 42 cell transformation assay in an in-house study by HRI [Sakai *et al.*, 2010]

		In vivo carcinogenicity		Total
		Carcinogen	Non-carcinogen	
Bhas 42 cell transformation assay	+	38	6	44
	-	14	31	45
	Total	52	37	89

Table 51.
The performance of Bhas 42 cell transformation assay for the prediction of chemical carcinogenicity [Sakai *et al.*, 2010]

Performance index	%	
Concordance	78	(69/89)
Sensitivity	73	(38/52)
Specificity	84	(31/37)
Positive predictivity	86	(38/44)
Negative predictivity	69	(31/45)
False negative	27	(14/52)
False positive	16	(6/37)

5.3 Quantification of transformation frequency

The endpoint of the Bhas 42 CTA, i.e., focus formation, is quantified by the number of transformed foci per well in the 6-well method, and by the number of wells having transformed foci in the 96-well method. This latter 96-well micro-plate method of quantifying transformation frequency is considerably more advantageous. In the 6-well method, all foci appearing in all wells have to be individually judged as to whether they are transformed or not and then the number of transformed foci/well is recorded. In contrast, in the 96-well method a given well is counted if it contains at least one transformed focus irrespective of the presence of other clearly transformed, marginally transformed and/or non-transformed foci in the same well: the transformation frequency is then expressed as the number of wells having transformed foci/number of wells treated with a test chemical at a given concentration. This latter method has proven to be much more expedient and efficient, thereby holding out promise for adaptation to a high throughput system for carcinogenesis screening. Based on the data generated in this study, it seems that the different scoring approaches do not affect the final results.

5.4 Negative and positive controls

Comparisons of the transformation frequencies for negative and positive controls, respectively, (Figures 3, 4, 17 and 18) demonstrate greater comparability of results as well as similar standard deviations between laboratories in the 96-well method than in the 6-well method. The improvement of between-laboratory reproducibility observed in the 96-well method validation study could be methodology related and/or experience related, among other possibilities. Thus, the more straightforward method of quantifying transformation frequency in the 96-well method, as described above in section 5.3 might be one contributing factor, while the accumulated experience of Bhas 42 CTA by the participating laboratories may be another. Nevertheless, it is clear that the 96-well micro-plate method offers advantages that warrant additional follow up.

Table 52.

Comparison of the results in all validation studies conducted on the Bhas 42 cell transformation assay

Compound	6-well pre-validation study [1][a]							6-well validation study [2]							96-well validation study [3]													Carcinogenicity *in vivo*
															Pre-validation study					Phase I study					Phase II study				
	Laboratory[b]						OA[c]	Laboratory						OA	Laboratory				OA	Laboratory				OA	Laboratory			OA	
	A	B	C	D	E	F		I	II	III	IV	V	VI		1	2	3	4		1	2	3	4		1	2	3		
2-Acetylaminofluorene																												+
Initiation[d]								+			+		+	+						-	+	+	+	+					
Promotion[e]								+			+		+	+						+	-	+	+	+					
Transformation[f]								+			+		+	**+**						+	+	+	+	**+**					
Benz[*a*]anthracene																												+
Initiation		+			+	+	+																						
Promotion		+			-	+	+																						
Transformation		+			+	+	**+**																						
Benzo[*a*]pyrene																												+
Initiation	+				+	+	+													+	+	+	+	+	+		+	+	
Promotion	-				-	-	-													-	-	-	-	-	-		-	-	
Transformation	+				+	+	**+**													+	+	+	+	**+**	+		+	**+**	
Cadmium chloride																												+
Initiation								-	-	-				-											-		-	-	
Promotion								+	+	+				+											+		+	+	
Transformation								+	+	+				**+**											+		+	**+**	
Dibenz[*a,h*]anthracene																												+
Initiation								+	+			+		+												+	+	+	
Promotion								-	-			-		-												-	-	-	
Transformation								+	+			+		**+**												+	+	**+**	
Lithocholic acid																												TP[g]
Initiation	-		-			-	-	-			-		-	-												-	-	-	
Promotion	+		+			+	+	+			+		+	+												+	+	+	
Transformation	+		+			+	**+**	+			+		+	**+**												+	+	**+**	
Methapyrilene HCl																												+, TP[h]
Initiation		-			-	+	-	-	-		-			-												-	-	-	
Promotion		+			+	+	+	+	+		+			+												+	+	+	
Transformation		+			+	+	**+**	+	+		+			**+**												+	+	**+**	
3-Methylcholanthrene																												+
Initiation															+	+	+	+	+	+	+	+	+	+					
Promotion															-	±[i]	-	-	-	-	-	-	±	-					
Transformation															+	+	+	+	**+**	+	+	+	+	**+**					
Mezerein																												TP
Initiation	-			-		-	-	-		±	±															+	+	+	
Promotion	+			+		+	+	+		+	+			+												+	+	+	
Transformation	+			+		+	**+**	+		+	+			**+**												+	+	**+**	
MNNG																												+
Initiation	+		-			+	+																		+		+	+	
Promotion	-		-			-	-																		-		-	-	
Transformation	+		-			+	**+**																		+		+	**+**	
Sodium arsenite																												+
Initiation								-		+			j												-		-	-	
Promotion								+		+			+	+											-		-	-	
Transformation								+		+			+	**+**											-		-	**-**	
o-Toluidine (HCl)[k]																												+
Initiation								-				+	+	+						-	-	-	-	-					
Promotion								-				+	+	+						-	-	-	-	-					
Transformation								-				+	+	**+**						-	-	-	-	**-**					
TPA																												TP
Initiation															-	-	-	-	-	-	-	-	-	-					
Promotion															+	+	+	+	+	+	+	+	+	+					
Transformation															+	+	+	+	**+**	+	+	+	+	**+**					

Table 52.
Continued.

Compound	6-well pre-validation study							6-well validation study							96-well validation study													Carcinogenicity *in vivo*
															Pre-validation study													
	Laboratory						OA	Laboratory						OA	Laboratory				OA	Phase I study						Phase II study				
																				Laboratory						Laboratory				
	A	B	C	D	E	F		I	II	III	IV	V	VI		1	2	3	4		1	2	3	4			1	2	3		
Ampicillin sodium																														-
Initiation																										-		-	-	
Promotion																										-		-	-	
Transformation																										-		-	-	
Anthracene																														-
Initiation	-		-			-	-	-	-				-	-						-	-	-	-	-						
Promotion	-		-			-	-	-	-				+	-						-	-	-	-	-						
Transformation	-		-			-	-	-	-				+	-						-	-	-	-	-						
L-Ascorbic acid																														-
Initiation								-	-		-			-												-		-	-	
Promotion								-	-		-			-												-		-	-	
Transformation								-	-		-			-												-		-	-	
Caffeine																														-
Initiation								-				-	i	-													-	-	-	
Promotion								-				-	-	-													-	-	-	
Transformation								-				-		-													-	-	-	
Caprolactam																														-
Initiation																										-l		-l	-	
Promotion																										-		-	-	
Transformation																										-		-	-	
Eugenol																														-
Initiation																											-	-	-	
Promotion																											-	-	-	
Transformation																											-	-	-	
D-Mannitol																														-
Initiation								-		-	-			-													-	-	-	
Promotion								-		-	-			-													-	-	-	
Transformation								-		-	-			-													-	-	-	
Phenanthrene																														-
Initiation																				-	-	-	-	-						
Promotion																				-	+	-	-	-						
Transformation																				-	+	-	-	-						
Phorbol[m]																														*
Initiation	i			i		i																				i		i		
Promotion	i			i		i																				i		i		
Transformation	i			i		i																				i		i		
Pyrene																														-
Initiation		+			+	+	+																				+	+	+	
Promotion		+			-	+	+																				+	+	+	
Transformation		+			+	+	+																				+	+	+	

[a] References: [1] *Tanaka et al.* (2009); [2] Sakai *et al.* (2001); [3] Sakai *et al.* (in preparation)
[b] Lab A, Lab IV and Lab 2 represent the same laboratory between the validation studies. Likewise, Lab B, Lab III and Lab 1 represent the same laboratory; Lab C, Lab II and Lab 4 represent the same laboratory; and Lab F, Lab I and Lab 3 represent the same laboratory.
[c] Overall judgment: Judgment by majority rule among laboratories.
[d] Judgment in the initiation assay.
[e] Judgment in the promotion assay.
[f] Judgment in the Bhas 42 CTA into which judgments in initiation and promotion assays were integrated.
[g] Tumor-promoter.
[h] [Lijinsky *et al.*, 1992]
i Equivocal:. there was a statistically significant increase in transformation frequency at only one concentration.
[j] Incomplete: unable to judge the results due to inadequate dosing.
[k] *o*-Toluidine hydrochloride was tested in the 6-well method validation study, and free *o*-toluidine was tested in the 96-well method validation study.
[l] <21.3 mM and <29.2 mM, negative in Lab 1 and 3, respectively; 25.5 mM< and 32.7 mM<, positive in Lab 1 and Lab 3
[m] Phorbol was unable to be judged for its transformation activity in the Bhas 42 CTA since the concentrations used were considered to be too low (see text, 4.3.5.15).
* Carcinogenicity in mice (leukaemogenic action) is positive or negative depending on the strains[Berenblum and Vlasta, 1970; Armuth, 1976]. Tumor-promoting activity in mouse skin is positive or negative depending on the strains [Baird and Boutwell, 1971; Slaga et al., 1976, 1980].

5.5 Similarity of results between the 6-well method and 96-well method

The results in all three validation studies on the Bhas 42 CTA, i.e., the pre-validation study of the 6-well method, the validation study of 6-well method and the validation study of 96-well method, all of which were carried out chronologically, are summarized and compared in Table 52. The data presented illustrate that the assay results derived from the 6-well method and those derived from the 96-well method were concordant with exceptions of *o*-toluidine and sodium arsenite.

Mezerein, which is a potent tumor-promoter in mouse skin, induced marked transformation of Bhas 42 cells in the promotion assay, both in the 6-well method and the 96-well method. In the initiation assay, the chemical resulted in positive calls with the 96-well method, while it caused a statistically significant increase of transformation frequency only at the highest concentration tested in two of three laboratories with 6-well method (Tables 9 and 37; Figures 10 and 33). These results of mezerein in the initiation assay appear to be discordant between the 6-well method and the 96-well method (see Table 52). However, this discordance may have simply resulted from the different maximum concentrations of mezerein used in the 6-well method validation study (0.01 μg/mL) and the 96-well method validation study (0.1 μg/mL), which were pre-assigned by the VMT. Rather, those data show the complete agreement of results in the initiation assay between both methods, provided the results are compared at the same doses. That is, (a) in the 6-well method, at a concentration of 0.01 μg/mL, the numbers of transformed foci in the initiation assay were statistically significantly increased in the two of three laboratories, and in the other laboratory the number was increased but the increase was not statistically significant (Table 9 and Fig. 10); (b) in the 96-well method, the increase of transformation frequency in the initiation assay started at 0.0125 μg/mL in one of the laboratories and at 0.03 μg/mL in the other (Table 37 and Fig. 33). Meanwhile, it was also reasoned that these restricted positive results obtained in the initiation assay could be explained by the possible failure to thoroughly remove residual mezerein present in the treatment medium used for the initiation stage of the assay, thereby continuing to remain available to the target cells during the promotion stage. Thus, the transformation positives observed may not have been "initiation positives" per se, but rather "promotion positives" that were incorrectly assumed to have been generated during the initiation stage.

5.6 Discrepancy of results between the 6-well method and 96-well method

o-Toluidine hydrochloride was positive in two of three laboratories in the 6-well method validation study, but free *o*-toluidine was negative in all four laboratories in the 96-well method validation study. HRI, which was the lead laboratory, found that *o*-toluidine often yielded varied or equivocal results, i.e., was borderline positive/negative in the Bhas 42 CTA. According to HRI's repeated experiments, *o*-toluidine, including its hydrochloride, is always negative in the initiation assay, but its results in the promotion assay are inconsistent, although more often tended to be negative. This trend is similar between the 6-well method and the 96-well method and also between hydrochloride and free amine..

In the 6-well method validation study, sodium arsenite was positive in the promotion assay in all the three laboratories, although results were ambiguous in the initiation

assay. In the 96-well method validation study, however, the chemical was negative in both the initiation and promotion assays. The consistent sodium arsenite results obtained in the HRI in-house studies corroborated the positive results in the promotion assay of the 6-well method [Muramatsu *et al.*, 2009; Sakai *et al.*, 2010] and the predominantly negative results in the promotion assay of the 96-well method. According to the information from HRI, sodium arsenite produces transformed foci mainly on the side-wall rather than on the bottom of the 6-well and 96-well micro-plates, and this tendency is more prevalent in the 96-well method (Fig. 44). Only the foci that formed on the bottom of the well were counted in the 96-well method because it is difficult to microscopically discern the morphology of foci attached to the side-wall of such a narrow well, as is the case in a 96-well micro-plate. Since such difficulties were not encountered in the 6-well method, the foci that formed both on the side-wall and the bottom of the well were counted. Consequently, sodium arsenite was judged as negative in the 96-well method. The other chemicals, including MCA and TPA, occasionally produced only a few foci on the side-wall, and therefore were not problematic. Currently there is no available information suggesting the mechanism that underlies the affinity for sodium arsenite-induced foci to preferentially collect on the side-wall of the micro-plates.

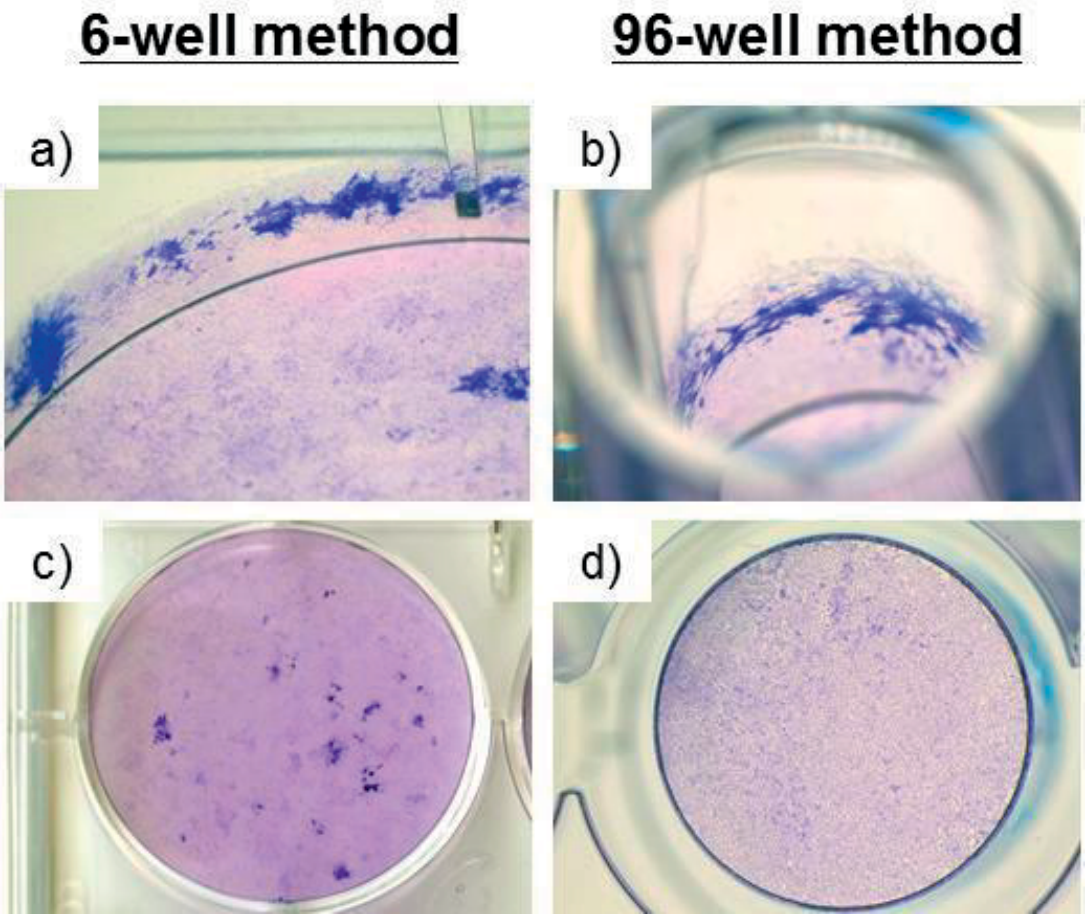

Fig. 44. Foci produced by 0.25 μg/mL of sodium arsenite with the 6-well method and the 96-well method (photos contributed byHRI).

5.7 Phorbol and TPA

Phorbol, the parent alcohol of TPA, was inactive at the concentrations tested in this validation study (Table 46 and Fig. 42). Because the chemical was expensive and scarce, the VMT limited the highest concentration to be ≤ 5 μg/mL, which was too low a concentration to achieve cytotoxicity, thereby precluding a valid judgment of transformation activity in the Bhas 42 cells. However, it was shown that the promotion activity of phorbol was much weaker, even if active, than that of TPA in the Bhas 42 CTA. That was likewise the case in the two-stage carcinogenesis system of mouse skin [Baird and Boutwell, 1971; Slaga et al., 1976]. In the Bhas 42 CTA, TPA was active at around 0.01 μg/mL, but phorbol was inactive at 5 μg/mL in the promotion assay (Tables 29 and 46, and Figures 25 and 42).

6 Recommended protocol

The protocols attached as Annex 2, and Annexes 6, 7 and 8 had been prepared for individual validation studies on the Bhas 42 CTA, the validation study of 6-well method, and the pre-validation, validation phase I and validation phase II studies of 96-well method, respectively. The integrated recommended protocol for the Bhas 42 CTA (protocol Ver. 5) has been developed on the basis of the outcome of this validation study, which was also partly complemented with historical data derived by HRI. Protocols for the 6-well method and the 96-well method have been combined into a single recommended protocol to reduce redundancy. The definitive recommended protocol is presented in Annex 12.

Assay acceptance criteria are essential components of any assay protocol. Although initially absent in the protocol for the 6-well method validation study (Annex 2), appropriate assay acceptance criteria have since been defined, emulating those for the 96-well method (see below). The acceptance criteria for the negative and positive controls have been determined based on the results of this validation study and historical data derived by HRI (see Table 57 of Annex 13). The acceptance criteria for the 6-well method provided in the recommended protocol are as follows (Annex 12, protocol Ver. 5):

> The following assay acceptance criteria must be fulfilled for a given assay to be considered valid. The initiation or promotion assay is repeated independently, as needed, to satisfy the assay acceptance criteria.
>
> 1. Concurrent cell growth assay
>   - When contamination or technical problems are observed, two undamaged wells are necessary at the minimum for each concentration for cell growth assessment.
>
> 2. Transformation assay
> - Initiation assay and promotion assay
>   - If a given chemical concentration results in excessive cell death and/or cells fail to reach confluence at the end of transformation assay because of chemical toxicity, the concentration is not valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment, and “toxicity” is recorded in the data sheet.
>   - When contamination or technical problems are observed, if, for a given concentration, the number of damaged wells is two or more, the concentration is not considered valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment. In such cases, “contamination”, “accident”, “technical error”, etc. are recorded in the data sheet.
>   - For the positive control, there must be a statistically significant increase in the number of transformed foci per well compared to the corresponding negative control (one-sided t-test or Aspin-Welch test, $p<0.05$).
> - Initiation assay
>   - In the negative control, the number of transformed foci must be ten or less per well.
>   - If there is no statistically significant increase in the number of transformed foci at any dose (in case of negative results), four valid test

chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and three concentrations in the range between the NOEL and IC_{90} in the concurrent cell growth assay.

- Promotion assay
  - In the negative control, the number of transformed foci must be twelve or less per well.
  - If there is no statistically significant increase in the number of transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increases cell density in the cell growth assay). When the chemical does not induce growth enhancement but induces cytotoxicity instead, the cytotoxicity observed in the concurrent cell growth assay may not be similar to that observed in the transformation assay, since the durations of chemical exposure to the cells are different between the cell growth assay (3 days) and the transformation assay (10 days). Consequently, chemical toxicity is sometimes accumulated over the 10 day duration of the transformation promotion assay and the otherwise valid plates may be lost because of chemical toxicity. In such cases, four valid plates are also necessary in the concentration range where cells are not killed and are confluent at the end of the transformation assay. If excessive toxicity is encountered, it may be necessary to repeat the assay in a lower concentration range.

The assay acceptance criteria for the transformation frequency of negative and positive controls in the 96-well method have been made more general in the recommended protocol in order to avoid any presuppositions that may result from use of specific assay components, materials and supplies. In this validation study, the batch of FBS used had been pre-screened. For the validation phase I and II studies, the acceptance criteria for the transformation frequency of negative and positive controls were based upon absolute numbers, which, in turn, were based on the results obtained from DMSO, MCA and TPA in the pre-validation phase, all of which presupposed the use of the specific lot of FBS (GIBCO; Lot no. 1391481) (see 4.2.6).

The transformation frequency in any CTA is known to be affected by FBS batch [Sakai et al., 2002]. Generally, FBS lots that induce lower transformation frequencies in the vehicle control tend to produce the fewer numbers of transformed foci in the chemically-treated cultures. As shown in Table 57 of Annex 13, Moregate FBS resulted in lower transformation frequencies of Bhas 42 cells than GIBCO FBS in both negative and positive controls, in both the initiation and promotion assays and in both the 6-well and 96-well methods. Meanwhile, most FBS lots tested have been found to be usable with the Bhas 42 CTA. Actually the absolute numbers of transformation frequency of negative and positive controls are less important in the transformation assays than the relative transformation frequencies of the positive and negative controls in each assay. Therefore, the acceptance criteria for positive controls in the 96-well method have been amended and no longer consider the absolute numbers of wells having transformed foci/plate. Rather, the recommended acceptance criteria now rely upon statistically significant increases in transformation frequency compared to the corresponding

negative control. The acceptance criteria for negative controls, which were established in 96-well method protocols, Ver. 3 (for the phase I study) and Ver. 4 (for the phase II study) (initiation assay, ≤15 wells/plate; promotion assay, ≤20 wells/plate), were incorporated into the recommended protocol. These criteria match the historical data derived at HRI, which were produced using GIBCO FBS lot no. 13914814 and Moregate FBS lot no. 7825120 (see Table 57 of Annex 13).
The acceptance criteria provided in the recommended protocol for the 96-well method are as follows (Annex 12, protocol Ver. 5):

> The following criteria must be fulfilled for an assay to be considered valid. The initiation or promotion assay is repeated independently, as needed, to satisfy the assay acceptance criteria.
>
> 1. Concurrent cell growth assay
>
> - When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration for cell growth assessment.
>
> 2. Transformation assay
>
> - Initiation assay and promotion assay
>   - If a given chemical concentration results in excessive cell death and/or cells fail to reach confluence at the end of transformation assay because of chemical toxicity, the concentration is not considered valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment, and "toxicity" is recorded in the data sheet.
>   - When contamination or technical problems are observed, such that for a given concentration, the number of damaged wells is ≥7, the concentration is not considered valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment. In such cases, "contamination", "accident", "technical error", etc. are recorded in the data sheet.
>   - For the positive control, there must be a statistically significant increase in the proportion of wells having transformed foci compared to the corresponding negative control (one-sided chi-square test, $p<0.05$, upper-sided).
> - Initiation assay
>   - In the negative control, the number of wells having transformed foci must be ≤15 wells/plate (if there exist damaged wells, ≤15.625% of undamaged wells).
>   - If there is no statistically significant increase in the proportion of wells having transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and three concentrations in the range between the NOEL and IC_{90} in the concurrent cell growth assay.
> - Promotion assay
>   - In the negative control, the number of wells having transformed foci must be ≤20 wells/plate (if there exist damaged wells, ≤20.833% of undamaged wells).

- If there is no statistically significant increase in the proportion of wells having transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increases cell density in the cell growth assay). When the chemical does not induce growth enhancement but induces cytotoxicity instead, the cytotoxicity observed in the concurrent cell growth assay may not be similar to that observed in the transformation assay, since the durations of chemical exposure to the cells are different between the cell growth assay (3 days) and the transformation assay (10 days). Consequently, chemical toxicity is sometimes accumulated over the 10 day duration of the transformation promotion assay and the otherwise valid plates may be lost because of chemical toxicity. In such cases, four valid plates are also necessary in the concentration range where cells are not killed and are confluent at the end of the transformation assay. If excessive toxicity is encountered, it may be necessary to repeat the assay in a lower concentration range.

The protocols for validation studies did not include instructions for FBS selection, since the batch of FBS used had been screened by the lead laboratory, HRI, prior to the studies, and the batch of FBS had been specified for each validation study of the 6-well method (Moregate lot no. 7825120) and the 96-well method (GIBCO lot no. 13914814). A section for the selection of FBS has been inserted into the recommended protocol.

The amendments in the recommended protocol relate only to (a) refinement of the assay description, (b) the amalgamation of overlapping aspects of the 6-well method and the 96-well method, and (c) the standardization of assay acceptance criteria among FBS lots. These revisions in no way undermine the validation of the Bhas 42 CTA.

7 Overall conclusion by the validation management team

The aim of the study was to validate the Bhas 42 CTA, in a formal inter-laboratory study, following the modular approach of Hartung et al. (2004) and concentrating on modules 1-5: test definition, within-laboratory reproducibility, transferability, between-laboratory reproducibility and predictive capacity.

Two protocol variations of the Bhas 42 CTA were evaluated in this validation study, i.e., that of the 6-well method and that of the 96-well method. In its guidance and assessment of the various phases of the study, the VMT noted minor differences in study design, conduct and assessment practices had been implemented in the two protocols, much of which were resultant subsequent improvements to previous methodologies. Despite these minor variances, the two methods were found to yield similar results. Experience gained from the 6-well method protocol helped guide the evolution of those procedures ultimately implemented in the 96-well method validation study. As a result, the latter protocol came to be more closely aligned with internationally acknowledged test method validation criteria, and consequently provided highly credible assay results that satisfied the established validation criteria.

Table 53 summarizes the conclusion by the VMT on the assessment of 6-well method and 96-well method in the Bhas 42 CTA.

Table 53 Conclusions of the Validation Management Team for the different modules

6-Well method			
Module		Summary & conclusion	
Module 1	Test definition	・Clear definition of the scientific basis of the method ・Description of the endpoint induced by genotoxic and non-genotoxic mechanisms ・Protocol available with clear definition of a valid study (acceptance criteria have been defined based on the data derived from this validation study and historical data derived by HRI, following those of the 96-well method)	yes
Module 2	Within-laboratory reproducibility	The within-laboratory reproducibility was shown to be satisfactory in all laboratories for ・the vehicle control ・the positive control and in the laboratories which had retrospective data in addition to those data obtained in this prospective validation study for ・the test chemicals*	yes
Module 3	Transferability	The test method is transferable between laboratories: ・basic cell cultivation experience is needed ・training in procedures and transformed-foci identification is essential ・photo catalog of foci is available	yes
Module 4	Between-laboratory reproducibility	The between-laboratory reproducibility was shown to be satisfactory for ・the vehicle control ・the positive control ・the test chemicals**	yes

Table 53 Continued

6-Well method			
Module		Summary & conclusion	
Module 5	Predictive capacity	The capacity to predict chemical carcinogenicity is satisfactory in ・this validation study (12 chemicals) ・the in-house HRI study in which 98 chemicals were tested	yes
96-Well method			
Module		Summary & conclusion	
Module 1	Test definition	・Clear definition of the scientific basis of the method ・Description of the endpoint induced by genotoxic and non-genotoxic mechanisms ・Protocol available with clear definition of a valid study (some acceptance criteria have been refined based on the data derived from this validation study and historical data derived by HRI	yes
Module 2	Within-laboratory reproducibility	The within-laboratory reproducibility was shown to be satisfactory in all laboratories for ・the vehicle control ・the positive control ・the test chemicals	yes
Module 3	Transferability	The test method is transferable between laboratories ・basic cell cultivation experience is needed ・training in procedures and transformed-foci identification is essential ・Photo catalog of foci is available	yes
Module 4	Between-laboratory reproducibility	The between-laboratory reproducibility was shown to be satisfactory for ・the vehicle control ・the positive control ・the test chemicals	yes
Module 5	Predictive capacity	The capacity to predict chemical carcinogenicity is satisfactory in ・this validation study	yes

* The results of one chemical (*o*-toluidine) obtained by HRI were not concordant between its in-house study and this validation study but the chemical was considered by the VMT to be equivocal in the Bhas 42 CTA.

** The results submitted for two out of twelve test chemicals were not concordant between the naive and experienced laboratories.

In addition to the conclusions drawn by the VMT regarding satisfaction of the modules cited, the VMT also considered the following issues noteworthy.

The Bhas 42 CTA has the following advantages:

1. The culture period (3 weeks) is shorter than that of a conventional BALB/c 3T3 CTA (6 weeks).
2. Less labor and reduced amounts of materials are necessary compared with those of the conventional CTAs.

3. Either the 6-well method or the 96-well method can be used with equal assurance.
4. Bhas 42 CTA can detect both tumor-initiators and tumor-promoters and discriminate them depending on a protocol modification.

The validation studies on the Bhas 42 CTA have verified the following:
1. The 6-well method and the 96-well method produced similar results.
2. The Bhas 42 CTA is transferable between laboratories.
3. The Bhas 42 CTA is reliable, i.e., is reproducible within and between laboratories.
4. The Bhas 42 CTA is relevant, i.e., is capable of correctly predicting chemical rodent carcinogenicity.

The in-house HRI study in which the Bhas 42 CTA was applied to 98 chemicals has shown the following:
1. The Bhas 42 CTA could detect a considerable number of Ames-negative and Ames discordant carcinogens.
2. The performance of the Bhas 42 CTA to predict the carcinogenicity of chemicals was superior or equal to that of conventional genotoxicity assays.

In view of the results reported herein and the conclusions asserted by the VMT, we consider that the overall accuracy of detecting potential chemical carcinogens would be improved and the tumor-promoting activity of chemicals could be better predicted by incorporating the Bhas 42 CTA into the battery of *in vitro* assays customarily employed. The Bhas 42 CTA can also conceivably help reduce the number of animals routinely used for *in vivo* chronic carcinogenicity testing by identifying likely positive *in vivo* carcinogens, and thus allow for the possible waiving of subsequent animal testing for carcinogenicity. In addition, the 96-well method has the potential to be easily adapted for automation with further relatively minimal refinement. The VMT recommends that an OECD Test Guideline for the Bhas 42 CTA to be drafted to encourage regulatory acceptance and universal implementation of this validated, reliable, cost- and time-efficient test method.

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Annex 1

Table 54.
Supplier, catalog number, and lot number of test chemicals in the 6-well method validation study.

Chemical	CAS no.[a]	Supplier	Catalog no.	Lot no.	
2-Acetylaminofluorene	53-96-3	Sigma	A7015	076K0677	
Cadmium chloride	10108-64-2	Aldrich	439800	11869TE	
Dibenz[*a,h*]anthracene	53-70-3	Wako	040-00901	CEL1090	
Lithocholic acid	434-13-9	Sigma	L6250	022K1392	
Methapyrilene HCl	135-23-9	Sigma	M9125	037F09291	
Mezerein	34807-41-5	Sigma	M5518	113K0993	
Sodium arsenite	7784-46-5	Wako	191-01241	LTM0420	
o-Toluidine HCl	636-21-3	Aldrich	327115	12915DD	
Anthracene	120-12-7	Aldrich	331481	12223EE	
L-Ascorbic acid	50-81-7	Sigma	A5960	106K0053	
Caffeine	58-08-2	Aldrich	C0750	126K0705	
D-Mannitol	69-65-8	Aldrich	M4125	086K0103	
3-Methylcholanthrene	56-49-5	Aldrich	213942	01115HE	Positive control
TPA[b]	16561-29-8	Sigma	P1585	037K1322	Positive control

[a] Chemical Abstract Service registry number.
[b] 12-*O*-tetradecanoylphorbol-13-acetate.

Annex 2

Protocol of Bhas 42 Cell Transformation Assay (2007.10.26)
6-Well Method Ver. 2

This protocol is described for the validation study of Bhas 42 cell transformation assay. Used materials and reagents with their catalogue numbers are listed in annex 2.1.

I. Preparation

1. Materials

1) Cell line

Bhas 42 cells (v-Ha-*ras*-transfected Balb/c 3T3 clone A31-1-1 cells)[1,2]: Free from bacteria, fungi and mycoplasma, supplied from HRI (Hatano Research Institute, Food and Drug Safety Center, Japan) Cell Bank.

2) Media

MEM: Minimum essential medium with 2.2 g/L $NaHCO_3$.

DMEM/F12: Dulbecco's modified Eagle's medium/F12 with 1.2 g/L $NaHCO_3$.

FBS: Fetal bovine serum should be selected showing a low spontaneous focus formation and a high focus formation in the positive control.

PS: Penicillin G sodium (10000 units/mL) and streptomycin sulfate (10 mg/mL).

M10F: MEM + 10% FBS + 1% PS (M10F is used for the expansion of provided cells, cell storage, and the first culture after thawing.).

DF5F: DMEM/F12 + 5% FBS + 1% PS (DF5F is used for routine passages, cell growth assays and transformation experiments.).

3) Chemicals

Test chemicals and solvent/vehicle: Test chemicals are dissolved or suspended in an appropriate solvent or vehicle and diluted with the solvent/vehicle to each individual concentration before added to culture media so that all chemical treatment media contain an equal concentration of the solvent/vehicle. The solvent/vehicle should neither interact with the test chemicals nor affect survival and focus formation of the cells. The final concentration of the solvent/vehicle in the medium is $\leq$5% with distilled water and $\leq$ 0.1% with DMSO (permissible up to 0.5% when a test chemical does not dissolve).

Negative and positive controls: Negative and positive control cultures are included in each experiment. The solvent/vehicle for a test chemical is used as the negative control. A known initiator, 3-methylcholanthrene (MCA: final concentration of 1 μg/mL), and a promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA: final concentration of 50 ng/mL), are used for the positive controls.

Blank control: Wells added with the medium alone are prepared for the blank control in cell growth assays.

4) Fixatives and staining solutions

Formalin (37% formaldehyde): Used for fixing cells in cell growth assays.

CV solution (0.1% crystal violet solution): One gram of crystal violet is dissolved in 50 mL of ethanol. The ethanol solution is diluted with distilled water to make a final concentration of 0.1%, and used for staining cells in

cell growth assays.
Extraction solution (containing 0.02 mol/L HCl and 50% ethanol): Used for extracting CV from the stained cells.
Methanol: Used for fixing cells in transformation assays.
5% Giemsa solution: Used for staining cells in transformation assays.

5) Culture vessels
ϕ100-mm Petri dishes: Used for routine passage.
6-well plates: Used for cell growth assays and transformation assays.

2. Cell culture and storage

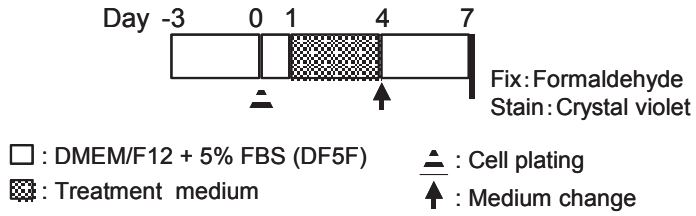
1) Cell passage
Bhas 42 cells are cultured in a humidified 5% CO_2 incubator at 37ºC. The passage should be done at about 70% confluence of cell growth.

2) Cell freezing
The provided cells are expanded with M10F and cryopreserved at an early passage generation. The cells are suspended to make 5 x 10^5 cells/mL in cold M10F containing 5% DMSO. Aliquots of the cell suspension are transferred into freezing tubes, frozen in a -80℃ deep freezer, and stored in liquid nitrogen. For each transformation experiment the cells are thawed from the frozen stock.

II. Experimental procedures

A. Initiation assay[3)]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels
The highest concentration is 5 mg/mL or 10 mM, whichever is the lowest[4,5)]. In the case of chemicals difficult to dissolve, the highest concentration may be one or two level higher than the concentration showing the utmost solubility. Five or more concentration levels are set by an appropriate serial dilution factor such as 10, square root 10 or 2. In some chemicals the cell growth assay should be repeated in a narrower concentration range. Three wells of 6-well plates are used for each concentration.

2) Procedure of cell growth assay
Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm Petri dishes. (Cells with a high passage number can be used for the cell growth assay.)
Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.2 x 10^4 cells/mL. The cell

suspension is transferred at a volume of 2 mL to each well of 6-well plates. Three wells are prepared for each treatment concentration.

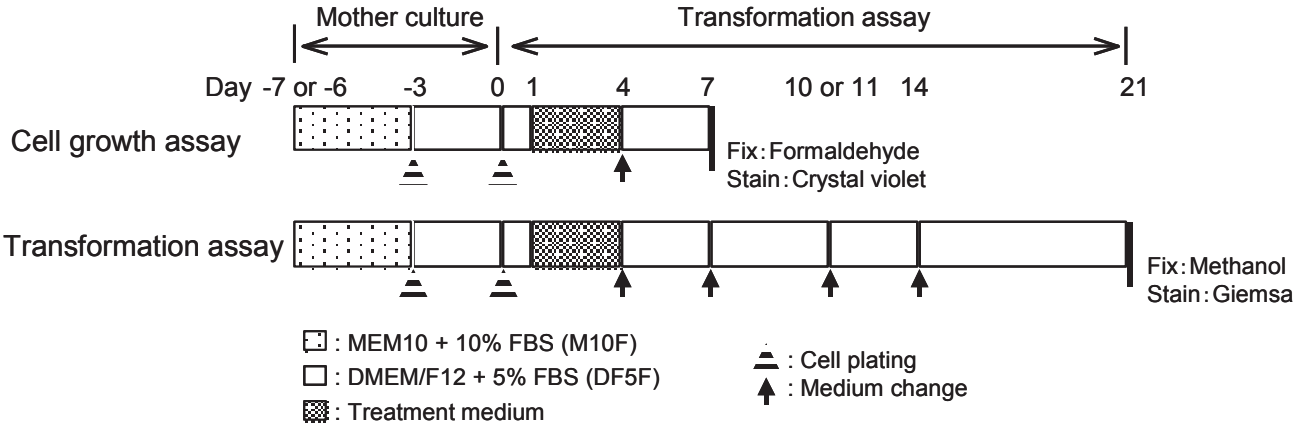
Day 1: Media containing various concentrations of test chemicals are prepared, and used for medium change. It is also practicable to prepare concentrated test chemical solutions and add them to wells without medium change.

Day 4: Medium is changed with fresh DF5F, and the cultivation is continued in the incubator.

Day 7: The cells are fixed with direct addition of 0.2 mL of formalin to the culture medium, or with 10 % formalin or methanol after medium is removed. After 30 min, the cells are washed and dried. The cells are stained with 1.5 mL of CV solution for 15 min, rinsed well with water and dried.

Measurement: The stained dye of each well is extracted with a constant volume of extraction solution (usually 2 mL) for 10 min, and the optical density of each extract is measured at a wavelength between 540 and 570 nm. Growth rates relative to the control culture are calculated from the absorbance.

2. Transformation assay



1) Test concentrations and used vessels

Five or more concentrations are set up based on the results of cell growth assays. These concentrations cover a range from highest toxicity (less than 20% survival compared to the control culture) to little or no toxicity. Ideally, one dose below NOEL, two doses between NOEL and IC50 and two doses between IC50 and IC90 are assessed in the initiation assay, as follows;

----------NOEL----------IC50----------IC90

One dose	Two doses	Two doses

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals the highest concentration is 5 mg/mL or 10 mM, whichever is the lowest[4,5].

At least six wells of 6-well plates for the transformation assay, and at least three wells of 6-well plates for the concurrent cell growth assay are prepared at each concentration.

2) Procedure of transformation assay and concurrent cell growth assay

Day -7 or -6: Frozen stock cells are thawed, suspended in M10F and cultured in ϕ100-mm Petri dishes at a volume of 10 mL.

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cells are cultured in ϕ100-mm Petri dishes.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.2 x 10^4 cells/mL. The cell suspension is distributed into each well of 6-well plates at a volume of 2 mL for the transformation assay and the concurrent cell growth assay. One plate is prepared for each treatment concentration of transformation assay. Three wells are prepared for each treatment concentration of concurrent cell growth assay.

Day 1: Media containing various concentrations of test chemicals are prepared, and used for medium change. It is also practicable to prepare concentrated test chemical solutions and add them to wells without medium change.

Day 4: The medium is changed with fresh DF5F.

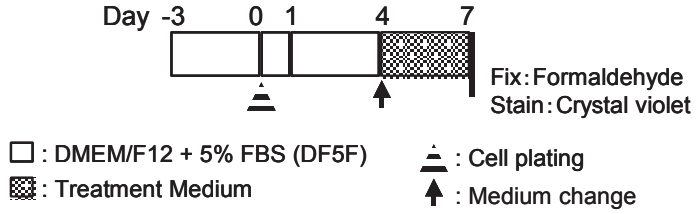
Day 7: The medium of transformation assay is changed with fresh DF5F. The cells for the concurrent growth assay are fixed and processed according to the procedure described above (**II.A.1.**2).

Day 10 or 11, and 14: The medium of the transformation assay is changed with fresh DF5F.

Day 21: The cells are fixed with methanol for 10 min and stained with 5% Giemsa solution for 30 min.

B. Promotion assay[6,7)]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

The highest concentration is 5 mg/mL or 10 mM, whichever is the lowest[4,5)]. In the case of chemicals difficult to dissolve, the highest concentration may be one or two level higher than the concentration showing the utmost solubility. Five or more concentration levels are set by an appropriate serial dilution factor such as 10, square root 10 or 2. In some chemicals the cell growth assay should be repeated in a narrower concentration range. Three wells of 6-well plates are used for each concentration.

2) Procedure of cell growth assay

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm Petri dishes. (Cells with a high passage number can be used for the cell growth assay.)

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 x 10^4 cells/mL. The cell suspension is transferred at a volume of 2 mL to each well of 6-well plates. Three wells are prepared for each treatment concentration.

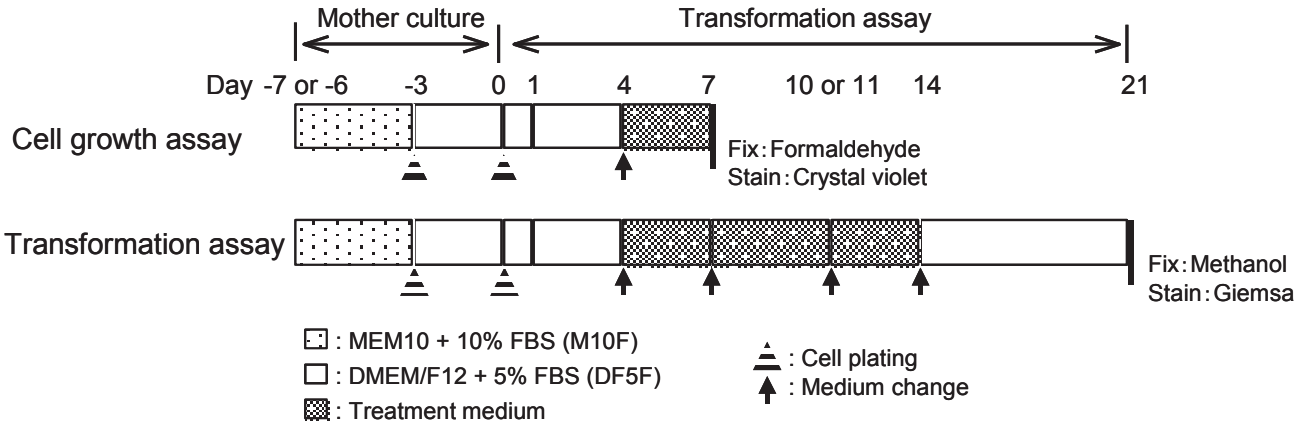
Day 4: Media containing various concentrations of test chemicals are prepared, and used for medium change.

Day 7: The cells are fixed with direct addition of 0.2 mL of formalin to the culture medium, or with 10 % formalin or methanol after medium is removed. After 30 min, the cells are washed and dried. The cells are stained with

1.5 mL of CV solution for 15 min, rinsed well with water and dried.

Measurement: The stained dye of each well is extracted with a constant volume of extraction solution (usually 2 mL) for 10 min, and the optical density of each extract is measured at a wavelength between 540 and 570 nm. Growth rates relative to the control culture are calculated from the absorbance.

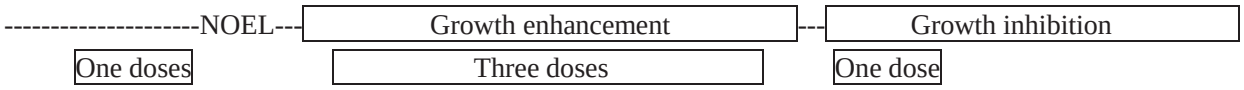
2. Transformation assay



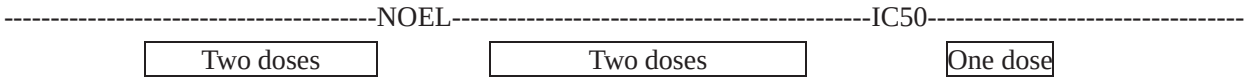
1) Test concentrations and used vessels

Five or more concentrations are set up based on the results of cell growth assays.

For the chemicals that exhibit marked growth enhancement, test concentrations are selected to cover from growth enhancement to little effect on cell growth. Ideally, one dose below NOEL, three doses in the range of growth enhancement, and one dose in the range of weak growth inhibition are assessed in the promotion assay, as follows;



For the chemicals that do not induce marked growth enhancement, test concentrations are selected ranging from a dose exhibiting below 50% growth level to that two or three levels lower than the non-effective concentration. Ideally, two doses below NOEL, two doses between NOEL and IC50 and one dose above IC50 are assessed, as follows;



For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For little cytotoxic compounds the highest concentration is 5 mg/mL or 10 mM, whichever is the lowest[4,5].

At least six wells of 6-well plates for the transformation assay, and at least three wells of 6-well plates for the concurrent cell growth assay are prepared at each concentration.

2) Procedure of transformation assay and concurrent cell growth assay

Day -7 or -6: Frozen stock cells are thawed, suspended in M10F and cultured in ϕ100-mm Petri dishes at a volume of 10 mL.

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cells are cultured in ϕ100-mm Petri dishes.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 x 10^4 cells/mL. The cell suspension is distributed into each well of 6-well plates at a volume of 2 mL for the transformation assay and the concurrent cell growth assay. One plate is prepared for each treatment concentration of transformation assay. Three wells are prepared for each treatment concentration of concurrent cell growth assay.

Day 4: Media containing various concentrations of test chemicals are prepared, and used for medium change.

Day 7: The medium of transformation assay is changed with media containing test chemicals. The cells for the concurrent cell growth assay are fixed and processed according to the procedure described above (**II.B.1.**2).

Day 10 or 11: The medium of transformation assay is changed with media containing test chemicals.

Day 14: The medium is changed with fresh DF5F without test chemicals.

Day 21: The cells are fixed with methanol for 10 min and stained with 5% Giemsa solution for 30 min.

C. Focus count

Transformed foci are featured by the following morphological characteristics; (a) more than 100 cells, (b) spindle-shaped cells different from the contact-inhibited monolayer cells (spindle-shaped), (c) deep basophilic staining (basophilic), (d) random orientation of cells at the edge of foci (criss-cross), (e) dense multilayering of cells (piling up) and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. There are transformed foci not prominent in some of these characteristics. The number of transformed foci is recorded for each well.

D. Judgment

The statistical analysis is carried out for increase in the number of transformed foci per well using the one-side Dunnett test with significant level of 5% ($p<0.05$). The test chemical is judged positive (+), when there exist two or more doses that induce statistically significant increases in the number of transformation foci. When the statistically significant increase is observed in only one dose, the first judgment for the chemical is equivocal (+/-). Then the transformation assay together with the concomitant cell growth assay is repeated in a narrower concentration range including the positive dose of the first assay. The chemical is judged positive (+) when in the second assay the chemical again produces statistically significant increases in the number of transformed foci at one ore more doses, and otherwise negative. When low transformation frequency in the positive control or high transformation frequency in the negative control is obtained, the experiment should be repeated using optimized test conditions. When biologically suspicious results are obtained, a re-experiment may be requested after discussion.

III. References

(1) K. Sasaki, H. Mizusawa and M. Ishidate, Isolation and characterization of ras-transfected BALB/3T3 clone showing morphplogical transformation by 12-*O*-tetradecanoyo-phorbol-13-acetate, Jpn. J. Cancer Res. 79 (1988) 921-930.

(2) K. Sasaki, H. Mizusawa, M. Ishidate and N. Tanaka, Establishment of a highly reproducible transformation assay of a ras-transfected BALB/3T3 clone by treatment with promoters, Basic Life Sci. 52 (1990) 411-416.

(3) S. Asada, K. Sasaki, N. Tanaka, K. Takeda, M. Hayashi and M. Umeda, Detection of initiating activities of

chemicals using v-Ha-ras-transfected BALB/c 3T3 cells (Bhas 42 cells), Mutat. Res. 588 (2005) 7-21.
(4) OECD Guidelines for the Testing of Chemicals. Test No. 473: *In vitro* Mammalian Chromosome Aberration Test.
(5) OECD Guidelines for the Testing of Chemicals. Test No. 476: *In vitro* Mammalian Cell Gene Mutation Test.
(6) K. Ohmori, K. Sasaki, S. Asada, N. Tanaka and M. Umeda, An assay method for the prediction of tumor promoting potential of chemicals by the use of Bhas 42 cells, Mutat. Res. 557 (2004) 191-202.
(7) K. Ohmori *et al*., Inter-laboratory collaborative study of cell transformation assay for tumor promoters using Bhas 42 cells by non-genotoxic carcinogen study group in Japan, ATRA 33 (2005) 1-21.

Annex 2.1 Materials and Reagents Used and their Catalogue Numbers (and Lot Numbers)

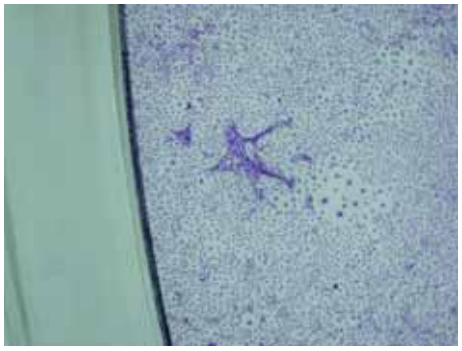
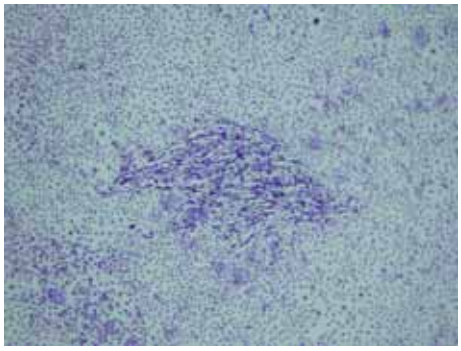
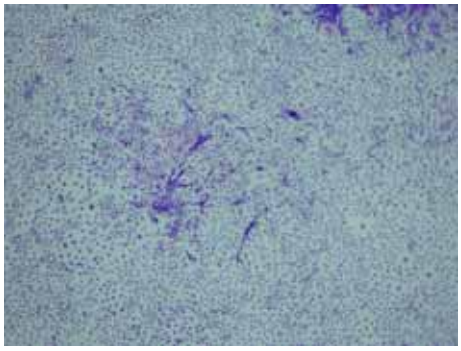
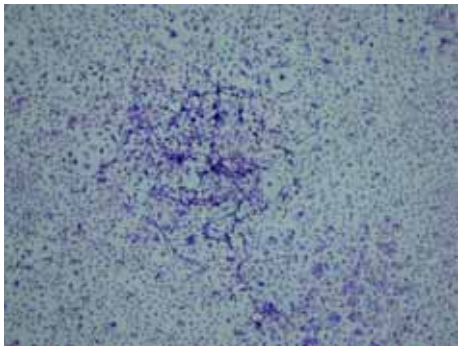
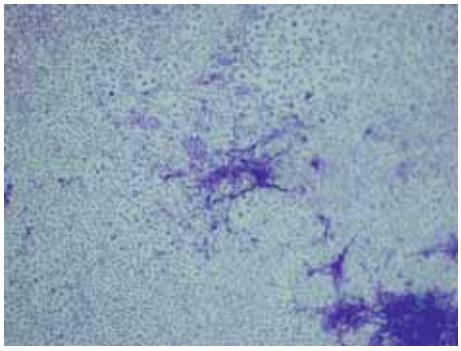
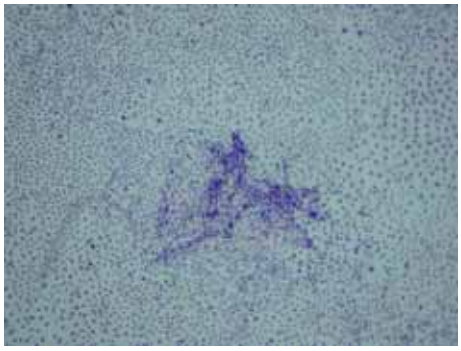
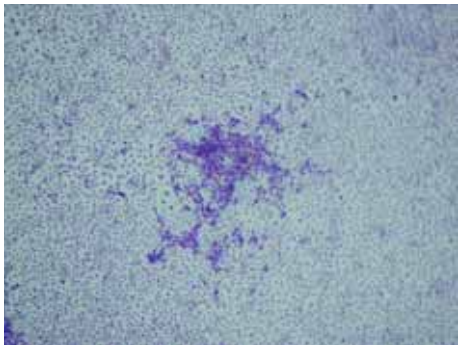
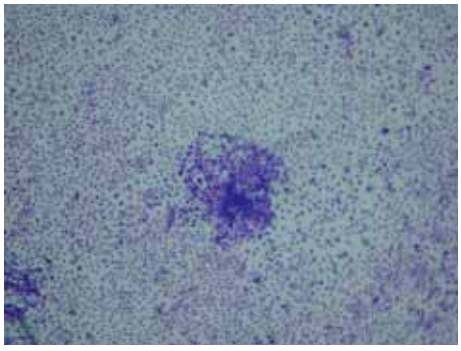
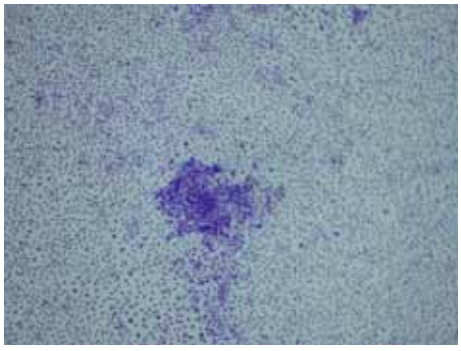
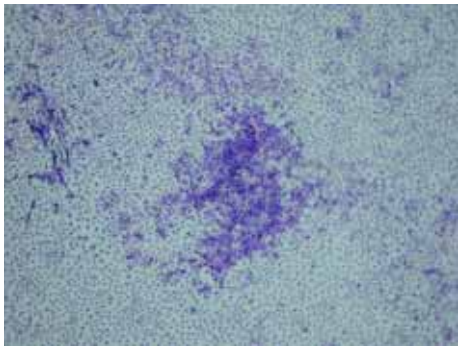
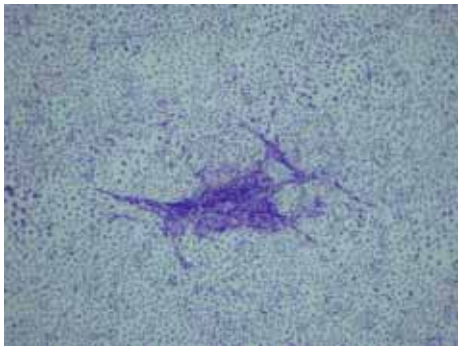
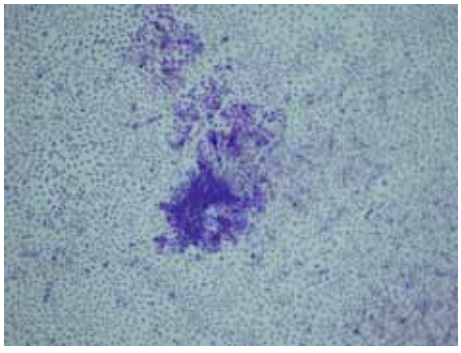
	Supplier	Cat. No.	Lot No.
Positive control			
3-Methylcholanthrene （MCA）	Aldrich	213942-100MG	01115HE
Phorbor 12-myristate 13-acetate (TPA)	SIGMA	P-1585-1MG	037K1322
Culture reagents			
Minimum Essential Medium	GIBCO	11095-080(500mL) (Japan) 11095-098(500mLx10) 31095-029 (Europe)	----
Fetal bovine serum	Moregate		7825120
Dulbecco's modified Eagle's medium/F12	GIBCO	11330-032(500mL) (Japan) 11330-057(500mLx10) (Japan) 31330-038 (Europe)	----
Penicillin(10000units/mL)-Streptomycin (10 mg/mL)	GIBCO	15140-122	----
Other reagent			
DMSO	SIGMA	D8418	----
Fixation and staining solutions			
Methanol	----	----	----
Giemsa solution *	MERK	1.09204	----
Giemsa solution (0.4%)*	Sigma	GS 500	----
Formalin (37% formaldehyde)	SIGMA	F8775	----
Crystal violet	SIGMA	C3886	----
Ethanol	----	----	----
HCl	----	----	----
Culture vessels			
6-well microplates	Falcon or Costar can be used		----

*: Giemsa solution purchased from Merck is used after X20 dilution. Either Giemsa solution can be used.

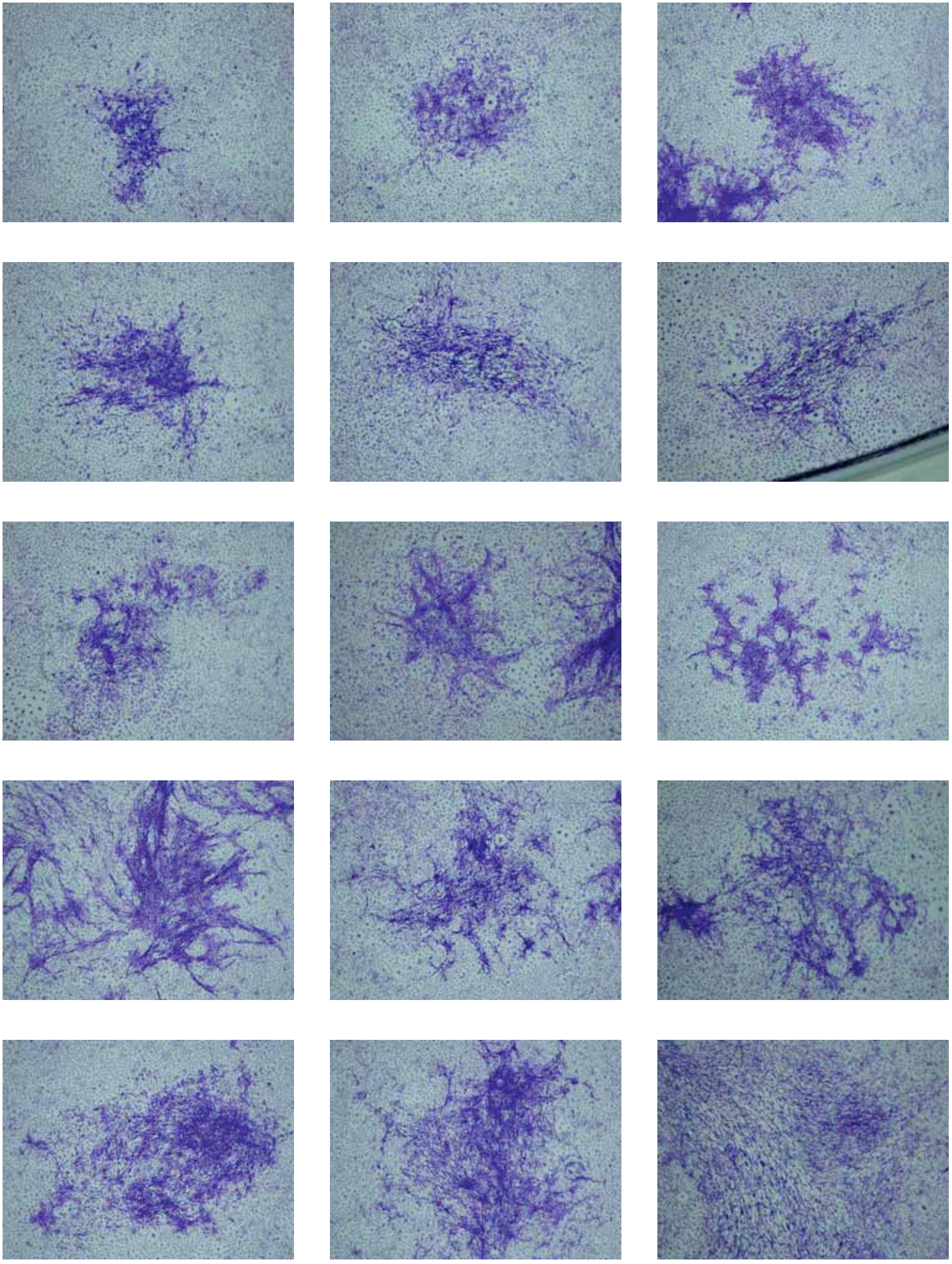
Annex 3

Photo Catalog for the Judgment of Foci in the Bhas 42 Cell Transformation Assay

Negative foci

Positive foci (sparse)



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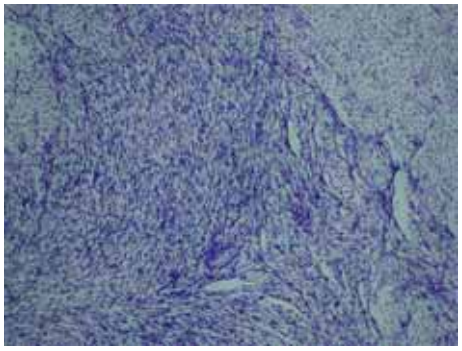
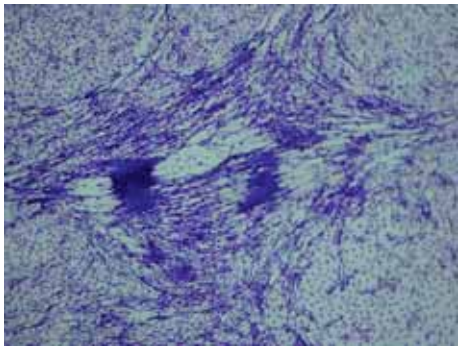
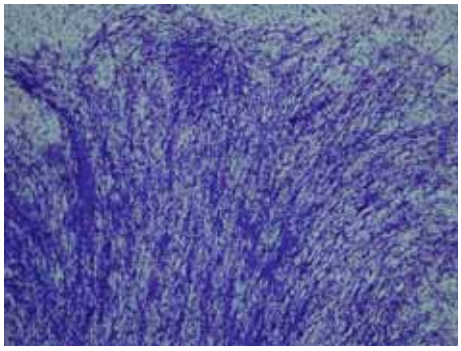
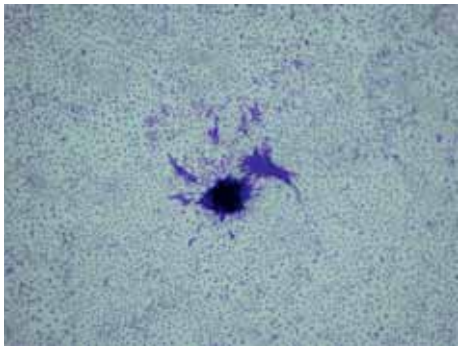
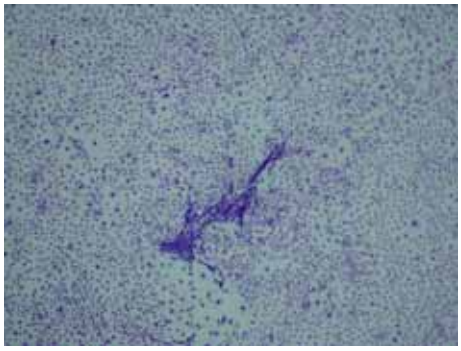
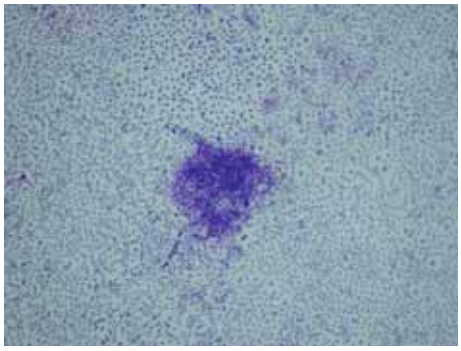
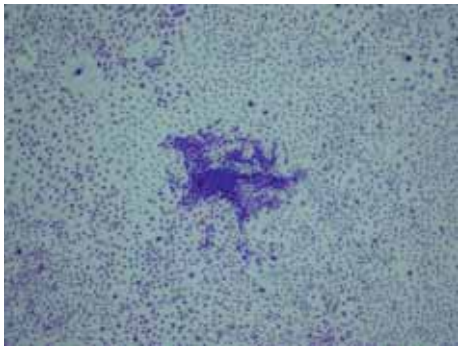
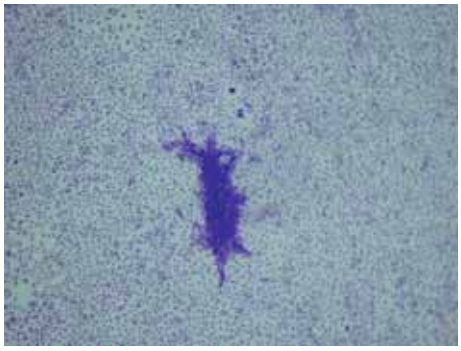
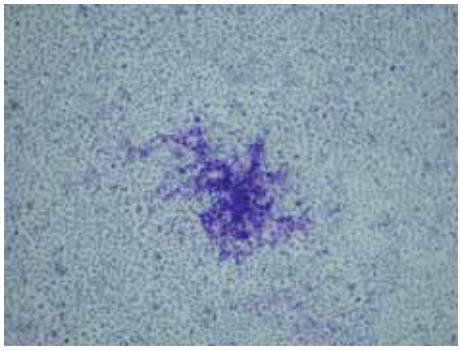
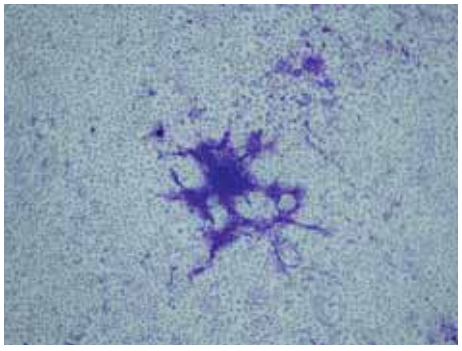
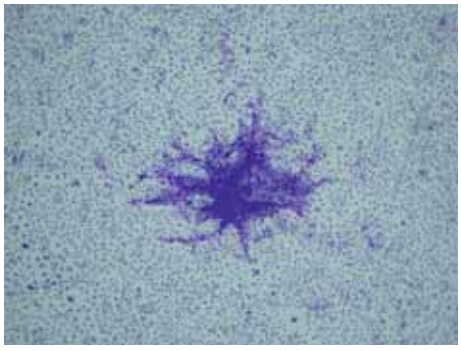
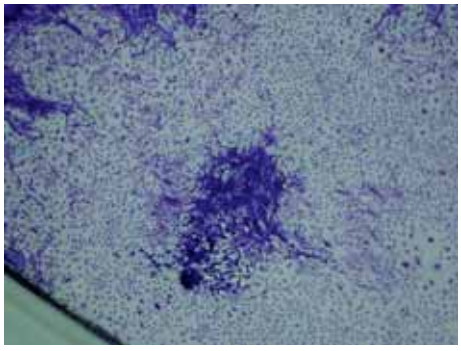
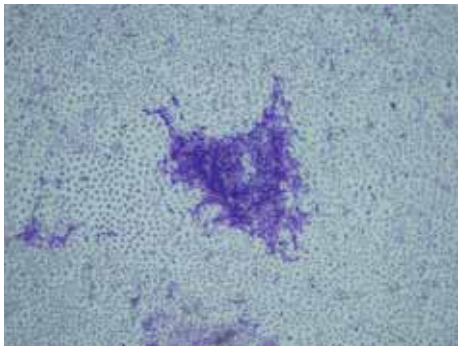
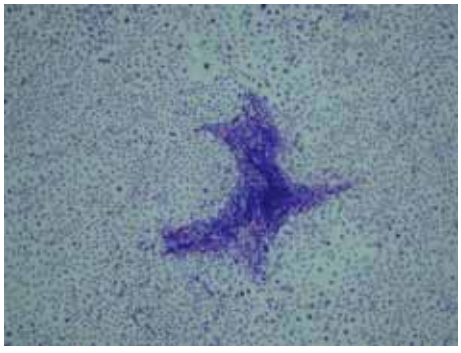
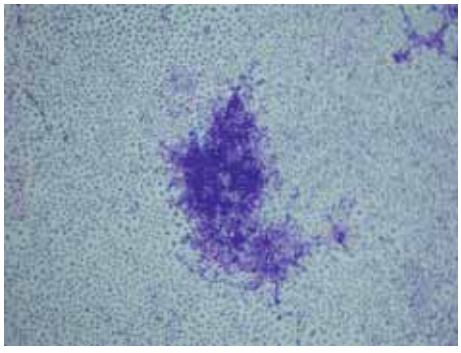
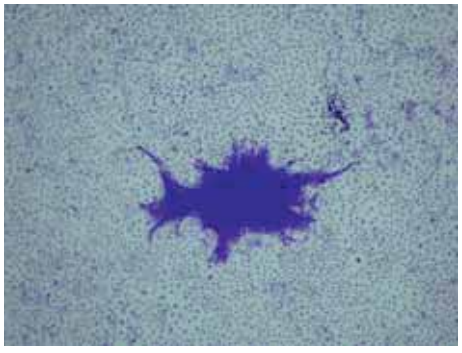
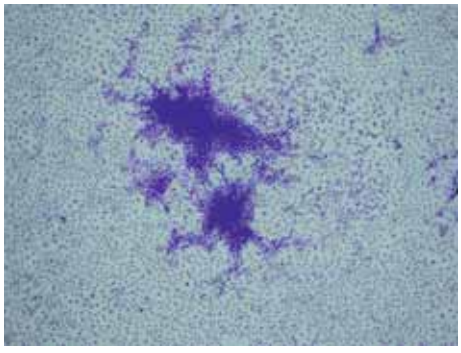
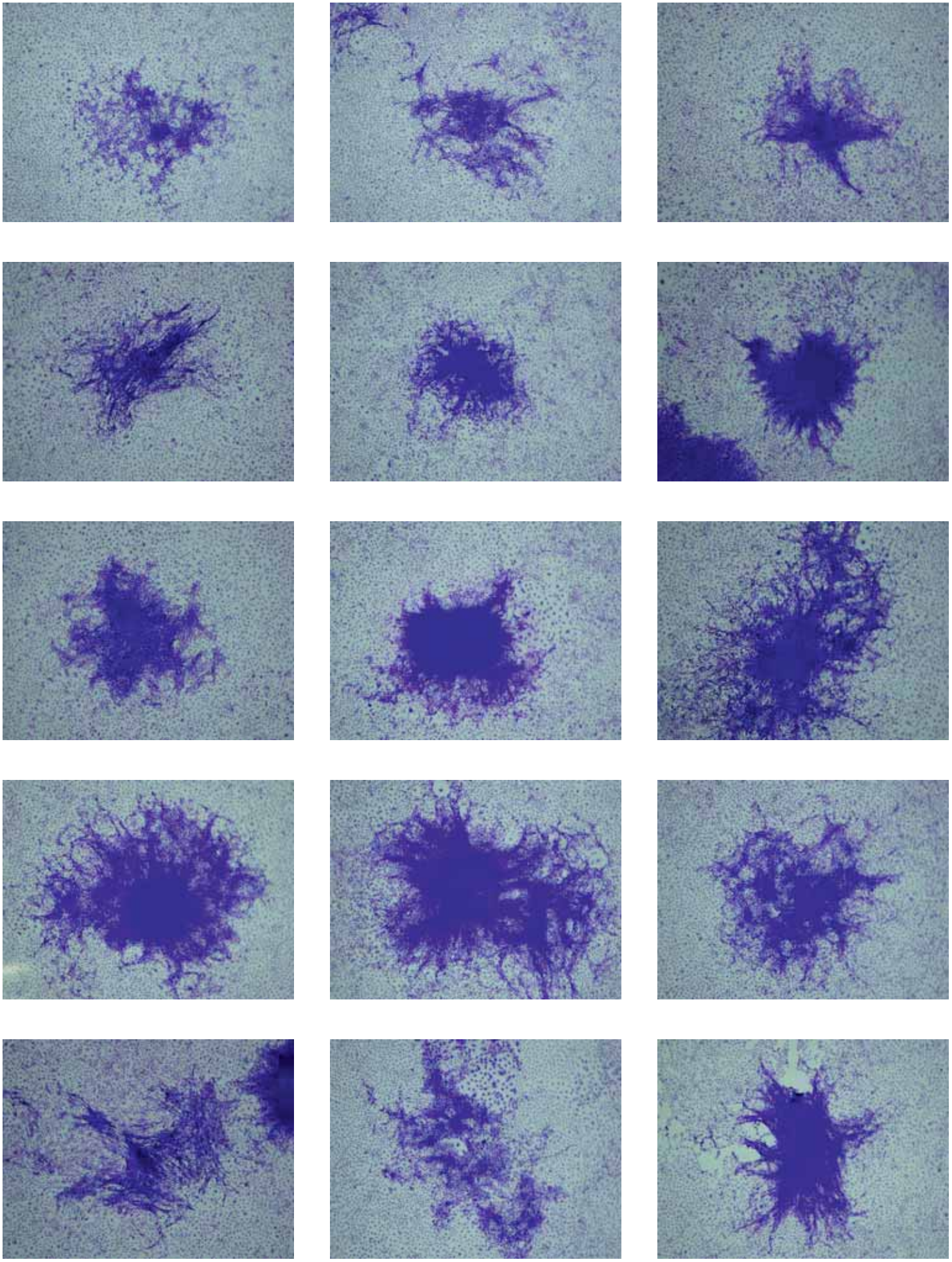




Positive foci (dense and small-sized)

Positive foci (dense and middle-sized)



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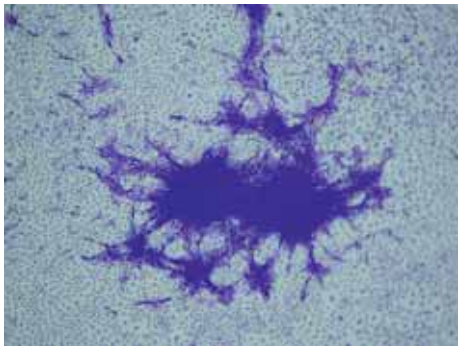
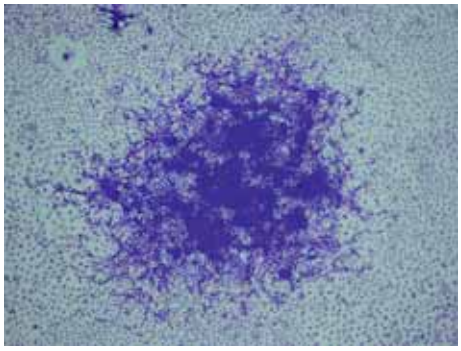
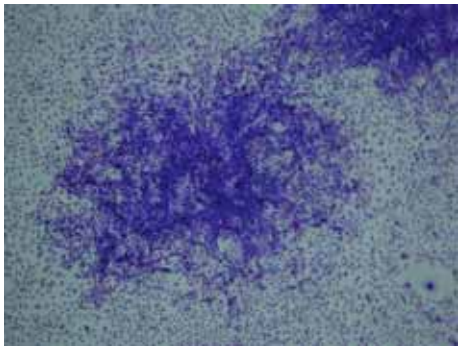
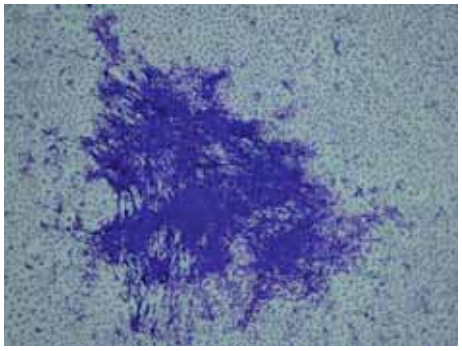
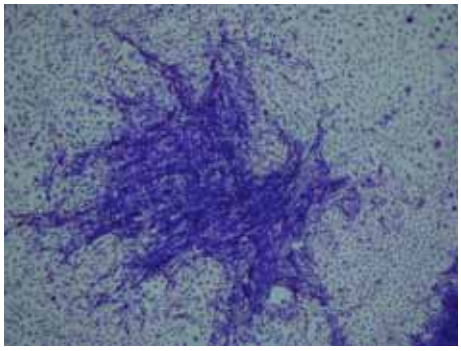
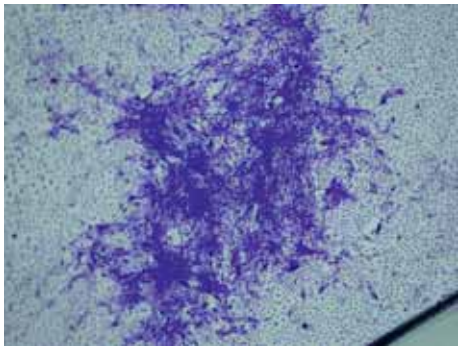
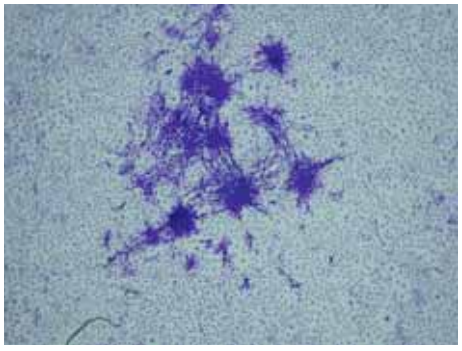
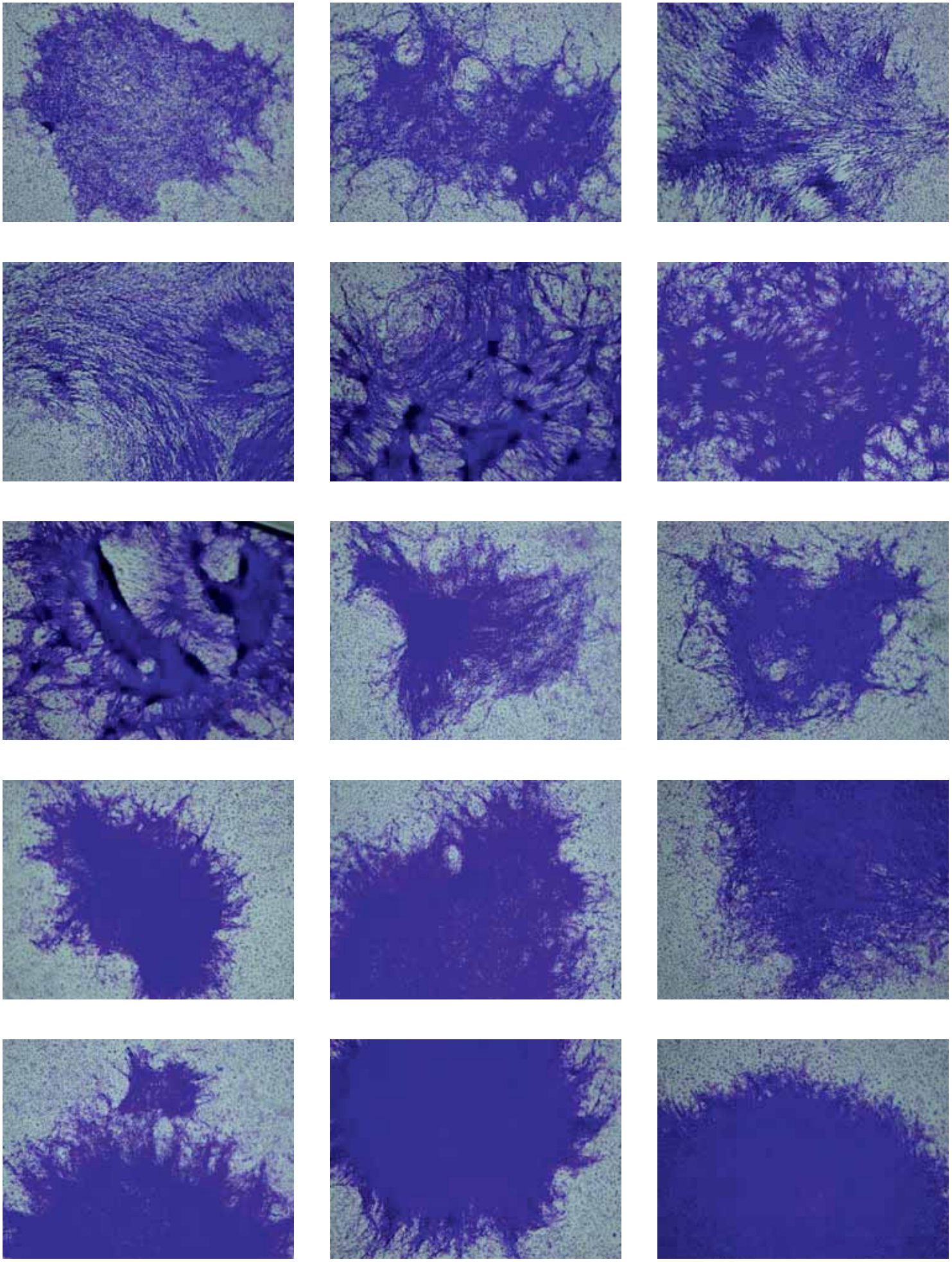








Positive foci (dense and large-sized)



Annex 4

Results Submitted from Laboratories in the Validation Study of 6-Well Method

Assay Laboratory:	Lab I

Compound:	2AAF

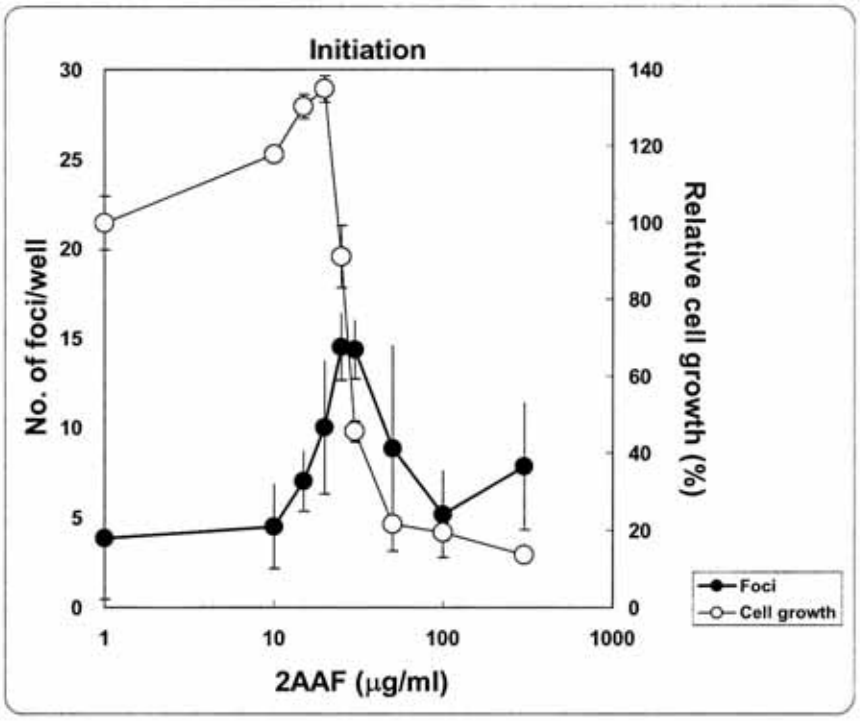
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	1	10	15	20	25	30	50	100	300	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	0.813	0.868	0.963	1.014	0.655	0.406	0.224	0.223	0.161	0.813	0.633
	0.073	0.742	0.896	0.991	0.972	0.762	0.369	0.222	0.192	0.173	0.742	0.625
	0.077	0.723	0.882	0.948	1.01	0.681	0.385	0.222	0.208	0.169	0.723	0.64
Average	0.074	0.759	0.882	0.967	0.999	0.699	0.387	0.223	0.208	0.168	0.759	0.633
SD	0.002	0.047	0.014	0.022	0.023	0.056	0.019	0.001	0.016	0.006	0.047	0.008
Average-Blank	0.000	0.685	0.808	0.893	0.924	0.625	0.312	0.148	0.133	0.093	0.685	0.558
Relative cell growth (%)		100.0	117.9	130.4	134.9	91.2	45.6	21.7	19.5	13.6	100.0	81.5
SD of relative cell growth(%)		6.9	2.0	3.2	3.4	8.1	2.7	0.2	2.3	0.9	6.9	1.1

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	1	10	15	20	25	30	50	100	300	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	5	4	7	9	12	14	10	8	14	5	43
	2	6	2	6	7	13	17	1	7	8	6	43
	3	9	8	7	14	14	14	18	7	4	9	81
	4	1	5	7	15	17	12	7	3	9	1	49
	5	1	2	5	9	16	15	6	3	5	1	46
	6	1	6	10	6	15	14	11	3	7	1	38
Average		3.8	4.5	7.0	10.0	14.5	14.3	8.8	5.2	7.8	3.8	50.0
SD		3.4	2.3	1.7	3.7	1.9	1.6	5.7	2.4	3.5	3.4	15.6
Dunnett test					*	***	***	*				
t-Test (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	2AAF

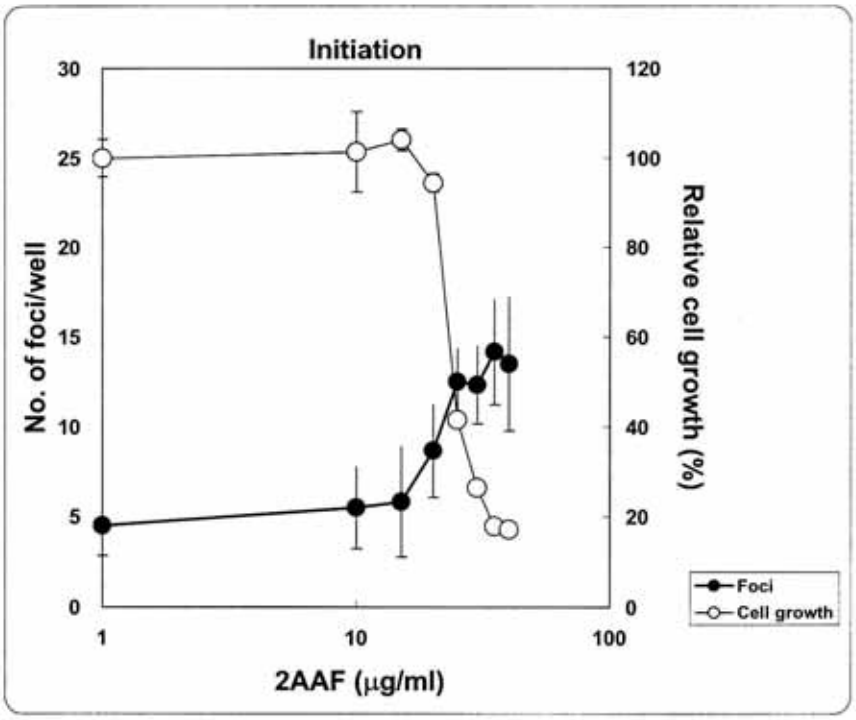
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Blank	1	10	15	20	25	30	35	40	DMSO 0.1%	MCA 1 ug/mL
OD	0.032	0.264	0.289	0.266	0.255	0.127	0.095	0.073	0.072	0.264	0.212
	0.035	0.271	0.253	0.277	0.248	0.13	0.096	0.076	0.073	0.271	0.204
	0.033	0.252	0.254	0.272	0.246	0.128	0.091	0.074	0.073	0.252	0.177
Average	0.033	0.262	0.265	0.272	0.250	0.128	0.094	0.074	0.073	0.262	0.198
SD	0.002	0.010	0.021	0.006	0.005	0.002	0.003	0.002	0.001	0.010	0.018
Average-Blank	0.000	0.229	0.232	0.238	0.216	0.095	0.061	0.041	0.039	0.229	0.164
Relative cell growth (%)		100.0	101.3	104.1	94.5	41.5	26.5	17.9	17.2	100.0	71.8
SD of relative cell growth(%)		4.2	9.0	2.4	2.1	0.7	1.2	0.7	0.3	4.2	8.0

Transformation Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Well No.	1	10	15	20	25	30	35	40	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	7	2	4	11	10	11	9	20	7	42
	2	4	7	9	9	15	15	14	9	4	38
	3	6	7	10	11	13	15	16	12	6	39
	4	3	4	3	5	12	12	13	15	3	44
	5	4	5	6	6	14	10	16	13	4	33
	6	3	8	3	10	11	11	17	12	3	35
Average		4.5	5.5	5.8	8.7	12.5	12.3	14.2	13.5	4.5	38.5
SD		1.6	2.3	3.1	2.6	1.9	2.2	2.9	3.7	1.6	4.1
Dunnett test				*	***	***	***	***			
t-Test (Aspin Welchi)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	2AAF

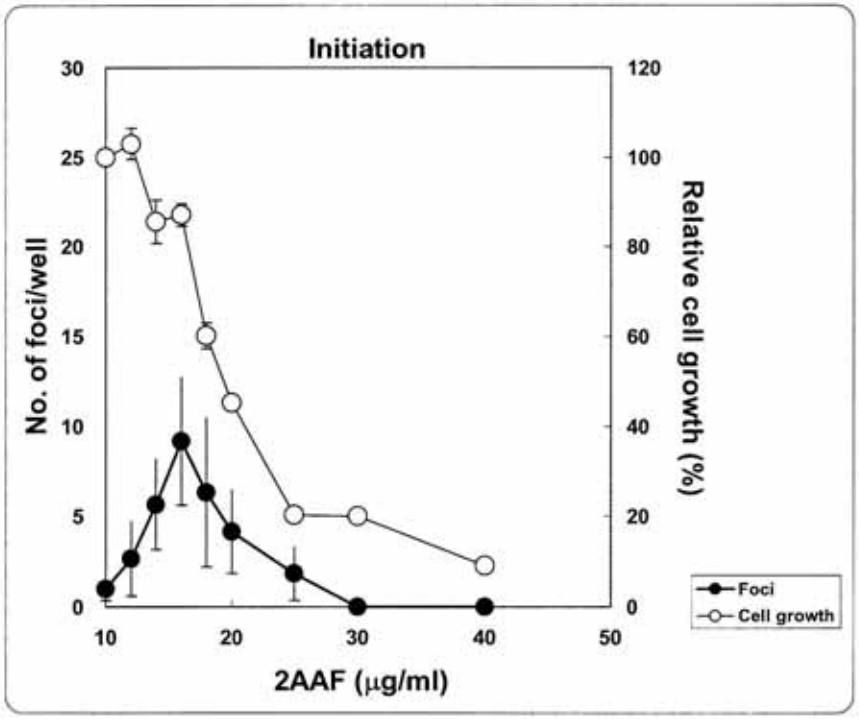
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Blank	10	12	14	16	18	20	25	30	40	DMSO 0.1%	MCA 1 ug/mL
OD	0.032	0.266	0.283	0.228	0.241	0.170	0.137	0.079	0.075	0.052	0.266	0.149
	0.033	0.265	0.267	0.246	0.230	0.181	0.139	0.080	0.081	0.053	0.265	0.161
	0.03	0.270	0.272	0.225	0.239	0.169	0.138	0.080	0.081	0.054	0.270	0.162
Average	0.032	0.267	0.274	0.233	0.237	0.173	0.138	0.080	0.079	0.053	0.267	0.157
SD	0.002	0.003	0.008	0.011	0.006	0.007	0.001	0.001	0.003	0.001	0.003	0.007
Average-Blank	0.000	0.235	0.242	0.201	0.205	0.142	0.106	0.048	0.047	0.021	0.235	0.126
Relative cell growth (%)		100.0	103.0	85.6	87.1	60.2	45.2	20.4	20.1	9.1	100.0	53.4
SD of relative cell growth(%)		1.1	3.5	4.8	2.5	2.8	0.4	0.2	1.5	0.4	1.1	3.1

Transformation Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Well No.	10	12	14	16	18	20	25	30	40	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	1	5	9	9	3	4	1	toxic	toxic	1	18
	2	1	0	8	10	14	4	4	toxic	toxic	1	18
	3	0	3	3	5	4	8	1	toxic	toxic	0	25
	4	1	2	6	10	8	5	0	toxic	toxic	1	27
	5	2	1	5	15	5	1	2	toxic	toxic	2	29
	6	1	5	3	6	4	3	3	toxic	toxic	1	25
Average		1.0	2.7	5.7	9.2	6.3	4.2	1.8	#DIV/0!	#DIV/0!	1.0	23.7
SD		0.6	2.1	2.5	3.5	4.1	2.3	1.5	#DIV/0!	#DIV/0!	0.6	4.6
Dunnett test				**	***	**						
t-Test (Aspin-Welchi)												***

The true value is zero. This value was temporarily put



Assay Laboratory:	Lab I

Compound:	2AAF

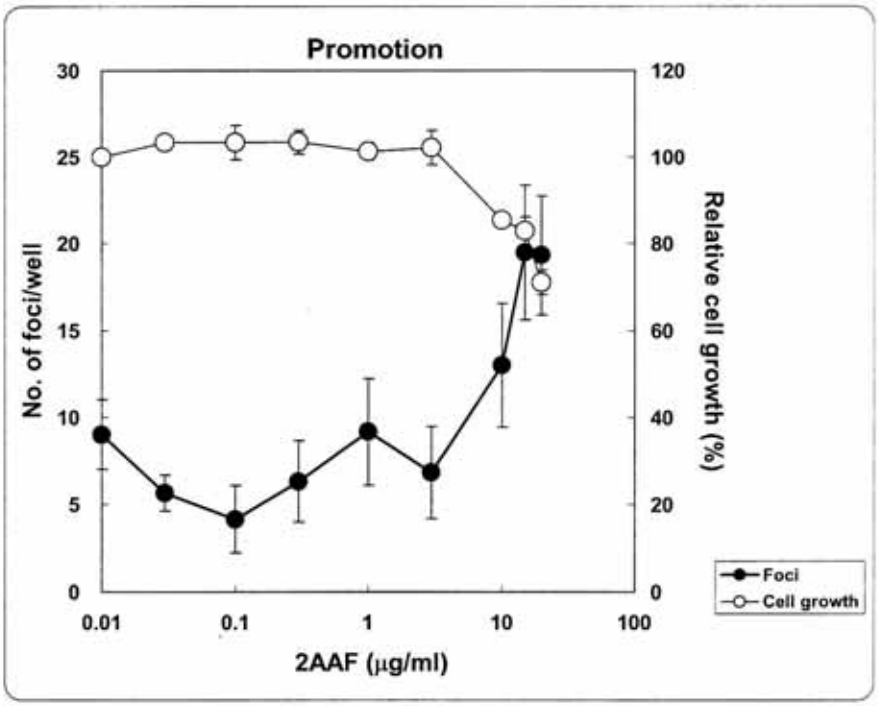
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	0.01	0.03	0.1	0.3	1	3	10	15	20	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	0.931	0.965	0.989	0.937	0.942	0.981	0.797	0.801	0.658	0.931	1.074
	0.073	0.922	0.939	0.923	0.944	0.916	0.927	0.794	0.749	0.704	0.922	1.172
	0.077	0.918	0.952	0.946	0.98	0.948	0.919	0.81	0.788	0.673	0.918	1.16
Average	0.074	0.924	0.952	0.953	0.954	0.935	0.942	0.800	0.779	0.678	0.924	1.135
SD	0.002	0.007	0.013	0.034	0.023	0.017	0.034	0.009	0.027	0.023	0.007	0.053
Average-Blank	0.000	0.849	0.878	0.878	0.879	0.861	0.868	0.726	0.705	0.604	0.849	1.061
Relative cell growth (%)		100.0	103.3	103.4	103.5	101.4	102.2	85.5	83.0	71.1	100.0	124.9
SD of relative cell growth(%)		0.8	1.5	3.9	2.7	2.0	4.0	1.0	3.2	2.8	0.8	6.3

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	0.01	0.03	0.1	0.3	1	3	10	15	20	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	11	6	2	10	7	6	19	23	18	11	39
	2	8	7	5	7	10	7	11	24	19	8	45
	3	12	6	7	6	14	12	13	13	14	12	41
	4	7	5	5	5	6	5	15	19	19	7	30
	5	8	4	2	3	11	5	11	19	22	8	39
	6	8	6	4	7	7	6	9	19	24	8	37
Average		9.0	5.7	4.2	6.3	9.2	6.8	13.0	19.5	19.3	9.0	38.5
SD		2.0	1.0	1.9	2.3	3.1	2.6	3.6	3.9	3.4	2.0	5.0
Dunnett test								*	***	***		
t-Test (Aspin-Welchi)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅳ
Compound:	2AAF

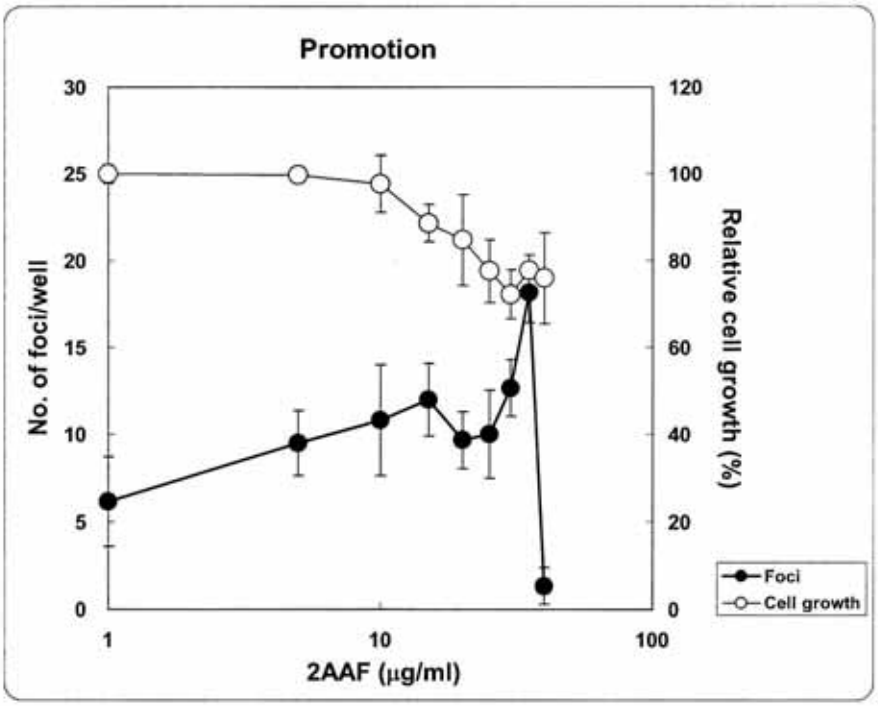
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	1	5	10	15	20	25	30	35	40	DMSO 0.1%	TPA 50 ng/mL
OD	0.032	0.18	0.174	0.179	0.163	0.162	0.144	0.142	0.146	0.16	0.18	0.393
	0.035	0.174	0.178	0.18	0.166	0.166	0.156	0.142	0.15	0.136	0.174	0.337
	0.033	0.178	0.179	0.163	0.154	0.138	0.135	0.128	0.14	0.132	0.178	0.316
Average	0.033	0.177	0.177	0.174	0.161	0.155	0.145	0.137	0.145	0.143	0.177	0.349
SD	0.002	0.003	0.003	0.010	0.006	0.015	0.011	0.008	0.005	0.015	0.003	0.040
Average-Blank	0.000	0.144	0.144	0.141	0.128	0.122	0.112	0.104	0.112	0.109	0.144	0.315
Relative cell growth (%)		100.0	99.8	97.7	88.7	84.7	77.5	72.2	77.8	75.9	100.0	219.0
SD of relative cell growth(%)		2.1	1.8	6.6	4.3	10.5	7.3	5.6	3.5	10.5	2.1	27.6

Transformation Assay	Solvent:											
Compound (ug/ml)	Well No.	1	5	10	15	20	25	30	35	40	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	10	6	15	8	14	10	18	1	3	23
	2	8	11	13	10	8	8	15	18	0	8	22
	3	7	10	9	11	9	10	13	19	1	7	29
	4	9	6	12	12	12	8	12	17	1	9	25
	5	3	9	10	10	11	8	13	21	2	3	28
	6	7	11	15	14	10	12	13	16	3	7	21
Average		6.2	9.5	10.8	12.0	9.7	10.0	12.7	18.2	1.3	6.2	24.7
SD		2.6	1.9	3.2	2.1	1.6	2.5	1.6	1.7	1.0	2.6	3.3
Dunnett test			*	**	***	*	*	***	***			
t-Test (Assuming Equal Variances)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	2AAF

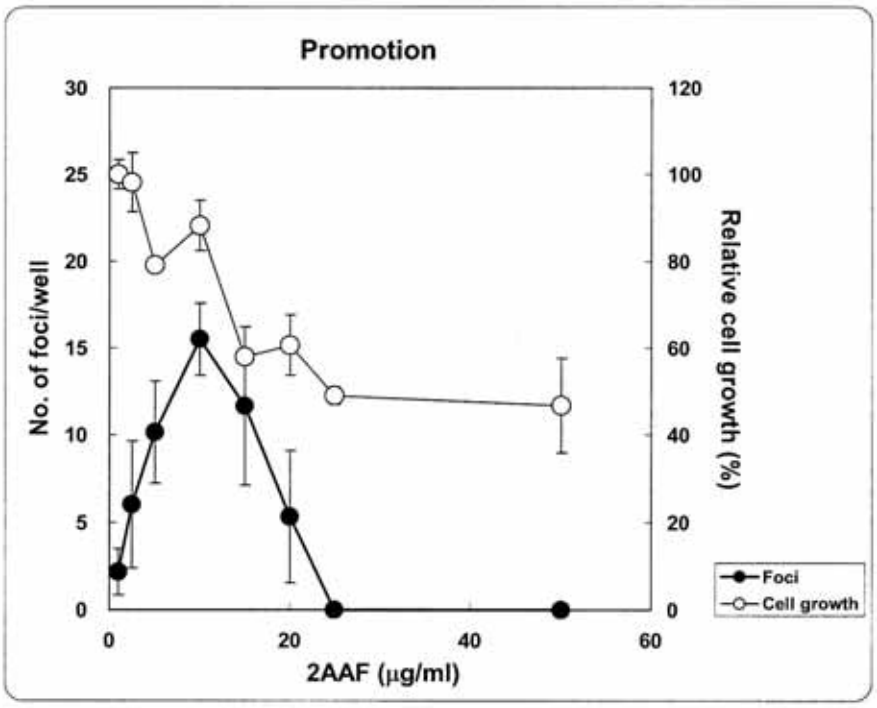
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%									
Compound (ug/ml)	Blank	1	2.5	5	10	15	20	25	50	DMSO 0.5%	TPA 50 ng/mL
OD	0.03	0.244	0.265	0.202	0.212	0.158	0.173	0.138	0.142	0.244	0.253
	0.032	0.259	0.241	0.206	0.228	0.156	0.174	0.144	0.153	0.259	0.277
	0.03	0.252	0.237	0.208	0.237	0.162	0.147	0.135	0.107	0.252	0.250
Average	0.031	0.252	0.248	0.205	0.226	0.159	0.165	0.139	0.134	0.252	0.260
SD	0.001	0.008	0.015	0.003	0.013	0.003	0.015	0.005	0.024	0.008	0.015
Average-Blank	0.000	0.221	0.217	0.175	0.195	0.128	0.134	0.108	0.103	0.221	0.229
Relative cell growth (%)		100.0	98.2	79.0	88.2	57.9	60.6	49.0	46.8	100.0	103.8
SD of relative cell growth(%)		3.4	6.9	1.4	5.7	1.4	6.9	2.1	10.9	3.4	6.7

Transformation Assay	Solvent:	DMSO 0.5%									
Compound (ug/ml)	Well No.	1	2.5	5	10	15	20	25	50	DMSO 0.5%	TPA 50 ng/mL
No of foci	1	4	4	7	17	14	12	Toxic	Toxic	4	16
	2	2	4	9	16	19	7	Toxic	Toxic	2	17
	3	2	4	12	18	8	2	Toxic	Toxic	2	23
	4	2	9	15	15	13	2	Toxic	Toxic	2	17
	5	0	12	8	15	7	5	Toxic	Toxic	0	20
	6	3	3	10	12	9	4	Toxic	Toxic	3	12
Average		2.2	6.0	10.2	15.5	11.7	5.3	#DIV/0!	#DIV/0!	2.2	17.5
SD		1.3	3.6	2.9	2.1	4.5	3.8	#DIV/0!	#DIV/0!	1.3	3.7
Dunnett test				***	***	***					
t-Test (Aspin-Welchi)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Cadmium chloride

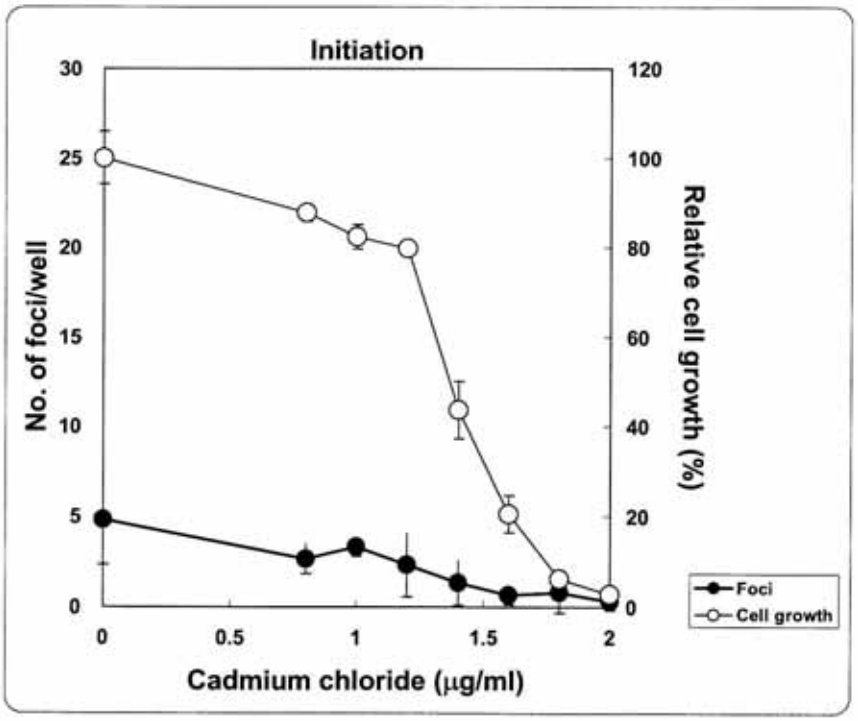
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	0	0.8	1	1.2	1.4	1.6	1.8	2	DMSO 0.1%	MCA 1ug/mL
OD	0.073	1.019	0.897	0.857	0.809	0.526	0.255	0.14	0.109	0.875	0.603
	0.073	0.931	0.862	0.807	0.817	0.49	0.305	0.119	0.097	0.835	0.667
	0.077	1.029	0.881	0.829	0.795	0.411	0.231	0.136	0.095	0.822	0.669
Average	0.074	0.993	0.880	0.831	0.807	0.476	0.264	0.132	0.100	0.844	0.646
SD	0.002	0.054	0.018	0.025	0.011	0.059	0.038	0.011	0.008	0.028	0.038
Average-Blank	0.000	0.919	0.806	0.757	0.733	0.401	0.189	0.057	0.026	0.770	0.572
Relative cell growth (%)		100.0	87.7	82.4	79.8	43.7	20.6	6.2	2.8	100.0	74.3
SD of relative cell growth(%)		5.9	1.9	2.7	1.2	6.4	4.1	1.2	0.8	3.6	4.9

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	0	0.8	1	1.2	1.4	1.6	1.8	2	DMSO 0.1%	MCA 1ug/mL
No of foci	1	6	2	3	3	1	1	3	1	5	42
	2	8	2	4	0	3	1	1	1	6	46
	3	3	3	3	3	2	1	1	0	4	42
	4	5	4	3	2	0	1	0	0	7	50
	5	1	2	3	5	2	0	0	0	2	41
	6	6	3	4	1	0	0	0	0	2	40
Average		4.8	2.7	3.3	2.3	1.3	0.7	0.8	0.3	4.3	43.5
SD		2.5	0.8	0.5	1.8	1.2	0.5	1.2	0.5	2.1	3.8
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Cadmium chloride

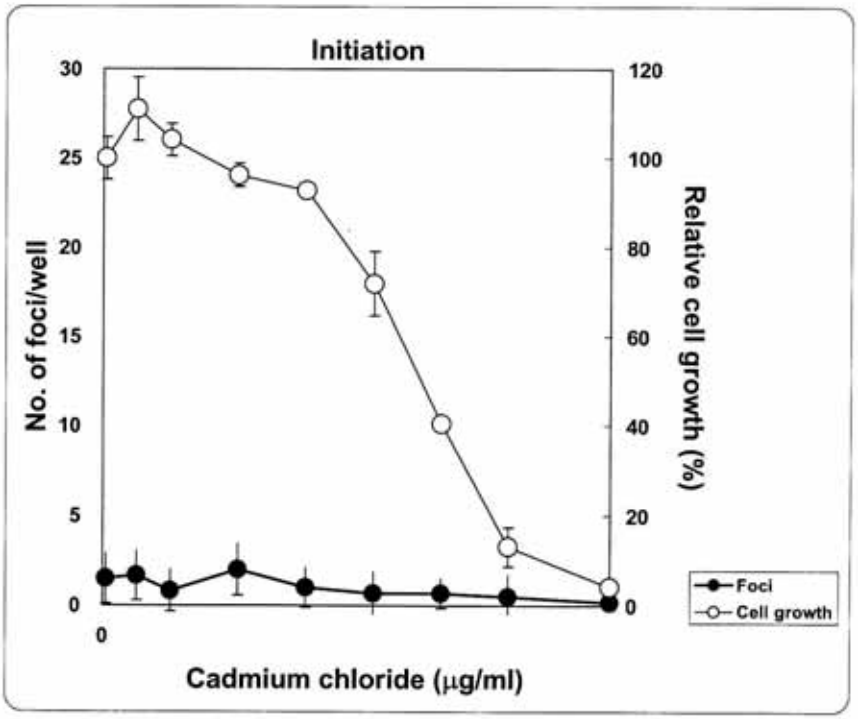
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%										
Compound (ug/ml)	Blank	0.01	0.1	0.2	0.4	0.6	0.8	1	1.2	1.5	DMSO 0.1%	MCA 1ug/mL
OD	0.0681	0.7008	0.7818	0.7161	0.6433	0.6334	0.4608	0.3233	0.122	0.0914		
	0.0709	0.6533	0.7194	0.6726	0.6315	0.6158	0.546	0.3037	0.1733	0.102		
	0.0711	0.6501	0.7009	0.6894	0.6622	0.6257	0.4924	0.3098	0.149	0.0904		
Average	0.070	0.668	0.734	0.693	0.646	0.625	0.500	0.312	0.148	0.095	#DIV/0!	#DIV/0!
SD	0.002	0.028	0.042	0.022	0.015	0.009	0.043	0.010	0.026	0.006	#DIV/0!	#DIV/0!
Average-Blank	0.000	0.598	0.664	0.623	0.576	0.555	0.430	0.242	0.078	0.025	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	111.0	104.1	96.3	92.8	71.9	40.5	13.1	4.1	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		4.7	7.1	3.7	2.6	1.5	7.2	1.7	4.3	1.1	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	Water 5%										
Compound (ug/ml)	Well No.	0.01	0.1	0.2	0.4	0.6	0.8	1	1.2	1.5	DMSO 0.1%	MCA 1ug/mL
No of foci	1	1	2	1	2	0	1	0	0	0	2	9
	2	3	4	3	0	0	0	1	3	0	2	16
	3	0	0	0	3	2	3	0	0	0	0	23
	4	2	2	0	2	2	0	2	0	0	1	16
	5	0	1	0	4	2	0	1	0	0	1	16
	6	3	1	1	1	0	0	0	0	1	0	15
Average		1.5	1.7	0.8	2.0	1.0	0.7	0.7	0.5	0.2	1.0	15.8
SD		1.4	1.4	1.2	1.4	1.1	1.2	0.8	1.2	0.4	0.9	4.4
Dunnett test												N.S.
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab III

Compound:	Cadmium chloride

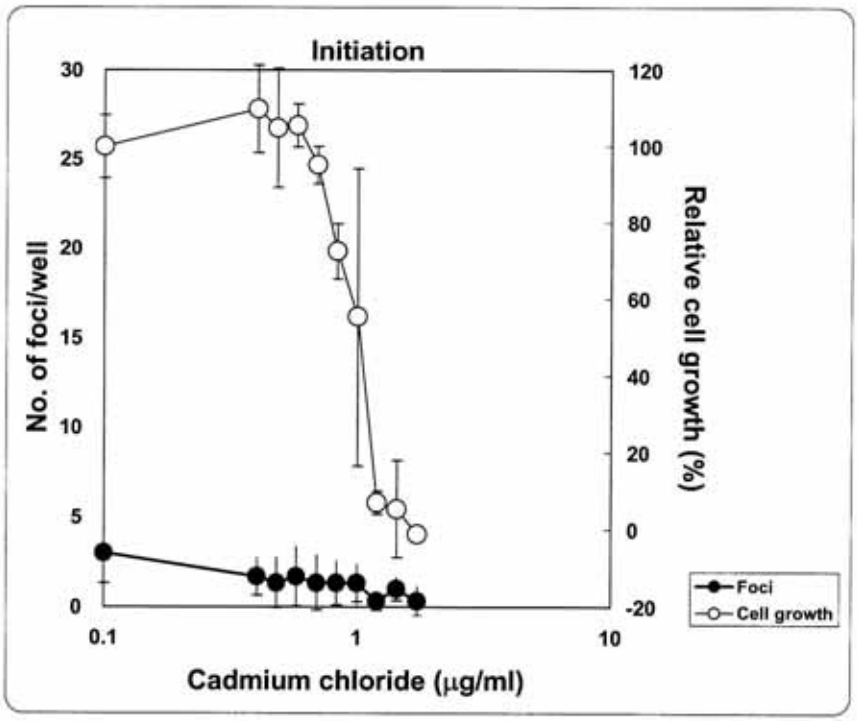
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	D.W. 5 %											
Compound (ug/ml)	Blank	0.1	0.402	0.482	0.579	0.694	0.833	1	1.2	1.44	1.73	DMSO 0.1%	MCA 1ug/mL
OD	0.176	1.509	1.879	1.859	1.722	1.561	1.225	1.556	0.268	0.412	0.14	–	0.366
	0.106	1.687	1.639	1.593	1.564	1.472	1.217	0.593	0.233	0.099	0.092	–	0.375
	0.102	1.466	1.565	1.415	1.615	1.423	1.043	0.602	0.182	0.102	0.101	–	0.253
Average	0.128	1.554	1.694	1.622	1.634	1.485	1.162	0.917	0.228	0.204	0.111	#DIV/0!	0.331
SD	0.042	0.117	0.164	0.223	0.081	0.070	0.103	0.553	0.043	0.180	0.026	#DIV/0!	0.068
Average-Blank	0.000	1.426	1.566	1.494	1.506	1.357	1.034	0.789	0.100	0.076	-0.017	#DIV/0!	0.203
Relative cell growth (%)		100.0	109.8	104.8	105.6	95.2	72.5	55.3	7.0	5.4	-1.2	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		8.2	11.5	15.7	5.7	4.9	7.2	38.8	3.0	12.6	1.8	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %											
Compound (ug/ml)	Well No.	0.1	0.402	0.482	0.579	0.694	0.833	1	1.2	1.44	1.73	DMSO 0.1%	MCA 1ug/mL
No of foci	1	5	2	0	4	2	2	3	0	1	0	–	11
	2	5	2	3	1	4	1	1	1	1	0	–	8
	3	1	1	0	2	0	3	0	1	2	0	–	7
	4	3	2	3	0	1	0	2	0	0	2	–	11
	5	2	0	1	3	1	0	1	0	1	0	–	11
	6	2	3	1	0	0	2	1	0	1	0	–	7
Average		3.0	1.7	1.3	1.7	1.3	1.3	1.3	0.3	1.0	0.3	#DIV/0!	9.2
SD		1.7	1.0	1.4	1.6	1.5	1.2	1.0	0.5	0.6	0.8	#DIV/0!	2.0
Dunnett test													N.S.
t-Test (Assuming Equal Variances) vs 5% Water													***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Cadmium chloride

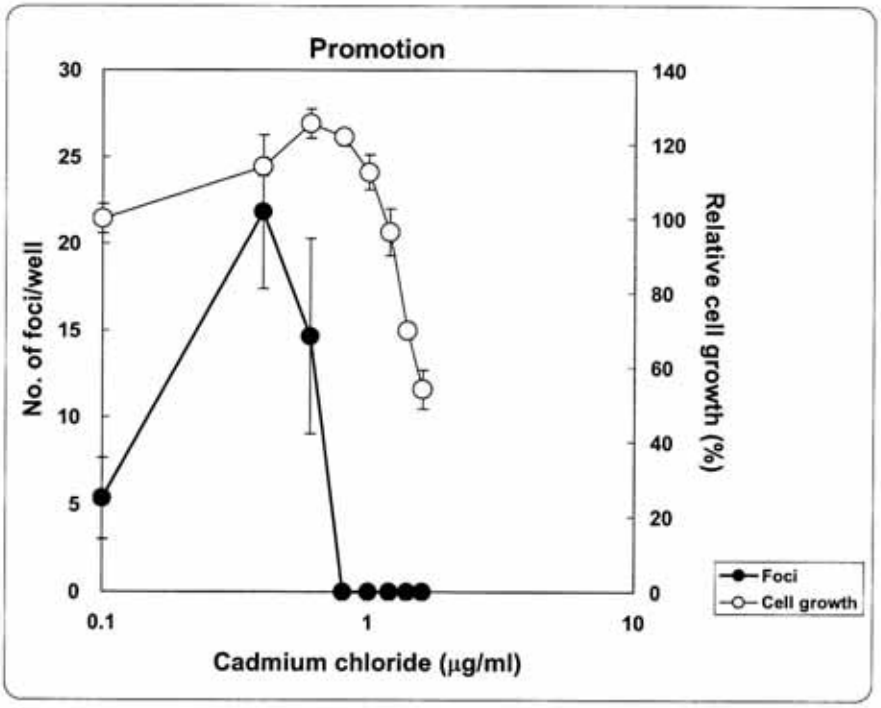
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	0.1	0.4	0.6	0.8	1	1.2	1.4	1.6	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	0.962	1.047	1.175	1.134	1.005	0.938	0.67	0.527	1.038	1.287
	0.073	0.897	1.077	1.116	1.1	1.086	0.931	0.66	0.589	1.032	1.281
	0.077	0.943	1.038	1.174	1.135	1.035	0.841	0.697	0.503	1.021	1.254
Average	0.074	0.934	1.054	1.155	1.123	1.042	0.903	0.676	0.540	1.030	1.274
SD	0.002	0.033	0.020	0.034	0.020	0.041	0.054	0.019	0.044	0.009	0.018
Average-Blank	0.000	0.860	0.980	1.081	1.049	0.968	0.829	0.601	0.465	0.956	1.200
Relative cell growth (%)		100.0	114.0	125.7	122.0	112.6	96.4	69.9	54.1	100.0	125.5
SD of relative cell growth(%)		3.9	2.4	3.9	2.3	4.8	6.3	2.2	5.2	0.9	1.8

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	0.1	0.4	0.6	0.8	1	1.2	1.4	1.6	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	6	20	18	toxic	toxic	toxic	toxic	toxic	11	46
	2	7	22	19	toxic	toxic	toxic	toxic	toxic	6	42
	3	8	15	17	toxic	toxic	toxic	toxic	toxic	12	31
	4	3	25	8	toxic	toxic	toxic	toxic	toxic	13	34
	5	6	21	7	toxic	toxic	toxic	toxic	toxic	5	42
	6	2	28	19	toxic	toxic	toxic	toxic	toxic	7	37
Average		5.3	21.8	14.7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	9.0	38.7
SD		2.3	4.4	5.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	3.4	5.6
Dunnett test			***	**							
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Cadmium chloride

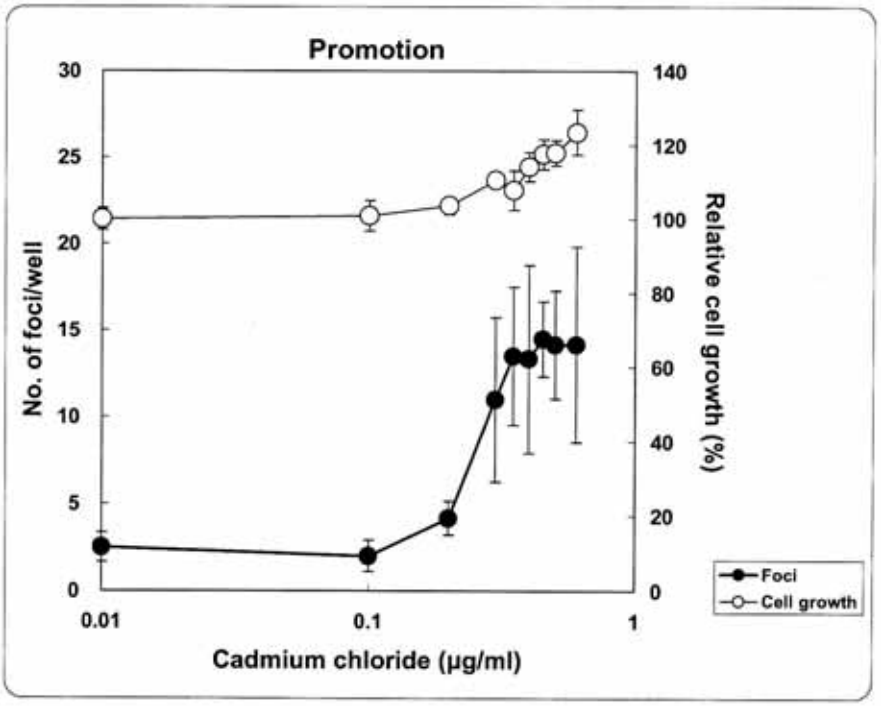
Toransformation Assay

Promotion

Cell Growth Assay	Solvent:	water 5%										
Compound (ug/ml)	Blank	0.01	0.1	0.2	0.3	0.35	0.4	0.45	0.5	0.6	DMSO 0.1%	TPA 50 ng/mL
OD	0.065	0.98	0.999	1.01	1.053	1.072	1.117	1.152	1.118	1.226	0.998	1.308
	0.065	0.956	0.964	0.975	1.049	1.022	1.078	1.092	1.142	1.14	0.956	1.265
	0.063	0.928	0.925	0.974	1.042	0.978	1.046	1.086	1.082	1.124	0.92	1.167
Average	0.064	0.955	0.963	0.986	1.048	1.024	1.080	1.110	1.114	1.163	0.958	1.247
SD	0.001	0.026	0.037	0.021	0.006	0.047	0.036	0.036	0.030	0.055	0.039	0.072
Average-Blank	0.000	0.890	0.898	0.922	0.984	0.960	1.016	1.046	1.050	1.099	0.894	1.182
Relative cell growth (%)		100.0	100.9	103.6	110.5	107.8	114.1	117.4	117.9	123.4	100.0	132.3
SD of relative cell growth(%)		2.9	4.2	2.3	0.6	5.3	4.0	4.1	3.4	6.2	4.4	8.1

Transformation Assay	Solvent:	water 5%										
Compound (ug/ml)	Well No.	0.01	0.1	0.2	0.3	0.35	0.4	0.45	0.5	0.6	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	2	5	12	14	14	13	17	17	10	21
	2	2	1	3	10	12	14	16	17	11	4	16
	3	2	3	5	17	12	16	14	16	24	5	28
	4	3	1	5	14	8	5	12	13	8	7	20
	5	2	3	4	3	15	21	14	9	12	0	11
	6	4	2	3	10	20	10	18	13	13	4	20
Average		2.5	2.0	4.2	11.0	13.5	13.3	14.5	14.2	14.2	5.0	19.3
SD		0.8	0.9	1.0	4.7	4.0	5.4	2.2	3.1	5.6	3.3	5.6
Dunnett test					**	***	***	***	***	***		
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Cadmium chloride

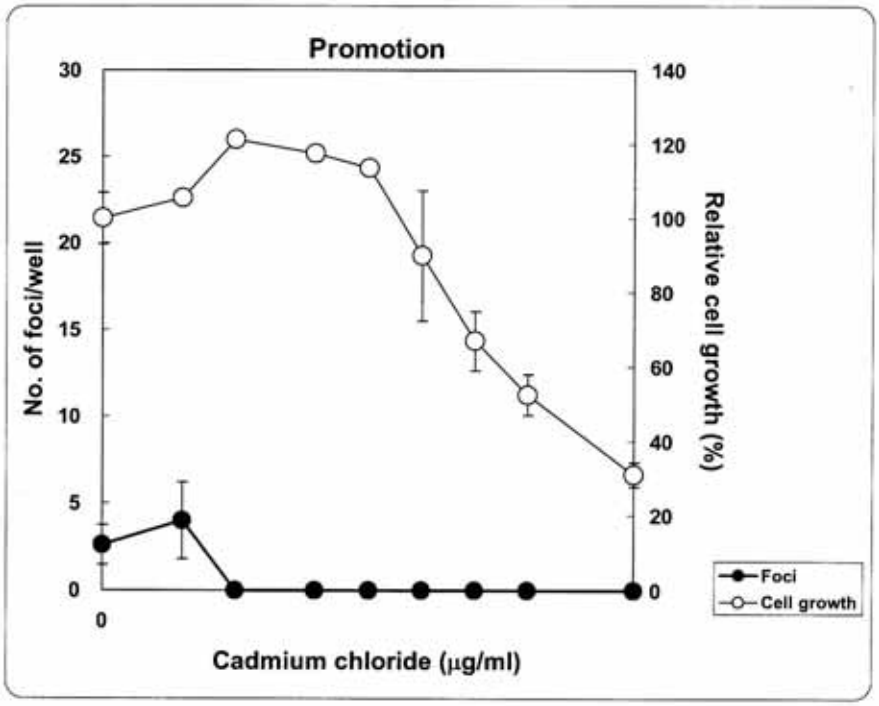
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%										
Compound (ug/ml)	Blank	0	0.3	0.5	0.8	1	1.2	1.4	1.6	2	DMSO 0.1%	TPA 50 ng/mL
OD	0.0727	0.5683	0.5679	0.6309	0.6153	0.6033	0.513	0.3993	0.3228	0.2062		
	0.0723	0.5318	0.5613	0.6265	0.6254	0.594	0.5543	0.4067	0.335	0.2082		
	0.0729	0.5042	0.5525	0.6436	0.606	0.5951	0.3975	0.34	0.2863	0.2334		
平均	0.073	0.535	0.561	0.634	0.616	0.597	0.488	0.382	0.315	0.216	#DIV/0!	#DIV/0!
SD	0.000	0.032	0.008	0.009	0.010	0.005	0.081	0.037	0.025	0.015	#DIV/0!	#DIV/0!
Average-Blank	0.000	0.462	0.488	0.561	0.543	0.525	0.416	0.309	0.242	0.143	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	105.6	121.4	117.5	113.6	89.9	66.9	52.4	31.0	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		7.0	1.7	1.9	2.1	1.1	17.6	7.9	5.5	3.3	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	Water 5%										
Compound (ug/ml)	Well No.	0	0.3	0.5	0.8	1	1.2	1.4	1.6	2	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	5	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	1	10
	2	3	3	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	4	4
	3	4	8	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	3	6
	4	1	2	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	2	13
	5	2	3	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	0	8
	6	-	3	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	4	9
Average		2.6	4.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	2.3	8.3
SD		1.1	2.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.6	3.1
t-Test for Unequal Variances (Aspin-Welch)			N.S.									
t-Test for Unequal Variances (Aspin-Welch)												**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II
Compound:	Cadmium chloride

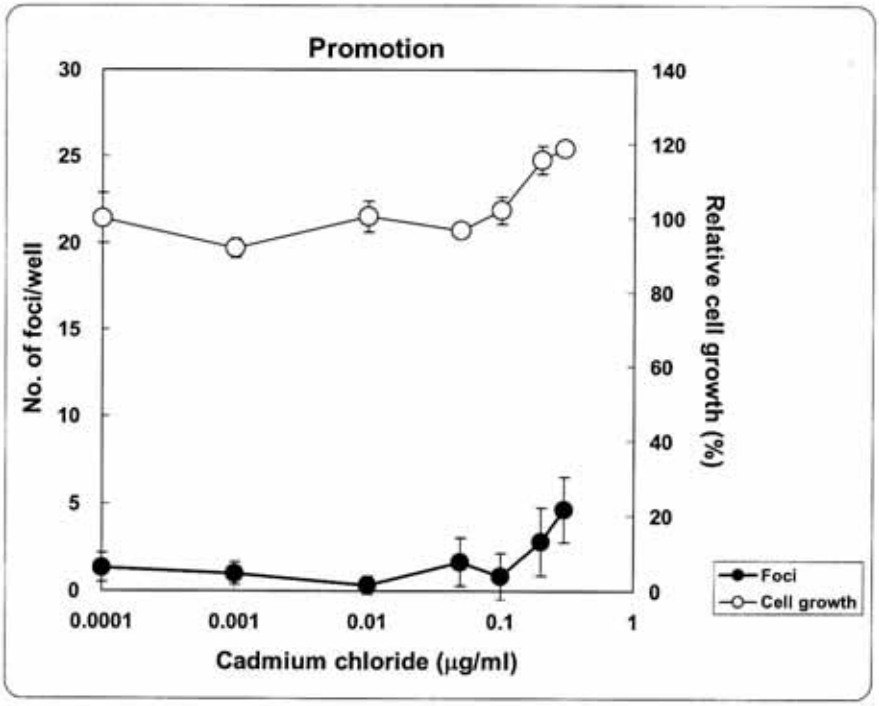
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%								
Compound (ug/ml)	Blank	0.0001	0.001	0.01	0.05	0.1	0.2	0.3	DMSO 0.1%	TPA 50 ng/mL
OD	0.0574	0.3741	0.3829	0.3906	0.3941	0.3946	0.445	0.4693	0.4138	0.5288
	0.0564	0.4182	0.3699	0.3975	0.3859	0.4096	0.4695	0.4646	0.4311	0.5402
	0.0567	0.4097	0.3655	0.4184	0.3876	0.4199	0.45	0.4626	0.4127	0.5479
Average	0.057	0.401	0.373	0.402	0.389	0.408	0.455	0.466	0.419	0.539
SD	0.001	0.023	0.009	0.014	0.004	0.013	0.013	0.003	0.010	0.010
Average-Blank	0.000	0.344	0.316	0.345	0.332	0.351	0.398	0.409	0.362	0.482
Relative cell growth (%)		100.0	91.9	100.4	96.7	102.1	115.8	118.9	100.0	133.1
SD of relative cell growth(%)		6.8	2.6	4.2	1.3	3.7	3.8	1.0	2.8	2.7

Transformation Assay	Solvent:	water 5%								
Compound (ug/ml)	Well No.	0.0001	0.001	0.01	0.05	0.1	0.2	0.3	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	2	1	4	3	2	5	1	14
	2	0	1	1	1	0	4	3	3	12
	3	2	1	0	2	0	6	2	2	11
	4	1	1	0	1	2	1	7	2	8
	5	2	1	0	0	0	1	6	3	15
	6	1	0	0	2	0	3	5	1	9
Average		1.3	1.0	0.3	1.7	0.8	2.8	4.7	2.0	11.5
SD		0.8	0.6	0.5	1.4	1.3	1.9	1.9	0.9	2.7
Dunnett test								***		
t-Test (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Cadmium chloride

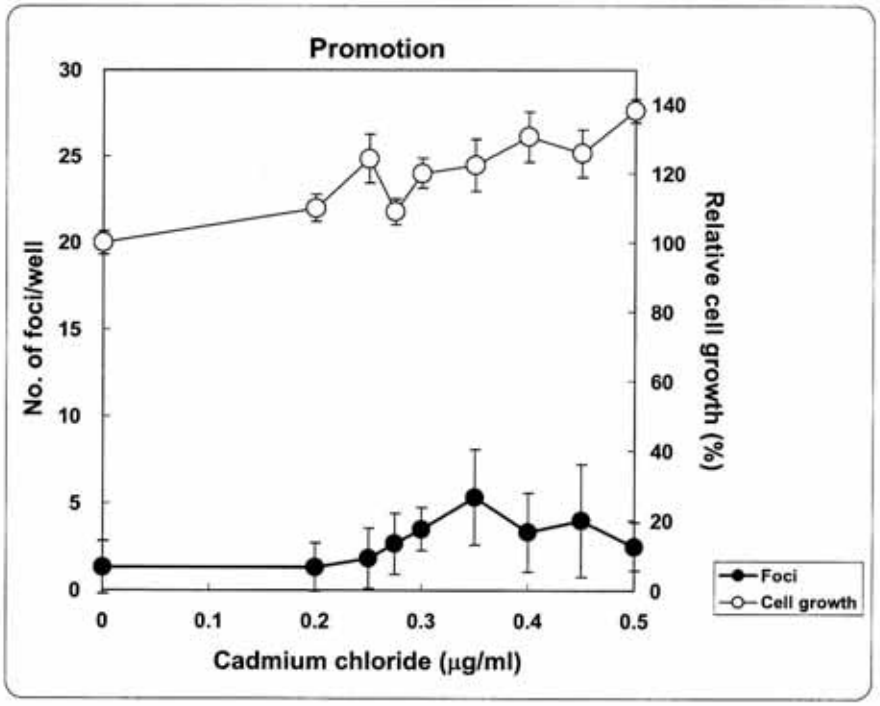
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DW 5%										
Compound (ug/ml)	Blank	0	0.2	0.25	0.275	0.3	0.35	0.4	0.45	0.5	DMSO 0.1%	TPA 50 ng/mL
OD	0.0618	0.5231	0.5837	0.6645	0.5625	0.639	0.677	0.7126	0.6848	0.7214	0.5434	0.7011
	0.059	0.5536	0.6021	0.6771	0.598	0.6478	0.6462	0.6885	0.6705	0.7317	0.5911	0.753
	0.0585	0.5306	0.5644	0.6138	0.5753	0.6082	0.6057	0.6439	0.622	0.6994	0.521	0.7044
Average	0.060	0.536	0.583	0.652	0.579	0.632	0.643	0.682	0.659	0.718	0.552	0.720
SD	0.002	0.016	0.019	0.034	0.018	0.021	0.036	0.035	0.033	0.016	0.036	0.029
Average-Blank	0.000	0.476	0.524	0.592	0.519	0.572	0.583	0.622	0.599	0.658	0.492	0.660
Relative cell growth (%)		100.0	110.0	124.4	109.0	120.1	122.5	130.7	125.9	138.2	100.0	134.1
SD of relative cell growth(%)		3.3	4.0	7.0	3.8	4.4	7.5	7.3	6.9	3.5	7.3	5.9

Transformation Assay	Solvent:	DW 5%										
Compound (ug/ml)	Well No.	0	0.2	0.25	0.275	0.3	0.35	0.4	0.45	0.5	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	1	1	1	3	3	1	2	0	2	14
	2	0	4	4	0	3	7	1	9	4	2	10
	3	0	1	0	3	2	4	5	7	2	2	11
	4	2	1	4	4	3	3	5	3	3	2	7
	5	3	0	1	4	5	5	2	1	3	1	8
	6	0	1	1	4	5	10	6	2	3	2	7
Average		1.3	1.3	1.8	2.7	3.5	5.3	3.3	4.0	2.5	1.8	9.5
SD		1.5	1.4	1.7	1.8	1.2	2.7	2.3	3.2	1.4	0.4	2.7
Dunnett test							**					
t-Test (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab III

Compound:	Cadmium chloride

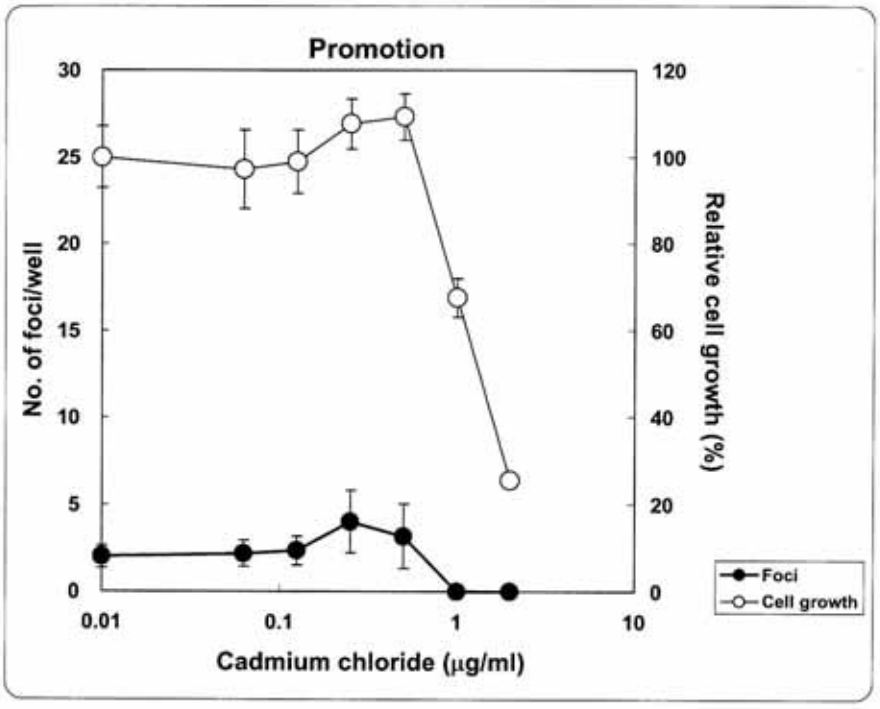
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Blank	0.01	0.0625	0.125	0.25	0.5	1	2	DMSO 0.1%	TPA 50 ng/mL
OD	0.183	1.954	1.856	1.935	2.128	2.172	1.459	0.701	-	3.007
	0.188	2.193	2.149	2.125	2.31	2.323	1.585	0.706	-	3.287
	0.225	2.183	2.164	2.209	2.33	2.367	1.423	0.648	-	3.212
平均	0.199	2.110	2.056	2.090	2.256	2.287	1.489	0.685	#DIV/0!	3.169
SD	0.023	0.135	0.174	0.140	0.111	0.102	0.085	0.032	#DIV/0!	0.145
Average-Blank	0.000	1.911	1.858	1.891	2.057	2.089	1.290	0.486	#DIV/0!	2.970
Relative cell growth (%)		100.0	97.2	98.9	107.6	109.3	67.5	25.4	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		7.1	9.1	7.3	5.8	5.4	4.5	1.7	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Well No.	0.01	0.0625	0.125	0.25	0.5	1	2	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	2	3	1	1	Tox	Tox	-	16
	2	3	3	2	4	2	Tox	Tox	-	17
	3	2	3	3	3	4	Tox	Tox	-	23
	4	1	1	3	5	6	Tox	Tox	-	12
	5	2	2	1	6	4	Tox	Tox	-	16
	6	2	2	2	5	2	Tox	Tox	-	10
Average		2.0	2.2	2.3	4.0	3.2	#DIV/0!	#DIV/0!	#DIV/0!	15.7
SD		0.6	0.8	0.8	1.8	1.8	#DIV/0!	#DIV/0!	#DIV/0!	4.5
Dunnett test					*					
t-Test for Unequal Variances (Aspin-Welch) vs 5% Water										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab III

Compound:	Cadmium chloride

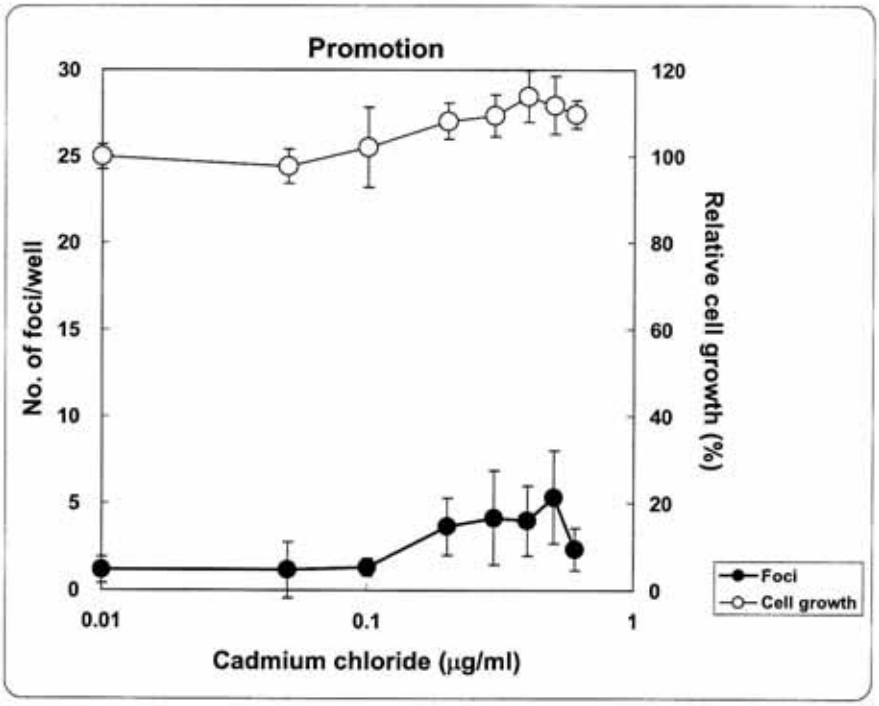
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Blank	0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.6	DMSO 0.1%	TPA 50 ng/mL
OD	0.267	2.091	2.006	1.992	2.351	2.192	2.473	2.193	2.267	1.984	3.004
	0.297	2.077	2.15	2.135	2.224	2.366	2.377	2.369	2.359	2.083	3.393
	0.281	2.174	2.061	2.331	2.216	2.304	2.255	2.429	2.251	2.195	3.415
Average	0.282	2.114	2.072	2.153	2.264	2.287	2.368	2.330	2.292	2.087	3.271
SD	0.015	0.052	0.073	0.170	0.076	0.088	0.109	0.123	0.058	0.106	0.231
Average-Blank	0.000	1.832	1.791	1.871	1.982	2.006	2.087	2.049	2.011	1.806	2.989
Relative cell growth (%)		100.0	97.7	102.1	108.2	109.5	113.9	111.8	109.7	100.0	165.5
SD of relative cell growth(%)		2.9	4.0	9.3	4.1	4.8	6.0	6.7	3.2	5.8	12.8

Transformation Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Well No.	0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.6	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	0	2	1	1	1	4	3	2	2	24
	2	1	0	1	4	3	3	7	1	6	22
	3	2	0	1	4	4	3	7	4	3	24
	4	1	4	2	6	3	3	9	3	4	18
	5	1	0	2	4	5	8	3	3	4	21
	6	2	1	1	3	9	3	3	1	4	13
Average		1.2	1.2	1.3	3.7	4.2	4.0	5.3	2.3	3.8	20.3
SD		0.8	1.6	0.5	1.6	2.7	2.0	2.7	1.2	1.3	4.2
Dunnett test						*	*	**			
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	DB[a,h]A

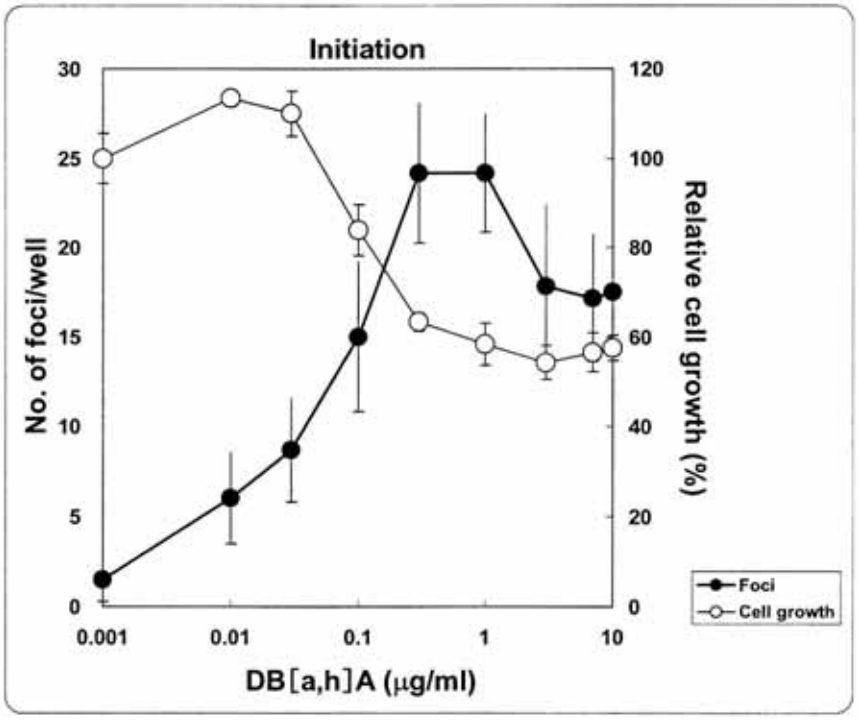
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	0.001	0.01	0.03	0.1	0.3	1	3	7	10	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	0.913	0.983	0.948	0.743	0.588	0.565	0.51	0.483	0.53	0.913	0.735
	0.073	0.847	0.972	0.899	0.777	0.577	0.546	0.47	0.53	0.505	0.847	0.695
	0.077	0.83	0.954	0.979	0.688	0.556	0.493	0.528	0.549	0.55	0.83	0.669
Average	0.074	0.863	0.970	0.942	0.736	0.574	0.535	0.503	0.521	0.528	0.863	0.700
SD	0.002	0.044	0.015	0.040	0.045	0.016	0.037	0.030	0.034	0.023	0.044	0.033
Average-Blank	0.000	0.789	0.895	0.868	0.662	0.499	0.460	0.428	0.446	0.454	0.789	0.625
Relative cell growth (%)		100.0	113.5	110.0	83.9	63.3	58.3	54.3	56.6	57.5	100.0	79.3
SD of relative cell growth(%)		5.6	1.9	5.1	5.7	2.1	4.7	3.8	4.3	2.9	5.6	4.2

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	0.001	0.01	0.03	0.1	0.3	1	3	7	10	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	2	10	7	19	27	23	25	21	14	2	16
	2	0	6	12	11	24	18	15	15	20	0	17
	3	2	4	12	17	30	25	15	16	22	2	24
	4	2	4	7	15	24	26	14	18	17	2	22
	5	3	4	5	9	20	27	22	12	13	3	25
	6	0	8	9	19	20	26	16	21	19	0	16
Average		1.5	6.0	8.7	15.0	24.2	24.2	17.8	17.2	17.5	1.5	20.0
SD		1.2	2.5	2.9	4.2	3.9	3.3	4.5	3.5	3.5	1.2	4.1
Dunnett test				**	***	***	***	***	***	***		
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	DB[a,h]A

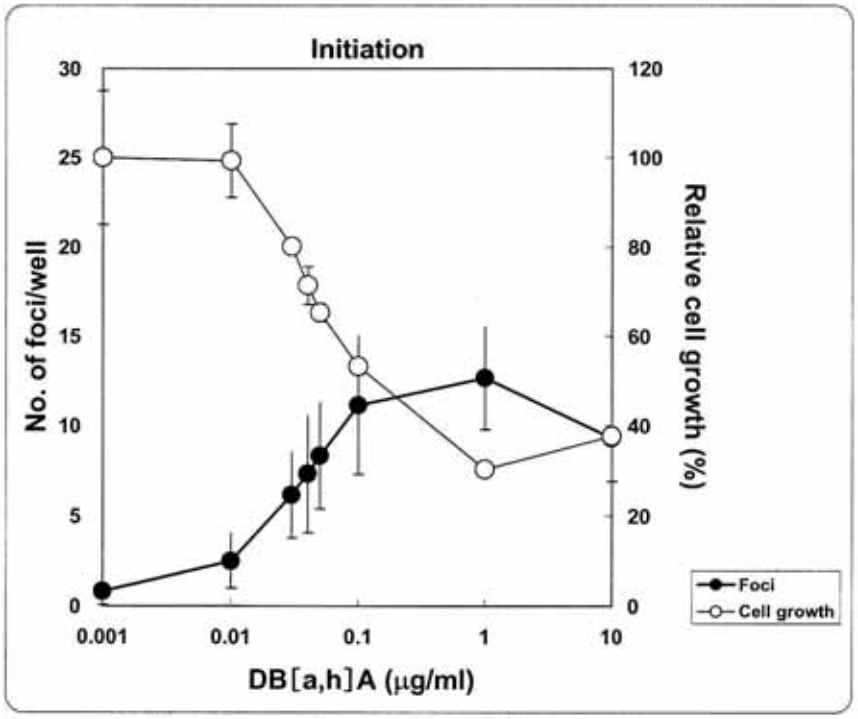
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.2%									
Compound (ug/ml)	Blank	0.001	0.01	0.03	0.04	0.05	0.1	1	10	DMSO 0.1%	MCA 1 ug/mL
OD	0.0726	0.6732	0.6899	0.5962	0.5762	0.4897	0.4299	0.268	0.3285	0.6529	0.3818
	0.0767	0.8473	0.705	0.6073	0.531	0.5126	0.4123	0.2737	0.325	0.67	0.389
	0.0755	0.6783	0.7907	0.603	0.5263	0.5114	0.4358	0.2814	0.3174	0.6404	0.3676
平均	0.075	0.733	0.729	0.602	0.545	0.505	0.426	0.274	0.324	0.654	0.379
SD	0.002	0.099	0.054	0.006	0.028	0.013	0.012	0.007	0.006	0.015	0.011
Average-Blank	0.000	0.658	0.654	0.527	0.470	0.430	0.351	0.199	0.249	0.580	0.305
Relative cell growth (%)		100.0	99.3	80.1	71.4	65.3	53.4	30.3	37.8	100.0	52.6
SD of relative cell growth(%)		15.1	8.3	0.9	4.2	2.0	1.9	1.0	0.9	2.6	1.9

Transformation Assay	Solvent:	DMSO 0.2%									
Compound (ug/ml)	Well No.	0.001	0.01	0.03	0.04	0.05	0.1	1	10	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	0	2	4	6	9	15	17	13	2	19
	2	2	4	4	4	4	8	11	7	3	16
	3	1	0	10	8	13	9	12	11	3	11
	4	1	2	5	4	8	8	9	8	0	20
	5	1	3	8	12	7	17	15	10	3	19
	6	0	4	6	10	9	10	12	7	1	15
Average		0.8	2.5	6.2	7.3	8.3	11.2	12.7	9.3	2.0	16.7
SD		0.8	1.5	2.4	3.3	2.9	3.9	2.9	2.4	1.3	3.4
Dunnett test				**	***	***	***	***	***		
t-Test (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V

Compound:	DB[a,h]A

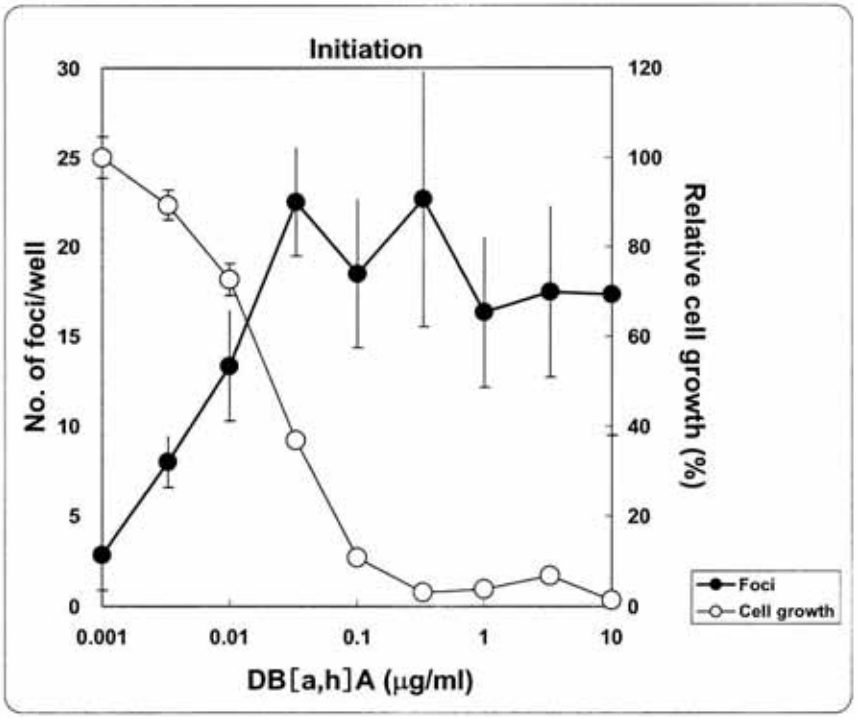
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	0.001	0.0033	0.01	0.033	0.1	0.33	1	3.3	10	DMSO 0.1%	MCA 1 ug/mL
OD	0.0828	0.4646	0.429	0.3666	0.2448	0.1455	0.1157	0.1165	0.1328	0.1091	0.4743	0.1833
	0.127	0.4991	0.4532	0.3935	0.2438	0.1436	0.1159	0.1169	0.1276	0.1091	0.4714	0.1885
	0.1006	0.4885	0.4485	0.3796	0.2421	0.1442	0.1143	0.1206	0.1279	0.1082	0.4669	0.1897
Average	0.103	0.484	0.444	0.380	0.244	0.144	0.115	0.118	0.129	0.109	0.471	0.187
SD	0.022	0.018	0.013	0.013	0.001	0.001	0.001	0.002	0.003	0.001	0.004	0.003
Average-Blank	0.000	0.381	0.340	0.276	0.140	0.041	0.012	0.015	0.026	0.005	0.367	0.084
Relative cell growth (%)		100.0	89.4	72.6	36.8	10.8	3.1	3.8	6.8	1.4	100.0	22.8
SD of relative cell growth(%)		4.6	3.4	3.5	0.4	0.3	0.2	0.6	0.8	0.1	1.0	0.9

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	0.001	0.0033	0.01	0.033	0.1	0.33	1	3.3	10	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	1	9	16	22	18	15	11	15	15	2	42
	2	3	7	10	25	17	13	19	27	14	1	43
	3	0	10	10	20	23	25	13	14	20	1	42
	4	4	8	17	22	14	29	14	16	11	1	40
	5	5	8	12	27	15	30	20	17	32	1	48
	6	4	6	15	19	24	24	21	16	12	1	33
Average		2.8	8.0	13.3	22.5	18.5	22.7	16.3	17.5	17.3	1.2	41.3
SD		1.9	1.4	3.1	3.0	4.1	7.1	4.2	4.8	7.8	0.4	4.9
Dunnett test				**	***	***	***	***	***	***		
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	DB[a,h]A

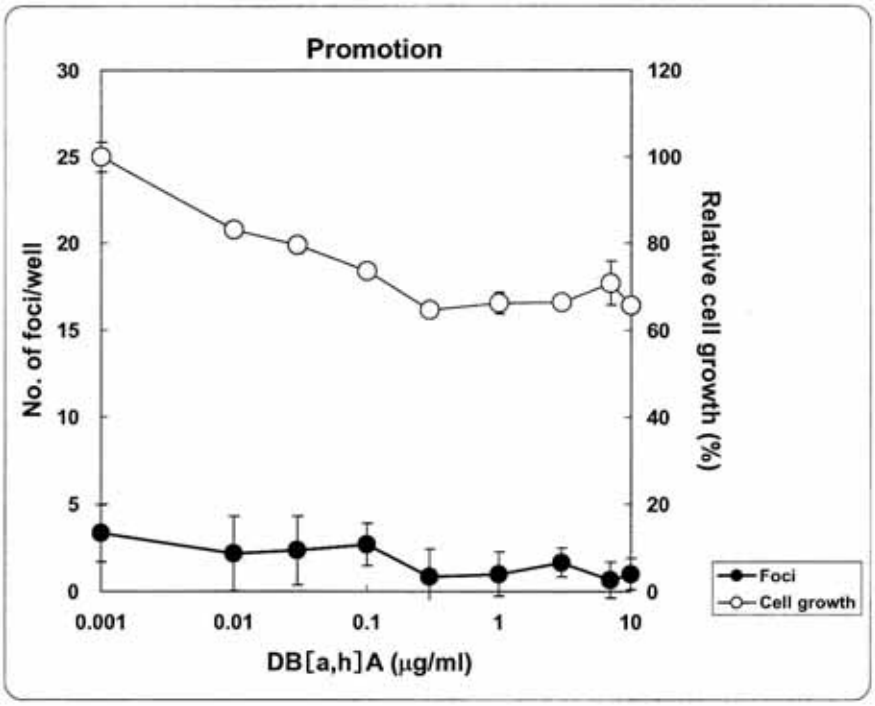
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	0.001	0.01	0.03	0.1	0.3	1	3	7	10	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	0.978	0.826	0.805	0.749	0.655	0.651	0.688	0.761	0.679	0.978	1.14
	0.073	1.012	0.835	0.804	0.736	0.655	0.675	0.678	0.717	0.662	1.012	1.148
	0.077	0.95	0.819	0.776	0.737	0.669	0.697	0.659	0.67	0.666	0.95	1.14
Average	0.074	0.980	0.827	0.795	0.741	0.660	0.674	0.675	0.716	0.669	0.980	1.143
SD	0.002	0.031	0.008	0.016	0.007	0.008	0.023	0.015	0.046	0.009	0.031	0.005
Average-Blank	0.000	0.906	0.752	0.721	0.666	0.585	0.600	0.601	0.642	0.595	0.906	1.068
Relative cell growth (%)		100.0	83.1	79.6	73.6	64.6	66.2	66.3	70.9	65.7	100.0	118.0
SD of relative cell growth(%)		3.4	0.9	1.8	0.8	0.9	2.5	1.6	5.0	1.0	3.4	0.5

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	0.001	0.01	0.03	0.1	0.3	1	3	7	10	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	2	4	2	0	1	2	0	1	3	21
	2	1	1	0	2	0	2	1	0	1	1	20
	3	3	3	4	2	4	3	1	0	2	3	18
	4	3	1	2	3	0	0	1	2	0	3	21
	5	4	6	4	2	0	0	2	0	2	4	16
	6	6	0	0	5	1	0	3	2	0	6	10
Average		3.3	2.2	2.3	2.7	0.8	1.0	1.7	0.7	1.0	3.3	17.7
SD		1.6	2.1	2.0	1.2	1.6	1.3	0.8	1.0	0.9	1.6	4.2
Dunnett test												N.S.
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	DB[a,h]A

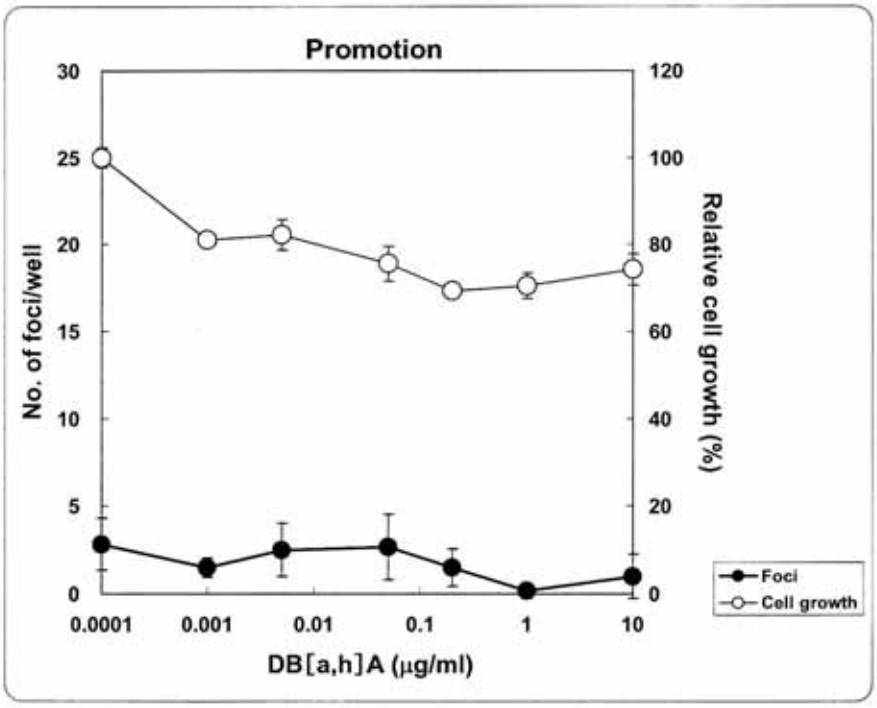
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.2%								
Compound (ug/ml)	Blank	0.0001	0.001	0.005	0.05	0.2	1	10	DMSO 0.1%	TPA 50 ng/mL
OD	0.0888	0.524	0.4486	0.468	0.4247	0.3949	0.3842	0.3992	0.5387	0.6938
	0.0847	0.5422	0.447	0.4488	0.4383	0.3988	0.4055	0.4185	0.5543	0.6414
	0.0813	0.5257	0.4436	0.4369	0.403	0.3887	0.4081	0.4304	0.4965	0.6272
平均	0.085	0.531	0.446	0.451	0.422	0.394	0.399	0.416	0.530	0.654
SD	0.004	0.010	0.003	0.016	0.018	0.005	0.013	0.016	0.030	0.035
Average-Blank	0.000	0.446	0.361	0.366	0.337	0.309	0.314	0.331	0.445	0.569
Relative cell growth (%)		100.0	81.1	82.2	75.6	69.4	70.5	74.3	100.0	127.9
SD of relative cell growth(%)		2.3	0.6	3.5	4.0	1.1	2.9	3.5	6.7	7.9

Transformation Assay	Solvent:	DMSO 0.2%								
Compound (ug/ml)	Well No.	0.0001	0.001	0.005	0.05	0.2	1	10	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	5	1	3	5	2	0	0	3	8
	2	1	2	2	2	1	0	2	0	13
	3	2	2	0	2	0	0	3	0	10
	4	4	1	4	1	3	0	0	4	11
	5	3	1	4	5	2	0	1	5	7
	6	2	2	2	1	1	1	0	3	4
Average		2.8	1.5	2.5	2.7	1.5	0.2	1.0	2.5	8.8
SD		1.5	0.5	1.5	1.9	1.0	0.4	1.3	2.1	3.2
Dunnnett test										N.S.
t-Test (Assuming Equal variance)										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V
Compound:	DB[a,h]A

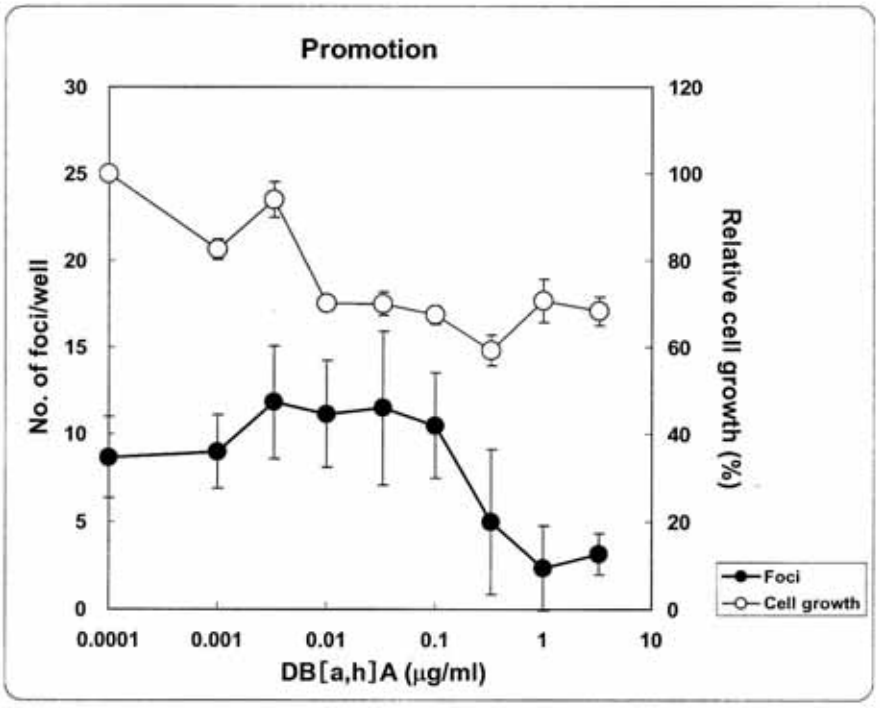
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Blank	0.0001	0.001	0.0033	0.01	0.033	0.1	0.33	1	3.3	DMSO 0.1%	TPA 1 ug/mL
OD	0.1064	0.5565	0.4771	0.5489	0.4208	0.4333	0.4194	0.3902	0.4454	0.4257	0.5395	0.6993
	0.1084	0.5556	0.4872	0.5155	0.4236	0.4199	0.4008	0.3596	0.401	0.3971	0.5138	0.6537
	0.1096	0.5521	0.4662	0.519	0.42	0.4091	0.4076	0.3689	0.425	0.4167	0.5176	0.6675
Average	0.108	0.555	0.477	0.528	0.421	0.421	0.409	0.373	0.424	0.413	0.524	0.674
SD	0.002	0.002	0.011	0.018	0.002	0.012	0.009	0.016	0.022	0.015	0.014	0.023
Average-Blank	0.000	0.447	0.369	0.420	0.313	0.313	0.301	0.265	0.316	0.305	0.416	0.565
Relative cell growth (%)		100.0	82.6	94.0	70.2	70.0	67.4	59.3	70.7	68.3	100.0	136.1
SD of relative cell growth(%)		0.5	2.4	4.1	0.4	2.7	2.1	3.5	5.0	3.3	3.3	5.6

Transformation Assay	Solvent:	DMSO 0.1%										
Compound (ug/ml)	Well No.	0.0001	0.001	0.0033	0.01	0.033	0.1	0.33	1	3.3	DMSO 0.1%	TPA1 ug/mL
No of foci	1	12	12	11	14	15	10	3	3	3	12	44
	2	8	11	16	9	11	13	5	6	4	8	42
	3	5	7	7	15	6	13	10	4	2	5	40
	4	8	7	11	10	8	5	1	0	5	8	39
	5	10	8	11	12	18	10	1	0	3	10	46
	6	9	9	15	7	11	12	10	1	2	9	50
Average		8.7	9.0	11.8	11.2	11.5	10.5	5.0	2.3	3.2	8.7	43.5
SD		2.3	2.1	3.3	3.1	4.4	3.0	4.1	2.4	1.2	2.3	4.1
Dunnett test												N.S.
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Methapyrilene HCl

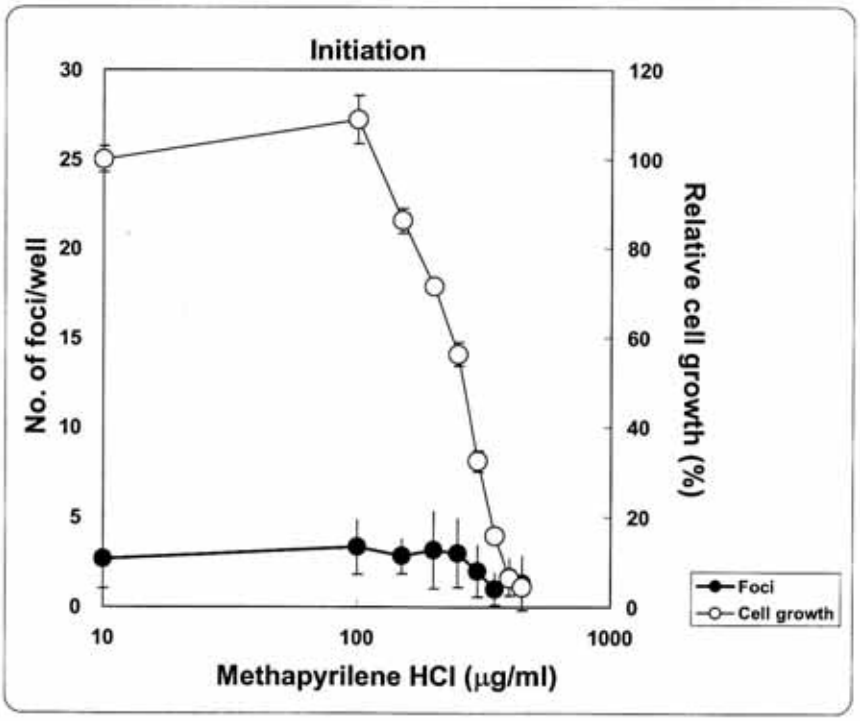
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%										
Compound (ug/ml)	Blank	10	100	150	200	250	300	350	400	450	DMSO 0.1%	MCA 1 ug/mL
OD	0.08	1.284	1.359	1.123	0.994	0.822	0.516	0.299	0.174	0.151	1.26	0.637
	0.088	1.342	1.475	1.15	0.959	0.761	0.489	0.266	0.155	0.138	1.248	0.599
	0.09	1.352	1.475	1.192	0.964	0.769	0.458	0.282	0.169	0.134	1.286	0.607
Average	0.086	1.326	1.436	1.155	0.972	0.784	0.488	0.282	0.166	0.141	1.265	0.614
SD	0.005	0.037	0.067	0.035	0.019	0.033	0.029	0.017	0.010	0.009	0.019	0.020
Average-Blank	0.000	1.240	1.350	1.069	0.886	0.698	0.402	0.196	0.080	0.055	1.179	0.528
Relative cell growth (%)		100.0	108.9	86.2	71.5	56.3	32.4	15.8	6.5	4.4	100.0	44.8
SD of relative cell growth(%)		3.0	5.4	2.8	1.5	2.7	2.3	1.3	0.8	0.7	1.6	1.7

Transformation Assay	Solvent:	water 5%										
Compound (ug/ml)	Well No.	10	100	150	200	250	300	350	400	450	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	1	3	4	2	5	0	1	2	4	1	26
	2	3	2	2	3	3	2	0	2	2	2	30
	3	2	6	2	1	0	4	1	2	1	3	35
	4	4	2	2	2	5	3	2	1	1	4	27
	5	5	4	4	7	2	1	0	0	0	3	26
	6	1	3	3	4	3	2	2	3	0	5	26
Average		2.7	3.3	2.8	3.2	3.0	2.0	1.0	1.7	1.3	3.0	28.3
SD		1.6	1.5	1.0	2.1	1.9	1.4	0.9	1.0	1.5	1.4	3.6
Dunnett test												N.S.
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Methapyrilene HCl

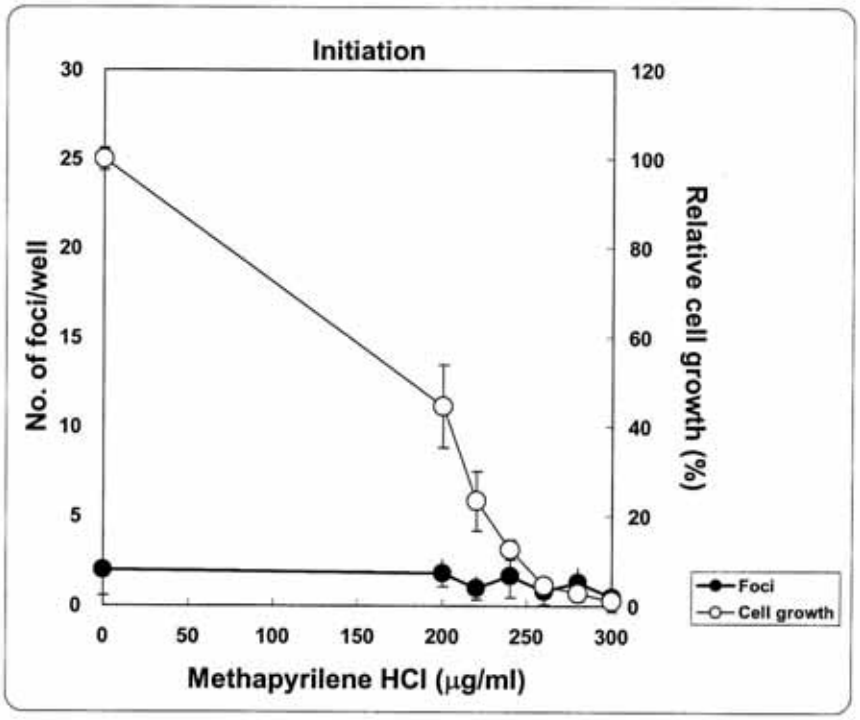
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%								
Compound (ug/ml)	Blank	0	200	220	240	260	280	300	DMSO 0.1%	MCA 1 ug/mL
OD	0.0715	0.802	0.3184	0.1948	0.1465	0.1029	0.081	0.0818	0.6566	0.3978
	0.0764	0.7666	0.446	0.2396	0.1758	0.1023	0.1021	0.0815	0.7332	0.421
	0.0793	0.7823	0.4083	0.2884	0.1704	0.1188	0.106	0.0877	0.6764	0.4037
Average	0.076	0.784	0.391	0.241	0.164	0.108	0.096	0.084	0.689	0.408
SD	0.004	0.018	0.066	0.047	0.016	0.009	0.013	0.003	0.040	0.012
Average-Blank	0.000	0.708	0.315	0.165	0.089	0.032	0.021	0.008	0.613	0.332
Relative cell growth (%)		100.0	44.5	23.3	12.5	4.6	2.9	1.1	100.0	54.1
SD of relative cell growth(%)		2.5	9.3	6.6	2.2	1.3	1.9	0.5	6.5	2.0

Transformation Assay	Solvent:	Water 5%								
Compound (ug/ml)	Well No.	0	200	220	240	260	280	300	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	3	2	1	2	1	2	0	2	19
	2	2	1	0	3	2	2	0	1	18
	3	0	1	1	3	0	1	0	0	19
	4	2	3	2	1	1	2	2	2	12
	5	1	2	1	1	1	1	0	3	16
	6	4	2	1	0	0	0	1	3	21
Average		2.0	1.8	1.0	1.7	0.8	1.3	0.5	1.8	17.5
SD		1.4	0.8	0.6	1.2	0.8	0.8	0.8	1.2	3.1
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Methapyrilene HCl

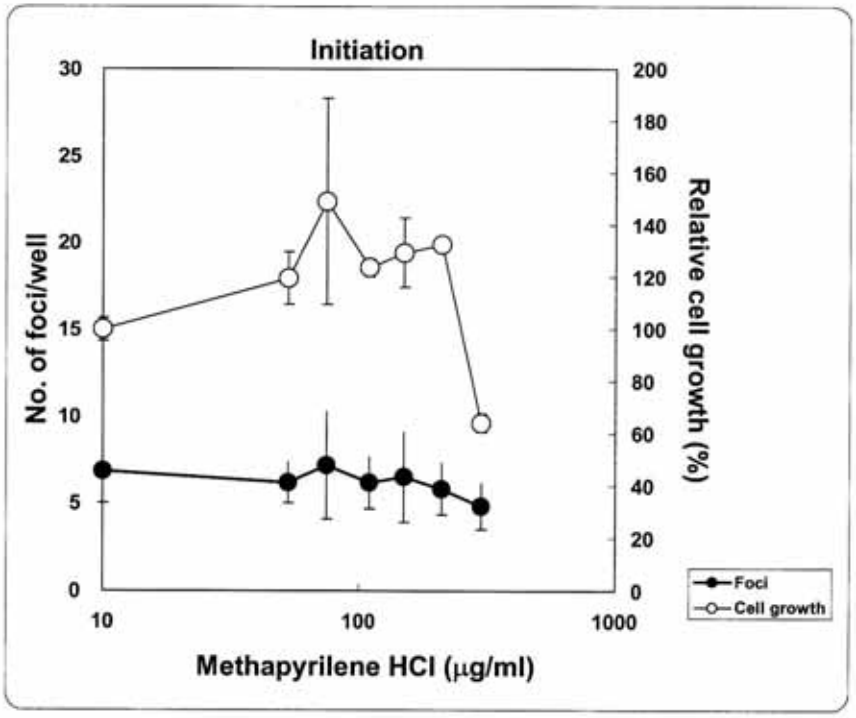
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%								
Compound (ug/ml)	Blank	10	53	75	110	150	210	300	DMSO 0.1%	MCA 1 ug/mL
OD	0.032	0.223	0.247	0.29	0.265	0.275	0.277	0.145	0.198	0.148
	0.035	0.207	0.271	0.383	0.253	0.289	0.273	0.157	0.215	0.153
	0.033	0.216	0.235	0.241	0.257	0.242	0.273	0.147	0.192	0.15
Average	0.033	0.215	0.251	0.305	0.258	0.269	0.274	0.150	0.202	0.150
SD	0.002	0.008	0.018	0.072	0.006	0.024	0.002	0.006	0.012	0.003
Average-Blank	0.000	0.182	0.218	0.271	0.225	0.235	0.241	0.116	0.168	0.117
Relative cell growth (%)		100.0	119.6	149.1	123.6	129.3	132.4	63.9	100.0	69.5
SD of relative cell growth(%)		4.4	10.1	39.6	3.4	13.3	1.3	3.5	7.1	1.5

Transformation Assay	Solvent:	Water 5%								
Compound (ug/ml)	Well No.	10	53	75	110	150	210	300	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	6	8	8	6	8	6	4	4	45
	2	8	5	6	7	5	5	7	6	40
	3	9	6	3	8	9	5	5	8	47
	4	8	6	11	7	7	7	5	4	41
	5	4	5	5	4	2	8	5	6	38
	6	6	7	10	5	8	4	3	5	40
Average		6.8	6.2	7.2	6.2	6.5	5.8	4.8	5.5	41.8
SD		1.8	1.2	3.1	1.5	2.6	1.5	1.3	1.5	3.4
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Methapyrilene HCl

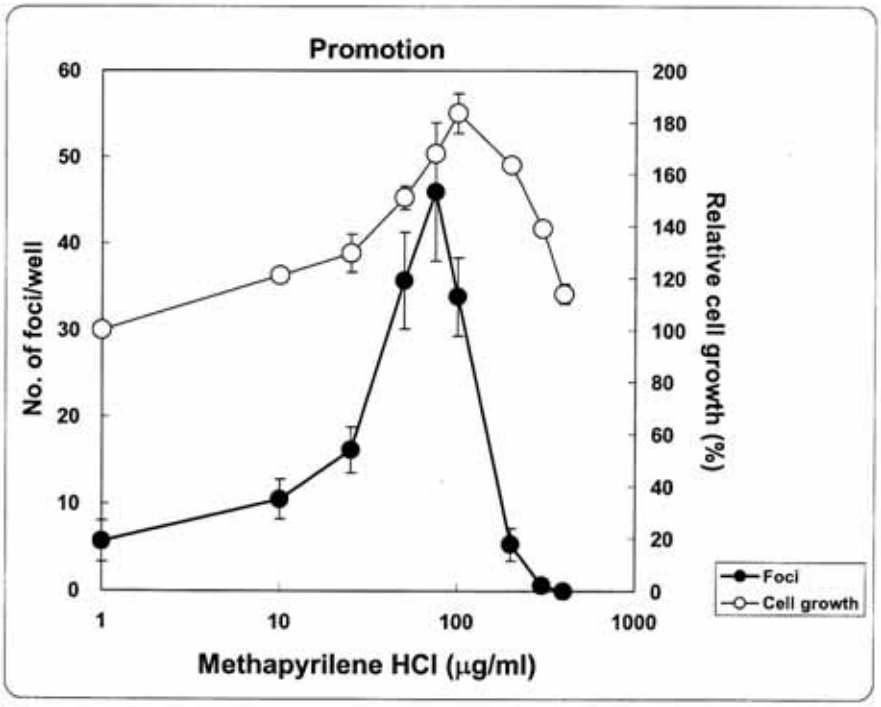
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%										
Compound (ug/ml)	Blank	1	10	25	50	75	100	200	300	400	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.047	1.277	1.415	1.6	1.72	1.954	1.678	1.458	1.229	1.006	1.416
	0.073	1.062	1.238	1.271	1.54	1.712	1.81	1.67	1.41	1.156	1.08	1.287
	0.077	1.048	1.259	1.337	1.513	1.72	1.844	1.673	1.438	1.179	1.094	1.258
Average	0.074	1.052	1.258	1.341	1.551	1.717	1.869	1.674	1.435	1.188	1.060	1.320
SD	0.002	0.008	0.020	0.072	0.045	0.005	0.075	0.004	0.024	0.037	0.047	0.084
Average-Blank	0.000	0.978	1.184	1.267	1.477	1.643	1.795	1.599	1.361	1.114	0.986	1.246
Relative cell growth (%)		100.0	121.0	129.5	151.0	168.0	183.5	163.5	139.2	113.9	100.0	126.4
SD of relative cell growth(%)		0.9	2.0	7.4	4.6	0.5	7.7	0.4	2.5	3.8	4.8	8.5

Transformation Assay	Solvent:	Water 5%										
Compound (ug/ml)	Well No.	1	10	25	50	75	100	200	300	400	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	7	12	15	40	56	26	7	1	Tox	6	35
	2	6	8	14	42	39	31	5	2	Tox	6	39
	3	3	11	20	30	41	35	8	1	Tox	3	47
	4	9	9	18	38	53	36	4	0	Tox	6	40
	5	3	14	13	36	50	38	5	0	Tox	6	41
	6	6	9	17	28	37	37	3	0	Tox	3	36
Average		5.7	10.5	16.2	35.7	46.0	33.8	5.3	0.7	#DIV/0!	5.0	39.7
SD		2.3	2.3	2.6	5.6	8.0	4.5	1.9	0.8	#DIV/0!	1.5	4.3
Dunnett test				***	***	***	***					
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Methapyrilene HCl

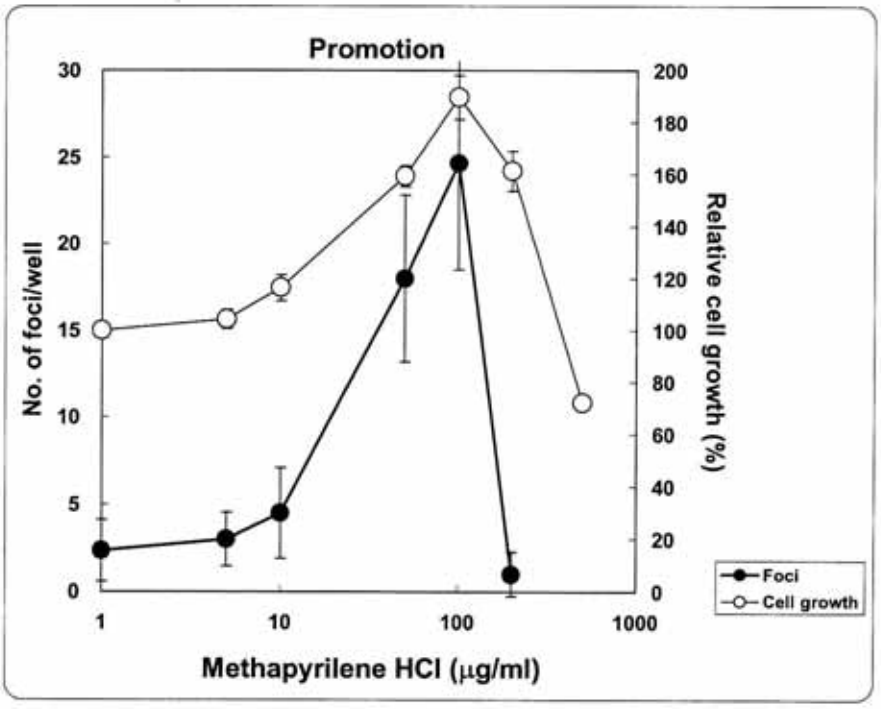
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%								
Compound (ug/ml)	Blank	1	5	10	50	100	200	500	DMSO 0.1%	TPA 50 ng/mL
OD	0.0736	0.5376	0.5797	0.611	0.8302	1.0048	0.8057	0.4192		
	0.0761	0.544	0.5608	0.6455	0.8329	0.9277	0.8709	0.4056		
	0.0721	0.5455	0.5462	0.6007	0.7989	0.9542	0.8123	0.4136		
平均	0.074	0.542	0.562	0.619	0.821	0.962	0.830	0.413	#DIV/0!	#DIV/0!
SD	0.002	0.004	0.017	0.023	0.019	0.039	0.036	0.007	#DIV/0!	#DIV/0!
Average-Blank	0.000	0.468	0.488	0.545	0.747	0.888	0.756	0.339	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	104.2	116.4	159.4	189.6	161.3	72.3	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		0.9	3.6	5.0	4.0	8.4	7.7	1.5	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	Water 5%								
Compound (ug/ml)	Well No.	1	5	10	50	100	200	500	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	5	2	5	22	26	3	Toxic	1	8
	2	0	2	8	15	15	0	Toxic	5	10
	3	1	3	7	15	20	1	Toxic	2	12
	4	3	2	3	15	26	0	Toxic	4	5
	5	3	3	2	15	32	2	Toxic	3	11
	6	2	6	2	26	29	0	Toxic	1	6
Average		2.3	3.0	4.5	18.0	24.7	1.0		2.7	8.7
SD		1.8	1.5	2.6	4.8	6.2	1.3		1.6	2.8
Dunnett test					***	***				
t-Test for Unequal Variances (Aspin-Welch)										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Methapyrilene HCl

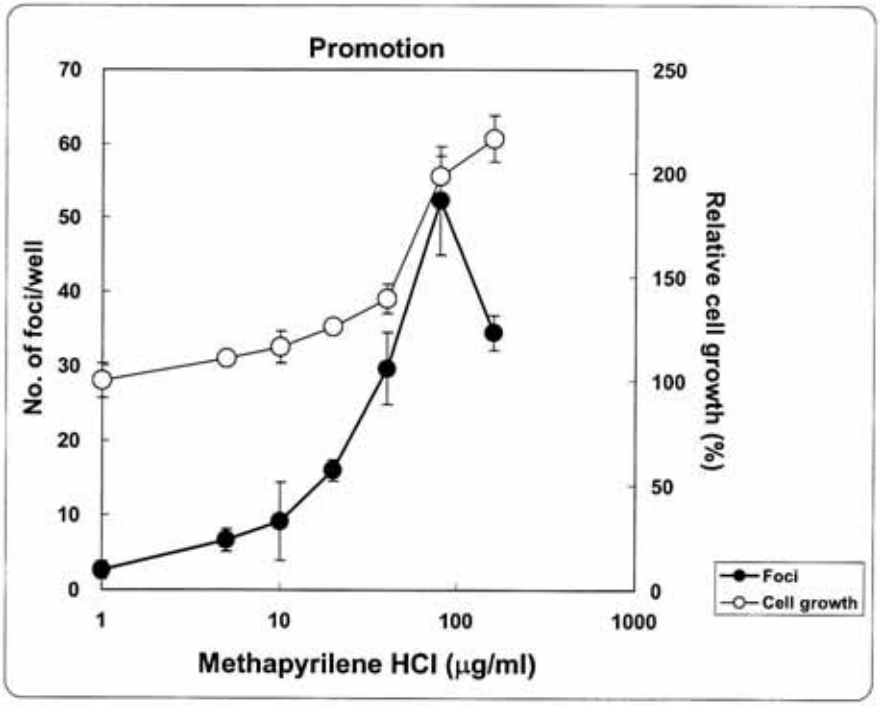
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DW 5%								
Compound (ug/ml)	Blank	1	5	10	20	40	80	160	DMSO 0.1%	TPA 50 ng/mL
OD	0.062	0.362	0.389	0.405	0.417	0.479	0.629	0.676		0.433
	0.061	0.32	0.379	0.369	0.416	0.439	0.603	0.654		0.391
	0.058	0.36	0.368	0.409	0.433	0.465	0.659	0.717		0.41
Average	0.060	0.347	0.379	0.394	0.422	0.461	0.630	0.682	#DIV/0!	0.411
SD	0.002	0.024	0.011	0.022	0.010	0.020	0.028	0.032	#DIV/0!	0.021
Average-Blank	0.000	0.287	0.318	0.334	0.362	0.401	0.570	0.622	#DIV/0!	0.351
Relative cell growth (%)		100.0	110.9	116.4	126.0	139.6	198.6	216.7	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		8.3	3.7	7.7	3.3	7.1	9.8	11.1	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	DW 5%								
Compound (ug/ml)	Well No.	1	5	10	20	40	80	160	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	7	8	18	33	59	32	3	22
	2	3	4	10	16	32	52	35	1	22
	3	1	6	19	17	30	48	32	3	13
	4	4	8	8	15	32	61	34	3	17
	5	2	7	6	16	20	53	36	2	15
	6	4	8	4	14	31	41	38	3	23
Average		2.7	6.7	9.2	16.0	29.7	52.3	34.5	2.5	18.7
SD		1.2	1.5	5.2	1.4	4.8	7.3	2.3	0.8	4.2
Dunnett test				*	***	***	***	***		
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Sodium arsenite

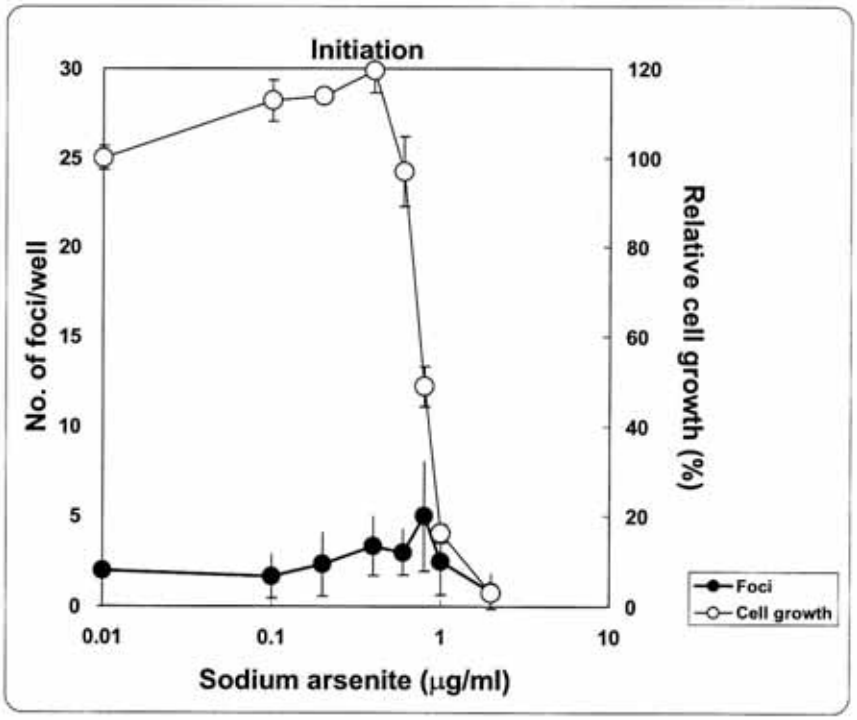
Toransformation Assay

Initiation-No.:	1

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	0.01	0.1	0.2	0.4	0.6	0.8	1	2	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	0.905	1.011	0.994	1.005	0.817	0.479	0.201	0.101	0.871	0.596
	0.073	0.863	1.011	0.988	1.041	0.832	0.43	0.191	0.096	0.934	0.584
	0.077	0.887	0.946	1.012	1.084	0.933	0.502	0.224	0.098	0.975	0.579
Average	0.074	0.885	0.989	0.998	1.043	0.861	0.470	0.205	0.098	0.927	0.586
SD	0.002	0.021	0.038	0.012	0.040	0.063	0.037	0.017	0.003	0.052	0.009
Average-Blank	0.000	0.811	0.915	0.924	0.969	0.786	0.396	0.131	0.024	0.852	0.512
Relative cell growth (%)		100.0	112.9	113.9	119.5	97.0	48.8	16.2	3.0	100.0	60.1
SD of relative cell growth(%)		2.6	4.6	1.5	4.9	7.8	4.5	2.1	0.3	6.1	1.0

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	0.01	0.1	0.2	0.4	0.6	0.8	1	2	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	1	2	4	3	2	5	1	2	3	39
	2	5	1	2	3	2	8	6	0	2	44
	3	1	3	1	6	4	3	2	0	5	43
	4	4	1	5	3	3	1	1	1	5	42
	5	0	3	1	1	2	4	3	2	2	42
	6	1	0	1	4	5	9	2	0	1	38
Average		2.0	1.7	2.3	3.3	3.0	5.0	2.5	0.8	3.0	41.3
SD		2.0	1.2	1.8	1.6	1.3	3.0	1.9	1.0	1.7	2.3
Dunnett test							*				
t-Test (Assuming Equal Variances)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Sodium arsenite

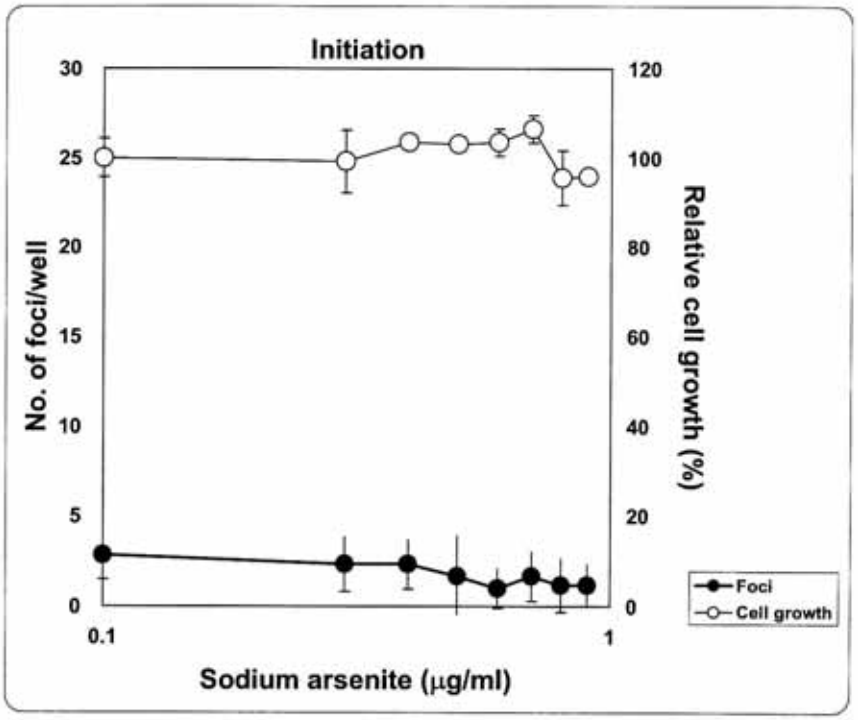
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	0.1	0.3	0.4	0.5	0.6	0.7	0.8	0.9	DMSO 0.1%	MCA 1 ug/mL
OD	0.08	1.44	1.48	1.441	1.407	1.465	1.506	1.312	1.324	1.24	0.639
	0.088	1.327	1.329	1.406	1.441	1.386	1.428	1.256	1.346	1.327	0.648
	0.09	1.388	1.315	1.445	1.426	1.438	1.472	1.412	1.322	1.526	0.773
Average	0.086	1.385	1.375	1.431	1.425	1.430	1.469	1.327	1.331	1.364	0.687
SD	0.005	0.057	0.091	0.021	0.017	0.040	0.039	0.079	0.013	0.147	0.075
Average-Blank	0.000	1.299	1.289	1.345	1.339	1.344	1.383	1.241	1.245	1.278	0.601
Relative cell growth (%)		100.0	99.2	103.5	103.1	103.4	106.4	95.5	95.8	100.0	47.0
SD of relative cell growth(%)		4.4	7.0	1.7	1.3	3.1	3.0	6.1	1.0	11.5	5.9

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	0.1	0.3	0.4	0.5	0.6	0.7	0.8	0.9	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	2	3	2	6	0	1	1	1	2	33
	2	2	2	2	2	3	2	0	0	2	33
	3	2	1	3	0	0	1	1	0	2	42
	4	5	1	0	1	1	0	0	1	5	31
	5	4	2	3	1	1	4	1	3	4	21
	6	2	5	4	0	1	2	4	2	2	32
Average		2.8	2.3	2.3	1.7	1.0	1.7	1.2	1.2	2.8	32.0
SD		1.3	1.5	1.4	2.3	1.1	1.4	1.5	1.2	1.3	6.7
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ

Compound:	Sodium arsenite

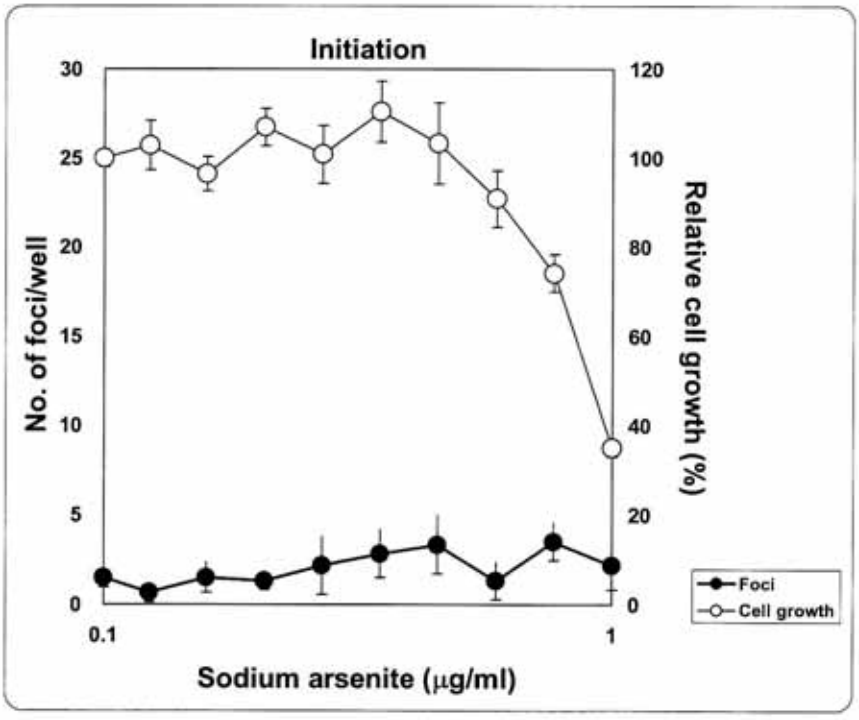
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	D.W. 5 %*											
Compound (ug/ml)	Blank	0.1	0.123	0.159	0.207	0.269	0.35	0.455	0.592	0.769	1	DMSO 0.1% MCA 1 ug/mL	
OD	0.226	2.617	2.501	2.562	2.633	2.43	2.643	2.418	2.2	2.049	1.013	-	0.677
	0.26	2.533	2.743	2.544	2.798	2.7	2.908	2.809	2.471	2.022	1.093	-	0.761
	0.257	2.603	2.708	2.399	2.811	2.68	2.934	2.76	2.44	1.868	1.084	-	0.855
Average	0.248	2.584	2.651	2.502	2.747	2.603	2.828	2.662	2.370	1.980	1.063	#DIV/0!	0.764
SD	0.019	0.045	0.131	0.089	0.099	0.150	0.161	0.213	0.148	0.098	0.044	#DIV/0!	0.089
Average-Blank	0.000	2.337	2.403	2.254	2.500	2.356	2.581	2.415	2.123	1.732	0.816	#DIV/0!	0.517
Relative cell growth (%)		100.0	102.8	96.5	107.0	100.8	110.4	103.3	90.8	74.1	34.9	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		1.9	5.6	3.8	4.2	6.4	6.9	9.1	6.3	4.2	1.9	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %*											
Compound (ug/ml)	Well No.	0.1	0.123	0.159	0.207	0.269	0.35	0.455	0.592	0.769	1	DMSO 0.1% MCA 1 ug/mL	
No of foci	1	1	1	3	1	2	2	2	0	3	2	-	15
	2	2	0	1	1	0	1	6	1	5	0	-	13
	3	1	1	1	1	4	4	2	2	4	3	-	19
	4	1	1	1	1	4	4	4	3	3	4	-	12
	5	2	0	2	2	2	4	2	1	2	2	-	12
	6	2	1	1	2	1	2	4	1	4	2	-	19
Average		1.5	0.7	1.5	1.3	2.2	2.8	3.3	1.3	3.5	2.2	#DIV/0!	15.0
SD		0.5	0.5	0.8	0.5	1.6	1.3	1.6	1.0	1.0	1.3	#DIV/0!	3.3
Parametric Dannett								*		*			
t-test for Unequal Variances (Aspin-Welch) vs 5% Water													***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ

Compound:	Sodium arsenite

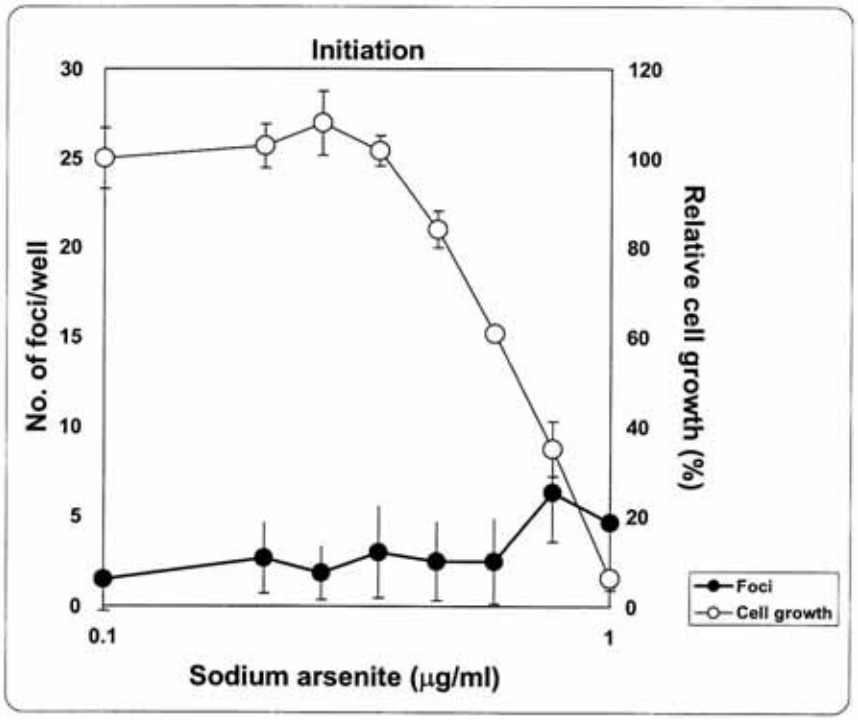
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Blank	0.1	0.207	0.269	0.35	0.455	0.592	0.769	1	DMSO 0.1%	MCA 1 ug/mL
OD	0.242	2.977	2.983	3.196	2.919	2.274	1.78	1.25	0.355	2.78	0.78
	0.239	2.75	2.854	2.854	2.744	2.482	1.807	0.96	0.447	2.985	0.825
	0.271	2.636	2.735	2.913	2.827	2.39	1.788	1.204	0.423	3.442	0.887
Average	0.251	2.788	2.857	2.988	2.830	2.382	1.792	1.138	0.408	3.069	0.831
SD	0.018	0.174	0.124	0.183	0.088	0.104	0.014	0.156	0.048	0.339	0.054
Average-Blank	0.000	2.537	2.607	2.737	2.579	2.131	1.541	0.887	0.158	2.818	0.580
Relative cell growth (%)		100.0	102.7	107.9	101.7	84.0	60.7	35.0	6.2	100.0	20.6
SD of relative cell growth(%)		6.8	4.9	7.2	3.5	4.1	0.5	6.1	1.9	12.0	1.9

Transformation Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Well No.	0.1	0.207	0.269	0.35	0.455	0.592	0.769	1	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	4	2	3	0	5	2	6	2	4	5
	2	0	5	2	1	1	1	4	8	4	8
	3	2	4	1	3	3	7	4	10	4	9
	4	3	0	0	6	5	3	11	3	3	10
	5	0	1	4	6	0	1	5	0	4	16
	6	0	4	1	2	1	1	8	5	1	10
Average		1.5	2.7	1.8	3.0	2.5	2.5	6.3	4.7	3.3	9.7
SD		1.8	2.0	1.5	2.5	2.2	2.3	2.7	3.8	1.2	3.6
Dunnett test								**			
t-Test for Unequal Variances (Aspin-Welch)											**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI
Compound:	Sodium arsenite

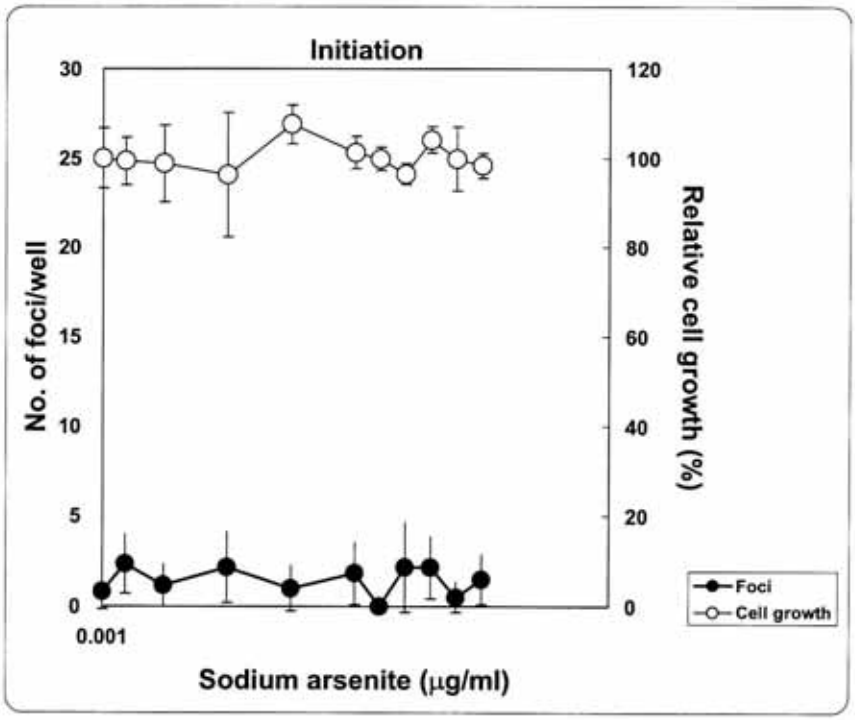
Toransformation Assay

Initiation

Cell Growth Assay	Solvent: DMSO 0.5%													
Compound (ug/ml)	Blank	0.001	0.01	0.025	0.05	0.075	0.1	0.11	0.12	0.13	0.14	0.15	DMSO 0.5%	MCA 1 ug/mL
OD	0.032	0.572	0.601	0.554	0.601	0.608	0.596	0.593	0.556	0.618	0.599	0.551	0.572	0.455
	0.033	0.617	0.578	0.624	0.601	0.646	0.596	0.567	0.572	0.588	0.600	0.577	0.617	0.475
	0.03	0.544	0.543	0.535	0.469	0.603	0.562	0.571	0.547	0.594	0.532	0.578	0.544	0.460
Average	0.032	0.578	0.574	0.571	0.557	0.619	0.585	0.577	0.558	0.600	0.577	0.569	0.578	0.463
SD	0.002	0.037	0.029	0.047	0.076	0.024	0.020	0.014	0.013	0.016	0.039	0.015	0.037	0.010
Average-Blank	0.000	0.546	0.542	0.539	0.525	0.587	0.553	0.545	0.527	0.568	0.545	0.537	0.546	0.432
Relative cell growth (%)		100.0	99.3	98.8	96.2	107.6	101.3	99.9	96.5	104.1	99.9	98.4	100.0	79.1
SD of relative cell growth(%)		6.7	5.3	8.6	14.0	4.3	3.6	2.6	2.3	2.9	7.1	2.8	6.7	1.9

Transformation Assay	Solvent: DMSO 0.5%													
Compound (ug/ml)	Well No.	0.001	0.01	0.025	0.05	0.075	0.1	0.11	0.12	0.13	0.14	0.15	DMSO 0.5%	MCA 1 ug/mL
No of foci	1	2	1	1	4	3	4	0	1	4	1	0	2	18
	2	1	2	0	0	0	4	0	7	3	2	3	1	14
	3	0	4	1	1	0	1	0	2	0	0	1	0	9
	4	0	3	2	2	0	1	0	1	3	0	3	0	12
	5	0	0	3	1	2	0	0	2	3	0	0	0	10
	6	2	4	0	5	1	1	0	0	0	0	2	2	9
Average		0.8	2.3	1.2	2.2	1.0	1.8	0.0	2.2	2.2	0.5	1.5	0.8	12.0
SD		1.0	1.6	1.2	1.9	1.3	1.7	0.0	2.5	1.7	0.8	1.4	1.0	3.5
Parametric Dunnett														N.S.
t-Test for Unequal Variances (Aspin-Welch)														***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Sodium arsenite

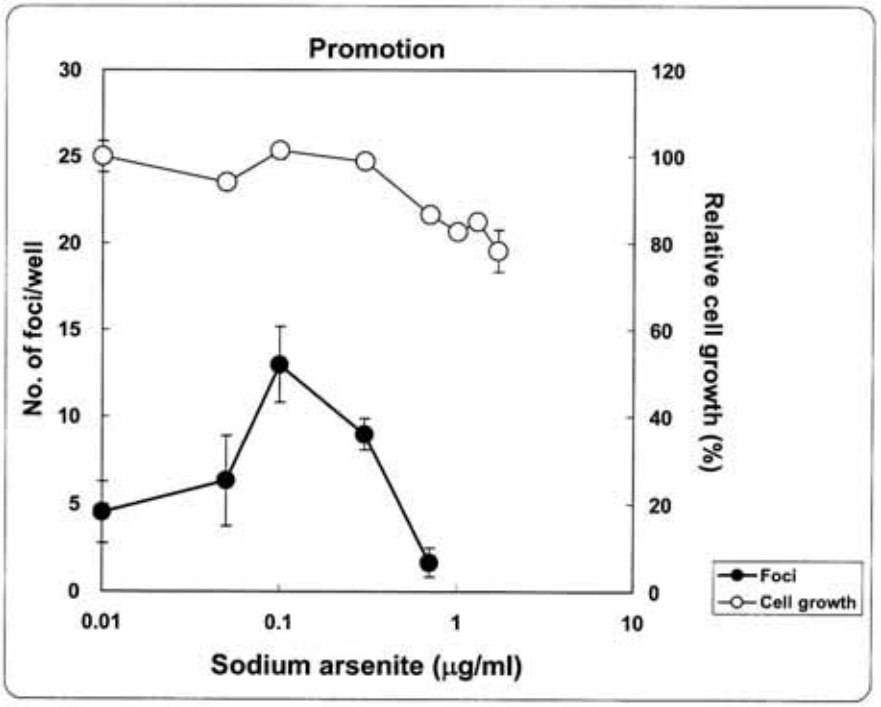
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%									
Compound (ug/ml)	Blank	0.01	0.05	0.1	0.3	0.7	1	1.3	1.7	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	0.954	0.871	0.948	0.922	0.82	0.771	0.787	0.773	1.003	1.21
	0.073	0.896	0.869	0.917	0.904	0.796	0.774	0.789	0.692	1.044	1.331
	0.077	0.911	0.87	0.929	0.907	0.802	0.773	0.802	0.743	1.057	1.369
Average	0.074	0.920	0.870	0.931	0.911	0.806	0.773	0.793	0.736	1.035	1.303
SD	0.002	0.030	0.001	0.016	0.010	0.012	0.002	0.008	0.041	0.028	0.083
Average-Blank	0.000	0.846	0.796	0.857	0.837	0.732	0.698	0.718	0.662	0.960	1.229
Relative cell growth (%)		100.0	94.1	101.3	98.9	86.5	82.5	84.9	78.2	100.0	128.0
SD of relative cell growth(%)		3.6	0.1	1.8	1.1	1.5	0.2	1.0	4.8	2.9	8.6

Transformation Assay	Solvent:	Water 5%									
Compound (ug/ml)	Well No.	0.01	0.05	0.1	0.3	0.7	1	1.3	1.7	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	6	3	13	8	3	Tox	Tox	Tox	3	32
	2	4	6	12	9	2	Tox	Tox	Tox	3	38
	3	6	10	12	8	1	Tox	Tox	Tox	4	42
	4	2	8	16	10	1	Tox	Tox	Tox	8	36
	5	3	7	10	9	2	Tox	Tox	Tox	4	35
	6	6	4	15	10	1	Tox	Tox	Tox	8	37
Average		4.5	6.3	13.0	9.0	1.7				5.0	36.7
SD		1.8	2.6	2.2	0.9	0.8				2.4	3.3
Dunnett t-Test (Assuming Equal Variances)				***	***						***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ

Compound:	Sodium arsenite

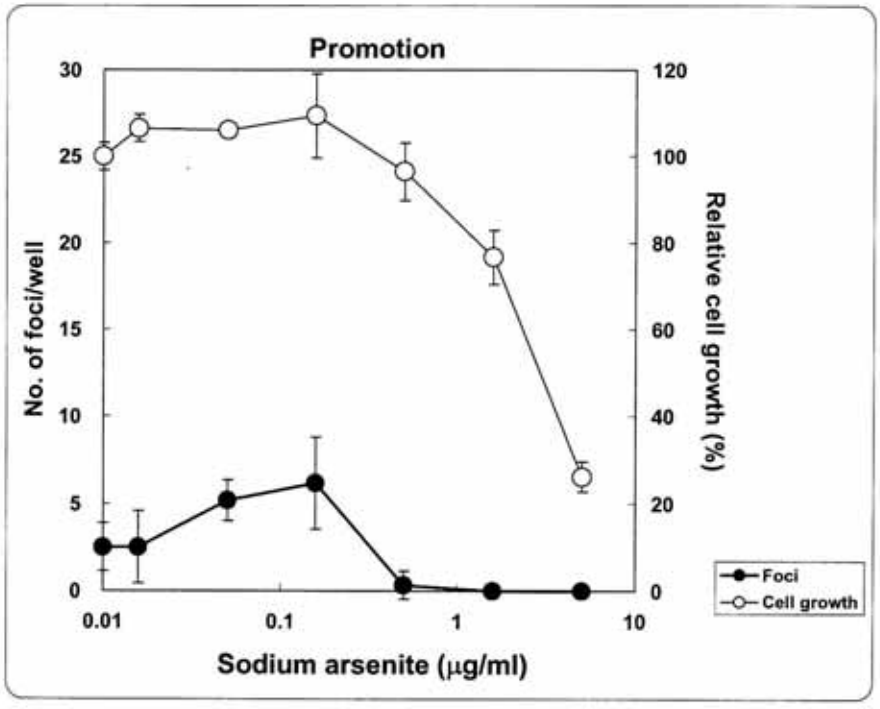
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	D.W. 5 %*								
Compound (ug/ml)	Blank	0.01	0.0158	0.05	0.158	0.5	1.58	5	DMSO 0.1%	TPA 50 ng/mL
OD	0.19	1.941	2.048	2.075	2.363	1.803	1.448	0.602	-	2.721
	0.206	1.986	2.153	2.094	2.084	2.04	1.619	0.724	-	3.017
	0.2	2.053	2.133	2.138	2.039	1.949	1.656	0.679	-	2.002
平均	0.199	1.993	2.111	2.102	2.162	1.931	1.574	0.668	#DIV/0!	2.580
SD	0.008	0.056	0.056	0.032	0.176	0.120	0.111	0.062	#DIV/0!	0.522
Average-Blank	0.000	1.795	1.913	1.904	1.963	1.732	1.376	0.470	#DIV/0!	2.381
Relative cell growth (%)		100.0	106.6	106.1	109.4	96.5	76.7	26.2	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		3.1	3.1	1.8	9.8	6.7	6.2	3.4	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %*								
Compound (ug/ml)	Well No.	0.01	0.0158	0.05	0.158	0.5	1.58	5	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	4	6	4	8	0	Tox	Tox	-	14
	2	4	2	7	10	2	Tox	Tox	-	26
	3	1	4	5	4	0	Tox	Tox	-	14
	4	3	1	4	5	0	Tox	Tox	-	13
	5	2	1	6	3	0	Tox	Tox	-	7
	6	1	1	5	7	0	Tox	Tox	-	8
Average		2.5	2.5	5.2	6.2	0.3	#DIV/0!	#DIV/0!	#DIV/0!	13.7
SD		1.4	2.1	1.2	2.6	0.8	#DIV/0!	#DIV/0!	#DIV/0!	6.8
Parametric Dannett				*	**					
t-Test for Unequal Variances (Aspin-Welch) vs 5% Water										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ

Compound:	Sodium arsenite

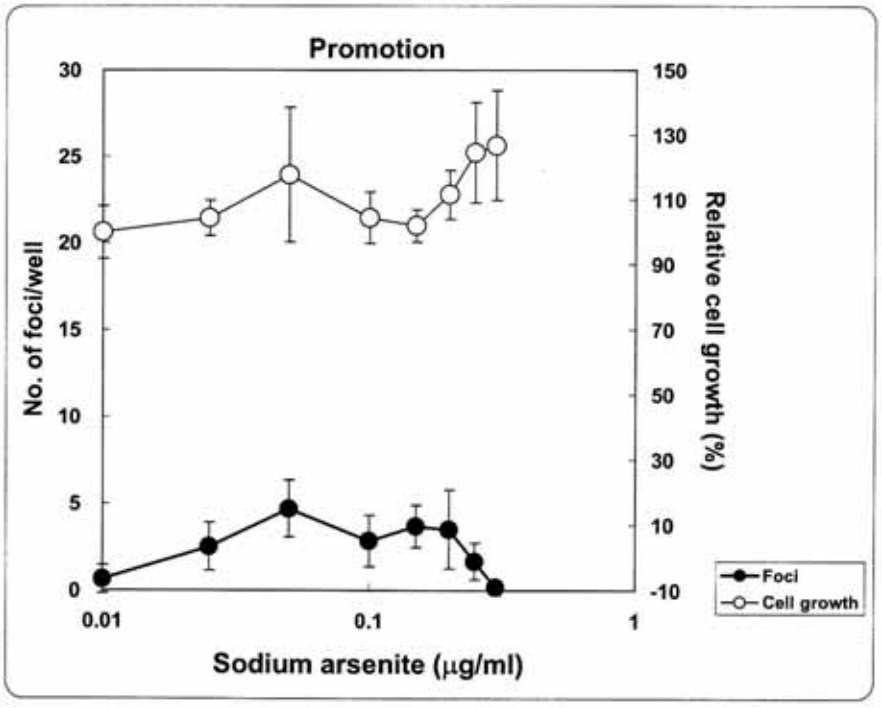
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Blank	0.01	0.025	0.05	0.1	0.15	0.2	0.25	0.3	DMSO 0.1%	TPA 50 ng/mL
OD	0.103	0.952	1.015	1.393	0.987	0.993	1.053	1.396	1.436	0.979	2.185
	0.219	1.095	1.112	1.095	1.095	1.07	1.175	1.199	1.176	1.25	2.067
	0.115	1.034	1.068	1.06	1.117	1.069	1.159	1.135	1.177	1.14	1.983
Average	0.146	1.027	1.065	1.183	1.066	1.044	1.129	1.243	1.263	1.123	2.078
SD	0.064	0.072	0.049	0.183	0.070	0.044	0.066	0.136	0.150	0.136	0.101
Average-Blank	0.000	0.881	0.919	1.037	0.921	0.898	0.983	1.098	1.117	0.977	1.933
Relative cell growth (%)		100.0	104.3	117.7	104.5	101.9	111.6	124.5	126.8	100.0	197.7
SD of relative cell growth(%)		8.1	5.5	20.8	7.9	5.0	7.5	15.4	17.0	13.9	10.4

Transformation Assay	Solvent:	D.W. 5%									
Compound (ug/ml)	Well No.	0.01	0.025	0.05	0.1	0.15	0.2	0.25	0.3	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	4	5	2	2	4	1	1	3	21
	2	0	3	5	1	5	2	2	0	1	14
	3	1	3	5	2	3	1	2	0	2	17
	4	1	0	4	3	4	5	0	0	3	19
	5	0	2	7	5	3	7	2	0	2	17
	6	0	3	2	4	5	2	3	0	4	21
Average		0.7	2.5	4.7	2.8	3.7	3.5	1.7	0.2	2.5	18.2
SD		0.8	1.4	1.6	1.5	1.2	2.3	1.0	0.4	1.0	2.7
Dunnett test				***	*	**	**				
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Sodium arsenite

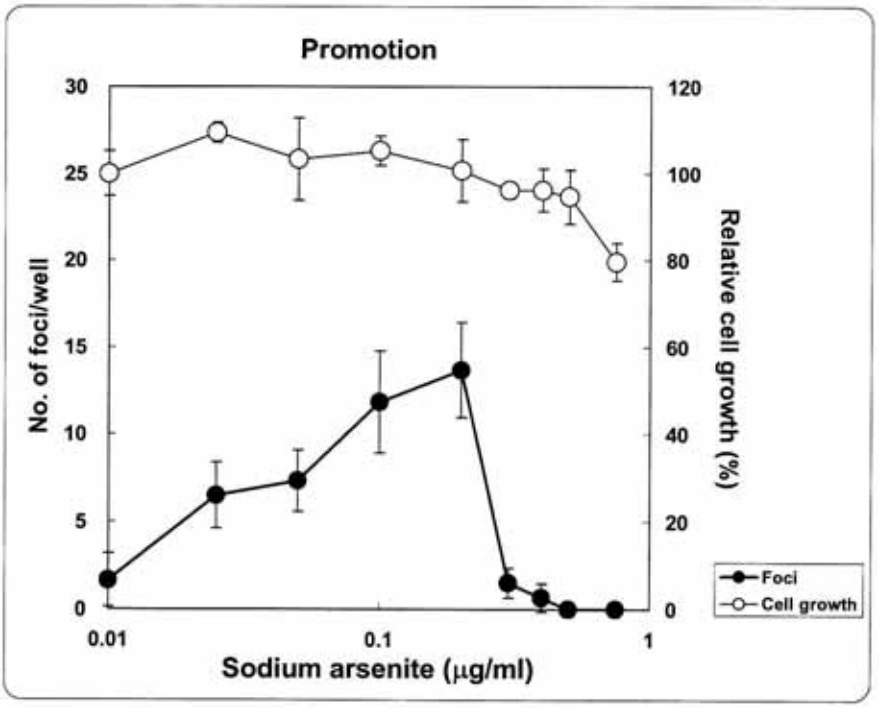
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Blank	0.01	0.025	0.05	0.1	0.2	0.3	0.4	0.5	0.75	DMSO 0.5%	TPA 50 ng/mL
OD	0.06	0.473	0.496	0.438	0.480	0.424	0.441	0.419	0.454	0.355	0.473	0.436
	0.062	0.456	0.496	0.453	0.483	0.469	0.430	0.440	0.438	0.378	0.456	0.498
	0.063	0.432	0.480	0.509	0.459	0.476	0.444	0.457	0.406	0.388	0.432	0.530
Average	0.062	0.454	0.491	0.467	0.474	0.456	0.438	0.439	0.433	0.374	0.454	0.488
SD	0.002	0.021	0.009	0.037	0.013	0.028	0.007	0.019	0.024	0.017	0.021	0.048
Average-Blank	0.000	0.392	0.429	0.405	0.412	0.395	0.377	0.377	0.371	0.312	0.392	0.426
Relative cell growth (%)		100.0	109.4	103.3	105.2	100.7	96.1	96.2	94.6	79.6	100.0	108.8
SD of relative cell growth(%)		5.3	2.4	9.5	3.3	7.2	1.9	4.9	6.2	4.3	5.3	12.2

Transformation Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Well No.	0.01	0.025	0.05	0.1	0.2	0.3	0.4	0.5	0.75	DMSO 0.5%	TPA 50 ng/mL
No of foci	1	0	5	8	13	15	1	0	0	0	0	13
	2	1	9	10	8	10	1	1	0	0	1	7
	3	3	7	7	16	13	1	1	0	0	3	16
	4	4	8	5	9	14	1	0	0	0	4	23
	5	1	6	8	12	12	2	0	0	0	1	16
	6	1	4	6	13	18	3	2	0	0	1	11
Average		1.7	6.5	7.3	11.8	13.7	1.5	0.7	0.0	0.0	1.7	14.3
SD		1.5	1.9	1.8	2.9	2.7	0.8	0.8	0.0	0.0	1.5	5.4
Dunnett test			***	***	***	***						
t-Test for Unequal Variances (Aspin-Welch)												**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	o-Toluidine HCL

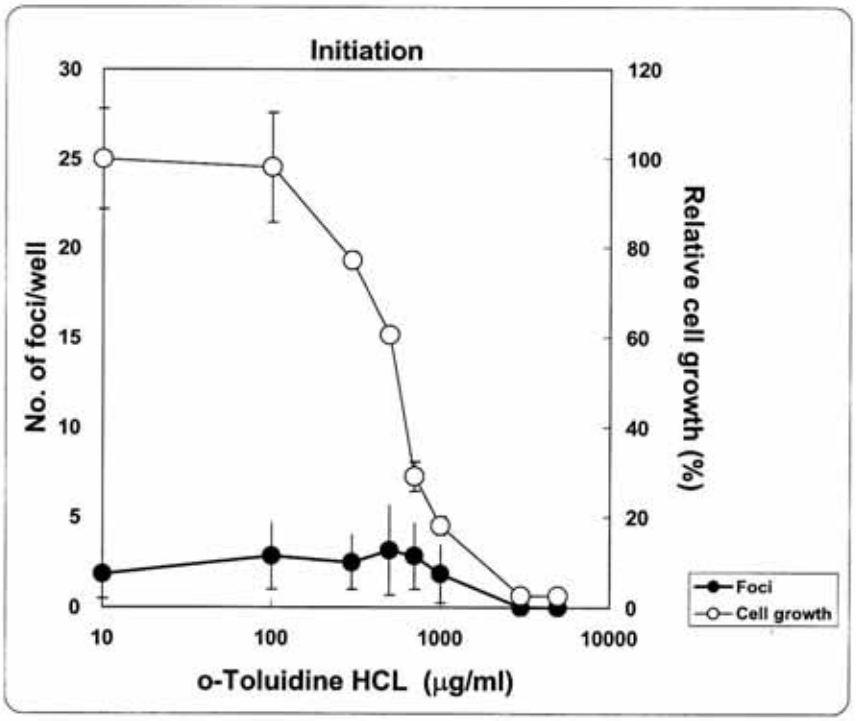
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	10	100	300	500	700	1000	3000	5000	DMSO 0.1%	MCA 1 ug/mL
OD	0.08	1.1	1.059	0.983	0.77	0.46	0.318	0.113	0.118	1.236	0.717
	0.088	1.228	1.222	0.979	0.778	0.399	0.281	0.112	0.115	1.332	0.699
	0.09	1.357	1.338	0.944	0.787	0.393	0.277	0.119	0.115	1.216	0.709
Average	0.086	1.228	1.206	0.969	0.778	0.417	0.292	0.115	0.116	1.261	0.708
SD	0.005	0.129	0.140	0.021	0.009	0.037	0.023	0.004	0.002	0.062	0.009
Average-Blank	0.000	1.142	1.120	0.883	0.692	0.331	0.206	0.029	0.030	1.175	0.622
Relative cell growth (%)		100.0	98.1	77.3	60.6	29.0	18.0	2.5	2.6	100.0	52.9
SD of relative cell growth(%)		11.2	12.3	1.9	0.7	3.2	2.0	0.3	0.2	5.3	0.8

Transformation Assay	Solvent:										
Compound (ug/ml)	Well No.	10	100	300	500	700	1000	3000	5000	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	0	2	4	1	4	2	toxic	toxic	2	20
	2	2	2	0	6	6	0	toxic	toxic	3	18
	3	4	2	2	0	1	4	toxic	toxic	1	22
	4	2	6	2	6	2	3	toxic	toxic	2	25
	5	1	1	3	3	2	2	toxic	toxic	1	17
	6	2	4	4	3	2	0	toxic	toxic	1	14
Average		1.8	2.8	2.5	3.2	2.8	1.8	#DIV/0!	#DIV/0!	1.7	19.3
SD		1.3	1.8	1.5	2.5	1.8	1.6	#DIV/0!	#DIV/0!	0.8	3.9
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V

Compound:	o-Toluidine HCL

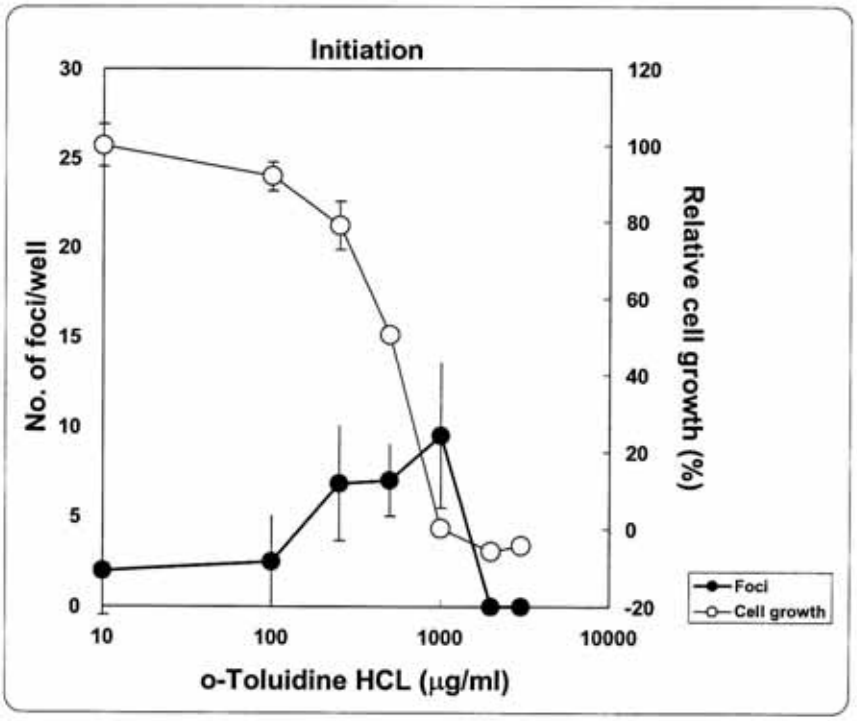
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%								
Compound (ug/ml)	Blank	10	100	250	500	1000	2000	3000	DMSO 0.1%	MCA 1 ug/mL
OD	0.0741	0.4239	0.4031	0.3485	0.2743	0.0967	0.0752	0.081	0.4903	0.2136
	0.1194	0.4581	0.4234	0.3919	0.2721	0.0962	0.0757	0.0815	0.4166	0.2132
	0.0943	0.4573	0.4279	0.3787	0.2718	0.0977	0.0758	0.0802	0.4501	0.2146
Average	0.096	0.446	0.418	0.373	0.273	0.097	0.076	0.081	0.452	0.214
SD	0.023	0.020	0.013	0.022	0.001	0.001	0.000	0.001	0.037	0.001
Average-Blank	0.000	0.351	0.322	0.277	0.177	0.001	-0.020	-0.015	0.356	0.118
Relative cell growth (%)		100.0	91.9	79.1	50.4	0.3	-5.8	-4.3	100.0	33.1
SD of relative cell growth(%)		5.6	3.8	6.3	0.4	0.2	0.1	0.2	10.4	0.2

Transformation Assay	Solvent:	Water5%								
Compound (ug/ml)	Well No.	10	100	250	500	1000	2000	3000	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	0	4	5	10	4	Toxic	Toxic	1	40
	2	0	0	11	6	10	Toxic	Toxic	3	53
	3	0	6	9	7	8	Toxic	Toxic	2	41
	4	3	0	8	7	13	Toxic	Toxic	3	46
	5	6	1	2	8	7	Toxic	Toxic	2	46
	6	3	4	6	4	15	Toxic	Toxic	3	36
Average		2.0	2.5	6.8	7.0	9.5	#DIV/0!	#DIV/0!	2.3	43.7
SD		2.4	2.5	3.2	2.0	4.0	#DIV/0!	#DIV/0!	0.8	6.0
Dunnett test				**	**	***				
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	o-Toluidine HCL

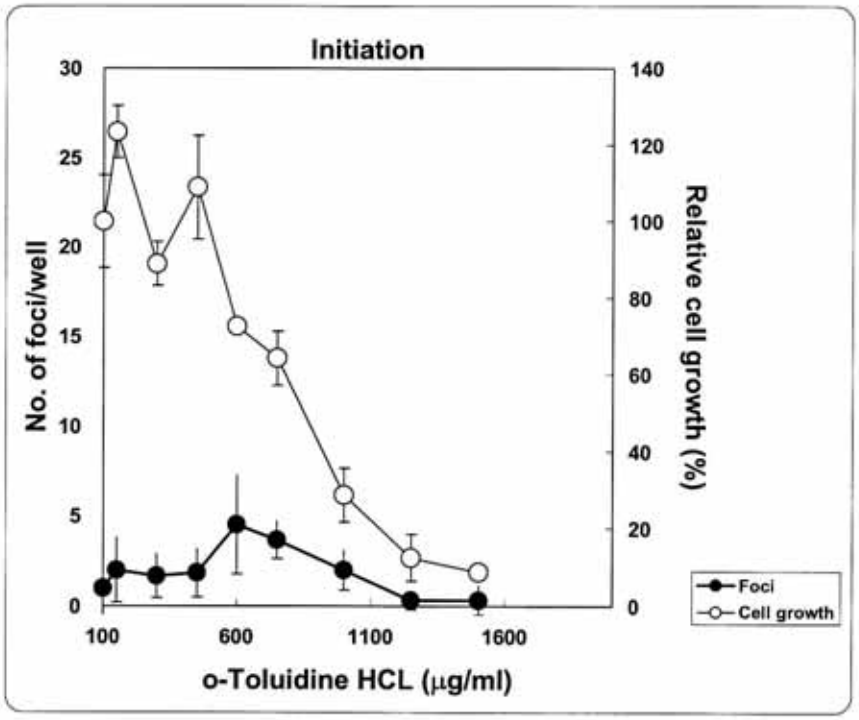
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Blank	100	150	300	450	600	750	1000	1250	1500	DMSO 0.5%	MCA 1 ug/mL
OD	0.032	0.228	0.291	0.221	0.237	0.197	0.184	0.097	0.076	0.059	0.228	0.125
	0.031	0.277	0.314	0.241	0.278	0.190	0.186	0.094	0.079	0.062	0.277	0.128
	0.071	0.245	0.289	0.220	0.290	0.195	0.160	0.120	0.056	0.067	0.245	0.130
Average	0.045	0.250	0.298	0.227	0.268	0.194	0.177	0.104	0.070	0.063	0.250	0.128
SD	0.023	0.025	0.014	0.012	0.028	0.004	0.014	0.014	0.013	0.004	0.025	0.003
Average-Blank	0.000	0.205	0.253	0.183	0.224	0.149	0.132	0.059	0.026	0.018	0.205	0.083
Relative cell growth (%)		100.0	123.4	89.0	108.9	72.7	64.3	28.7	12.5	8.8	100.0	40.4
SD of relative cell growth(%)		12.1	6.8	5.8	13.5	1.8	7.0	6.9	6.1	2.0	12.1	1.2

Transformation Assay	Solvent:	DMSO 0.5%										
Compound (ug/ml)	Well No.	100	150	300	450	600	750	1000	1250	1500	DMSO 0.5%	MCA 1 ug/mL
No of foci	1	0	3	3	2	3	4	4	0	0	0	25
	2	0	5	1	1	3	3	1	0	0	0	20
	3	0	0	1	2	3	5	2	0	0	0	14
	4	1	1	2	0	4	4	2	1	0	1	22
	5	4	1	3	4	10	2	2	1	0	4	32
	6	1	2	0	2	4	4	1	0	2	1	17
Average		1.0	2.0	1.7	1.8	4.5	3.7	2.0	0.3	0.3	1.0	21.7
SD		1.5	1.8	1.2	1.3	2.7	1.0	1.1	0.5	0.8	1.5	6.3
Dunnett test						**	*					
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	o-Toluidine HCL

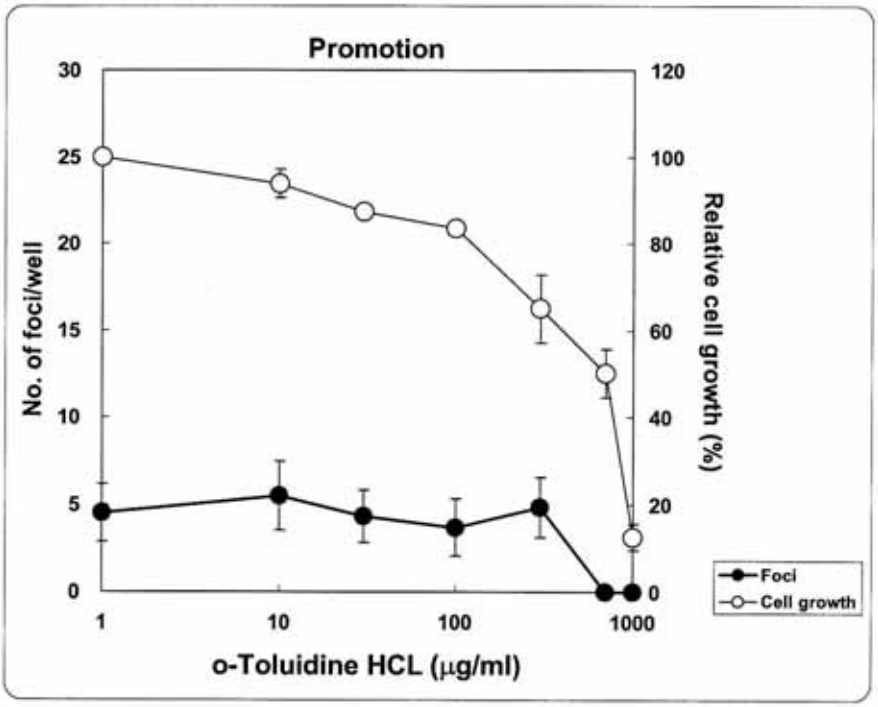
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%								
Compound (ug/ml)	Blank	1	10	30	100	300	700	1000	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	0.964	0.92	0.861	0.833	0.58	0.484	0.186	1.028	1.225
	0.073	0.958	0.875	0.848	0.806	0.657	0.5	0.159	1.028	1.255
	0.077	0.969	0.931	0.843	0.811	0.72	0.576	0.212	1.002	1.331
Average	0.074	0.964	0.909	0.851	0.817	0.652	0.520	0.186	1.019	1.270
SD	0.002	0.006	0.030	0.009	0.014	0.070	0.049	0.027	0.015	0.055
Average-Blank	0.000	0.889	0.834	0.776	0.742	0.578	0.446	0.111	0.945	1.196
Relative cell growth (%)		100.0	93.8	87.3	83.5	65.0	50.1	12.5	100.0	126.6
SD of relative cell growth(%)		0.6	3.3	1.0	1.6	7.9	5.5	3.0	1.6	5.8

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	1	10	30	100	300	700	1000	DMSO 0.1%	TPA 50 ng/mL	
No of foci	1	5	4	6	4	2	Toxic	Toxic	6	37	
	2	3	4	5	2	6	Toxic	Toxic	11	46	
	3	5	8	5	3	4	Toxic	Toxic	7	44	
	4	6	8	5	2	7	Toxic	Toxic	9	39	
	5	2	5	2	5	5	Toxic	Toxic	5	40	
	6	6	4	3	6	5	Toxic	Toxic	9	40	
Average		4.5	5.5	4.3	3.7	4.8	#DIV/0!	#DIV/0!	7.8	41.0	
SD		1.6	2.0	1.5	1.6	1.7	#DIV/0!	#DIV/0!	2.2	3.3	
Dunnet test											N.S.
t-Test (Assuming Equal Variances)										***	

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V
Compound:	o-Toluidine HCL

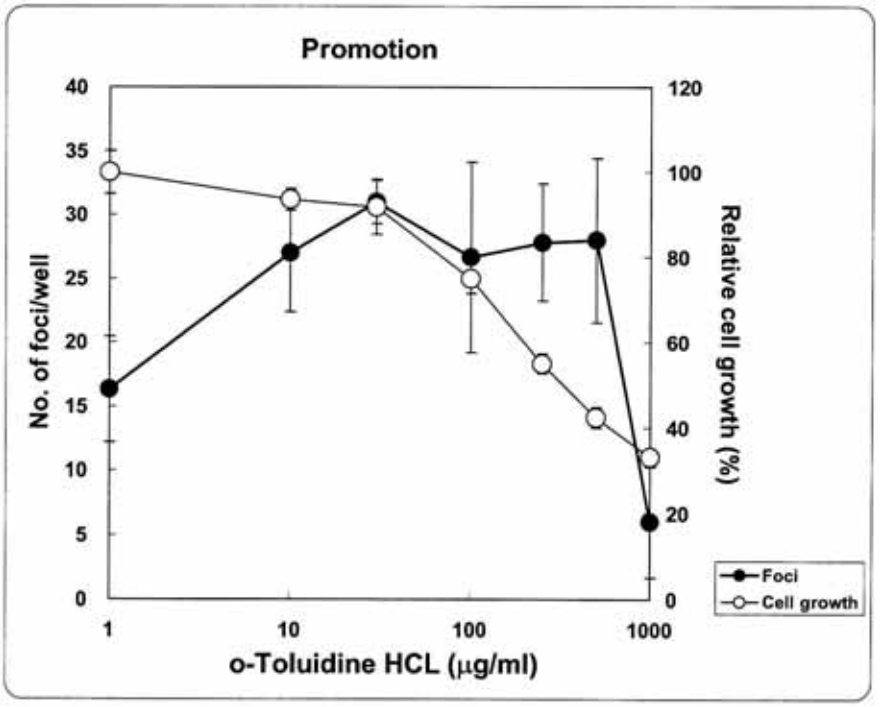
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water								
Compound (ug/ml)	Blank	1	10	30	100	250	500	1000	DMSO 0.1%	TPA 50 ng/mL
OD	0.0859	0.5773	0.5363	0.5481	0.4552	0.3553	0.2922	0.2486	0.5831	0.7353
	0.0875	0.5508	0.5183	0.5067	0.4257	0.3406	0.2719	0.2309	0.5275	0.698
	0.0886	0.5303	0.5144	0.4892	0.4278	0.334	0.2897	0.2442	0.5404	0.6873
Average	0.087	0.553	0.523	0.515	0.436	0.343	0.285	0.241	0.550	0.707
SD	0.001	0.024	0.012	0.030	0.016	0.011	0.011	0.009	0.029	0.025
Average-Blank	0.000	0.465	0.436	0.427	0.349	0.256	0.197	0.154	0.463	0.620
Relative cell growth (%)		100.0	93.6	91.8	75.0	55.0	42.4	33.1	100.0	133.8
SD of relative cell growth(%)		5.1	2.5	6.5	3.5	2.3	2.4	2.0	6.3	5.4

Transformation Assay	Solvent:	Water								
Compound (ug/ml)	Well No.	1	10	30	100	250	500	1000	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	12	36	31	26	26	31	2	11	40
	2	14	25	29	19	22	20	1	21	40
	3	12	27	34	36	31	21	11	21	45
	4	21	25	30	35	28	27	5	17	38
	5	19	23	31	19	25	34	6	9	60
	6	20	26	31	25	35	35	11	10	39
Average		16.3	27.0	31.0	26.7	27.8	28.0	6.0	14.8	43.7
SD		4.1	4.6	1.7	7.4	4.6	6.4	4.3	5.5	8.4
Dunnett test			**	***	**	**	**			
t-Test(Assuming Equal Variances										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	o-Toluidine HCL

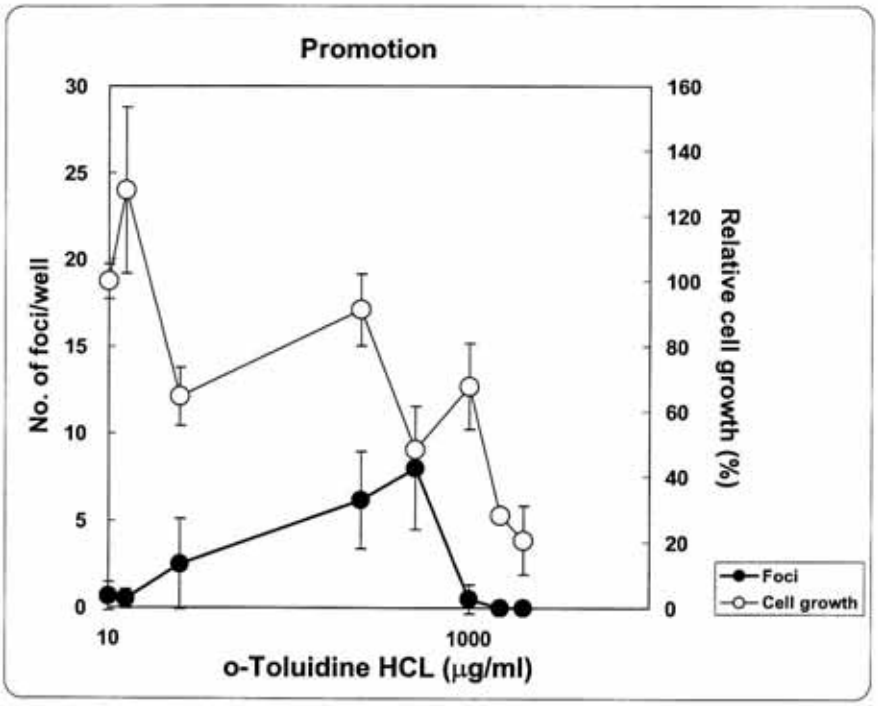
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%									
Compound (ug/ml)	Blank	10	12.5	25	250	500	1000	1500	2000	DMSO 0.5%	TPA 50 ng/mL
OD	0.032	0.226	0.296	0.145	0.197	0.128	0.141	0.090	0.085	0.226	0.234
	0.031	0.247	0.350	0.181	0.221	0.134	0.194	0.089	0.088	0.247	0.268
	0.031	0.243	0.244	0.170	0.243	0.132	0.180	0.090	0.049	0.243	0.236
Average	0.031	0.239	0.297	0.165	0.220	0.131	0.172	0.090	0.074	0.239	0.246
SD	0.001	0.011	0.053	0.018	0.023	0.003	0.027	0.001	0.022	0.011	0.019
Average-Blank	0.000	0.207	0.265	0.134	0.189	0.100	0.140	0.058	0.043	0.207	0.215
Relative cell growth (%)		100.0	128.0	64.6	91.2	48.2	67.7	28.1	20.6	100.0	103.5
SD of relative cell growth(%)		5.4	25.6	8.9	11.1	1.5	13.2	0.3	10.5	5.4	9.2

Transformation Assay	Solvent:	DMSO 0.5%									
Compound (ug/ml)	Well No.	10	12.5	25	250	500	1000	1500	2000	DMSO 0.5%	TPA 50 ng/mL
No of foci	1	1	0	0	2	6	0	Toxic	Toxic	1	17
	2	0	0	2	5	6	0	Toxic	Toxic	0	16
	3	2	1	7	10	11	0	Toxic	Toxic	2	16
	4	1	1	3	5	3	1	Toxic	Toxic	1	14
	5	0	0	3	8	10	0	Toxic	Toxic	0	12
	6	0	1	0	7	12	2	Toxic	Toxic	0	18
Average		0.7	0.5	2.5	6.2	8.0	0.5	#DIV/0!	#DIV/0!	0.7	15.5
SD		0.8	0.5	2.6	2.8	3.5	0.8	#DIV/0!	#DIV/0!	0.8	2.2
Dunnett test					***	***					
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Lithocholic acid

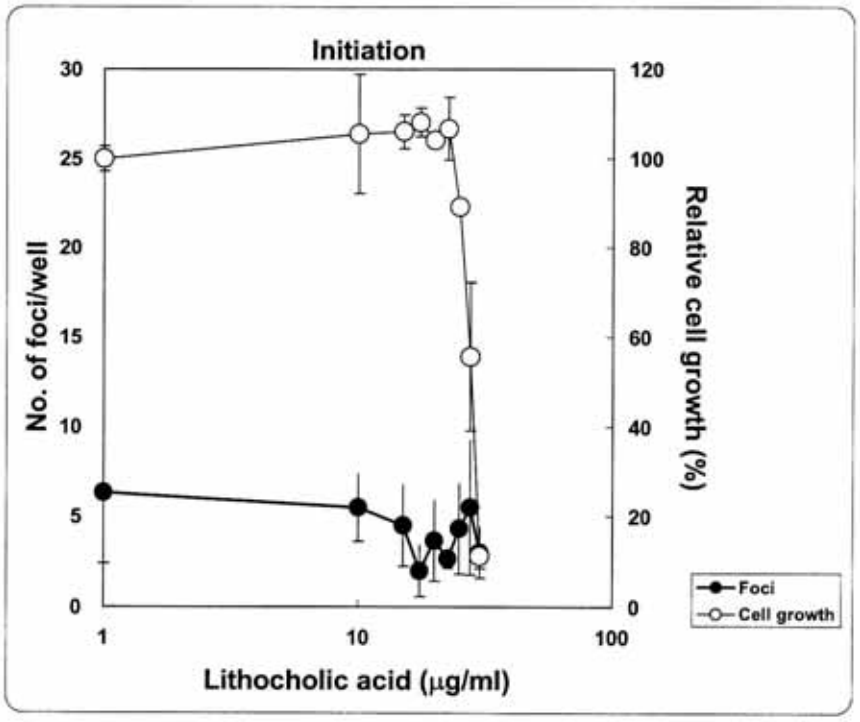
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%										
Compound E (ug/ml)	Blank	1	10	15	17.5	20	22.5	25	27.5	30	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	0.882	1.055	0.901	0.979	0.919	0.874	0.807	0.434	0.151	0.882	0.558
	0.073	0.865	0.879	0.959	0.928	0.918	0.973	0.801	0.463	0.19	0.865	0.53
	0.077	0.911	0.857	0.944	0.948	0.921	0.973	0.786	0.68	0.155	0.911	0.914
Average	0.074	0.886	0.930	0.935	0.952	0.919	0.940	0.798	0.526	0.165	0.886	0.667
SD	0.002	0.023	0.109	0.030	0.026	0.002	0.057	0.011	0.134	0.021	0.023	0.214
Average-Blank	0.000	0.812	0.856	0.860	0.877	0.845	0.866	0.724	0.451	0.091	0.812	0.593
Relative cell growth (%)		100.0	105.5	106.0	108.1	104.1	106.7	89.2	55.6	11.2	100.0	73.1
SD of relative cell growth(%)		2.9	13.4	3.7	3.2	0.2	7.0	1.3	16.6	2.6	2.9	26.4

Transformation Assay	Solvent:	DMSO 0.1%										
Compound E (ug/ml)	Well No.	1	10	15	17.5	20	22.5	25	27.5	30	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	6	5	7	1	6	3	4	2	4	6	40
	2	2	6	5	2	1	3	9	12	3	2	44
	3	10	7	2	4	4	3	5	7	2	10	48
	4	12	2	4	0	6	2	2	5	3	12	49
	5	5	6	2	3	1	3	3	5	1	5	43
	6	3	7	7	2	4	2	3	2	5	3	41
Average		6.3	5.5	4.5	2.0	3.7	2.7	4.3	5.5	3.0	6.3	44.2
SD		3.9	1.9	2.3	1.4	2.3	0.5	2.5	3.7	1.4	3.9	3.7
Dunnett test												N.S.
t-Test (Assuming Equal Variances)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV
Compound:	Lithocholic acid

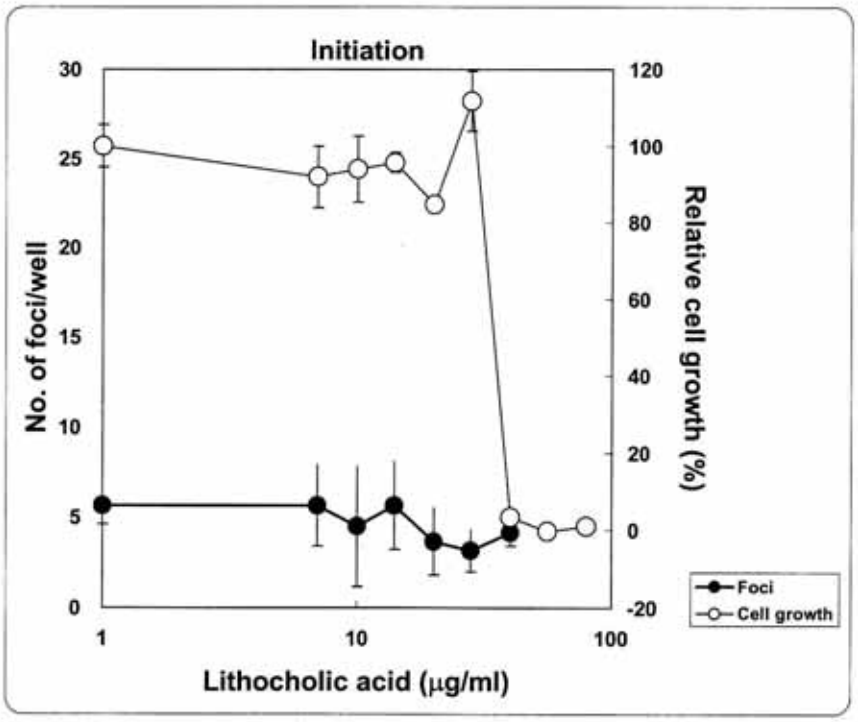
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%									DMSO 0.1%	MCA 1 ug/mL
Compound (ug/ml)	Blank	1	7	10	14	20	28	40	56	80		
OD	0.059	0.37	0.372	0.388	0.381	0.339	0.441	0.075	0.061	0.069	0.37	0.225
	0.067	0.406	0.381	0.381	0.365	0.333	0.44	0.076	0.062	0.067	0.406	0.271
	0.063	0.388	0.332	0.336	0.376	0.343	0.397	0.071	0.063	0.063	0.388	0.253
Average	0.063	0.388	0.362	0.368	0.374	0.338	0.426	0.074	0.062	0.066	0.388	0.250
SD	0.004	0.018	0.026	0.028	0.008	0.005	0.025	0.003	0.001	0.003	0.018	0.023
Average-Blank	0.000	0.325	0.299	0.305	0.311	0.275	0.363	0.011	-0.001	0.003	0.325	0.187
Relative cell growth (%)		100.0	91.9	93.9	95.7	84.7	111.7	3.4	-0.3	1.0	100.0	57.4
SD of relative cell growth(%)		5.5	8.0	8.7	2.5	1.5	7.7	0.8	0.3	0.9	5.5	7.1

Transformation Assay	Solvent:	DMSO 0.1%									DMSO 0.1%	MCA 1 ug/mL	
Compound (ug/ml)	Well No.	1	7	10	14	20	28	40	56	80			
No of foci	1	6	2	1	2	4	2	4	Toxic	Toxic	6	37	
	2	6	5	1	5	2	3	5	Toxic	Toxic	6	39	
	3	4	6	7	8	4	2	5	Toxic	Toxic	4	41	
	4	7	5	9	8	7	5	4	Toxic	Toxic	7	43	
	5	5	8	3	4	2	3	3	Toxic	Toxic	5	36	
	6	6	8	6	7	3	4	4	Toxic	Toxic	6	38	
Average		5.7	5.7	4.5	5.7	3.7	3.2	4.2			5.7	39.0	
SD		1.0	2.3	3.3	2.4	1.9	1.2	0.8			1.0	2.6	
Dunnett test													N.S.
t-Test (Aspin-Welch)												***	

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Lithocholic acid

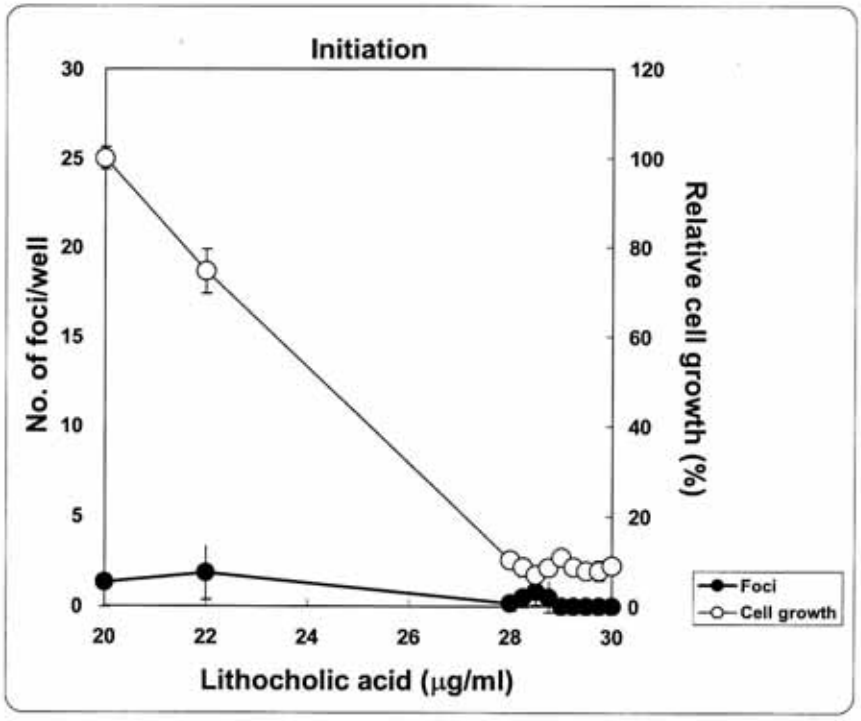
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%												
Compound (ug/ml)	Blank	20	22	28	28.25	28.5	28.75	29	29.25	29.5	29.75	30	DMSO 0.5%	MCA 1 ug/mL
OD	0.033	0.303	0.226	0.061	0.054	0.051	0.053	0.059	0.060	0.051	0.051	0.056	0.303	0.176
	0.034	0.316	0.236	0.061	0.054	0.050	0.058	0.062	0.056	0.058	0.052	0.057	0.316	0.193
	0.033	0.305	0.253	0.062	0.063	0.056	0.059	0.068	0.056	0.056	0.062	0.061	0.305	0.196
Average	0.033	0.308	0.238	0.061	0.057	0.052	0.057	0.063	0.057	0.055	0.055	0.058	0.308	0.188
SD	0.001	0.007	0.014	0.001	0.005	0.003	0.003	0.005	0.002	0.004	0.006	0.003	0.007	0.011
Average-Blank	0.000	0.275	0.205	0.028	0.024	0.019	0.023	0.030	0.024	0.022	0.022	0.025	0.275	0.155
Relative cell growth (%)		100.0	74.6	10.2	8.6	6.9	8.5	10.8	8.7	7.9	7.9	9.0	100.0	56.4
SD of relative cell growth(%)		2.5	5.0	0.2	1.9	1.2	1.2	1.7	0.8	1.3	2.2	1.0	2.5	3.9

Transformation Assay	Solvent:	DMSO 0.5%												
Compound (ug/ml)	Well No.	20	22	28	28.25	28.5	28.75	29	29.25	29.5	29.75	30	DMSO 0.5%	MCA 1 ug/mL
No of foci	1	3	4	1	1	1	0	0	Toxic	Toxic	Toxic	Toxic	3	8
	2	0	2	0	0	0	0	0	Toxic	Toxic	Toxic	Toxic	0	15
	3	1	1	0	0	1	0	0	Toxic	Toxic	Toxic	Toxic	1	16
	4	0	0	0	1	1	0	0	Toxic	Toxic	Toxic	Toxic	0	5
	5	1	1	0	0	0	2	0	Toxic	Toxic	Toxic	Toxic	1	9
	6	3	3	0	1	2	1	0	Toxic	Toxic	Toxic	Toxic	3	10
Average		1.3	1.8	0.2	0.5	0.8	0.5	0.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.3	10.5
SD		1.4	1.5	0.4	0.5	0.8	0.8	0.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1.4	4.2
Dunnett test														N.S.
t-Test (Aspin-Welch)														**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Lithocholic acid

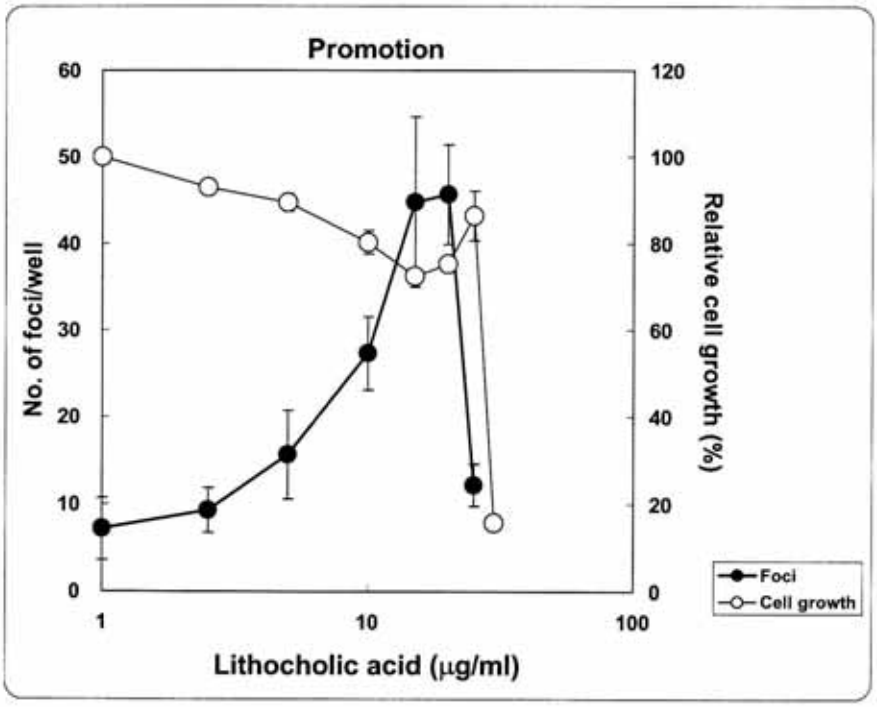
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%									
Compound E (ug/ml)	Blank	1	2.5	5	10	15	20	25	30	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.001	0.923	0.891	0.78	0.732	0.765	0.839	0.219	1.001	1.123
	0.073	0.974	0.917	0.872	0.812	0.73	0.741	0.827	0.228	0.974	1.224
	0.077	0.985	0.927	0.909	0.829	0.743	0.775	0.922	0.207	0.985	1.283
Average	0.074	0.987	0.922	0.891	0.807	0.735	0.760	0.863	0.218	0.987	1.210
SD	0.002	0.014	0.005	0.019	0.025	0.007	0.017	0.052	0.011	0.014	0.081
Average-Blank	0.000	0.912	0.848	0.816	0.733	0.661	0.686	0.788	0.144	0.912	1.136
Relative cell growth (%)		100.0	92.9	89.5	80.3	72.4	75.2	86.4	15.7	100.0	124.5
SD of relative cell growth(%)		1.5	0.6	2.0	2.7	0.8	1.9	5.7	1.2	1.5	8.9

Transformation Assay	Solvent:	DMSO 0.1%									
Compound E (ug/ml)	Well No.	1	2.5	5	10	15	20	25	30	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	5	13	16	33	38	49	16 tox		5	43
	2	2	9	21	29	48	35	11 tox		2	43
	3	7	8	17	23	53	51	13 tox		7	41
	4	7	12	13	27	50	45	9 tox		7	45
	5	10	7	7	22	52	49	13 tox		10	42
	6	12	7	20	30	28	45	11 tox		12	40
Average		7.2	9.3	15.7	27.3	44.8	45.7	12.2		7.2	42.3
SD		3.5	2.6	5.1	4.2	9.8	5.8	2.4		3.5	1.8
Dunnett test				*	***	***	***				
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV
Compound:	Lithocholic acid

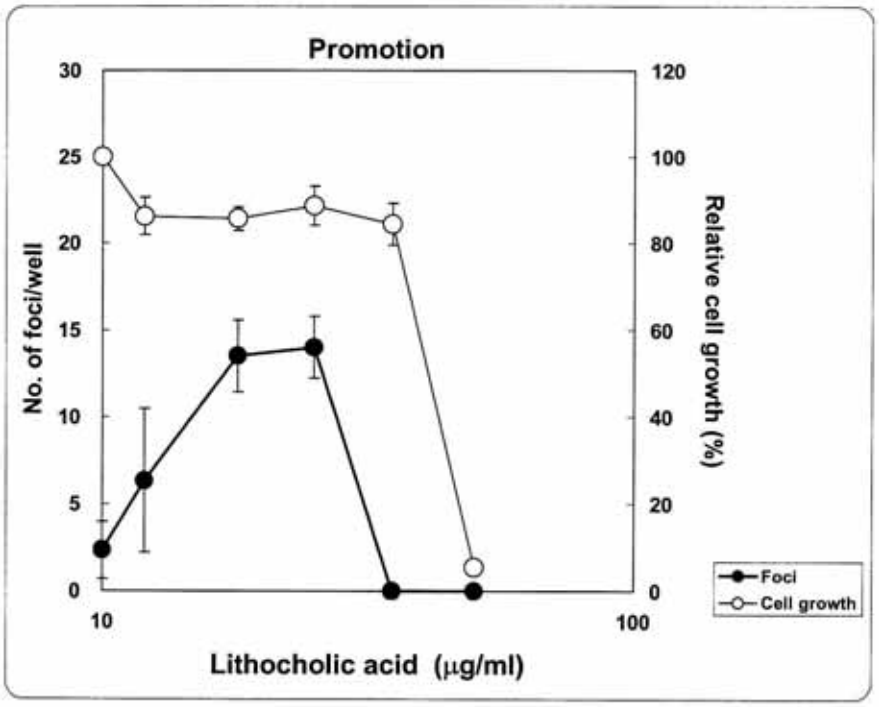
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%							
Compound (ug/ml)	Blank	10	12	18	25	35	50	DMSO 0.1%	TPA 50 ng/mL
OD	0.062	0.359	0.334	0.326	0.319	0.313	0.074	0.359	0.474
	0.061	0.362	0.312	0.31	0.342	0.299	0.08	0.362	0.447
	0.06	0.359	0.311	0.316	0.318	0.328	0.078	0.359	0.479
Average	0.061	0.360	0.319	0.317	0.326	0.313	0.077	0.360	0.467
SD	0.001	0.002	0.013	0.008	0.014	0.015	0.003	0.002	0.017
Average-Blank	0.000	0.299	0.258	0.256	0.265	0.252	0.016	0.299	0.406
Relative cell growth (%)		100.0	86.3	85.7	88.7	84.4	5.5	100.0	135.7
SD of relative cell growth(%)		0.6	4.3	2.7	4.5	4.9	1.0	0.6	5.8

Transformation Assay	Solvent:	DMSO 0.1%							
Compound (ug/ml)	Well No.	10	12	18	25	35	50	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	4	1	12	17	Toxic	Toxic	4	9
	2	0	7	12	12	Toxic	Toxic	0	12
	3	4	2	12	13	Toxic	Toxic	4	8
	4	2	12	17	15	Toxic	Toxic	2	4
	5	1	8	15	13	Toxic	Toxic	1	14
	6	3	8	13	14	Toxic	Toxic	3	10
Average		2.3	6.3	13.5	14.0	#DIV/0!	#DIV/0!	2.3	9.5
SD		1.6	4.1	2.1	1.8	#DIV/0!	#DIV/0!	1.6	3.4
Dunnett test			*	***	***				
t-Test for Unequal Variances (Aspin-Welch)									**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Lithocholic acid

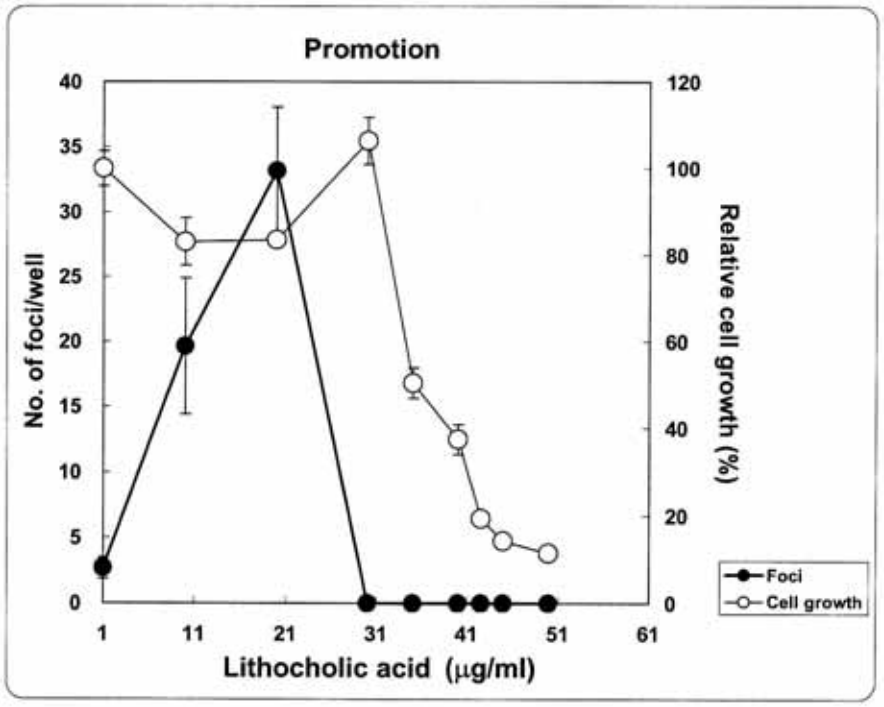
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0,5%										
Compound (ug/ml)	Blank	1	10	20	30	35	40	42.5	45	50	DMSO 0,5% TPA 50 ng/mL	
OD	0.033	0.223	0.174	0.181	0.215	0.125	0.093	0.070	0.057	0.054	0.223	0.286
	0.031	0.212	0.183	0.186	0.233	0.130	0.104	0.066	0.059	0.052	0.212	0.302
	0.031	0.209	0.194	0.186	0.231	0.117	0.104	0.065	0.057	0.052	0.209	0.304
Average	0.032	0.215	0.184	0.184	0.226	0.124	0.100	0.067	0.058	0.053	0.215	0.297
SD	0.001	0.007	0.010	0.003	0.010	0.007	0.006	0.003	0.001	0.001	0.007	0.010
Average-Blank	0.000	0.183	0.152	0.153	0.195	0.092	0.069	0.035	0.026	0.021	0.183	0.266
Relative cell growth (%)		100.0	83.1	83.4	106.4	50.5	37.5	19.3	14.2	11.5	100.0	145.2
SD of relative cell growth(%)		4.0	5.5	1.6	5.4	3.6	3.5	1.4	0.6	0.6	4.0	5.4

Transformation Assay	Solvent:	DMSO 0,5%										
Compound (ug/ml)	Well No.	1	10	20	30	35	40	42.5	45	50	DMSO 0,5% TPA 50 ng/mL	
No of foci	1	2	17	31	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	2	14
	2	2	14	33	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	2	17
	3	4	18	26	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	4	18
	4	2	29	37	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	2	12
	5	3	22	32	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	3	24
	6	3	18	40	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic	3	16
Average		2.7	19.7	33.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	2.7	16.8
SD		0.8	5.2	4.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.8	4.1
Dunnett test			***	***								
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Mezerein

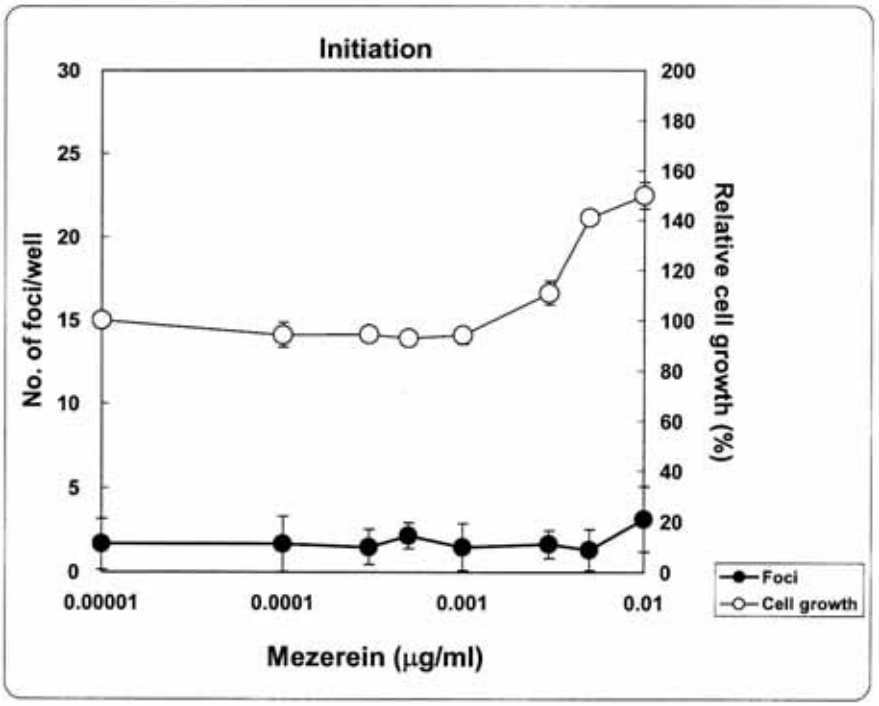
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Blank	0.00001	0.0001	0.0003	0.0005	0.001	0.003	0.005	0.01	DMSO 0.1%	MCA 1ug/mL
OD	0.073	1.1	0.962	1.007	0.996	1.051	1.132	1.518	1.523	1.1	0.641
	0.073	1.059	1.041	1.004	1.019	0.981	1.211	1.466	1.579	1.059	0.631
	0.077	1.07	1.053	1.047	0.998	1.019	1.217	1.483	1.631	1.07	0.62
Average	0.074	1.076	1.019	1.019	1.004	1.017	1.187	1.489	1.578	1.076	0.631
SD	0.002	0.021	0.049	0.024	0.013	0.035	0.047	0.027	0.054	0.021	0.011
Average-Blank	0.000	1.002	0.944	0.945	0.930	0.943	1.112	1.415	1.503	1.002	0.556
Relative cell growth (%)		100.0	94.2	94.3	92.8	94.1	111.0	141.2	150.0	100.0	55.5
SD of relative cell growth(%)		2.1	4.9	2.4	1.3	3.5	4.7	2.6	5.4	2.1	1.0

Transformation Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Well No.	0.00001	0.0001	0.0003	0.0005	0.001	0.003	0.005	0.01	DMSO 0.1%	MCA 1ug/mL
No of foci	1	3	3	3	3	3	1	1	6	3	17
	2	0	2	1	3	2	1	0	3	0	25
	3	0	0	2	2	0	2	2	1	0	19
	4	1	1	1	2	3	3	0	4	1	22
	5	3	0	0	2	1	1	3	4	3	20
	6	3	4	2	1	0	2	2	1	3	30
Average		1.7	1.7	1.5	2.2	1.5	1.7	1.3	3.2	1.7	22.2
SD		1.5	1.6	1.0	0.8	1.4	0.8	1.2	1.9	1.5	4.7
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab III

Compound:	Mezerein

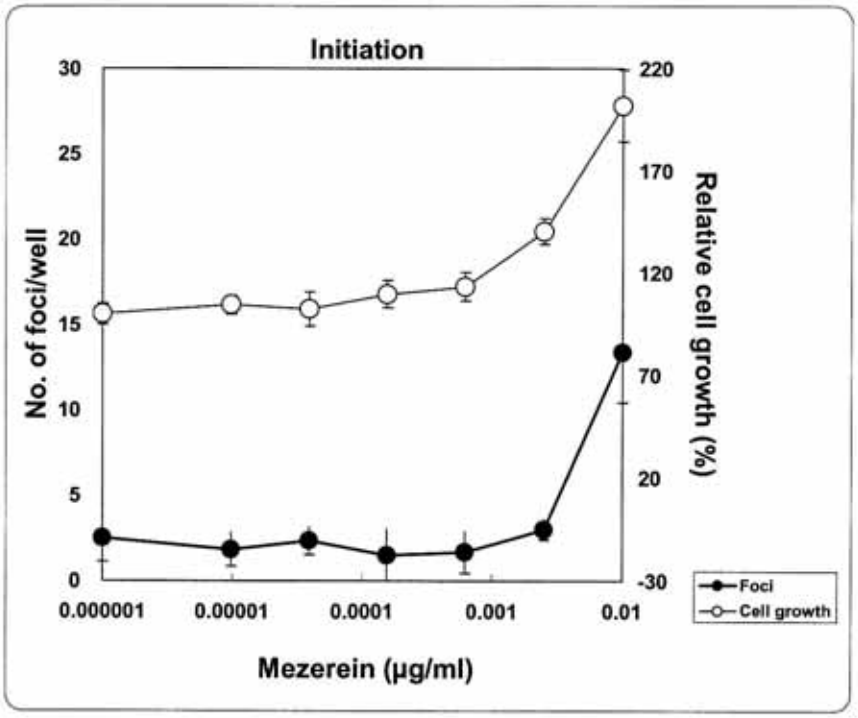
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Blank	0.000001	0.00000977	0.0000391	0.000156	0.000625	0.0025	0.01	DMSO 0.1%	MCA 1 ug/mL
OD	0.112	1.091	1.144	1.09	1.168	1.209	1.495	2.19	1.091	0.386
	0.134	1.169	1.23	1.261	1.288	1.295	1.625	2.366	1.169	0.399
	0.145	1.19	1.21	1.169	1.287	1.352	1.561	2.009	1.19	0.449
Average	0.130	1.150	1.195	1.173	1.248	1.285	1.560	2.188	1.150	0.411
SD	0.017	0.052	0.045	0.086	0.069	0.072	0.065	0.179	0.052	0.033
Average-Blank	0.000	1.020	1.064	1.043	1.117	1.155	1.430	2.058	1.020	0.281
Relative cell growth (%)		100.0	104.4	102.3	109.6	113.3	140.2	201.8	100.0	27.6
SD of relative cell growth(%)		5.1	4.4	8.4	6.8	7.1	6.4	17.5	5.1	3.3

Transformation Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Well No.	0.000001	0.00000977	0.0000391	0.000156	0.000625	0.0025	0.01	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	2	2	3	2	3	3	14	2	14
	2	3	0	1	2	2	4	12	3	12
	3	1	3	2	1	3	3	13	1	7
	4	1	2	2	0	0	2	18	1	9
	5	4	2	3	4	1	3	9	4	4
	6	4	2	3	0	1	3	14	4	9
Average		2.5	1.8	2.3	1.5	1.7	3.0	13.3	2.5	9.2
SD		1.4	1.0	0.8	1.5	1.2	0.6	2.9	1.4	3.5
Dunnett test								***		
t-Test (Aspin-Welch)										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Mezerein

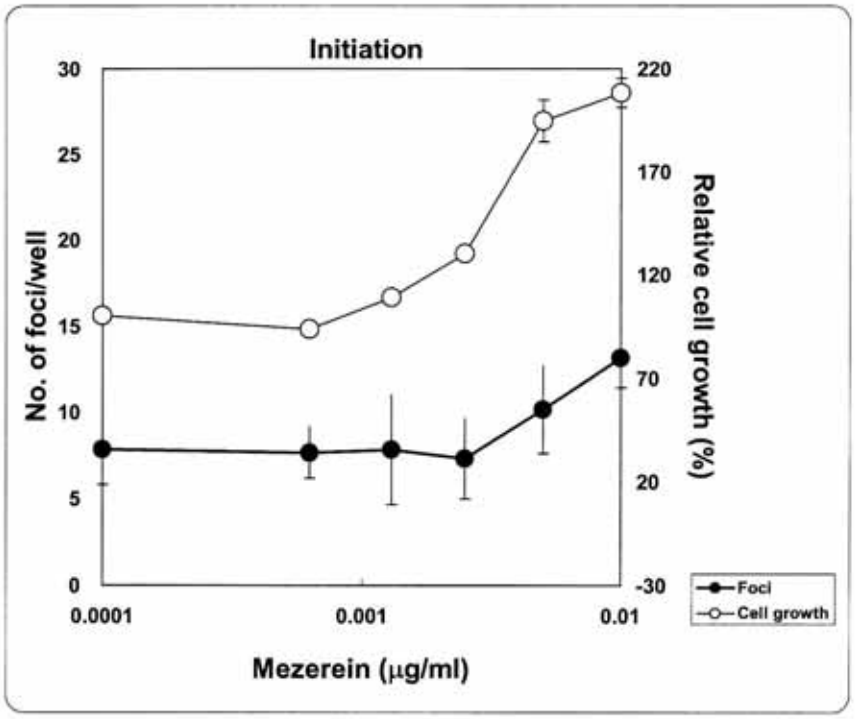
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.1%							
Compound (ug/ml)	Blank	0.0001	0.00063	0.0013	0.0025	0.005	0.01	DMSO 0.1%	MCA 1 ug/mL
OD	0.069	0.356	0.337	0.383	0.433	0.622	0.67	0.356	0.25
	0.068	0.364	0.332	0.378	0.438	0.597	0.641	0.364	0.269
	0.069	0.343	0.339	0.379	0.451	0.655	0.68	0.343	0.292
Average	0.069	0.354	0.336	0.380	0.441	0.625	0.664	0.354	0.270
SD	0.001	0.011	0.004	0.003	0.009	0.029	0.020	0.011	0.021
Average-Blank	0.000	0.286	0.267	0.311	0.372	0.556	0.595	0.286	0.202
Relative cell growth (%)		100.0	93.6	109.0	130.2	194.6	208.3	100.0	70.6
SD of relative cell growth(%)		3.7	1.3	0.9	3.3	10.2	7.1	3.7	7.4

Transformation Assay	Solvent:	DMSO 0.1%							
Compound (ug/ml)	Well No.	0.0001	0.00063	0.0013	0.0025	0.005	0.01	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	5	9	11	9	9	14	5	42
	2	10	5	2	6	11	11	10	43
	3	10	9	10	9	9	16	10	47
	4	8	7	9	4	8	13	8	46
	5	6	8	7	6	15	12	6	44
	6	8	8	8	10	9	13	8	52
Average		7.8	7.7	7.8	7.3	10.2	13.2	7.8	45.7
SD		2.0	1.5	3.2	2.3	2.6	1.7	2.0	3.6
Dunnett test							**		
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Mezerein

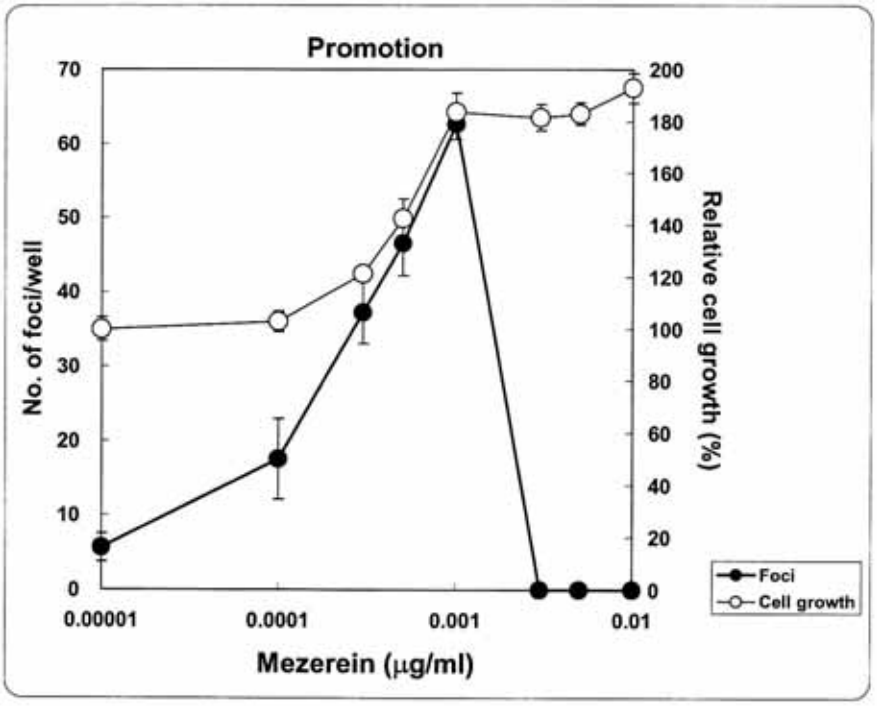
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Blank	0.00001	0.0001	0.0003	0.0005	0.001	0.003	0.005	0.01	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.085	1.066	1.276	1.54	1.937	1.789	1.88	2.007	1.085	1.258
	0.073	1.05	1.111	1.227	1.41	1.814	1.827	1.801	1.921	1.05	1.235
	0.077	0.997	1.037	1.248	1.418	1.816	1.886	1.863	1.903	0.997	1.343
Average	0.074	1.044	1.071	1.250	1.456	1.856	1.834	1.848	1.944	1.044	1.279
SD	0.002	0.044	0.037	0.025	0.073	0.070	0.049	0.042	0.056	0.044	0.057
Average-Blank	0.000	0.970	0.997	1.176	1.382	1.781	1.760	1.774	1.869	0.970	1.204
Relative cell growth (%)		100.0	102.8	121.3	142.5	183.7	181.5	182.9	192.8	100.0	124.2
SD of relative cell growth(%)		4.6	3.8	2.5	7.5	7.3	5.0	4.3	5.7	4.6	5.9

Transformation Assay	Solvent:	DMSO 0.1%									
Compound (ug/ml)	Well No.	0.00001	0.0001	0.0003	0.0005	0.001	0.003	0.005	0.01	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	4	20	39	49	60	Toxic	Toxic	Toxic	4	31
	2	6	7	36	48	62	Toxic	Toxic	Toxic	6	32
	3	6	22	36	50	66	Toxic	Toxic	Toxic	6	31
	4	7	20	42	50	62	Toxic	Toxic	Toxic	7	42
	5	3	17	40	40	64	Toxic	Toxic	Toxic	3	33
	6	8	19	30	42	62	Toxic	Toxic	Toxic	8	43
Average		5.7	17.5	37.2	46.5	62.7	#DIV/0!	#DIV/0!	#DIV/0!	5.7	35.3
SD		1.9	5.4	4.2	4.4	2.1	#DIV/0!	#DIV/0!	#DIV/0!	1.9	5.6
Dunnett test			***	***	***	***					
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ

Compound:	Mezerein

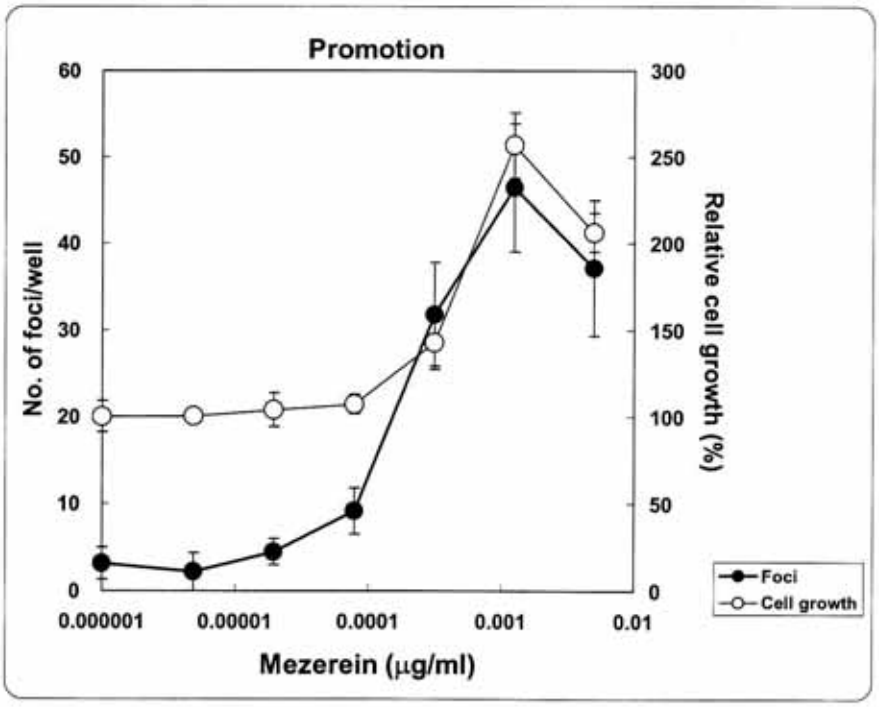
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Blank	0.000001	0.00000488	0.0000195	0.0000781	0.000313	0.00125	0.005	DMSO 0.1%	TPA 50 ng/mL
OD	0.111	0.936	0.986	0.967	1.041	1.322	2.269	1.885	0.936	1.301
	0.115	1.08	1.042	1.094	1.106	1.358	2.528	2.053	1.08	1.506
	0.121	1.073	1.066	1.138	1.145	1.587	2.594	2.071	1.073	1.456
平均	0.116	1.030	1.031	1.066	1.097	1.422	2.464	2.003	1.030	1.421
SD	0.005	0.081	0.041	0.089	0.053	0.144	0.172	0.103	0.081	0.107
Average-Blank	0.000	0.914	0.916	0.951	0.982	1.307	2.348	1.887	0.914	1.305
Relative cell growth (%)		100.0	100.2	104.0	107.4	143.0	256.9	206.5	100.0	142.8
SD of relative cell growth(%)		8.9	4.5	9.7	5.7	15.7	18.8	11.2	8.9	11.7

Transformation Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Well No.	0.000001	0.00000488	0.0000195	0.0000781	0.000313	0.00125	0.005	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	6	6	7	8	34	40	33	6	11
	2	5	1	5	14	37	50	35	5	24
	3	2	0	3	7	36	53	47	2	8
	4	2	3	3	7	34	41	43	2	12
	5	2	1	4	10	29	39	25	2	11
	6	2	2	5	9	21	56	40	2	7
Average		3.2	2.2	4.5	9.2	31.8	46.5	37.2	3.2	12.2
SD		1.8	2.1	1.5	2.6	6.0	7.4	7.9	1.8	6.1
Dunnett test						***	***	***		
t-Test for Unequal Variances (Aspin-Welch)										*

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Mezerein

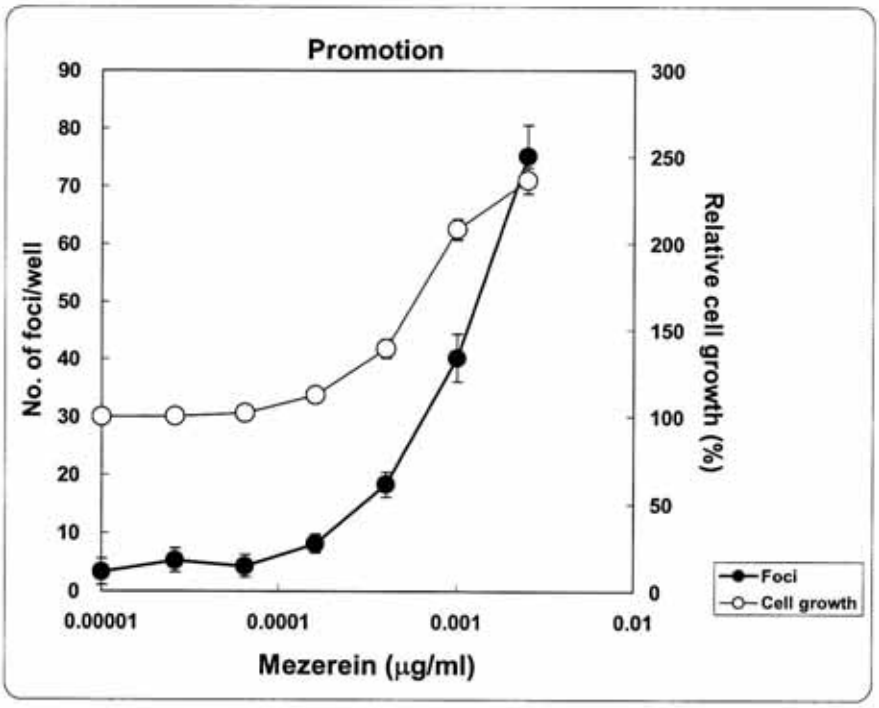
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Blank	0.00001	0.000026	0.000064	0.00016	0.0004	0.001	0.0025	DMSO 0.1%	TPA 50 ng/mL
OD	0.062	0.359	0.36	0.366	0.412	0.479	0.68	0.808	0.359	0.483
	0.061	0.377	0.364	0.373	0.393	0.474	0.692	0.763	0.377	0.49
	0.06	0.362	0.378	0.38	0.408	0.505	0.716	0.775	0.362	0.507
Average	0.061	0.366	0.367	0.373	0.404	0.486	0.696	0.782	0.366	0.493
SD	0.001	0.010	0.009	0.007	0.010	0.017	0.018	0.023	0.010	0.012
Average-Blank	0.000	0.305	0.306	0.312	0.343	0.425	0.635	0.721	0.305	0.432
Relative cell growth (%)		100.0	100.4	102.3	112.6	139.3	208.2	236.4	100.0	141.7
SD of relative cell growth(%)		3.2	3.1	2.3	3.3	5.5	6.0	7.6	3.2	4.0

Transformation Assay	Solvent:	DMSO 0.1%								
Compound (ug/ml)	Well No.	0.00001	0.000026	0.000064	0.00016	0.0004	0.001	0.0025	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	7	6	6	8	18	36	79	7	8
	2	2	3	7	8	19	39	72	2	6
	3	5	4	5	8	20	46	73	5	8
	4	3	5	3	8	17	44	84	3	13
	5	2	5	3	11	21	40	69	2	14
	6	1	9	2	6	15	36	74	1	10
Average		3.3	5.3	4.3	8.2	18.3	40.2	75.2	3.3	9.8
SD		2.3	2.1	2.0	1.6	2.2	4.1	5.4	2.3	3.1
Dunnett test					*	***	***	***		
t-Test (Assuming Equal Variances)										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Anthracene

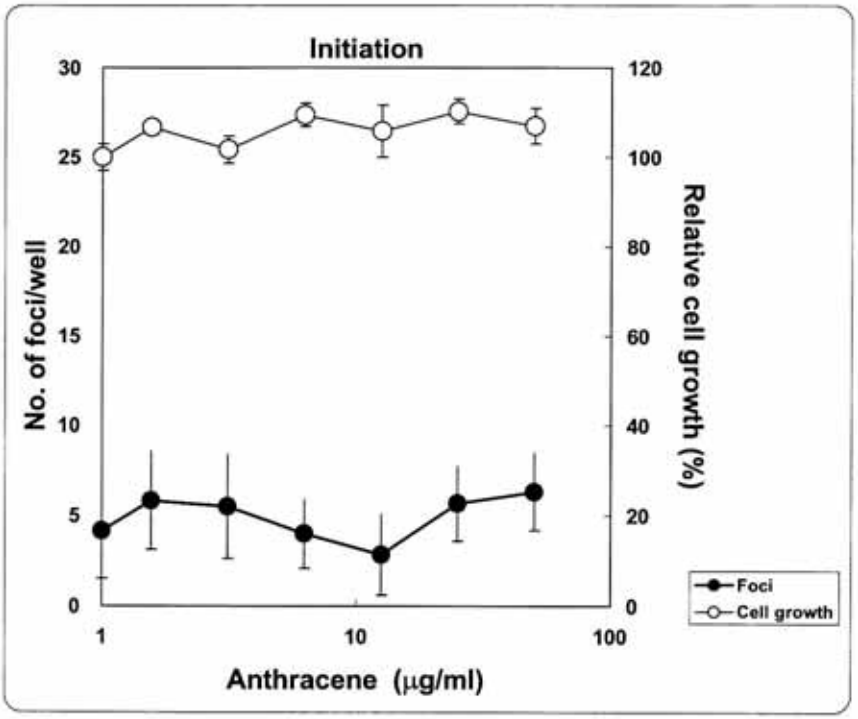
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%								
Compound (ug/ml)	Blank	1	1.5625	3.125	6.25	12.5	25	50	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	1.24	1.288	1.182	1.34	1.193	1.3	1.234	1.185	0.581
	0.073	1.193	1.286	1.248	1.307	1.295	1.355	1.323	1.137	0.601
	0.076	1.176	1.26	1.234	1.28	1.316	1.301	1.286	1.182	0.609
Average	0.074	1.203	1.278	1.221	1.309	1.268	1.319	1.281	1.168	0.597
SD	0.002	0.033	0.016	0.035	0.030	0.066	0.031	0.045	0.027	0.014
Average-Blank	0.000	1.129	1.204	1.147	1.235	1.194	1.245	1.207	1.094	0.523
Relative cell growth (%)		100.0	106.6	101.6	109.4	105.8	110.2	106.9	100.0	47.8
SD of relative cell growth(%)		2.9	1.4	3.1	2.7	5.8	2.8	4.0	2.5	1.3

Transformation Assay	Solvent:	DMSO 0.5%								
Compound (ug/ml)	Well No.	1	1.5625	3.125	6.25	12.5	25	50	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	8	7	8	3	1	4	5	2	24
	2	5	6	5	2	3	3	5	3	14
	3	3	5	3	5	5	8	10	2	24
	4	2	1	4	2	6	6	7	4	28
	5	6	7	3	6	1	8	4	3	30
	6	1	9	10	6	1	5	7	2	26
Average		4.2	5.8	5.5	4.0	2.8	5.7	6.3	2.7	24.3
SD		2.6	2.7	2.9	1.9	2.2	2.1	2.2	0.8	5.6
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Anthracene

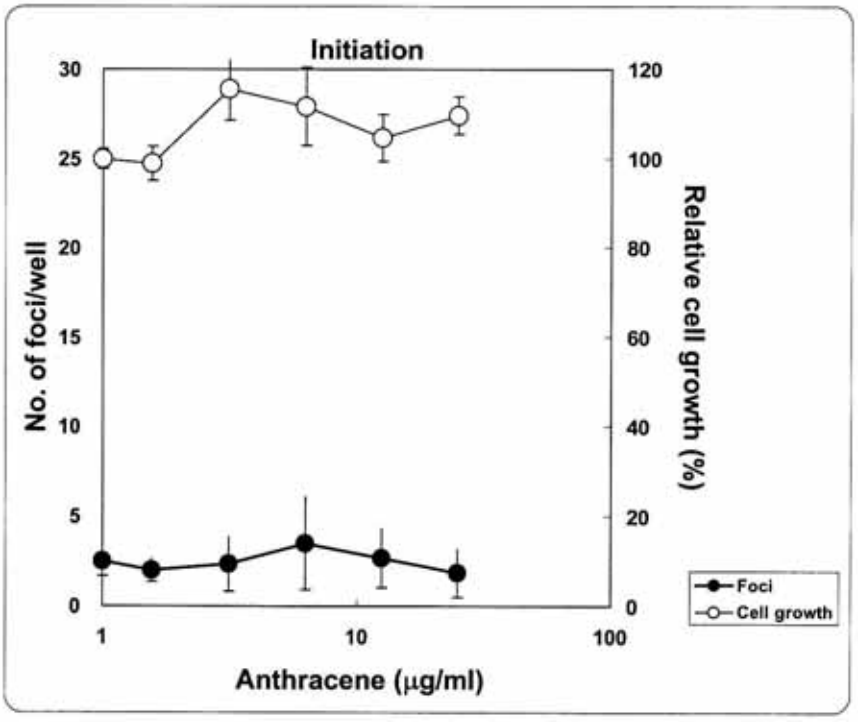
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%						DMSO 0.1%	MCA 1 ug/mL
Compound (ug/ml)	Blank	1	1.56	3.13	6.25	12.5	25		
OD	0.0669	0.7751	0.7566	0.9402	0.888	0.8361	0.8315	0.7033	0.4616
	0.0733	0.8065	0.7844	0.9293	0.9342	0.8582	0.8628	0.7356	0.4519
	0.0665	0.7957	0.8123	0.8469	0.8082	0.7839	0.8925	0.6301	0.4411
Average	0.069	0.792	0.784	0.905	0.877	0.826	0.862	0.690	0.452
SD	0.004	0.016	0.028	0.051	0.064	0.038	0.031	0.054	0.010
Average-Blank	0.000	0.724	0.716	0.837	0.808	0.757	0.793	0.621	0.383
Relative cell growth (%)		100.0	98.9	115.6	111.7	104.6	109.7	100.0	61.6
SD of relative cell growth(%)		2.2	3.8	7.1	8.8	5.3	4.2	8.7	1.7

Transformation Assay	Solvent:	DMSO 0.5%						DMSO 0.1%	MCA 1 ug/mL	
Compound (ug/ml)	Well No.	1	1.56	3.13	6.25	12.5	25			
No of foci	1	2	3	3	3	3	1	5	15	
	2	2	2	3	7	1	3	1	18	
	3	2	2	1	0	5	3	4	18	
	4	4	1	4	6	4	3	2	15	
	5	2	2	3	3	2	1	2	18	
	6	3	2	0	2	1	0	4	17	
Average		2.5	2.0	2.3	3.5	2.7	1.8	3.0	16.8	
SD		0.8	0.6	1.5	2.6	1.6	1.3	1.5	1.5	
Parametric Dannett t-Test (Assuming Equal Variances)									***	N.S.

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Anthracene

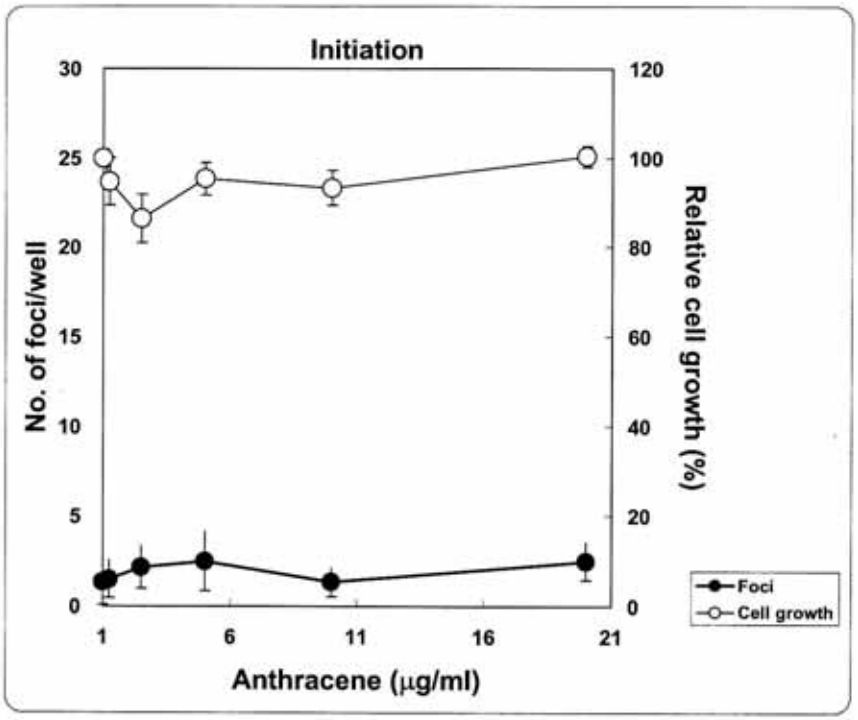
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Blank	1	1.25	2.5	5	10	20	DMSO 0.5%	MCA 1 ug/mL
OD	0.033	0.305	0.295	0.257	0.306	0.281	0.311	0.305	0.185
	0.031	0.316	0.311	0.274	0.300	0.291	0.318	0.316	0.198
	0.03	0.310	0.281	0.287	0.286	0.303	0.305	0.310	0.209
Average	0.031	0.310	0.296	0.273	0.297	0.292	0.311	0.310	0.197
SD	0.002	0.006	0.015	0.015	0.010	0.011	0.007	0.006	0.012
Average-Blank	0.000	0.279	0.264	0.241	0.266	0.260	0.280	0.279	0.166
Relative cell growth (%)		100.0	94.7	86.5	95.3	93.3	100.4	100.0	59.5
SD of relative cell growth(%)		2.0	5.4	5.4	3.7	3.9	2.3	2.0	4.3

Transformation Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Well No.	1	1.25	2.5	5	10	20	DMSO 0.5%	MCA 1 ug/mL
No of foci	1	3	1	3	4	1	2	3	13
	2	2	3	0	1	2	2	2	14
	3	0	1	3	3	0	3	0	17
	4	2	0	2	0	2	1	2	16
	5	0	2	3	3	2	3	0	10
	6	1	2	2	4	1	4	1	16
Average		1.3	1.5	2.2	2.5	1.3	2.5	1.3	14.3
SD		1.2	1.0	1.2	1.6	0.8	1.0	1.2	2.6
Dunnett test									N.S.
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Anthracene

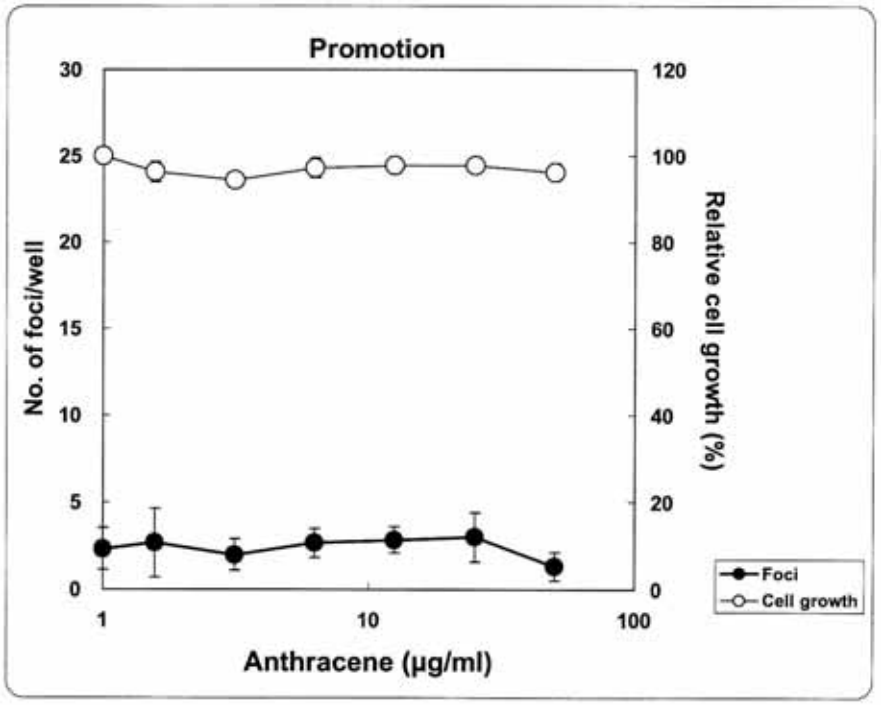
Toransformation Assay

Promotion

Cell Growth Assay	Solvent:	DMSO 0.5%								
Compound (ug/ml)	Blank	1	1.5625	3.125	6.25	12.5	25	50	DMSO 0.1%	TPA 50 ng/mL
OD	0.07	0.907	0.88	0.861	0.894	0.886	0.862	0.879	0.957	1.269
	0.067	0.886	0.871	0.845	0.863	0.885	0.891	0.867	0.969	1.239
	0.068	0.893	0.843	0.841	0.86	0.86	0.88	0.846	0.962	1.223
Average	0.068	0.895	0.865	0.849	0.872	0.877	0.878	0.864	0.963	1.244
SD	0.002	0.011	0.019	0.011	0.019	0.015	0.015	0.017	0.006	0.023
Average-Blank	0.000	0.827	0.796	0.781	0.804	0.809	0.809	0.796	0.894	1.175
Relative cell growth (%)		100.0	96.3	94.4	97.2	97.8	97.9	96.2	100.0	131.4
SD of relative cell growth(%)		1.3	2.3	1.3	2.3	1.8	1.8	2.0	0.7	2.6

Transformation Assay	Solvent:	DMSO 0.5%								
Compound (ug/ml)	Well No.	1	1.5625	3.125	6.25	12.5	25	50	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	2	1	3	3	3	3	2	2	9
	2	3	1	2	3	2	4	0	4	23
	3	4	2	2	3	2	2	2	6	15
	4	1	2	1	3	3	1	1	2	17
	5	1	4	3	1	4	3	1	2	27
	6	3	6	1	3	3	5	2	6	14
Average		2.3	2.7	2.0	2.7	2.8	3.0	1.3	3.7	17.5
SD		1.2	2.0	0.9	0.8	0.8	1.4	0.8	2.0	6.5
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II
Compound:	Anthracene

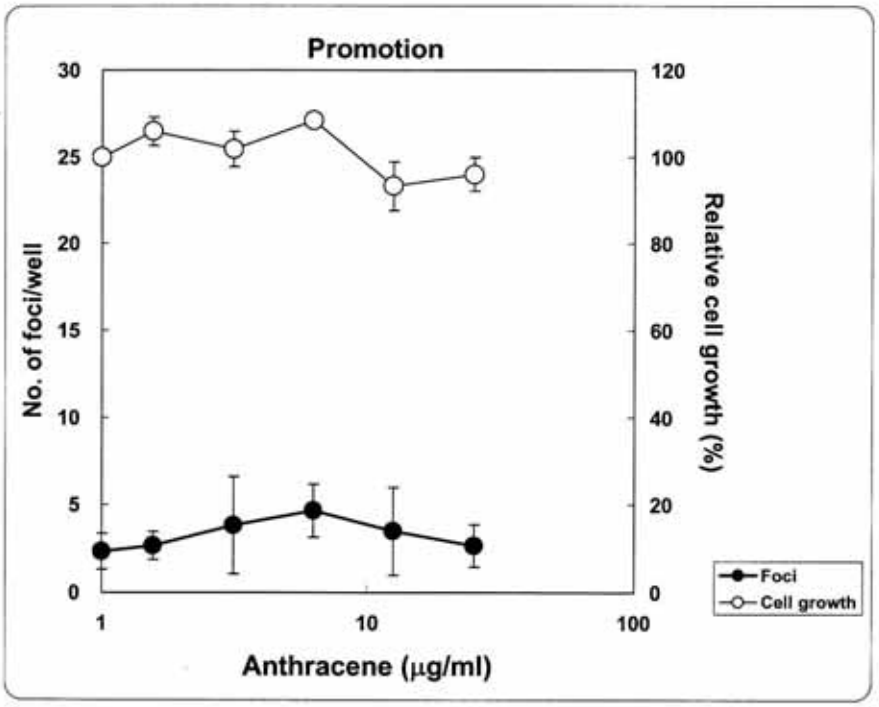
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Blank	1	1.56	3.13	6.25	12.5	25	DMSO 0.1%	TPA 50 ng/mL
OD	0.0726	0.5185	0.558	0.5402	0.5602	0.506	0.5221	0.5127	0.6579
	0.0775	0.5245	0.552	0.5379	0.5522	0.5048	0.4981	0.5317	0.6761
	0.0777	0.5187	0.5303	0.5078	0.5617	0.4621	0.4891	0.4817	0.6676
平均	0.076	0.521	0.547	0.529	0.558	0.491	0.503	0.509	0.667
SD	0.003	0.003	0.015	0.018	0.005	0.025	0.017	0.025	0.009
Average-Blank	0.000	0.445	0.471	0.453	0.482	0.415	0.427	0.433	0.591
Relative cell growth (%)		100.0	105.9	101.8	108.4	93.3	96.1	100.0	136.6
SD of relative cell growth(%)		0.8	3.3	4.1	1.1	5.6	3.8	5.8	2.1

Transformation Assay	Solvent:	DMSO 0.5								
Compound (ug/ml)	Well No.	1	1.56	3.13	6.25	12.5	25	DMSO 0.1%	TPA 50 ng/mL	
No of foci	1	3	3	1	5	1	2	2	12	
	2	2	4	4	7	7	1	3	12	
	3	2	2	3	3	6	4	3	10	
	4	4	2	2	5	1	4	8	8	
	5	2	3	9	3	3	3	3	11	
	6	1	2	4	5	3	2	3	12	
Average		2.3	2.7	3.8	4.7	3.5	2.7	3.7	10.8	
SD		1.0	0.8	2.8	1.5	2.5	1.2	2.2	1.6	
Parametric Dannett t-Test (Assuming Equal Variances)								***		N.S.

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Anthracene

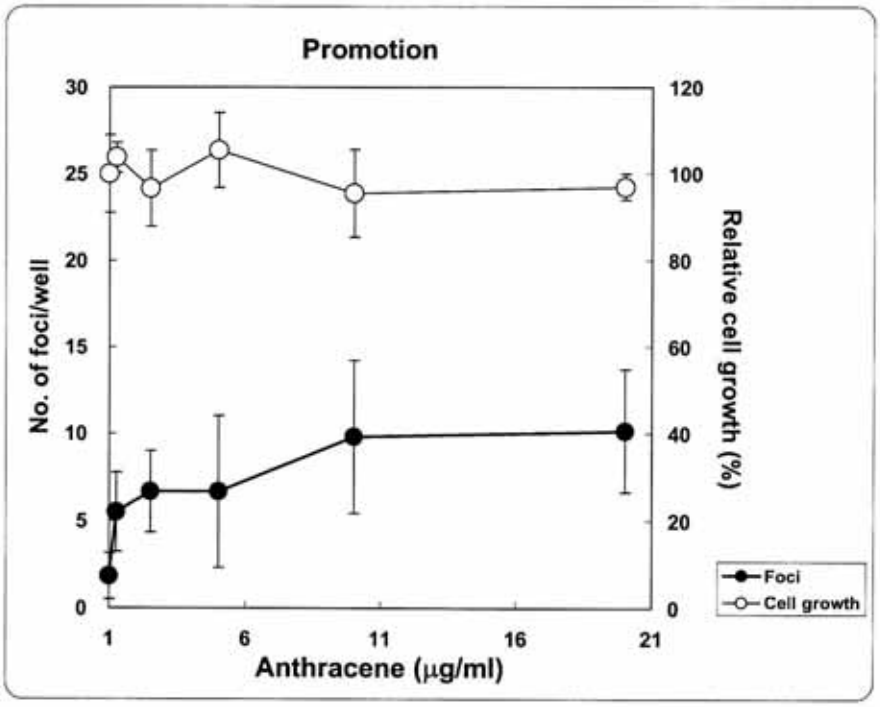
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Blank	1	1.25	2.5	5	10	20	DMSO 0.5%	TPA 50 ng/mL
OD	0.033	0.216	0.242	0.208	0.245	0.207	0.229	0.216	0.272
	0.031	0.252	0.248	0.23	0.262	0.22	0.233	0.252	0.314
	0.032	0.233	0.234	0.243	0.227	0.247	0.221	0.233	0.334
Average	0.032	0.234	0.241	0.227	0.245	0.225	0.228	0.234	0.307
SD	0.001	0.018	0.007	0.018	0.018	0.020	0.006	0.018	0.032
Average-Blank	0.000	0.202	0.209	0.195	0.213	0.193	0.196	0.202	0.275
Relative cell growth (%)		100.0	103.8	96.7	105.5	95.5	97.0	100.0	136.2
SD of relative cell growth(%)		8.9	3.5	8.8	8.7	10.1	3.0	8.9	15.7

Transformation Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Well No.	1	1.25	2.5	5	10	20	DMSO 0.5%	TPA 50 ng/mL
No of foci	1	3	5	10	13	13	14	3	15
	2	3	5	4	4	4	13	3	14
	3	0	8	9	10	6	13	0	19
	4	1	5	6	8	14	8	1	16
	5	1	2	5	2	14	7	1	10
	6	3	8	6	3	8	6	3	14
Average		1.8	5.5	6.7	6.7	9.8	10.2	1.8	14.7
SD		1.3	2.3	2.3	4.4	4.4	3.5	1.3	2.9
Dunnett test				*	*	***	***		
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Mannitol

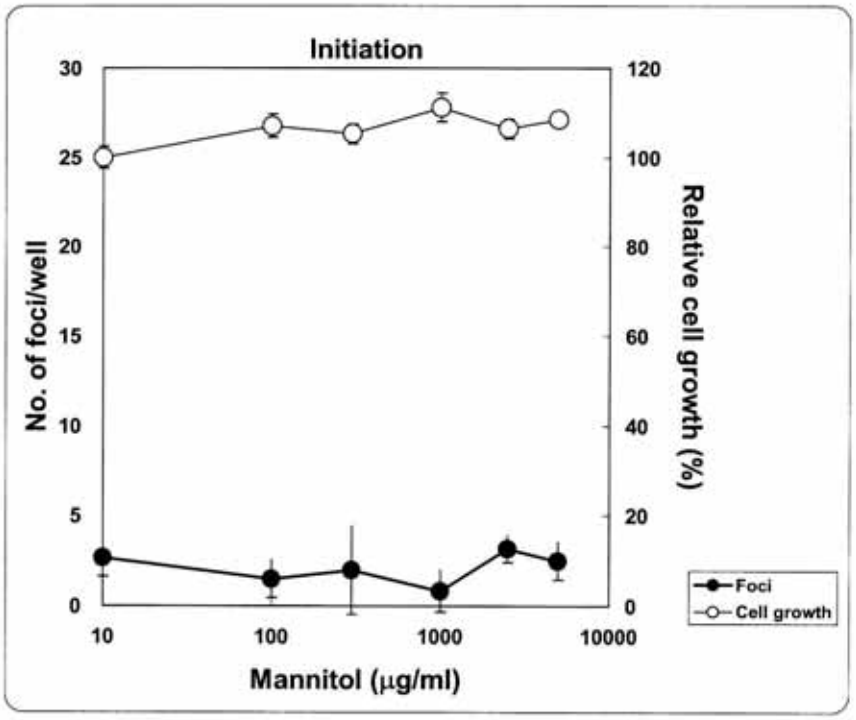
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%							
Compound (ug/ml)	Blank	10	100	300	1000	2500	5000	DMSO 0.1%	MCA 1 ug/mL
OD	0.073	0.937	1.027	1.003	1.061	0.996	1.035	1.12	0.702
	0.073	0.973	0.984	1.009	1.064	1.027	1.024	1.023	0.698
	0.077	0.935	1.02	0.973	1.015	0.991	1.009	1.022	0.72
Average	0.074	0.948	1.010	0.995	1.047	1.005	1.023	1.055	0.707
SD	0.002	0.021	0.023	0.019	0.027	0.020	0.013	0.056	0.012
Average-Blank	0.000	0.874	0.936	0.921	0.972	0.930	0.948	0.981	0.632
Relative cell growth (%)		100.0	107.1	105.3	111.3	106.4	108.5	100.0	64.5
SD of relative cell growth(%)		2.4	2.6	2.2	3.1	2.2	1.5	5.7	1.2

Transformation Assay	Solvent:	water 5%							
Compound (ug/ml)	Well No.	10	100	300	1000	2500	5000	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	3	0	1	3	3	3	3	33
	2	2	2	0	1	3	2	5	39
	3	1	3	6	1	2	4	3	35
	4	3	2	1	0	4	3	0	39
	5	3	1	0	0	4	1	2	28
	6	4	1	4	0	3	2	3	28
Average		2.7	1.5	2.0	0.8	3.2	2.5	2.7	33.7
SD		1.0	1.0	2.4	1.2	0.8	1.0	1.6	5.0
Dunnett test									N.S.
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab III

Compound:	Mannitol

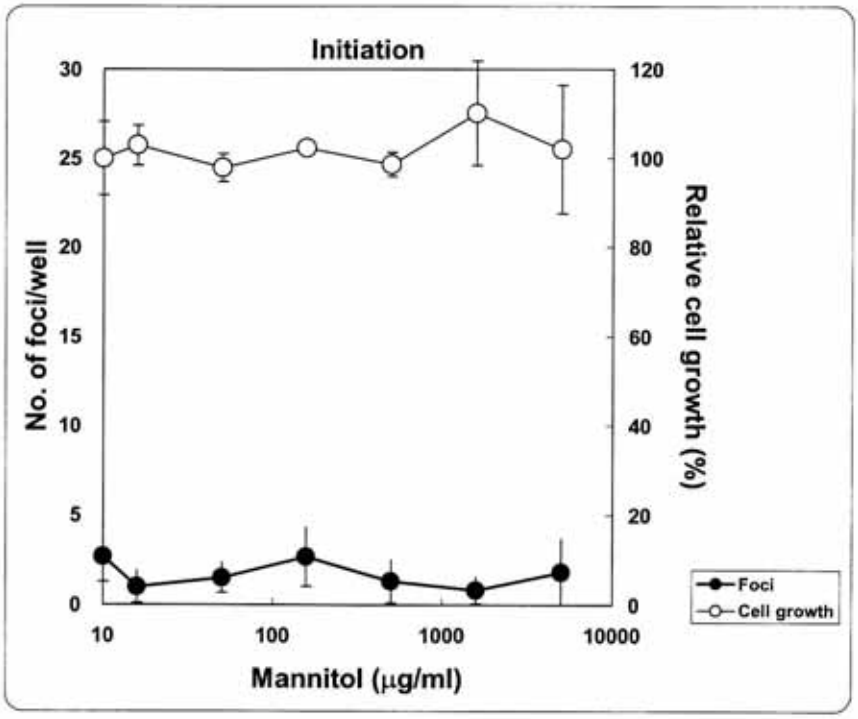
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Blank	10	15.8	50	158	500	1580	5000	DMSO 0.1%	MCA 1ug/mL
OD	0.198	2.694	2.45	2.382	2.567	2.411	3.029	2.925	-	0.937
	0.221	2.488	2.647	2.523	2.565	2.534	2.656	2.331	-	0.966
	0.227	2.318	2.606	2.449	2.525	2.459	2.51	2.385	-	1
Average	0.215	2.500	2.568	2.451	2.552	2.468	2.732	2.547	#DIV/0!	0.968
SD	0.015	0.188	0.104	0.071	0.024	0.062	0.268	0.328	#DIV/0!	0.032
Average-Blank	0.000	2.285	2.352	2.236	2.337	2.253	2.516	2.332	#DIV/0!	0.752
Relative cell growth (%)		100.0	103.0	97.9	102.3	98.6	110.1	102.1	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		8.2	4.5	3.1	1.0	2.7	11.7	14.4	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Well No.	10	15.8	50	158	500	1580	5000	DMSO 0.1%	MCA 1ug/mL
No of foci	1	2	2	2	5	0	1	0	-	11
	2	3	1	2	3	2	1	2	-	20
	3	1	0	2	2	3	0	5	-	19
	4	2	0	1	0	1	0	2	-	13
	5	5	1	0	3	2	2	2	-	10
	6	3	2	2	3	0	1	0	-	11
Average		2.7	1.0	1.5	2.7	1.3	0.8	1.8	#DIV/0!	14.0
SD		1.4	0.9	0.8	1.6	1.2	0.8	1.8	#DIV/0!	4.4
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch) vs 5% Water										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Mannitol

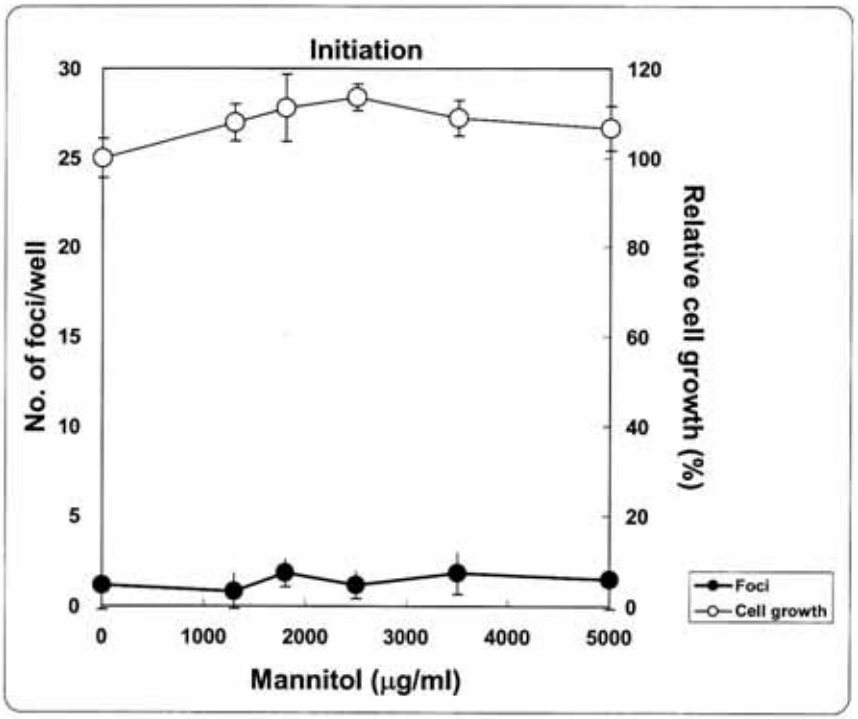
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DW 5%							
Compound (ug/ml)	Blank	0	1300	1800	2500	3500	5000	DMSO 0.1%	MCA 1 ug/mL
OD	0.063	0.285	0.324	0.307	0.336	0.322	0.321		0.224
	0.06	0.3	0.305	0.342	0.322	0.324	0.317		0.208
	0.059	0.305	0.317	0.32	0.328	0.307	0.299		0.226
Average	0.061	0.297	0.315	0.323	0.329	0.318	0.312	#DIV/0!	0.219
SD	0.002	0.010	0.010	0.018	0.007	0.009	0.012	#DIV/0!	0.010
Average-Blank	0.000	0.236	0.255	0.262	0.268	0.257	0.252	#DIV/0!	0.159
Relative cell growth (%)		100.0	107.9	111.2	113.6	108.9	106.6	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		4.4	4.1	7.5	3.0	3.9	5.0	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	DW 5%							
Compound (ug/ml)	Well No.	0	1300	1800	2500	3500	5000	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	3	2	1	1	3	4	2	33
	2	0	0	2	2	0	3	2	39
	3	2	1	1	2	3	1	0	38
	4	0	2	3	0	1	0	1	35
	5	0	0	2	1	2	0	1	43
	6	2	0	2	1	2	1	0	55
Average		1.2	0.8	1.8	1.2	1.8	1.5	1.0	40.5
SD		1.3	1.0	0.8	0.8	1.2	1.6	0.9	7.9
Dunnett test									N.S.
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Mannitol

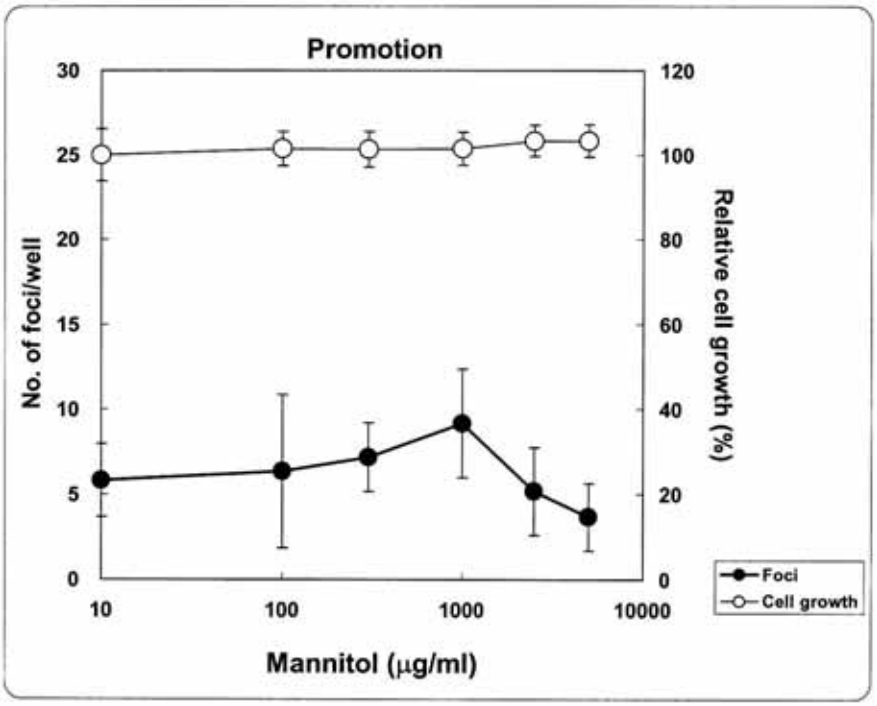
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%							
Compound (ug/ml)	Blank	10	100	300	1000	2500	5000	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.098	1.092	1.09	1.091	1.102	1.107	1.12	1.415
	0.073	0.983	1.021	1.04	1.032	1.032	1.039	1.048	1.388
	0.077	1.018	1.029	1.01	1.02	1.062	1.049	1.06	1.446
Average	0.074	1.033	1.047	1.047	1.048	1.065	1.065	1.076	1.416
SD	0.002	0.059	0.039	0.040	0.038	0.035	0.037	0.039	0.029
Average-Blank	0.000	0.959	0.973	0.972	0.973	0.991	0.991	1.002	1.342
Relative cell growth (%)		100.0	101.5	101.4	101.5	103.4	103.3	100.0	134.0
SD of relative cell growth(%)		6.1	4.1	4.2	4.0	3.7	3.8	3.9	2.9

Transformation Assay	Solvent:	water 5%								
Compound (ug/ml)	Well No.	10	100	300	1000	2500	5000	DMSO 0.1%	TPA 50 ng/mL	
No of foci	1	9	8	7	13	3	5	12	51	
	2	6	2	8	13	7	1	5	48	
	3	3	14	10	8	3	2	10	42	
	4	4	2	4	5	9	6	4	52	
	5	7	5	6	8	3	5	12	53	
	6	6	7	8	8	6	3	6	55	
Average		5.8	6.3	7.2	9.2	5.2	3.7	8.2	50.2	
SD		2.1	4.5	2.0	3.2	2.6	2.0	3.6	4.6	
Dunnett test										N.S.
t-Test (Assuming Equal Variances)									***	

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab Ⅲ
Compound:	Mannitol

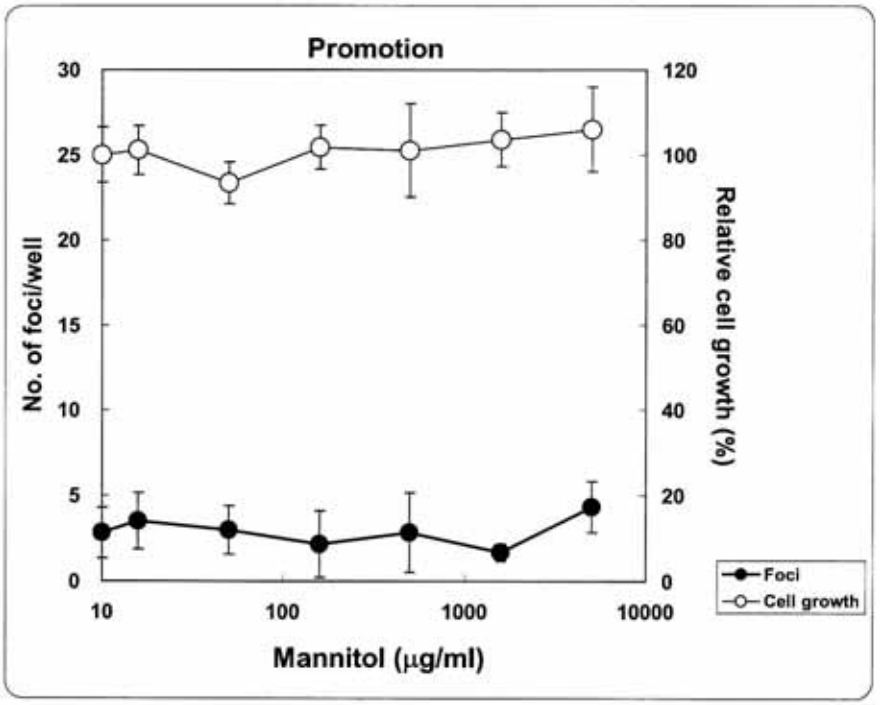
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Blank	10	15.8	50	158	500	1580	5000	DMSO 0.1%	TPA 50 ng/mL
OD	0.171	2.388	2.393	1.984	2.151	2.513	2.172	2.59	-	3.491
	0.196	2.172	2.209	2.164	2.336	2.099	2.431	2.259	-	3.369
	0.218	2.146	2.172	2.154	2.33	2.158	2.325	2.222	-	3.339
平均	0.195	2.235	2.258	2.101	2.272	2.257	2.309	2.357	#DIV/0!	3.400
SD	0.024	0.133	0.118	0.101	0.105	0.224	0.130	0.203	#DIV/0!	0.081
Average-Blank	0.000	2.040	2.063	1.906	2.077	2.062	2.114	2.162	#DIV/0!	3.205
Relative cell growth (%)		100.0	101.1	93.4	101.8	101.0	103.6	106.0	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		6.5	5.8	5.0	5.2	11.0	6.4	9.9	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	D.W. 5 %								
Compound (ug/ml)	Well No.	10	15.8	50	158	500	1580	5000	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	3	5	0	3	1	4	-	12
	2	2	1	3	1	3	1	3	-	12
	3	2	3	4	2	0	2	5	-	10
	4	1	6	2	1	2	2	4	-	6
	5	4	4	3	4	2	2	7	-	9
	6	5	4	1	5	7	2	3	-	3
Average		2.8	3.5	3.0	2.2	2.8	1.7	4.3	#DIV/0!	8.7
SD		1.5	1.6	1.4	1.9	2.3	0.5	1.5	#DIV/0!	3.6
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch) vs 5% Water										**

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV
Compound:	Mannitol

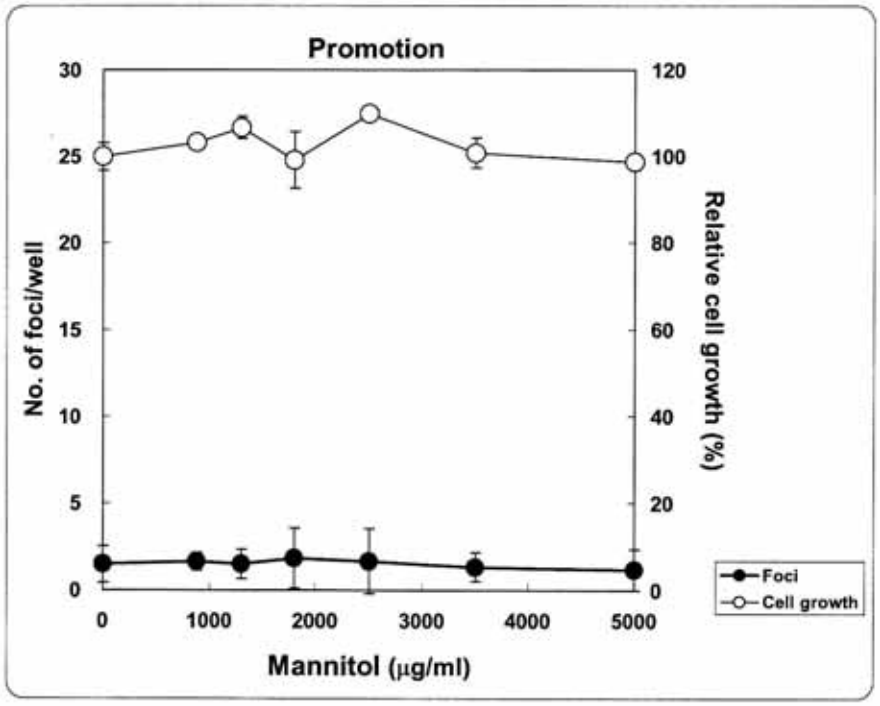
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DW 5%								
Compound (ug/ml)	Blank	0	880	1300	1800	2500	3500	5000	DMSO 0.1%	TPA 50 ng/mL
OD	0.063	0.272	0.273	0.282	0.275	0.286	0.275	0.267		0.305
	0.06	0.259	0.274	0.282	0.267	0.282	0.262	0.261		0.305
	0.059	0.265	0.269	0.273	0.249	0.289	0.264	0.26		0.297
Average	0.061	0.265	0.272	0.279	0.264	0.286	0.267	0.263	#DIV/0!	0.302
SD	0.002	0.007	0.003	0.005	0.013	0.004	0.007	0.004	#DIV/0!	0.005
Average-Blank	0.000	0.205	0.211	0.218	0.203	0.225	0.206	0.202	#DIV/0!	0.242
Relative cell growth (%)		100.0	103.3	106.7	99.2	109.9	100.8	98.7	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		3.2	1.3	2.5	6.5	1.7	3.4	1.8	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	DW 5%								
Compound (ug/ml)	Well No.	0	880	1300	1800	2500	3500	5000	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	1	2	2	2	0	2	1	4	9
	2	0	2	1	1	2	1	3	1	11
	3	3	1	2	0	1	0	0	2	12
	4	2	1	0	1	0	2	1	3	8
	5	1	2	2	2	5	2	0	2	13
	6	2	2	2	5	2	1	2	2	8
Average		1.5	1.7	1.5	1.8	1.7	1.3	1.2	2.3	10.2
SD		1.0	0.5	0.8	1.7	1.9	0.8	1.2	1.0	2.1
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I

Compound:	Caffeine

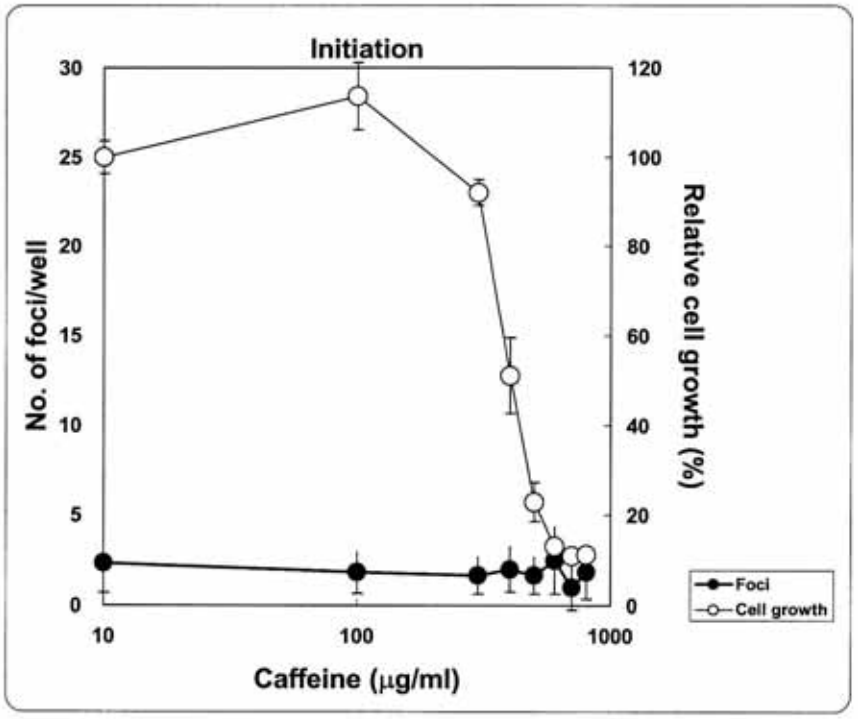
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	10	100	300	400	500	600	700	800	DMSO 0.1%	MCA 1 ug/mL
OD	0.08	1.009	1.258	1	0.667	0.341	0.213	0.202	0.204	1.159	0.639
	0.088	1.052	1.119	0.966	0.554	0.318	0.211	0.183	0.189	1.173	0.654
	0.09	1.079	1.155	0.944	0.51	0.26	0.213	0.189	0.188	1.243	0.529
Average	0.086	1.047	1.177	0.970	0.577	0.306	0.212	0.191	0.194	1.192	0.607
SD	0.005	0.035	0.072	0.028	0.081	0.042	0.001	0.010	0.009	0.045	0.068
Average-Blank	0.000	0.961	1.091	0.884	0.491	0.220	0.126	0.105	0.108	1.106	0.521
Relative cell growth (%)		100.0	113.6	92.0	51.1	22.9	13.2	11.0	11.2	100.0	47.2
SD of relative cell growth(%)		3.7	7.5	2.9	8.4	4.3	0.1	1.0	0.9	4.1	6.2

Transformation Assay	Solvent:	water 5%									
Compound (ug/ml)	Well No.	10	100	300	400	500	600	700	800	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	1	1	2	4	2	1	0	4	0	19
	2	3	3	1	2	0	2	2	1	0	15
	3	5	2	3	0	1	2	0	3	1	17
	4	3	2	2	2	2	3	0	0	3	18
	5	1	3	0	2	2	1	1	1	2	25
	6	1	0	2	2	3	6	3	2	2	24
Average		2.3	1.8	1.7	2.0	1.7	2.5	1.0	1.8	1.3	19.7
SD		1.6	1.2	1.0	1.3	1.0	1.9	1.3	1.5	1.2	4.0
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V

Compound:	Caffeine

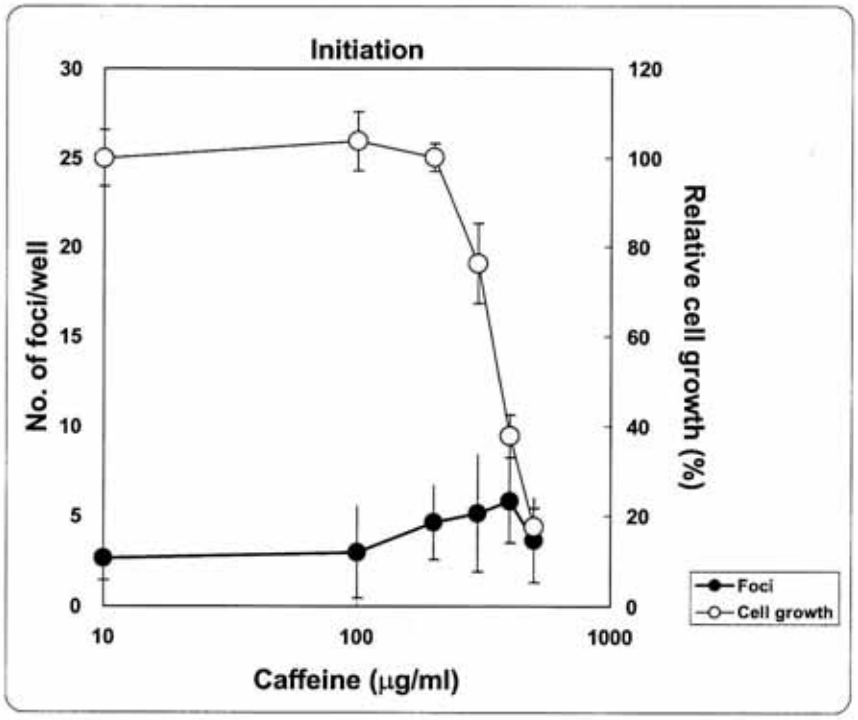
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%							
Compound (ug/ml)	Blank	10	100	200	300	400	500	DMSO 0.1%	MCA 1 ug/mL
OD	0.0628	1.0052	1.0452	1.0831	0.8255	0.4196	0.2911	0.8554	0.5062
	0.0707	1.1261	1.1784	1.1084	0.9364	0.4261	0.2435	0.9557	0.5617
	0.0747	1.1027	1.1232	1.0451	0.7562	0.5056	0.2084	0.9029	0.4859
Average	0.069	1.078	1.116	1.079	0.839	0.450	0.248	0.905	0.518
SD	0.006	0.064	0.067	0.032	0.091	0.048	0.042	0.050	0.039
Average-Blank	0.000	1.009	1.046	1.009	0.770	0.381	0.178	0.835	0.449
Relative cell growth (%)		100.0	103.7	100.1	76.3	37.8	17.7	100.0	53.7
SD of relative cell growth(%)		6.4	6.6	3.2	9.0	4.7	4.1	6.0	4.7

Transformation Assay	Solvent:	Water 5%							
Compound (ug/ml)	Well No.	10	100	200	300	400	500	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	4	6	7	7	8	5	4	31
	2	1	2	5	0	9	4	2	45
	3	2	1	3	7	3	0	1	28
	4	4	3	7	5	6	3	4	49
	5	3	0	2	3	4	7	6	45
	6	2	6	4	9	5	3	3	50
Average		2.7	3.0	4.7	5.2	5.8	3.7	3.3	41.3
SD		1.2	2.5	2.1	3.3	2.3	2.3	1.8	9.4
Dunnett test									N.S.
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI
Compound:	Caffeine

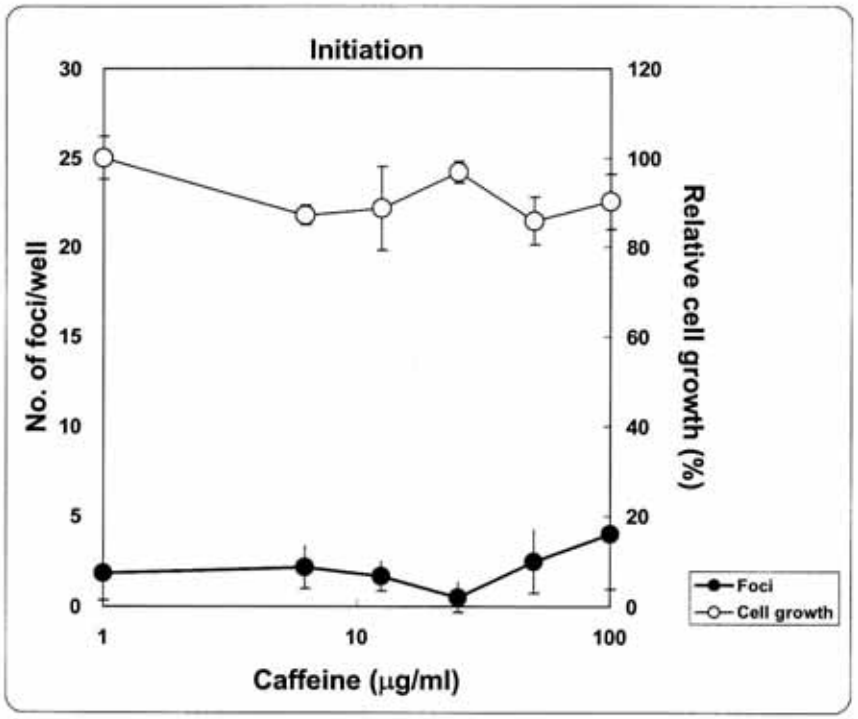
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DMSO 0.5%							DMSO 0.5%	MCA 1 ug/mL
Compound (ug/ml)	Blank	1	6.25	12.5	25	50	100			
OD	0.033	0.342	0.293	0.267	0.316	0.28	0.303		0.342	0.181
	0.032	0.316	0.291	0.29	0.31	0.272	0.312		0.316	0.203
	0.031	0.32	0.281	0.322	0.324	0.302	0.277		0.32	0.202
Average	0.032	0.326	0.288	0.293	0.317	0.285	0.297	#DIV/0!	0.326	0.195
SD	0.001	0.014	0.006	0.028	0.007	0.016	0.018	#DIV/0!	0.014	0.012
Average-Blank	0.000	0.294	0.256	0.261	0.285	0.253	0.265	#DIV/0!	0.294	0.163
Relative cell growth (%)		100.0	87.2	88.8	96.8	85.9	90.2	#DIV/0!	100.0	55.6
SD of relative cell growth(%)		4.8	2.2	9.4	2.4	5.3	6.2	#DIV/0!	4.8	4.2

Transformation Assay	Solvent:	DMSO 0.5%							DMSO 0.5%	MCA 1 ug/mL	
Compound (ug/ml)	Well No.	1	6.25	12.5	25	50	100				
No of foci	1	3	3	2	0	5	3		3	19	
	2	1	1	2	0	0	2		1	16	
	3	0	2	1	0	1	10		0	17	
	4	1	4	3	0	3	2		1	23	
	5	4	2	1	1	3	3		4	15	
	6	2	1	1	2	3	4		2	13	
Average		1.8	2.2	1.7	0.5	2.5	4.0	#DIV/0!	1.8	17.2	
SD		1.5	1.2	0.8	0.8	1.8	3.0	#DIV/0!	1.5	3.5	
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Caffeine

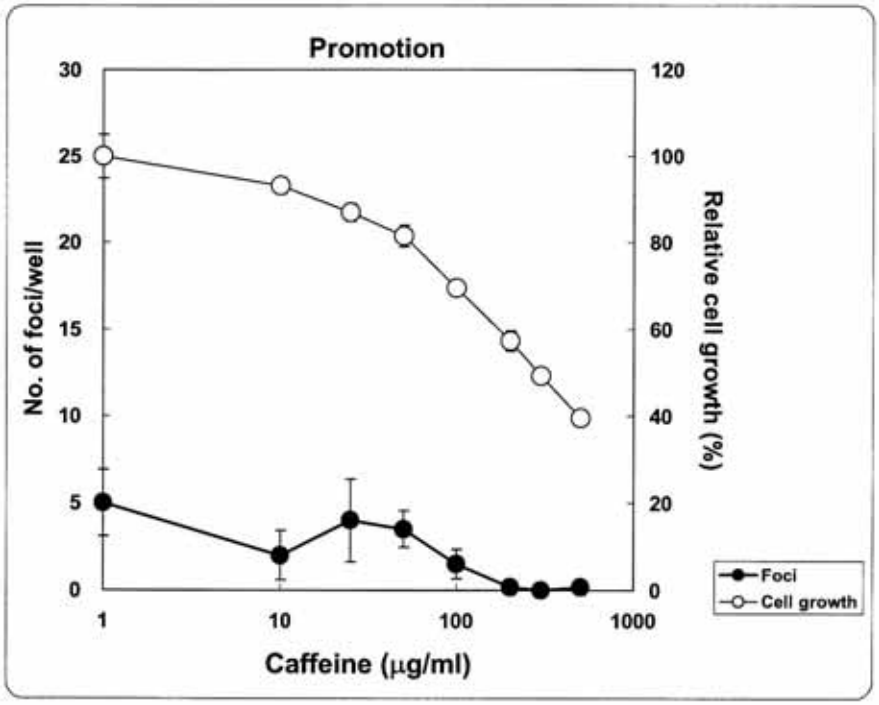
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%									
Compound (ug/ml)	Blank	1	10	25	50	100	200	300	500	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.096	0.999	0.943	0.894	0.759	0.625	0.56	0.473	1.069	1.257
	0.073	1.061	0.964	0.927	0.874	0.757	0.661	0.55	0.452	1.048	1.238
	0.077	0.998	0.991	0.902	0.846	0.745	0.617	0.555	0.456	1.029	1.271
Average	0.074	1.052	0.985	0.924	0.871	0.754	0.634	0.555	0.460	1.049	1.255
SD	0.002	0.050	0.018	0.021	0.024	0.008	0.023	0.005	0.011	0.020	0.017
Average-Blank	0.000	0.977	0.910	0.850	0.797	0.679	0.560	0.481	0.386	0.974	1.181
Relative cell growth (%)		100.0	93.1	86.9	81.5	69.5	57.3	49.2	39.5	100.0	121.2
SD of relative cell growth(%)		5.1	1.9	2.1	2.5	0.8	2.4	0.5	1.1	2.1	1.7

Transformation Assay	Solvent:	water 5%										
Compound (ug/ml)	Well No.	1	10	25	50	100	200	300	500	DMSO 0.1%	TPA 50 ng/mL	
No of foci	1	3	1	3	3	2	0	0	0	4	29	
	2	3	0	1	4	0	0	0	1	4	31	
	3	6	3	8	3	2	1	0	0	9	46	
	4	5	2	3	4	1	0	0	0	6	33	
	5	5	4	4	2	2	0	0	0	8	37	
	6	8	2	5	5	2	0	0	0	9	37	
Average		5.0	2.0	4.0	3.5	1.5	0.2	0.0	0.2	6.7	35.5	
SD		1.9	1.4	2.4	1.0	0.8	0.4	0.0	0.4	2.3	6.1	
Dunnet test												N.S.
t-Test for Unequal Variances (Aspin-Welch)											***	

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab V

Compound:	Caffeine

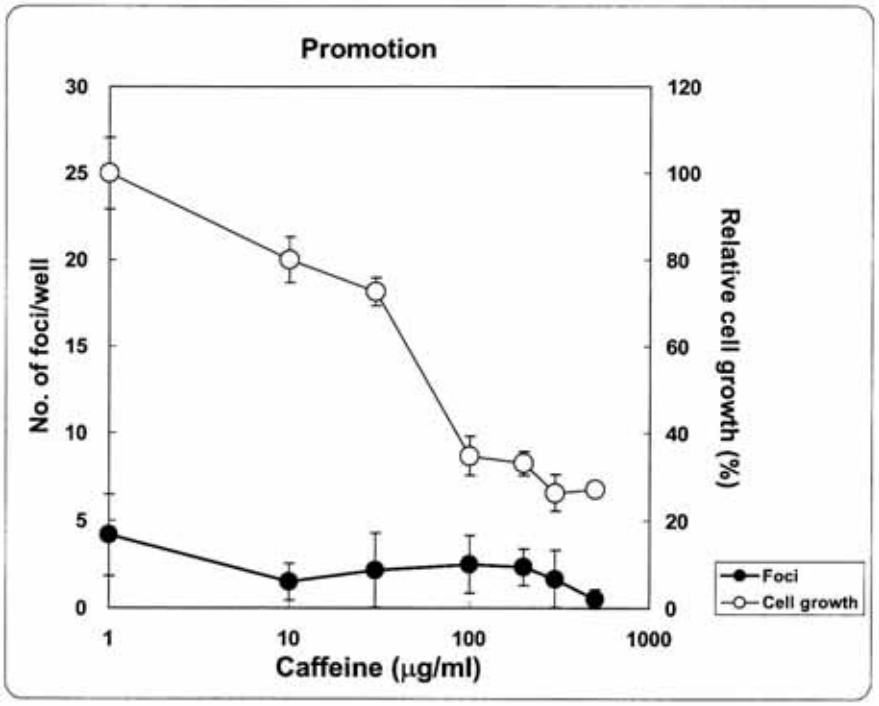
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%								
Compound (ug/ml)	Blank	1	10	30	100	200	300	500	DMSO 0.1%	TPA 50 ng/mL
OD	0.0659	0.9931	0.794	0.7031	0.3734	0.3739	0.3161	0.2948	0.7554	0.7851
	0.0703	0.9022	0.7417	0.6978	0.323	0.349	0.2525	0.3135	0.7473	0.8139
	0.0744	0.8544	0.7052	0.6533	0.3971	0.3285	0.3106	0.2929	0.7076	0.7309
Average	0.070	0.917	0.747	0.685	0.365	0.350	0.293	0.300	0.737	0.777
SD	0.004	0.070	0.045	0.027	0.038	0.023	0.035	0.011	0.026	0.042
Average-Blank	0.000	0.846	0.677	0.615	0.294	0.280	0.223	0.230	0.667	0.706
Relative cell growth (%)		100.0	80.0	72.6	34.8	33.1	26.3	27.2	100.0	106.0
SD of relative cell growth(%)		8.3	5.3	3.2	4.5	2.7	4.2	1.3	3.8	6.3

Transformation Assay	Solvent:	Water 5%								
Compound (ug/ml)	Well No.	1	10	30	100	200	300	500	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	4	1	5	3	1	2	1	3	17
	2	1	2	4	2	3	0	0	2	25
	3	6	3	0	0	3	1	1	2	22
	4	2	0	3	5	3	0	0	1	26
	5	7	2	1	3	1	4	0	2	20
	6	5	1	0	2	3	3	1	6	19
Average		4.2	1.5	2.2	2.5	2.3	1.7	0.5	2.7	21.5
SD		2.3	1.0	2.1	1.6	1.0	1.6	0.5	1.8	3.5
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab VI

Compound:	Caffeine

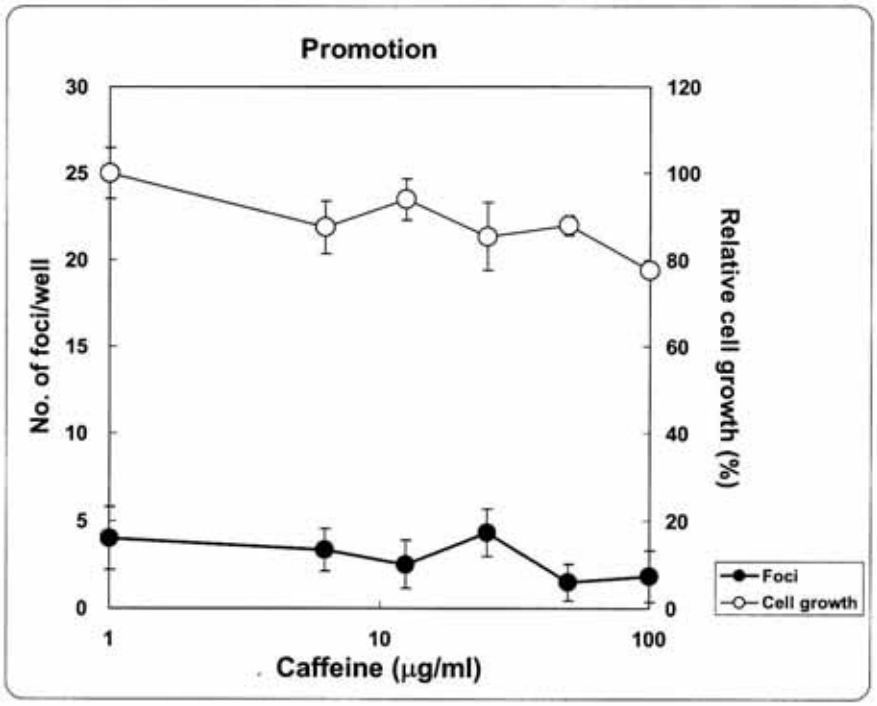
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Blank	1	6.25	12.5	25	50	100	DMSO 0.5%	TPA 50 ng/mL
OD	0.033	0.268	0.234	0.229	0.231	0.227	0.205	0.268	0.315
	0.032	0.253	0.235	0.249	0.233	0.233	0.206	0.253	0.377
	0.032	0.242	0.211	0.245	0.202	0.223	0.203	0.242	0.342
Average	0.032	0.254	0.227	0.241	0.222	0.228	0.205	0.254	0.345
SD	0.001	0.013	0.014	0.011	0.017	0.005	0.002	0.013	0.031
Average-Blank	0.000	0.222	0.194	0.209	0.190	0.195	0.172	0.222	0.312
Relative cell growth (%)		100.0	87.5	94.0	85.4	88.0	77.6	100.0	140.7
SD of relative cell growth(%)		5.9	6.1	4.8	7.8	2.3	0.7	5.9	14.0

Transformation Assay	Solvent:	DMSO 0.5%							
Compound (ug/ml)	Well No.	1	6.25	12.5	25	50	100	DMSO 0.5%	TPA 50 ng/mL
No of foci	1	5	2	2	2	1	2	5	18
	2	4	3	2	6	2	0	4	15
	3	2	4	1	4	2	4	2	11
	4	3	5	2	5	0	1	3	14
	5	3	4	3	5	1	1	3	25
	6	7	2	5	4	3	3	7	16
Average		4.0	3.3	2.5	4.3	1.5	1.8	4.0	16.5
SD		1.8	1.2	1.4	1.4	1.0	1.5	1.8	4.8
Dunnnett test									N.S.
t-Test for Unequal Variances (Aspin-Welch)									***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Ascorbic acid

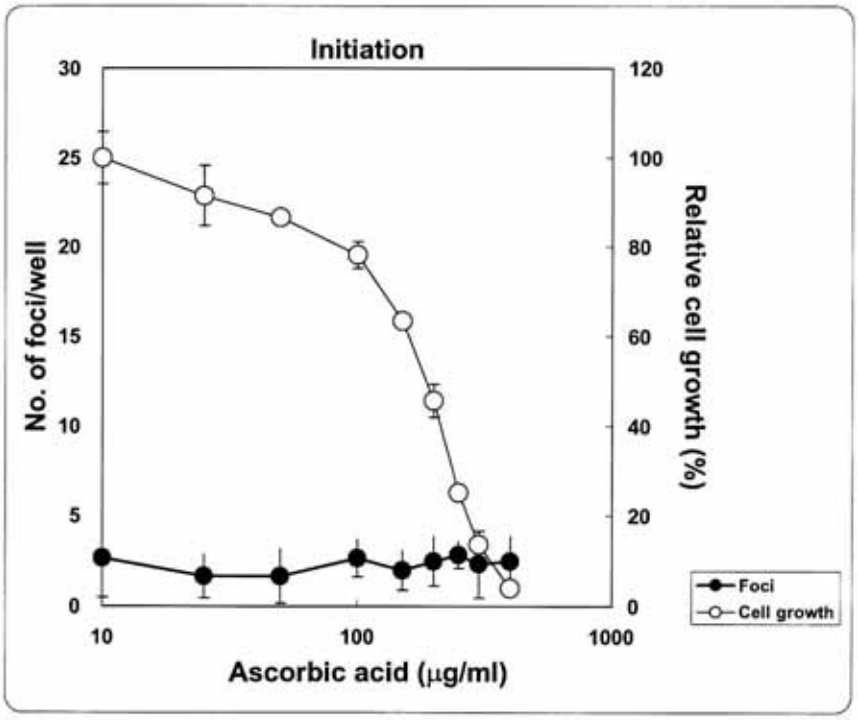
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%										
Compound (ug/ml)	Blank	10	25	50	100	150	200	250	300	400	DMSO 0.1%	MCA 1 ug/mL
OD	0.08	1.339	1.26	1.1	1.045	0.847	0.677	0.398	0.209	0.14	1.265	0.613
	0.088	1.278	1.105	1.126	1.023	0.849	0.595	0.369	0.256	0.132	1.245	0.636
	0.09	1.201	1.15	1.115	0.975	0.822	0.609	0.387	0.279	0.13	1.163	0.65
Average	0.086	1.273	1.172	1.114	1.014	0.839	0.627	0.385	0.248	0.134	1.224	0.633
SD	0.005	0.069	0.080	0.013	0.036	0.015	0.044	0.015	0.036	0.005	0.054	0.019
Average-Blank	0.000	1.187	1.086	1.028	0.928	0.753	0.541	0.299	0.162	0.048	1.138	0.547
Relative cell growth (%)		100.0	91.5	86.6	78.2	63.5	45.6	25.2	13.7	4.0	100.0	48.1
SD of relative cell growth(%)		5.8	6.7	1.1	3.0	1.3	3.7	1.2	3.0	0.4	4.7	1.6

Transformation Assay	Solvent:	Water										
Compound (ug/ml)	Well No.	10	25	50	100	150	200	250	300	400	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	4	1	4	3	0	3	3	1	4	5	35
	2	0	1	1	3	2	1	3	6	1	2	25
	3	4	2	0	2	2	4	3	2	4	1	27
	4	3	0	1	4	3	4	2	1	2	3	23
	5	5	3	1	1	3	1	4	2	1	1	20
	6	0	3	3	3	2	2	2	2	3	2	30
Average		2.7	1.7	1.7	2.7	2.0	2.5	2.8	2.3	2.5	2.3	26.7
SD		2.2	1.2	1.5	1.0	1.1	1.4	0.8	1.9	1.4	1.5	5.3
Dunnett test												N.S.
t-Test for Unequal Variances (Aspin-Welch)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Ascorbic acid

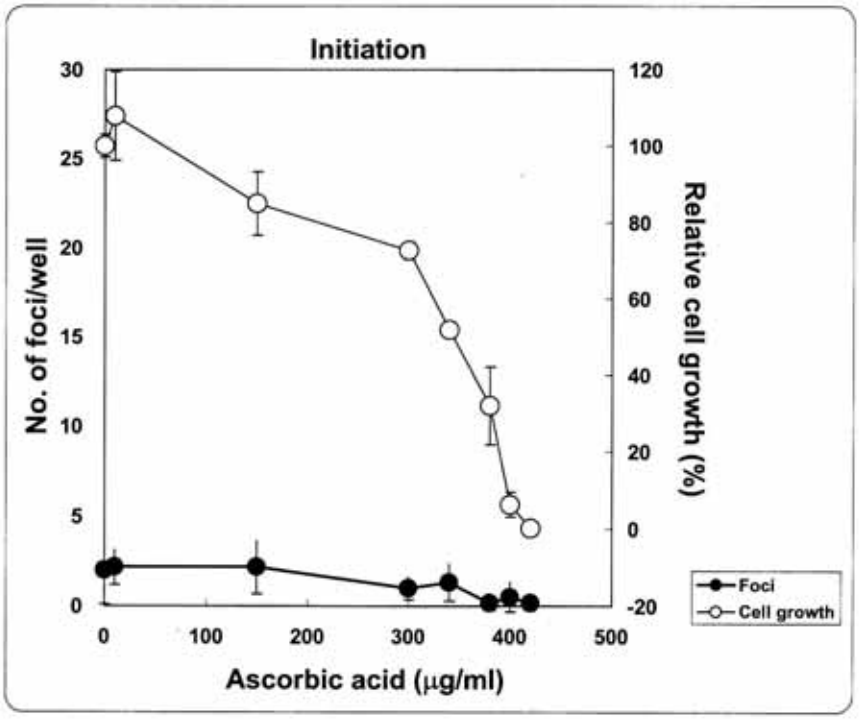
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	Water 5%									
Compound (ug/ml)	Blank	0	10	150	300	340	380	400	420	DMSO 0.1%	MCA 1 ug/mL
OD	0.0818	0.8058	0.7894	0.6374	0.5769	0.4324	0.3673	0.1482	0.0848	0.6681	0.365
	0.0852	0.7641	0.8019	0.6593	0.6011	0.4616	0.2297	0.1034	0.0909	0.6958	0.4466
	0.0854	0.7924	0.9369	0.7478	0.6074	0.4524	0.3323	0.1332	0.0809	0.65	0.4105
Average	0.084	0.787	0.843	0.682	0.595	0.449	0.310	0.128	0.086	0.671	0.407
SD	0.002	0.021	0.082	0.058	0.016	0.015	0.072	0.023	0.005	0.023	0.041
Average-Blank	0.000	0.703	0.759	0.597	0.511	0.365	0.226	0.044	0.001	0.587	0.323
Relative cell growth (%)		100.0	107.9	84.9	72.7	51.9	32.1	6.3	0.2	100.0	55.0
SD of relative cell growth(%)		3.0	11.6	8.3	2.3	2.1	10.2	3.2	0.7	3.9	7.0

Transformation Assay	Solvent:	Water 5%									
Compound (ug/ml)	Well No.	0	10	150	300	340	380	400	420	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	2	1	2	1	1	0	0	0	2	13
	2	3	2	3	1	0	0	1	0	1	8
	3	0	3	1	1	3	0	2	1	1	22
	4	2	3	3	0	1	1	0	0	3	14
	5	0	1	4	2	2	0	0	0	0	17
	6	5	3	0	1	1	0	0	0	1	21
Average		2.0	2.2	2.2	1.0	1.3	0.2	0.5	0.2	1.3	15.8
SD		1.9	1.0	1.5	0.6	1.0	0.4	0.8	0.4	1.0	5.3
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV
Compound:	Ascorbic acid

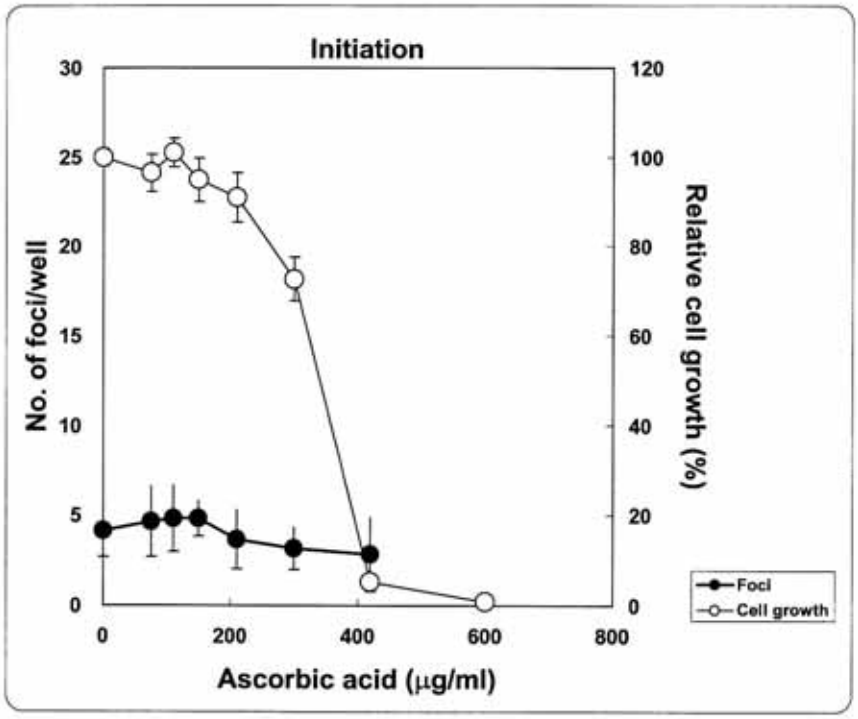
Toransformation Assay

Initiation

Cell Growth Assay	Solvent:	DW 5%									
Compound (ug/ml)	Blank	0	75	110	150	210	300	420	600	DMSO 0.1%	MCA 1 ug/mL
OD	0.039	0.355	0.354	0.37	0.355	0.342	0.291	0.057	0.041		0.114
	0.039	0.36	0.36	0.352	0.326	0.342	0.261	0.06	0.043		0.105
	0.04	0.367	0.334	0.37	0.352	0.311	0.267	0.052	0.042		0.105
Average	0.039	0.361	0.349	0.364	0.344	0.332	0.273	0.056	0.042	#DIV/0!	0.108
SD	0.001	0.006	0.014	0.010	0.016	0.018	0.016	0.004	0.001	#DIV/0!	0.005
Average-Blank	0.000	0.321	0.310	0.325	0.305	0.292	0.234	0.017	0.003	#DIV/0!	0.069
Relative cell growth (%)		100.0	96.5	101.0	94.9	91.0	72.7	5.3	0.8	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		1.9	4.2	3.2	5.0	5.6	4.9	1.3	0.3	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	DW 5%									
Compound (ug/ml)	Well No.	0	75	110	150	210	300	420	600	DMSO 0.1%	MCA 1 ug/mL
No of foci	1	3	8	3	4	2	3	1	Toxic	3	48
	2	3	5	5	4	6	4	5	Toxic	7	40
	3	4	5	7	5	3	2	4	Toxic	4	41
	4	6	4	3	4	4	5	5	Toxic	6	44
	5	6	2	4	6	2	3	1	Toxic	8	46
	6	3	4	7	6	5	2	1	Toxic	5	43
Average		4.2	4.7	4.8	4.8	3.7	3.2	2.8		5.5	43.7
SD		1.5	2.0	1.8	1.0	1.6	1.2	2.0		1.9	3.0
Parametric Dunnett t-Test (Assuming Equal Variances)											N.S. ***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab I
Compound:	Ascorbic acid

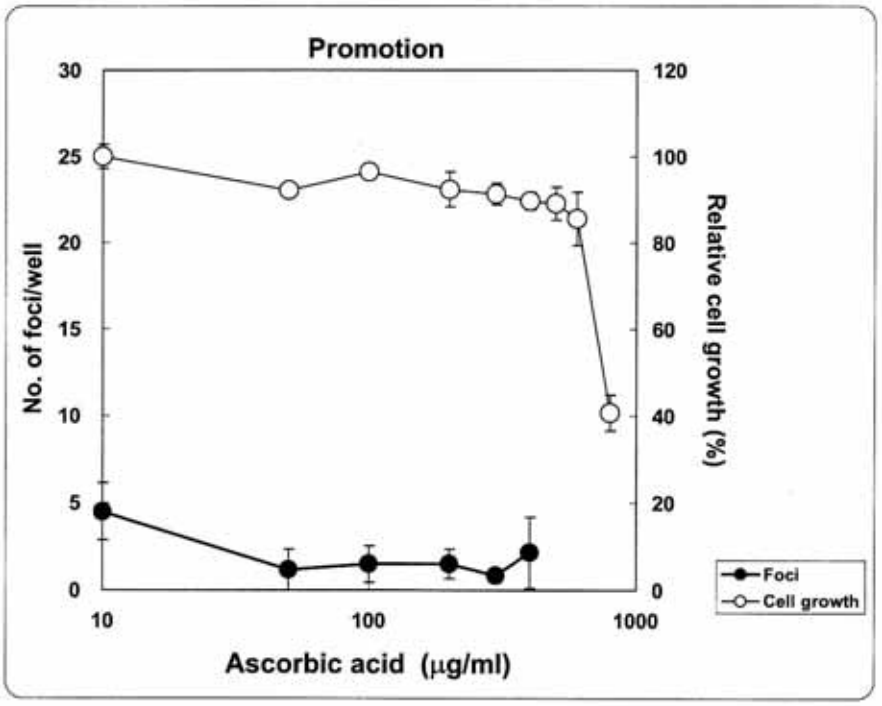
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%										
Compound (ug/ml)	Blank	10	50	100	200	300	400	500	600	800	DMSO 0.1%	TPA 50 ng/mL
OD	0.073	1.148	1.068	1.12	1.04	1.063	1.044	0.99	0.949	0.557	1.108	1.409
	0.073	1.121	1.049	1.107	1.048	1.027	1.015	1.036	0.965	0.512	1.058	1.298
	0.077	1.182	1.082	1.109	1.118	1.08	1.059	1.072	1.071	0.469	1.076	1.231
Average	0.074	1.150	1.066	1.112	1.069	1.057	1.039	1.033	0.995	0.513	1.081	1.313
SD	0.002	0.031	0.017	0.007	0.043	0.027	0.022	0.041	0.066	0.044	0.025	0.090
Average-Blank	0.000	1.076	0.992	1.038	0.994	0.982	0.965	0.958	0.921	0.438	1.006	1.238
Relative cell growth (%)		100.0	92.2	96.4	92.4	91.3	89.7	89.1	85.6	40.7	100.0	123.1
SD of relative cell growth(%)		2.8	1.5	0.7	4.0	2.5	2.1	3.8	6.2	4.1	2.5	8.9

Transformation Assay	Solvent:	Water 5%										
Compound (ug/ml)	Well No.	10	50	100	200	300	400	500	600	800	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	4	0	2	1	1	6	Tox	ToX	ToX	3	35
	2	5	1	3	1	1	1	Tox	ToX	ToX	9	30
	3	5	1	1	1	1	1	Tox	ToX	ToX	12	43
	4	2	3	2	2	0	1	Tox	ToX	ToX	4	35
	5	4	0	0	1	1	3	Tox	ToX	ToX	5	40
	6	7	2	1	3	1	1	Tox	ToX	ToX	12	40
Average		4.5	1.2	1.5	1.5	0.8	2.2				7.5	37.2
SD		1.6	1.2	1.0	0.8	0.4	2.0				4.0	4.7
Dunnett test												N.S.
t-Test (Assuming Equal Variances)												***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II
Compound:	Ascorbic acid

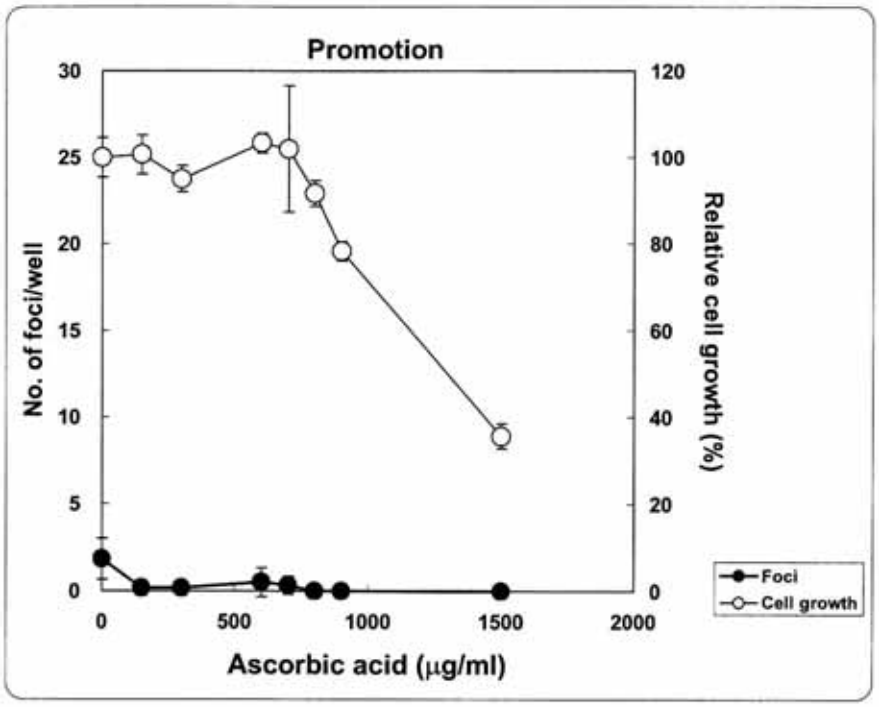
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	Water 5%									
Compound (ug/ml)	Blank	0	150	300	600	700	800	900	1500	DMSO 0.1%	TPA 50 ng/mL
OD	0.075	0.5394	0.5639	0.5204	0.5621	0.5078	0.4843	0.4388	0.2523	0.5052	0.7007
	0.0765	0.5567	0.5306	0.524	0.554	0.6241	0.5111	0.4453	0.24	0.5333	0.7167
	0.0739	0.5151	0.5263	0.4986	0.5416	0.5067	0.5007	0.4253	0.2259	0.4879	0.68
平均	0.075	0.537	0.540	0.514	0.553	0.546	0.499	0.436	0.239	0.509	0.699
SD	0.001	0.021	0.021	0.014	0.010	0.067	0.014	0.010	0.013	0.023	0.018
Average-Blank	0.000	0.462	0.465	0.439	0.477	0.471	0.424	0.361	0.164	0.434	0.624
Relative cell growth (%)		100.0	100.7	95.1	103.4	102.0	91.7	78.2	35.6	100.0	143.9
SD of relative cell growth(%)		4.5	4.5	3.0	2.2	14.6	2.9	2.2	2.9	5.3	4.2

Transformation Assay	Solvent:	Water 5%									
Compound (ug/ml)	Well No.	0	150	300	600	700	800	900	1500	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	3	1	0	0	0	0	0	0	3	11
	2	1	0	0	2	0	0	0	0	4	11
	3	2	0	0	0	0	0	0	0	1	12
	4	3	0	0	0	1	0	0	0	6	10
	5	2	0	1	0	0	0	0	0	4	12
	6	0	0	0	1	1	0	0	0	1	11
Average		1.8	0.2	0.2	0.5	0.3	0.0	0.0	0.0	3.2	11.2
SD		1.2	0.4	0.4	0.8	0.5	0.0	0.0	0.0	1.9	0.8
Dunnett test											N.S.
t-Test for Unequal Variances (Aspin-Welch)											***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab II

Compound:	Ascorbic acid

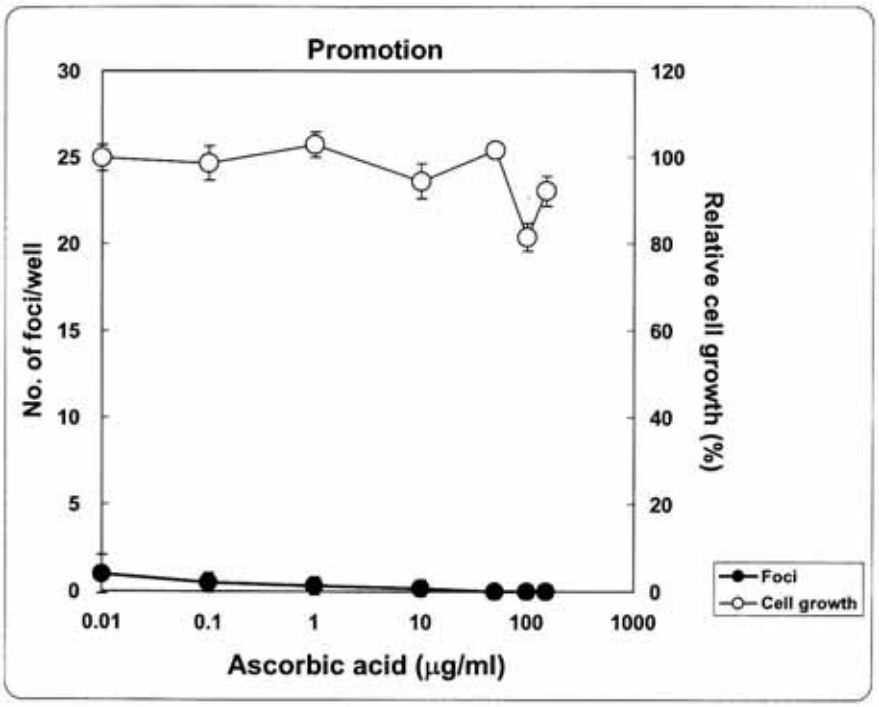
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	water 5%								
Compound (ug/ml)	Blank	0.01	0.1	1	10	50	100	150	DMSO 0.1%	TPA 50 ng/mL
OD	0.0595	0.358	0.3564	0.3605	0.3561	0.361	0.3122	0.3374	0.35	0.4836
	0.0598	0.3715	0.3695	0.3728	0.3463	0.3669	0.3098	0.3483	0.3514	0.5203
	0.0597	0.3542	0.3457	0.3774	0.3316	0.3706	0.2944	0.3272	0.3301	0.5025
Average	0.060	0.361	0.357	0.370	0.345	0.366	0.305	0.338	0.344	0.502
SD	0.000	0.009	0.012	0.009	0.012	0.005	0.010	0.011	0.012	0.018
Average-Blank	0.000	0.302	0.298	0.311	0.285	0.307	0.246	0.278	0.284	0.442
Relative cell growth (%)		100.0	98.7	103.0	94.5	101.6	81.5	92.2	100.0	155.7
SD of relative cell growth(%)		3.0	4.0	2.9	4.1	1.6	3.2	3.5	4.2	6.5

Transformation Assay	Solvent:	water 5%								
Compound (ug/ml)	Well No.	0.01	0.1	1	10	50	100	150	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	0	1	0	0	0	0	0	1	9
	2	1	0	1	0	0	0	0	0	12
	3	1	0	0	0	0	0	0	3	10
	4	1	1	0	1	0	0	0	2	14
	5	3	1	1	0	0	0	0	0	11
	6	0	0	0	-	0	0	0	0	13
Average		1.0	0.5	0.3	0.2	0.0	0.0	0.0	1.0	11.5
SD		1.1	0.5	0.5	0.4	0.0	0.0	0.0	1.3	1.9
Dunnett test										N.S.
t-Test (Assuming Equal Variances)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Assay Laboratory:	Lab IV

Compound:	Ascorbic acid

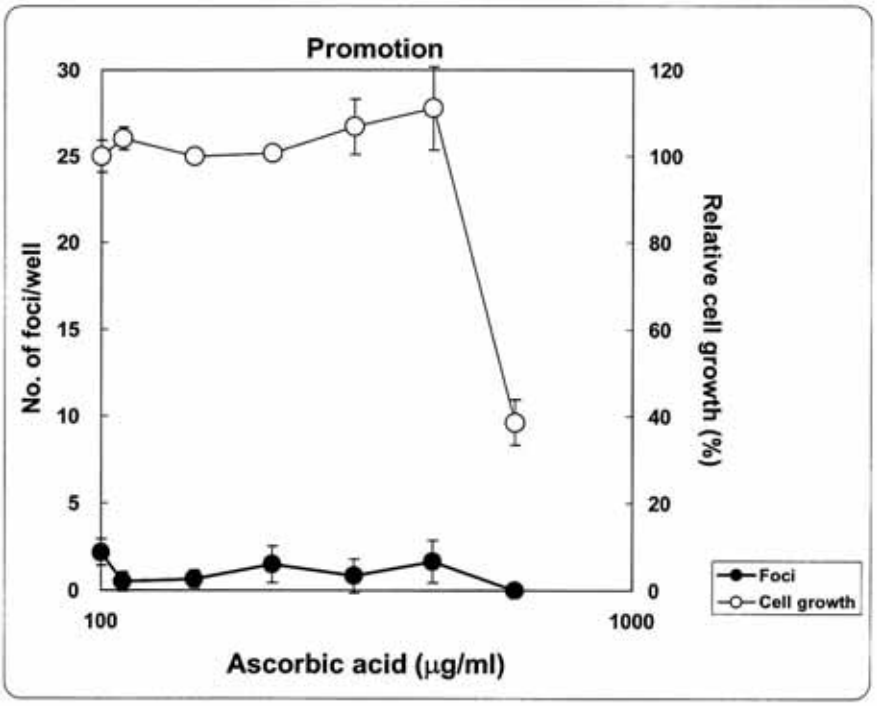
Toransformation Assay

Promotiom

Cell Growth Assay	Solvent:	DW 5%								
Compound (ug/ml)	Blank	100	110	150	210	300	420	600	DMSO 0.1%	TPA 50 ng/mL
OD	0.062	0.312	0.321	0.318	0.322	0.317	0.328	0.158		0.388
	0.061	0.313	0.332	0.315	0.315	0.348	0.337	0.147		0.402
	0.058	0.329	0.333	0.321	0.323	0.342	0.375	0.174		0.457
Average	0.060	0.318	0.329	0.318	0.320	0.336	0.347	0.160	#DIV/0!	0.416
SD	0.002	0.010	0.007	0.003	0.004	0.016	0.025	0.014	#DIV/0!	0.036
Average-Blank	0.000	0.258	0.268	0.258	0.260	0.275	0.286	0.099	#DIV/0!	0.355
Relative cell growth (%)		100.0	104.1	100.0	100.8	106.9	111.1	38.6	#DIV/0!	#DIV/0!
SD of relative cell growth(%)		3.7	2.6	1.2	1.7	6.4	9.7	5.3	#DIV/0!	#DIV/0!

Transformation Assay	Solvent:	DW 5%								
Compound (ug/ml)	Well No.	100	110	150	210	300	420	600	DMSO 0.1%	TPA 50 ng/mL
No of foci	1	1	1	1	2	2	0	TOX	2	26
	2	2	1	1	2	2	2		1	20
	3	3	0	0	1	0	1		2	17
	4	3	1	0	3	0	3		3	17
	5	2	0	1	1	1	1		3	19
	6	2	0	1	0	0	3		5	21
Average		2.2	0.5	0.7	1.5	0.8	1.7	#DIV/0!	2.7	20.0
SD		0.8	0.5	0.5	1.0	1.0	1.2	#DIV/0!	1.4	3.3
Dunnett test										N.S.
t-Test for Unequal Variances (Aspin-Welch)										***

The true value is zero. This value was temporarily put to make logarithm graph.



Annex 5

Table 55
Supplier, catalog number and lot number of test chemicals in the phase I of 96-well method validation study.

Chemical	CAS no.[a]	Supplier	Catalog no.	Lot no.	
3-Methylcholanthrene	56-49-5	Supelco	442388	LB61087	
Benzo[*a*]pyrene	50-32-8	Wako	020-13591	TSL8636	
2-Acetylaminofluorene	53-96-3	Ultra Scientific	Rcc-002	NTO1276	
TPA[b]	16561-29-8	Wako	545-00261	UIL0088	
o-Toluidine	95-53-4	Wako	209-01992	PEH9805	
Anthracene	120-12-7	Wako	010-04222	PEG1159	
Phenanthrene	85-01-8	Wako	162-00852	PEN0411	
3-Methylcholanthrene	56-49-5	Aldrich	213942	11303JE	Positive control
TPA	16561-29-8	Sigma	P8139	038K1501	Positive control

[a] Chemical Abstract Service registry number.
[b] 12-*O*-tetradecanoylphorbol-13-acetate.

Table 56
Supplier, catalog number and lot number of test chemicals in the phase II of 96-well method validation study.

Chemical	CAS no.[a]	Supplier	Catalog no.	Lot no.
N-Methyl-*N*'-nitro-*N*-nitrosoguanidine	70-25-7	TCI	M0527	2VXVI
Benzo[*a*]pyrene	50-32-8	Wako	020-13591	KWP9839
Dibenz[*a,h*]anthracene	53-70-3	TCI	D0145	JF01
Sodium arsenite	7784-46-5	Wako	199-01242	CDJ5162
Cadmium chloride	10108-64-2	Wako	032-00122	PEE3332
Methapyrilene HCl	135-23-9	Sigma	M9125-25G	037F09291
Mezerein	34807-41-5	Wako	544-01691	GNM1691
Lithocholic acid	434-13-9	Wako	127-04982	DPF1761
Pyrene	129-00-0	Wako	167-05302	KWM0171
Caprolactam	105-60-2	Wako	036-01122	CDL2706
Ampicillin sodium	69-52-3	Wako	016-10373	CDM1299
L-Ascorbic acid	50-81-7	Wako	012-04802	CDG0167
D-Mannitol	69-65-8	Wako	139-00842	CDK3426
Caffeine	58-08-2	Wako	031-06792	CDK3686
Phorbol	17673-25-5	MP Biomedicals	151849	8218F
Eugenol	97-53-0	Wako	053-03932	KWJ3327
3-Methylcholanthrene	56-49-5	Aldrich	11303JE	Positive control
TPA[b]	16561-29-8	Sigma	038K1501	Positive control

[a] Chemical Abstract Service registry number.
[b] 12-*O*-tetradecanoylphorbol-13-acetate.

Annex 6

Protocol of Bhas 42 Cell Transformation Assay (2008.11.18)
96-Well Method Ver. 2

This protocol is described for the international validation study of Bhas 42 cell transformation assay using 96-well plates. Materials and reagents with their catalogue numbers are listed in annex 1.

I. Preparation

1. Materials

1) Cell line

Bhas 42 cells (v-Ha-*ras*-transfected Balb/c 3T3 clone A31-1-1 cells)[1,2]: Free from bacteria, fungi and mycoplasma, supplied from HRI (Hatano Research Institute, Food and Drug Safety Center, Japan) Cell Bank.

2) Media

MEM: Minimum essential medium with 2.2 g/L $NaHCO_3$.

DMEM/F12: Dulbecco's modified Eagle's medium/F12 with 1.2 g/L $NaHCO_3$.

FBS: Fetal bovine serum should be selected showing a low spontaneous focus formation and a high focus formation in the positive control.

PS: Penicillin G sodium (10000 units/mL) and streptomycin sulfate (10 mg/mL).

M10F: MEM + 10% FBS + 1% PS (500 mL MEM + 56 mL FBS + 5 mL PS): Used for the expansion of provided cells, cell storage, and the first culture after thawing.

DF5F: DMEM/F12 + 5% FBS + 1% PS (500 mL DMEM/F12 + 26.5 mL FBS + 5 mL PS): Used for routine passages, cell growth assays and transformation assays.

3) Chemicals

Test chemicals and solvent/vehicle: Test chemicals are dissolved or suspended in an appropriate solvent or vehicle and diluted with the solvent/vehicle to each individual concentration before added to culture media so that all chemical treatment media contain an equal concentration of the solvent/vehicle. The final concentration of the solvent/vehicle in the medium is 5% with distilled water/ultra pure water and 0.1% with DMSO (permissible up to 0.5% when a test chemical does not dissolve).

Negative and positive controls: The solvent/vehicle for a test chemical is used as the negative control. A known initiator, 3-methylcholanthrene (MCA: final concentration of 1 μg/mL), and a promoter, 12-*O*-tetradecanoylphorbol-13- acetate (TPA: final concentration of 50 ng/mL), are used for the positive controls.

4) Fixatives and staining solutions

Formalin (37% formaldehyde): Used for fixing cells.

Methanol: Used for fixing cells.

0.1% crystal violet (CV) solution: Used for staining cells in cell growth assays. First, 1 g of crystal violet is dissolved in 50 mL of ethanol, and the total volume is adjusted to 1 L with distilled water/ultra pure water.

Extraction solution (0.02 mol/L HCl and 50% ethanol): Used for extracting CV in cell growth assays. 480 mL distilled water/ultra pure water + 500 mL ethanol + 20 mL 1 M HCl.

5% Giemsa solution: Used for staining cells in transformation assays.

5) Culture vessels
ϕ100-mm dishes: Used for routine passage.
96-well plates: Used for cell growth and transformation assays.

6) Micropipettes
Electronic multichannel pipettes are convenient.

2. Cell culture

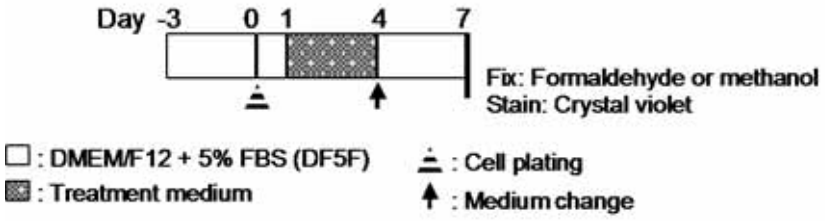
1) Cell culture and passage
Bhas 42 cells are cultured in a humidified 5% CO_2 incubator at 37ºC. The passage should be done at about 70% confluence of cell growth.

2) Preparation of cell stock
The provided cells are expanded in M10F. The regular method of preparation of cell stock is as follows:
One tube (2.5 x 10^5 cells) is thawed and suspended in 50 mL of M10F. The cell suspension is distributed into 5 of 100-mm tissue culture dishes at a volume of 10 mL/dish and cultured. Usually the cultures become to be 50-70% confluent in 4 days under these conditions. The cultures at 50-70% confluence are trypsinized with 0.25% trypsin after washed once with 0.02% EDTA-PBS(-). The cells are resuspended at a cell density of 5 x 10^5 cells/mL in cold fresh M10F containing 5% DMSO, frozen in 0.5 mL aliquots (2.5 x 10^5 cells/tube) at - 80℃ and stored in liquid nitrogen. Every transformation experiment is started from this frozen stock.
For this validation study Bhas 42 cells are provided at passage 17 (2006.12.16, P17) from HRI. In this validation study Bhas 42 cells must be used at passage 18 for the transformation assays. But for cell growth assays the cells at higher passages can be used and should be used to save frozen cell stocks for subsequent transformation assays.

II. Experimental procedures

A. Initiation assay[3)]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels
The highest concentration is 5 mg/mL[4,5)]. In the case of chemicals difficult to dissolve, the highest concentration may be one or two level higher than the concentration showing the utmost solubility. Five or more concentration levels are set by an appropriate serial dilution factor such as 10, square root 10 or 2. In some chemicals the cell

growth assay should be repeated in a narrower concentration range. The solvent/vehicle for a test chemical is used as the control. Each cell growth assay should include each solvent/vehicle control. Eight wells of 96-well plates are used for each concentration.

Blank control: Wells added with the medium alone are prepared for the blank control in cell growth assays. The blank control can be shared among different experiments conducted at the same time.

2) Procedure of cell growth assay

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes. The cells with a high passage number can be used for the cell growth assay.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL (200 cells/well). Because the volume is very small, the cell suspension is spread on the bottom of well by tapping the plates lightly. After keeping the plates at room temperature for 15 min until the cells are attached to the bottom, they are put into a incubator. Eight wells are prepared for each treatment condition.

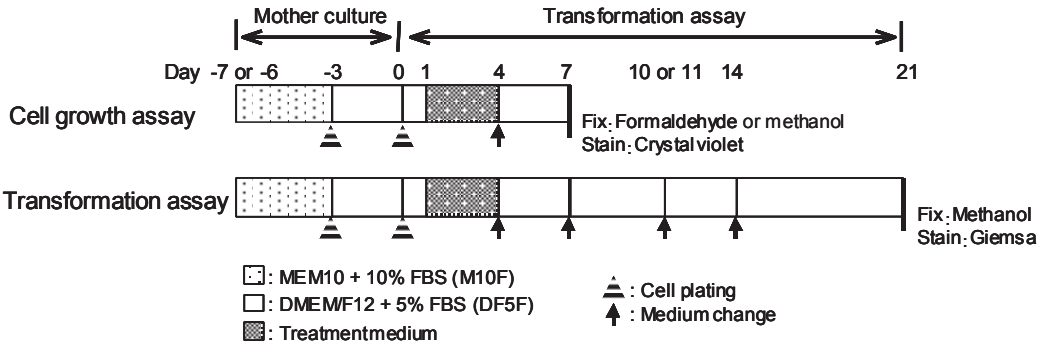
Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in the wells.

Day 4: Medium is changed with 0.1 mL of fresh DF5F.

Day 7: After fixing, the cells are stained with 0.1 mL of CV solution for 15 min or more, rinsed well with water and dried.

Measurement: The stained dye of each well is extracted with 0.1 mL of extraction solution, gently shaking for 10 min or more, and the optical density of each well is measured at a wavelength between 540 and 570 nm. Before measurement, the plates are shaken for several seconds (a microplate reader with mixing function is recommended). Bubbles, if any, are broken by touching with lightly heated tweezers. Growth rates relative to the solvent/vehicle control culture are calculated.

2. Transformation assay



1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays. These concentrations cover a range from highest toxicity (less than 20% survival compared to the control culture) to little or no toxicity. Ideally, one dose below NOEL, two doses between NOEL and IC50 and two doses between IC50 and IC90 are assessed in the initiation assay, as follows;

----------NOEL----------IC50----------IC90

One dose	Two doses	Two doses

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.

Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control for MCA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay

Day -7 or -6: Frozen stock cells are thawed, and cultured in 10 mL of M10F in ϕ100-mm dishes. Transformation assays must be started from the frozen stock cells but not from the cells with a high passage number.

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL for the transformation assay and the concurrent cell growth assay (200 cells/well). One plate is prepared for each treatment concentration of transformation assay. Eight wells are prepared for each treatment concentration of concurrent cell growth assay.

Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in wells.

Day 4: The medium is changed with 0.1 mL of fresh DF5F.

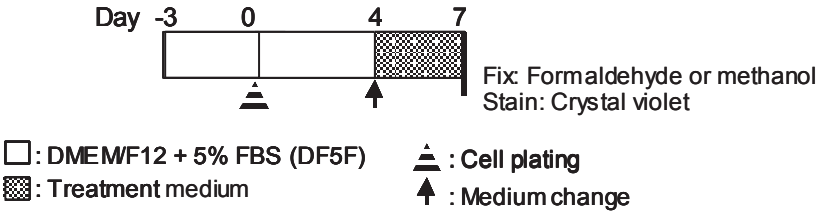
Day 7: The medium of transformation assay is changed with fresh DF5F. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.1.**2).

Day 10 or 11, and 14: The medium of the transformation assay is changed with fresh DF5F.

Day 21: The cells are fixed and stained with 5% Giemsa solution for 30 min or more.

B. Promotion assay[6,7]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

Same as the initiation assay (**II.A.1.**1).

2) Procedure of cell growth assay

Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.1.**2).

Day 0: The cells are replated in the same manner as the initiation assay (**II.A.1.**2) except that 0.1 mL of the cell

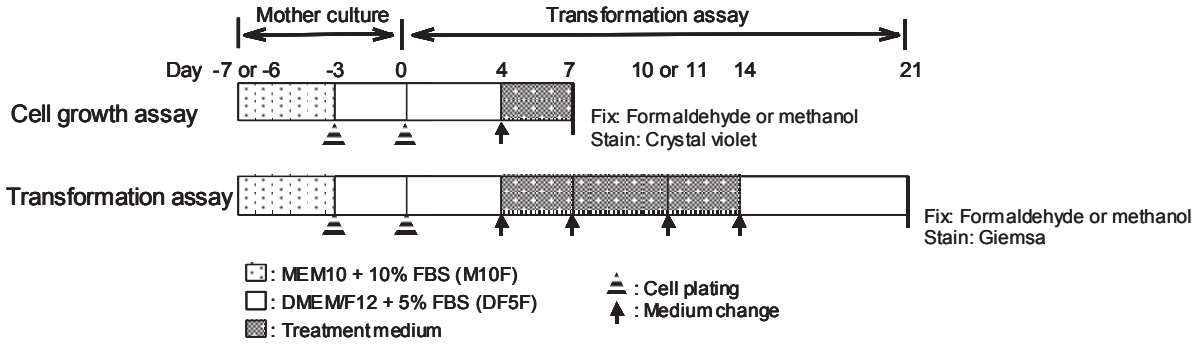
suspension are plated (400 cells/well).

Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).

Day 7: The cells are fixed and processed according to the procedures described above (**II.A.1.**2).

Measurement: The optical density is measured in the same way as the initiation assay (**II.A.1.**2). Growth rates relative to the solvent/vehicle control culture are calculated.

2. Transformation assay



1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays.

For the chemicals that exhibit marked growth enhancement, test concentrations are selected to cover from growth enhancement to little effect on cell growth. Ideally, one doses bellow NOEL, three doses in the range of growth enhancement, and one dose in the range of weak growth inhibition are assessed in the promotion assay, as follows;

----------NOEL---	Growth enhancement	---	Growth inhibition
One doses	Three doses		One dose

For the chemicals that do not induce marked growth enhancement, test concentrations are selected ranging from a dose exhibiting below 50% growth level to that two or three levels lower than the non-effective concentration. Ideally, two doses below NOEL, two doses between NOEL and IC50 and one dose above IC50 are assessed, as follows;

----------NOEL----------	----------IC50----------
Two doses	Two doses	One dose

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.

Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control of TPA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay

Day -7 or -6: The cells are thawed in the same manner as the initiation assay (**II.A.2.**2).

Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.2.**2).

Day 0: The cells are replated in the same manner as the initiation assay (**II.A.2.**2) except that 0.1 mL of the cell suspension are plated (400 cells/well).

Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).

Day 7: The medium of transformation assay is changed with a media containing a test chemical. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.2.**2).

Day 10 or 11: The medium of transformation assay is changed with a media containing a test chemical.

Day 14: The medium is changed with 0.1 mL of fresh DF5F.

Day 21: The cells are fixed and stained in the same manner as the initiation assay (**II.A.2.**2).

C. Medium change

The medium is removed by inverting the plates, i.e. paper towels or diapers are laid to catch the medium and the plates are swing and shake three times to drain off the medium completely. The medium is gently dispensed to the cells from tips which are touched to the upper walls of the wells.

D. Cell fixation

The cells are fixed with direct addition of 0.01 mL of formalin to the culture medium, or with 10 % formalin or methanol after the medium is removed. After 30 min or more (when fixed with formalin) or 10 min or more (when fixed with methanol), the cells are washed with tap water and dried.

E. Focus count

Transformed foci are featured by the following morphological characteristics; (a) more than 100 cells, (b) spindle-shaped cells different from the contact-inhibited monolayer cells (spindle-shaped), (c) deep basophilic staining (basophilic), (d) random orientation of cells at the edge of foci (criss-cross), (e) dense multilayering of cells (piling up) and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. There are transformed foci not prominent in some of these characteristics. The number of wells having transformed foci is recorded for each plate: a well having one focus is counted as one and a well having two or more foci is also counted as one.

Transformed foci are counted on the bottom surface but not on the wall of a well.

F. Test acceptance of criteria

The initiation or promotion assay is repeated independently, as needed, to satisfy acceptance criteria.

1. Concurrent cell growth assay

- At least should exist one concentration near NOEL which gives 80-120% cell growth compared to solvent/vehicle control.
- When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration.

2. Transformation assay

1) Initiation assay and promotion assay

- If the cells are killed and/or not confluent at the end of transformation assay, the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and “toxicity” is written in the data sheet.

- When contamination or technical problems are observed, if the number of damaged wells are 7 or more the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and “contamination”, “accident”, “technical error” and so on are written in the data sheet.

2) Initiation assay
- Negative control: The number of wells having transformed foci should be 10 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 30 wells/plate or more.
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: at least one concentration near the NOEL and three concentrations in the range between the NOEL and IC90.

3) Promotion assay
- Negative control: The number of wells having transformed foci should be 20 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 30 wells/plate or more. Furthermore, the proportion of wells having transformed foci should be significantly increased in the positive control plate compared to the solvent control plate (chi-square test, $p<0.05$).
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increase cell density). The durations of chemical exposure to the cells are different between the cell growth assay and the transformation assay. Then chemical toxicity is sometimes accumulated during 10 days in the promotion assay and plates may be lost because of chemical toxicity. In such cases four valid plates are also requested in the concentration range where cells are not killed and confluent at the end of transformation assay. It may be required to repeat the assay in a lower concentration range.

(The parts underlined are tentative and may be shifted with change in the batches of FBS from Moregate, Lot no. 7825120 to GIBCO, Lot no. 1391481.)

G. Three independent control data

To evaluate interlaboratory and intralaboratory variability, three independent data, at least, of positive and negative controls are needed in each laboratory. Independent data mean data obtained from the cells thawed on different days.

An example of three independent control data by plating cells on different days

Lab	Chemicals for each lab	Chemicals evaluated at the same time	Thawing frozen cells
Lab-A	a, b, c, d, e, f	a, b	1 Dec 2008
		c, d	8 Dec 2008
		e, f	15 Dec 2008
Lab-B	a, b, c, d, e, f	a	8 Dec 2008
		b, c	25 Dec 2008
		d, e, f	5 Jan 2009
Lab-C	a, b, c, d, e, f	a, b	4 Dec 2008
		c, d, e	18 Dec 2008

H. Statistical analysis

The proportion of wells with transformed foci in the plate treated with a test chemical at each concentration is compared with that in the solvent/vehicle control plate by chi-square test ($p<0.05$). For multiplicity, the number of concentrations that satisfy "Test acceptance criteria" (**II.F**) is considered by Bonferroni method.

H. Judgment

The result is considered positive if significant increases in the proportion of wells with transformed foci are observed at two serial concentrations when analyzed by chi-square test ($p<0.05$, multiple comparison analysis). If the increase is significant at only one concentration, the transformation assay should be repeated in a narrower concentration range and judged based on the repeated assay.

Judgment of results

Results		Judgment
1st experiment	Repeated experiment	
Significant at two and more serial concentrations	-	Positive
Significant at one concentration	No significant concentration	Negative
	Significant at one or more concentrations	Positive
No significant concentration	-	Negative

The final judgment is based on a comprehensive evaluation that also takes into account the biological significance.

All results are entered in the data sheet given and sent to Arai (arai.s@fdsc.or.jp, Hadano Research Institute).
Transformation plates must be stored and archived in each lab until the termination of validation study.

III. References

(1) K. Sasaki, H. Mizusawa and M. Ishidate, Isolation and characterization of ras-transfected BALB/3T3 clone showing morphplogical transformation by 12-*O*-tetradecanoyo-phorbol-13-acetate, Jpn. J. Cancer Res. 79 (1988) 921-930.
(2) K. Sasaki, H. Mizusawa, M. Ishidate and N. Tanaka, Establishment of a highly reproducible transformation assay of a ras-transfected BALB/3T3 clone by treatment with promoters, Basic Life Sci. 52 (1990) 411-416.
(3) S. Asada, K. Sasaki, N. Tanaka, K. Takeda, M. Hayashi and M. Umeda, Detection of initiating activities of chemicals using v-Ha-ras-transfected BALB/c 3T3 cells (Bhas 42 cells), Mutat. Res. 588 (2005) 7-21.
(4) OECD Guidelines for the Testing of Chemicals. Test No. 473: *In vitro* Mammalian Chromosome Aberration

Test.
(5) OECD Guidelines for the Testing of Chemicals. Test No. 476: *In vitro* Mammalian Cell Gene Mutation Test.
(6) K. Ohmori, K. Sasaki, S. Asada, N. Tanaka and M. Umeda, An assay method for the prediction of tumor promoting potential of chemicals by the use of Bhas 42 cells, Mutat. Res. 557 (2004) 191-202.
(7) K. Ohmori *et al*., Inter-laboratory collaborative study of cell transformation assay for tumor promoters using Bhas 42 cells by non-genotoxic carcinogen study group in Japan, ATRA 33 (2005) 1-21.

Annex 1. Materials and Reagents Used and their Catalogue Numbers (and Lot Numbers)

	Supplier	Cat. No.	Lot No.
Positive control			
3-Methylcholanthrene （MCA）	Aldrich	213942-100MG	11303JE
Phorbor 12-myristate 13-acetate (TPA)	SIGMA	P-1585-1MG	038K1501
Culture reagents			
Minimum Essential Medium	GIBCO	11095-080(500mL) (Japan) 11095-098(500mLx10) 31095-029(500mL) (Europe) 31095-052(500mLx10)(Europe)	----
Fetal bovine serum	GIBCO		1391481
Dulbecco's modified Eagle's medium/F12	GIBCO	11330-032(500mL) (Japan) 11330-057(500mLx10) (Japan) 31330-038(500mL) (Europe) 31330-095(500mLx10)(Europe)	----
Penicillin(10000units/mL)-Streptomycin (10 mg/mL)	GIBCO	15140-122	----
Trypsin, 0.25%	GIBCO	15050-065	
EDTA(0.02%)-PBS(-)	----		
Other reagent			
DMSO	SIGMA	D8418	----
Fixation and staining solutions			
Methanol	----	----	----
Giemsa solution *	MERK	1.09204	----
Giemsa solution (0.4%)*	SIGMA	GS 500	----
Formalin (37% formaldehyde)	SIGMA	F8775	----
Crystal violet	SIGMA	C3886	----
Ethanol	----	----	----
HCl	----	----	----
Culture vessels			
96-well microplates	CORNING (COSTAR) (Do not use FALCON)	3598	----
100-mm tissue culture dish	CORNING	430167	

*: Giemsa solution purchased from Merck is used after ×20 dilution. Either Giemsa solution can be used.

Annex 7

Protocol of Bhas 42 Cell Transformation Assay (2009.1.26)
96-Well Method Ver. 3

This protocol is described for the international validation study of Bhas 42 cell transformation assay using 96-well plates. Materials and reagents with their catalogue numbers are listed in annex 1.

I. Preparation

1. Materials

1) Cell line

Bhas 42 cells (v-Ha-*ras*-transfected Balb/c 3T3 clone A31-1-1 cells)[1,2]: Free from bacteria, fungi and mycoplasma, supplied from HRI (Hatano Research Institute, Food and Drug Safety Center, Japan) Cell Bank.

2) Media

MEM: Minimum essential medium with 2.2 g/L $NaHCO_3$ and 0.292 g/L L-glutamine.

DMEM/F12: Dulbecco's modified Eagle's medium/F12 with 1.2 g/L $NaHCO_3$.

FBS: Fetal bovine serum should be selected showing a low spontaneous focus formation and a high focus formation in the positive control.

PS: Penicillin G sodium (10000 units/mL) and streptomycin sulfate (10 mg/mL).

M10F: MEM + 10% FBS + 1% PS (500 mL MEM + 56 mL FBS + 5 mL PS): Used for the expansion of provided cells, cell storage, and the first culture after thawing.

DF5F: DMEM/F12 + 5% FBS + 1% PS (500 mL DMEM/F12 + 26.5 mL FBS + 5 mL PS): Used for routine passages, cell growth assays and transformation assays.

3) Chemicals

Test chemicals and solvent/vehicle: Test chemicals are dissolved or suspended in an appropriate solvent or vehicle and diluted with the solvent/vehicle to each individual concentration before added to culture media so that all chemical treatment media contain an equal concentration of the solvent/vehicle. The final concentration of the solvent/vehicle in the medium is 5% with distilled water/ultra pure water and 0.1% with DMSO (permissible up to 0.5% when a test chemical does not dissolve).

Negative and positive controls: The solvent/vehicle for a test chemical is used as the negative control. A known initiator, 3-methylcholanthrene (MCA: final concentration of 1 μg/mL), and a promoter, 12-*O*-tetradecanoylphorbol-13- acetate (TPA: final concentration of 50 ng/mL), are used for the positive controls.

Preparation and storage of test-chemical solutions: Test-chemical solutions are prepared before use as a general rule. Working solutions may be preserved in aliquots at -20°C for less than 10 days and thawed before use, but must not be re-frozen. When the test chemical supplied is too little to be weighed at every assay, a stock solution is prepared at a concentration as high as possible or at an appropriate concentration and stored in aliquots at -20℃ for a year and thawed before use. The stock solution is never re-frozen. The suspensions of test chemicals must be prepared before use and cannot be stored for the future use. The stock solutions of MCA and TPA in DMSO can be stored at -20 ℃ for two years at least, if they are not thawed.

4) Fixatives and staining solutions

Formalin (37% formaldehyde): Used for fixing cells.

Methanol: Used for fixing cells.

0.1% crystal violet (CV) solution: Used for staining cells in cell growth assays. First, 1 g of crystal violet is dissolved in 50 mL of ethanol, and the total volume is adjusted to 1 L with distilled water/ultra pure water.

Extraction solution (0.02 mol/L HCl and 50% ethanol): Used for extracting CV in cell growth assays. 480 mL distilled water/ultra pure water + 500 mL ethanol + 20 mL 1 M HCl.

5% Giemsa solution: Used for staining cells in transformation assays.

5) Culture vessels

ϕ100-mm dishes: Used for routine passage.

96-well plates: Used for cell growth and transformation assays.

6) Micropipettes

Electronic multichannel pipettes are convenient.

2. Cell culture

1) Cell culture and passage

Bhas 42 cells are cultured in a humidified 5% CO_2 incubator at 37ºC. The passage should be done at about 70% confluence of cell growth.

2) Preparation of cell stock

The provided cells are expanded in M10F. The regular method of preparation of cell stock is as follows:

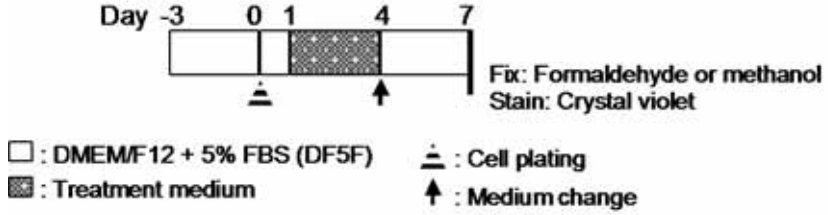
One tube (2.5 x 10^5 cells) is thawed and suspended in 50 mL of M10F. The cell suspension is distributed into 5 of 100-mm tissue culture dishes at a volume of 10 mL/dish and cultured. Usually the cultures become to be 50-70% confluent in 4 days under these conditions. The cultures at 50-70% confluence are trypsinized with 0.25% trypsin after washed once with 0.02% EDTA-PBS(-). The cells are resuspended at a cell density of 5 x 10^5 cells/mL in cold fresh M10F containing 5% DMSO, frozen in 0.5 mL aliquots (2.5 x 10^5 cells/tube) at - 80℃ and stored in liquid nitrogen. Every transformation experiment is started from this frozen stock.

For this validation study Bhas 42 cells are provided at passage 17 (2006.12.16, P17) from HRI. In this validation study Bhas 42 cells must be used at passage 18 for the transformation assays. But for cell growth assays the cells at higher passages can be used and should be used to save frozen cell stocks for subsequent transformation assays.

II. Experimental procedures

A. Initiation assay[3)]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

The highest concentration is 5 mg/mL[4,5]. In the case of chemicals difficult to dissolve, the highest concentration may be one or two level higher than the concentration showing the utmost solubility. Five or more concentration levels are set by an appropriate serial dilution factor such as 10, square root 10 or 2. In some chemicals the cell growth assay should be repeated in a narrower concentration range. The solvent/vehicle for a test chemical is used as the control. Each cell growth assay should include each solvent/vehicle control. Eight wells of 96-well plates are used for each concentration.

Blank control: Wells added with the medium alone are prepared for the blank control in cell growth assays. The blank control can be shared among different experiments conducted at the same time.

2) Procedure of cell growth assay

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes. The cells with a high passage number can be used for the cell growth assay.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL (200 cells/well). Because the volume is very small, the cell suspension is spread on the bottom of well by tapping the plates lightly. After keeping the plates at room temperature for 15 min until the cells are attached to the bottom, they are put into a incubator. Eight wells are prepared for each treatment condition.

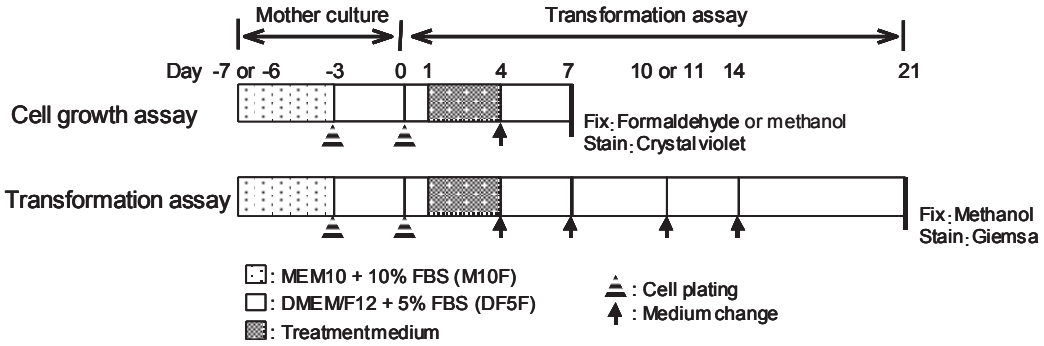
Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in the wells.

Day 4: Medium is changed with 0.1 mL of fresh DF5F.

Day 7: After fixing, the cells are stained with 0.1 mL of CV solution for 15 min or more, rinsed well with water and dried.

Measurement: The stained dye of each well is extracted with 0.1 mL of extraction solution, occasionally gently shaking for 10 min or more, and the optical density of each well is measured at a wavelength between 540 and 570 nm. Before measurement, the plates are shaken for several seconds (a microplate reader with mixing function is recommended). Bubbles, if any, are broken by touching with lightly heated tweezers. Growth rates relative to the solvent/vehicle control culture are calculated.

2. Transformation assay



1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays. These concentrations cover a range from highest toxicity (less than 20% survival compared to the control culture) to little or no toxicity. Ideally,

one dose below NOEL, two doses between NOEL and IC50 and two doses between IC50 and IC90 are assessed in the initiation assay, as follows;

----------NOEL----------IC50----------IC90

One dose	Two doses	Two doses

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.

Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control for MCA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay

Day -6 or -7: Frozen stock cells (2.5 x 10^5 cells) are thawed, and cultured in 20 to 50 mL of M10F in 2 to 5 of ϕ100-mm dishes. Transformation assays must be started from the frozen stock cells but not from the cells with a high passage number.

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL for the transformation assay and the concurrent cell growth assay (200 cells/well). One plate is prepared for each treatment concentration of transformation assay. Eight wells are prepared for each treatment concentration of concurrent cell growth assay.

Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in wells.

Day 4: The medium is changed with 0.1 mL of fresh DF5F.

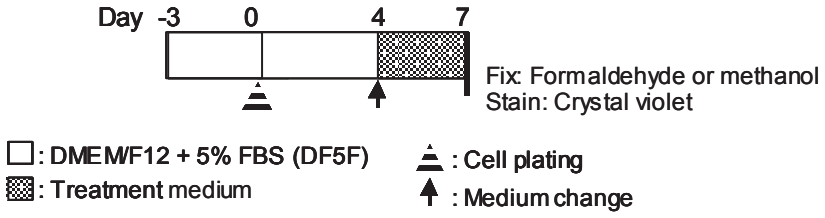
Day 7: The medium of transformation assay is changed with fresh DF5F. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.1.**2).

Day 10 or 11, and 14: The medium of the transformation assay is changed with fresh DF5F.

Day 21: The cells are fixed and stained with 5% Giemsa solution for 30 min or more.

B. Promotion assay[6,7]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

Same as the initiation assay (**II.A.1.**1).

2) Procedure of cell growth assay

Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.1.**2).

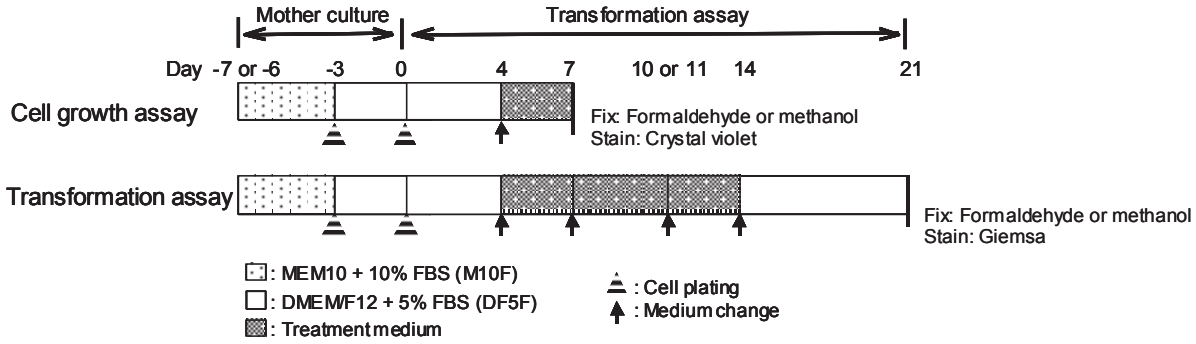
Day 0: The cells are replated in the same manner as the initiation assay (**II.A.1.**2) except that 0.1 mL of the cell suspension are plated (400 cells/well).

Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).

Day 7: The cells are fixed and processed according to the procedures described above (**II.A.1.**2).

Measurement: The optical density is measured in the same way as the initiation assay (**II.A.1.**2). Growth rates relative to the solvent/vehicle control culture are calculated.

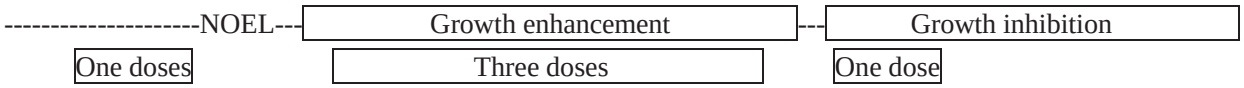
2. Transformation assay



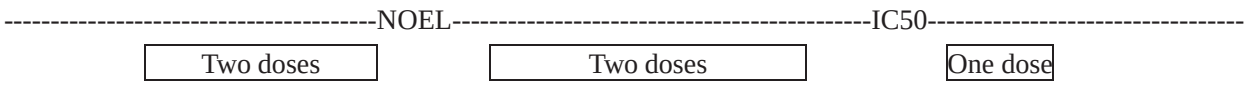
1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays.

For the chemicals that exhibit marked growth enhancement, test concentrations are selected to cover from growth enhancement to little effect on cell growth. Ideally, one doses bellow NOEL, three doses in the range of growth enhancement, and one dose in the range of weak growth inhibition are assessed in the promotion assay, as follows;



For the chemicals that do not induce marked growth enhancement, test concentrations are selected ranging from a dose exhibiting below 50% growth level to that two or three levels lower than the non-effective concentration. Ideally, two doses below NOEL, two doses between NOEL and IC50 and one dose above IC50 are assessed, as follows;



For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.
Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control of TPA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay
Day -7 or -6: The cells are thawed in the same manner as the initiation assay (**II.A.2.**2).
Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.2.**2).
Day 0: The cells are replated in the same manner as the initiation assay (**II.A.2.**2) except that 0.1 mL of the cell suspension are plated (400 cells/well).
Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).
Day 7: The medium of transformation assay is changed with a media containing a test chemical. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.2.**2).
Day 10 or 11: The medium of transformation assay is changed with a media containing a test chemical.
Day 14: The medium is changed with 0.1 mL of fresh DF5F.
Day 21: The cells are fixed and stained in the same manner as the initiation assay (**II.A.2.**2).

C. Medium change

The medium is removed by inverting the plates, i.e. paper towels or diapers are laid to catch the medium and the plates are swing and shake three times to drain off the medium completely. The medium is gently dispensed to the cells from tips which are touched to the upper walls of the wells.

D. Cell fixation

The cells are fixed with direct addition of 0.01 mL of formalin to the culture medium, or with 10 % formalin or methanol after the medium is removed. After 30 min or more (when fixed with formalin) or 10 min or more (when fixed with methanol), the cells are washed with tap water and dried.

E. Focus count

Transformed foci are featured by the following morphological characteristics; (a) more than 100 cells, (b) spindle-shaped cells different from the contact-inhibited monolayer cells (spindle-shaped), (c) deep basophilic staining (basophilic), (d) random orientation of cells at the edge of foci (criss-cross), (e) dense multilayering of cells (piling up) and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. There are transformed foci not prominent in some of these characteristics. The number of wells having transformed foci is recorded for each plate: a well having one focus is counted as one and a well having two or more foci is also counted as one.
Transformed foci are counted on the bottom surface but not on the wall of a well.

F. Test acceptance of criteria

The initiation or promotion assay is repeated independently, as needed, to satisfy acceptance criteria.

1. Concurrent cell growth assay

- When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration.

2. Transformation assay

1) Initiation assay and promotion assay

- If the cells are killed and/or not confluent at the end of transformation assay, the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and "toxicity" is written in the data sheet..
- When contamination or technical problems are observed, if the number of damaged wells are 7 or more the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and "contamination", "accident", "technical error" and so on are written in the data sheet.

2) Initiation assay

- Negative control: The number of wells having transformed foci should be 15 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 40 wells/plate or more.
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: at least one concentration near the NOEL (80 – 120 %) and three concentrations in the range between the NOEL and IC90 in the concurrent cell growth assay.

3) Promotion assay

- Negative control: The number of wells having transformed foci should be 20 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 40 wells/plate or more.
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: in the concurrent assay, at least one concentration near the NOEL and two concentrations in the range of growth enhancement, when the chemical enhances cell growth (increase cell density). The durations of chemical exposure to the cells are different between the cell growth assay and the transformation assay. Then chemical toxicity is sometimes accumulated during 10 days in the promotion assay and plates may be lost because of chemical toxicity. In such cases four valid plates are also requested in the concentration range where cells are confluent and not killed at the end of transformation assay. It may be required to repeat the assay in a lower concentration range.

(The parts underlined were tentative in the protocol for the pre-validation phase and have been shifted with change in the batches of FBS from Moregate, Lot no. 7825120 to GIBCO, Lot no. 1391481, based on the results of the pre-validation study.)

G. Three independent control data

To evaluate interlaboratory and intralaboratory variability, three independent data, at least, of positive and negative controls are needed in each laboratory. Independent data mean data obtained from the cells thawed on different days.

An example of three independent control data by plating cells on different days

Lab	Chemicals for each lab	Chemicals evaluated at the same time	Thawing frozen cells
Lab-A	a, b, c, d, e, f	a, b	1 Dec 2008
		c, d	8 Dec 2008
		e, f	15 Dec 2008
Lab-B	a, b, c, d, e, f	a	8 Dec 2008
		b, c	25 Dec 2008
		d, e, f	5 Jan 2009

Lab-C	a, b, c, d, e, f	a, b	4 Dec 2008
		c, d, e	18 Dec 2008
		f, (repeated a)	8 Jan 2009

H. Statistical analysis

The proportion of wells with transformed foci in the plate treated with a test chemical at each concentration is compared with that in the solvent/vehicle control plate by chi-square test with Bonferroni adjustment (p-value<0.025, upper-sided)[8]. For multiplicity, the number of concentrations that satisfy "TEST acceptance criteria" (**II.F**) is considered by this methodology.

H. Judgment

The result is considered positive if significant increases in the proportion of wells with transformed foci are observed at two serial concentrations when analyzed by chi-square test (p-value<0.025, upper-sided, multiple comparison analysis). If the increase is significant at only one or non-serial concentrations, the transformation assay should be repeated in a narrower concentration range and judged based on the repeated assay.

Judgment of results

Results		Judgment
1st experiment	Repeated experiment	
Significant at two and more serial concentrations	-	Positive
Significant at one or non-serial concentrations	No significant concentration	Negative
	Significant at one or more concentrations	Positive
No significant concentration	-	Negative

The final judgment is based on a comprehensive evaluation that also takes into account the biological significance.

All results are entered in the data sheet given and sent to both Shoko Arai (arai.s@fdsc.or.jp, Hadano Research Institute) and Masaya Suzuki (masaya@anpyo.or.jp, Biosafety Research Center, Foods, Drugs and Pesticides).
Transformation plates must be stored and archived in each lab until the termination of validation study.

III. References

(1) K. Sasaki, H. Mizusawa and M. Ishidate, Isolation and characterization of ras-transfected BALB/3T3 clone showing morphplogical transformation by 12-*O*-tetradecanoyo-phorbol-13-acetate, Jpn. J. Cancer Res. 79 (1988) 921-930.

(2) K. Sasaki, H. Mizusawa, M. Ishidate and N. Tanaka, Establishment of a highly reproducible transformation

assay of a ras-transfected BALB/3T3 clone by treatment with promoters, Basic Life Sci. 52 (1990) 411-416.
(3) S. Asada, K. Sasaki, N. Tanaka, K. Takeda, M. Hayashi and M. Umeda, Detection of initiating activities of chemicals using v-Ha-ras-transfected BALB/c 3T3 cells (Bhas 42 cells), Mutat. Res. 588 (2005) 7-21.
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(5) OECD Guidelines for the Testing of Chemicals. Test No. 476: *In vitro* Mammalian Cell Gene Mutation Test.
(6) K. Ohmori, K. Sasaki, S. Asada, N. Tanaka and M. Umeda, An assay method for the prediction of tumor promoting potential of chemicals by the use of Bhas 42 cells, Mutat. Res. 557 (2004) 191-202.
(7) K. Ohmori *et al.*, Inter-laboratory collaborative study of cell transformation assay for tumor promoters using Bhas 42 cells by non-genotoxic carcinogen study group in Japan, ATLA 33 (2005) 1-21.
(8) G. W. Snedecor and W. G. Cochran, Statistical Methods (8th Edition), Iowa State University Press, Ames, 1989, pp.125-128.

Annex 1. Materials and Reagents Used and their Catalogue Numbers (and Lot Numbers)

	Supplier	Cat. No.	Lot No.
Positive control			
3-Methylcholanthrene （MCA）	Aldrich	213942-100MG	11303JE
Phorbor 12-myristate 13-acetate (TPA)	SIGMA	P-1585-1MG	038K1501
Culture reagents			
Minimum Essential Medium	GIBCO	11095-080(500mL) (Japan) 11095-098(500mLx10) 31095-029(500mL) (Europe) 31095-052(500mLx10)(Europe)	----
Fetal bovine serum	GIBCO		1391481
Dulbecco's modified Eagle's medium/F12	GIBCO	11330-032(500mL) (Japan) 11330-057(500mLx10) (Japan) 31330-038(500mL) (Europe) 31330-095(500mLx10)(Europe)	----
Penicillin(10000units/mL)-Streptomycin (10 mg/mL)	GIBCO	15140-122	----
Trypsin, 0.25%	GIBCO	15050-065	
EDTA(0.02%)-PBS(-)	----		
Other reagent			
DMSO	SIGMA	D8418	----
Fixation and staining solutions			
Methanol	----	----	----
Giemsa solution *	MERK	1.09204	----
Giemsa solution (0.4%)*	SIGMA	GS 500	----
Formalin (37% formaldehyde)	SIGMA	F8775	----
Crystal violet	SIGMA	C3886	----
Ethanol	----	----	----
HCl	----	----	----
Culture vessels			
96-well microplates	CORNING (COSTAR) (Do not use FALCON)	3598	----
100-mm tissue culture dish	CORNING	430167	

*: Giemsa solution purchased from Merck is used after ×20 dilution. Either Giemsa solution can be used.

Annex 8

Protocol of Bhas 42 Cell Transformation Assay (2009.10.28)
96-Well Method Ver. 4

This protocol is described for the international validation study of Bhas 42 cell transformation assay using 96-well plates. Materials and reagents with their catalogue numbers are listed in annex 1.

I. Preparation

1. Materials

1) Cell line

Bhas 42 cells (v-Ha-*ras*-transfected Balb/c 3T3 clone A31-1-1 cells)[1,2]: Free from bacteria, fungi and mycoplasma, supplied from HRI (Hatano Research Institute, Food and Drug Safety Center, Japan) Cell Bank.

2) Media

MEM: Minimum essential medium with 2.2 g/L $NaHCO_3$ and 0.292 g/L L-glutamine.

DMEM/F12: Dulbecco's modified Eagle's medium/F12 with 1.2 g/L $NaHCO_3$.

FBS: Fetal bovine serum should be selected showing a low spontaneous focus formation and a high focus formation in the positive control.

PS: Penicillin G sodium (10000 units/mL) and streptomycin sulfate (10 mg/mL).

M10F: MEM + 10% FBS + 1% PS (500 mL MEM + 56 mL FBS + 5 mL PS): Used for the expansion of provided cells, cell storage, and the first culture after thawing.

DF5F: DMEM/F12 + 5% FBS + 1% PS (500 mL DMEM/F12 + 26.5 mL FBS + 5 mL PS): Used for routine passages, cell growth assays and transformation assays.

3) Chemicals

Test chemicals and solvent/vehicle: Test chemicals are dissolved or suspended in an appropriate solvent or vehicle and diluted with the solvent/vehicle to each individual concentration before added to culture media so that all chemical treatment media contain an equal concentration of the solvent/vehicle. The final concentration of the solvent/vehicle in the medium is 5% with distilled water/ultra pure water and 0.1% with DMSO (permissible up to 0.5% when a test chemical does not dissolve).

Negative and positive controls: The solvent/vehicle for a test chemical is used as the negative control. A known initiator, 3-methylcholanthrene (MCA: final concentration of 1 μg/mL), and a promoter, 12-*O*-tetradecanoylphorbol-13- acetate (TPA: final concentration of 50 ng/mL), are used for the positive controls.

Preparation and storage of test-chemical solutions: Test-chemical solutions are prepared before use as a general rule. Working solutions may be preserved in aliquots at -20°C for less than 10 days and thawed before use, but must not be re-frozen. When the test chemical supplied is too little to be weighed at every assay, a stock solution is prepared at a concentration as high as possible or at an appropriate concentration and stored in aliquots at -20℃ for a year and thawed before use. The stock solution is never re-frozen. The suspensions of test chemicals must be prepared before use and cannot be stored for the future use. The stock solutions of MCA and TPA in DMSO can be stored at -20 ℃ for two years at least, if they are not thawed.

4) Fixatives and staining solutions

Formalin (37% formaldehyde): Used for fixing cells.
Methanol: Used for fixing cells.
0.1% crystal violet (CV) solution: Used for staining cells in cell growth assays. First, 1 g of crystal violet is dissolved in 50 mL of ethanol, and the total volume is adjusted to 1 L with distilled water/ultra pure water.
Extraction solution (0.02 mol/L HCl and 50% ethanol): Used for extracting CV in cell growth assays. 480 mL distilled water/ultra pure water + 500 mL ethanol + 20 mL 1 M HCl.
5% Giemsa solution: Used for staining cells in transformation assays.

5) Culture vessels
ϕ100-mm dishes: Used for routine passage.
96-well plates: Used for cell growth and transformation assays.

6) Micropipettes
Electronic multichannel pipettes are convenient.

2. Cell culture

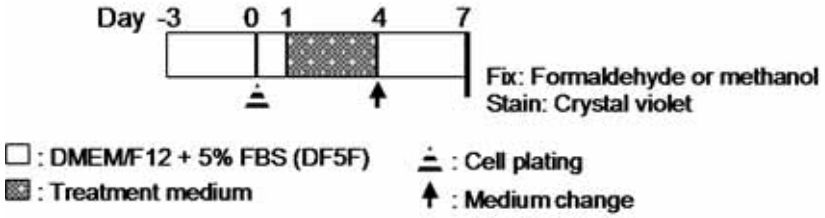
1) Cell culture and passage
Bhas 42 cells are cultured in a humidified 5% CO_2 incubator at 37ºC. The passage should be done at about 70% confluence of cell growth.

2) Preparation of cell stock
The provided cells are expanded in M10F. The regular method of preparation of cell stock is as follows:
One tube (2.5 x 10^5 cells) is thawed and suspended in 50 mL of M10F. The cell suspension is distributed into 5 of 100-mm tissue culture dishes at a volume of 10 mL/dish and cultured. Usually the cultures become to be 50-70% confluent in 4 days under these conditions. The cultures at 50-70% confluence are trypsinized with 0.25% trypsin after washed once with 0.02% EDTA-PBS(-). The cells are resuspended at a cell density of 5 x 10^5 cells/mL in cold fresh M10F containing 5% DMSO, frozen in 0.5 mL aliquots (2.5 x 10^5 cells/tube) at - 80℃ and stored in liquid nitrogen. Every transformation experiment is started from this frozen stock.
For this validation study Bhas 42 cells are provided at passage 17 (2006.12.16, P17) from HRI. In this validation study Bhas 42 cells must be used at passage 18 for the transformation assays. But for cell growth assays the cells at higher passages can be used and should be used to save frozen cell stocks for subsequent transformation assays.

II. Experimental procedures

A. Initiation assay[3)]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

The highest concentration is 5 mg/mL in medium[4,5]. In the case of chemicals difficult to dissolve in a solvent/vehicle, the highest concentration may be one or two level higher than the concentration showing the utmost solubility. Five or more concentration levels are set by an appropriate serial dilution factor such as 10, square root 10 or 2. In some chemicals the cell growth assay should be repeated in a narrower concentration range. The solvent/vehicle for a test chemical is used as the control. Each cell growth assay should include each solvent/vehicle control. Eight wells of 96-well plates are used for each concentration.

Blank control: Wells added with the medium alone are prepared for the blank control in cell growth assays. The blank control can be shared among different experiments conducted at the same time.

2) Procedure of cell growth assay

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes. The cells with a high passage number can be used for the cell growth assay.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL (200 cells/well). Because the volume is very small, the cell suspension is spread on the bottom of well by tapping the plates lightly. After keeping the plates at room temperature for 15 min until the cells are attached to the bottom, they are put into a incubator. Eight wells are prepared for each treatment condition.

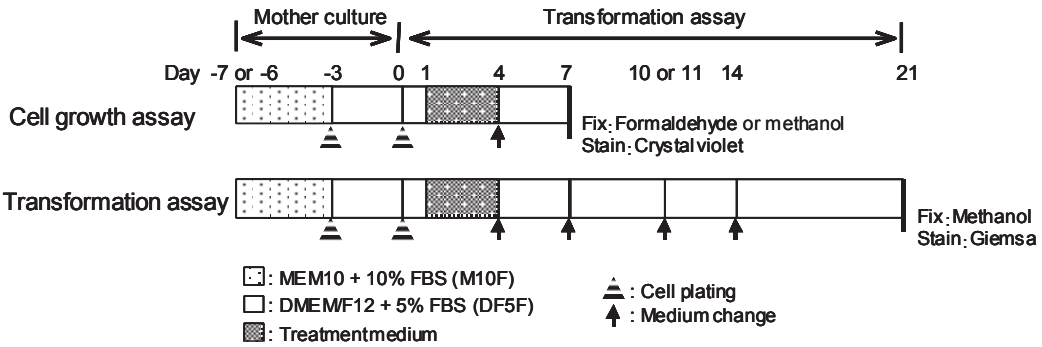
Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in the wells.

Day 4: Medium is changed with 0.1 mL of fresh DF5F.

Day 7: After fixing, the cells are stained with 0.1 mL of CV solution for 15 min or more, rinsed well with water and dried.

Measurement: The stained dye of each well is extracted with 0.1 mL of extraction solution, occasionally gently shaking for 10 min or more, and the optical density of each well is measured at a wavelength between 540 and 570 nm. Before measurement, the plates are shaken for several seconds (a microplate reader with mixing function is recommended). Bubbles, if any, are broken by touching with lightly heated tweezers. Growth rates relative to the solvent/vehicle control culture are calculated.

2. Transformation assay



1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays. These concentrations cover a range from highest toxicity (less than 20% survival compared to the control culture) to little or no toxicity. Ideally, one dose below NOEL, two doses between NOEL and IC50 and two doses between IC50 and IC90 are assessed in the initiation assay, as follows;

----------NOEL----------IC50----------IC90

One dose	Two doses	Two doses

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.

Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control for MCA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay

Day -6 or -7: Frozen stock cells (2.5 x 10^5 cells) are thawed, and cultured in 20 to 50 mL of M10F in 2 to 5 of ϕ100-mm dishes. Transformation assays must be started from the frozen stock cells but not from the cells with a high passage number.

Day -3: The cells at about 70% confluence are trypsinized and suspended in DF5F at 0.7 to 1 x 10^4 cells/mL. The cell suspension is transferred at a volume of 10 mL to ϕ100-mm dishes.

Day 0: The cells at about 70% confluence are trypsinized and suspended in DF5F at 4000 cells/mL. The cell suspension is distributed into each well of 96-well plates at a volume of 0.05 mL for the transformation assay and the concurrent cell growth assay (200 cells/well). One plate is prepared for each treatment concentration of transformation assay. Eight wells are prepared for each treatment concentration of concurrent cell growth assay.

Day 1: Media containing test chemicals at two times the final concentration are prepared, and 0.05 mL of the preparations are added to the wells without medium change. The plate is tapped to mix media in wells.

Day 4: The medium is changed with 0.1 mL of fresh DF5F.

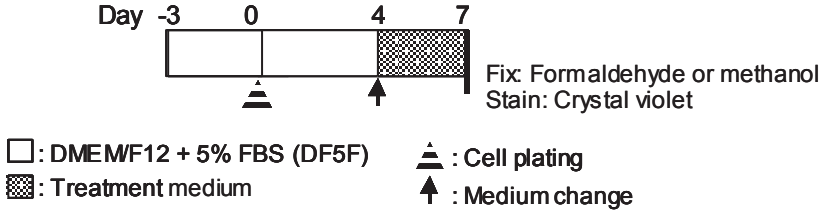
Day 7: The medium of transformation assay is changed with fresh DF5F. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.1.**2).

Day 10 or 11, and 14: The medium of the transformation assay is changed with fresh DF5F.

Day 21: The cells are fixed and stained with 5% Giemsa solution for 30 min or more.

B. Promotion assay[6,7]

1. Cell growth assay for determination of test concentrations (crystal violet method)



1) Test concentrations and used vessels

Same as the initiation assay (**II.A.1.**1).

2) Procedure of cell growth assay

Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.1.**2).

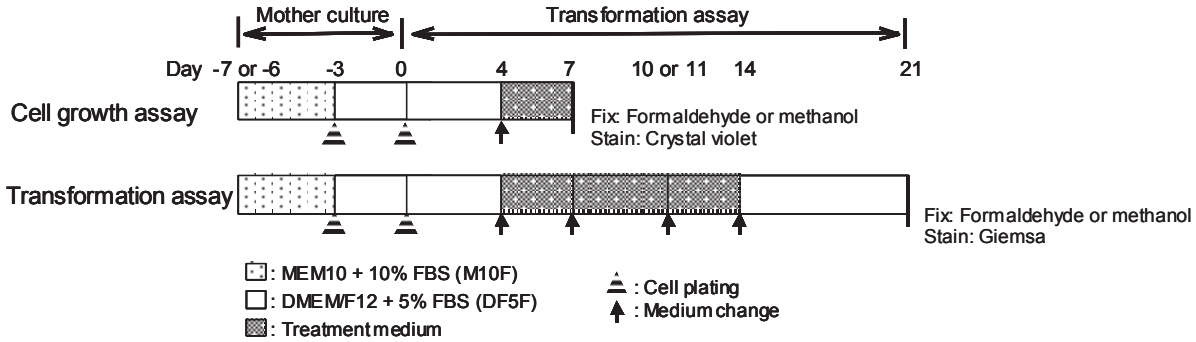
Day 0: The cells are replated in the same manner as the initiation assay (**II.A.1.**2) except that 0.1 mL of the cell suspension are plated (400 cells/well).

Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).

Day 7: The cells are fixed and processed according to the procedures described above (**II.A.1.**2).

Measurement: The optical density is measured in the same way as the initiation assay (**II.A.1.**2). Growth rates relative to the solvent/vehicle control culture are calculated.

2. Transformation assay



1) Test concentrations and used vessels

Five to nine concentrations are set up based on the results of cell growth assays.

For the chemicals that exhibit marked growth enhancement, test concentrations are selected to cover from growth enhancement to little effect on cell growth. Ideally, one doses bellow NOEL, three doses in the range of growth enhancement, and one dose in the range of weak growth inhibition are assessed in the promotion assay, as follows;

----------NOEL--- Growth enhancement --- Growth inhibition

One dose | Three doses | One dose

For the chemicals that do not induce marked growth enhancement, test concentrations are selected ranging from a dose exhibiting below 50% growth level to that two or three levels lower than the non-effective concentration. Ideally, two doses below NOEL, two doses between NOEL and IC50 and one dose above IC50 are assessed, as follows;

----------NOEL----------IC50----------

Two doses | Two doses | One dose

For a chemical which gives the sharp decline of cell growth within a narrow concentration range, one or two more doses above or below the predicted concentration range may be set up as a precaution against the fluctuation of cell response among experiments.

For low cytotoxic chemicals, the assay is performed at 0.5, 1, 2, 3, 4 and 5 mg/mL[4,5].

One plate for the transformation assay (96 wells), and eight wells for the concurrent cell growth assay are prepared at each concentration.

Each assay of a chemical should include negative and positive controls (when the solvent of the test chemical is 5% water, DMSO control is also necessary as the control of TPA). The control data cannot be shared among experiments of different chemicals even if they are carried out at the same time.

2) Procedure of transformation assay and concurrent cell growth assay

Day -7 or -6: The cells are thawed in the same manner as the initiation assay (**II.A.2.**2).

Day -3: The cells are subcultured in the same manner as the initiation assay (**II.A.2.**2).

Day 0: The cells are replated in the same manner as the initiation assay (**II.A.2.**2) except that 0.1 mL of the cell suspension are plated (400 cells/well).

Day 4: Media containing test chemicals at the final concentrations are prepared, and used for medium change (0.1 mL/well).

Day 7: The medium of transformation assay is changed with a media containing a test chemical. The cells for the concurrent growth assay are fixed and processed according to the procedures described above (**II.A.2.**2).

Day 10 or 11: The medium of transformation assay is changed with a media containing a test chemical.

Day 14: The medium is changed with 0.1 mL of fresh DF5F.

Day 21: The cells are fixed and stained in the same manner as the initiation assay (**II.A.2.**2).

C. Medium change

The medium is removed by inverting the plates, i.e. paper towels or diapers are laid to catch the medium and the plates are swing and shake three times to drain off the medium completely. The medium is gently dispensed to the cells from tips which are touched to the upper walls of the wells.

D. Cell fixation

The cells are fixed with direct addition of 0.01 mL of formalin to the culture medium, or with 10 % formalin or methanol after the medium is removed. After 30 min or more (when fixed with formalin) or 10 min or more (when fixed with methanol), the cells are washed with tap water and dried.

E. Focus count

Transformed foci are featured by the following morphological characteristics; (a) more than 100 cells, (b) spindle-shaped cells different from the contact-inhibited monolayer cells (spindle-shaped), (c) deep basophilic staining (basophilic), (d) random orientation of cells at the edge of foci (criss-cross), (e) dense multilayering of cells (piling up) and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. There are transformed foci not prominent in some of these characteristics. The number of wells having transformed foci is recorded for each plate: a well having one focus is counted as one and a well having two or more foci is also counted as one.

Transformed foci are counted on the bottom surface but not on the wall of a well.

F. Test acceptance of criteria

The initiation or promotion assay is repeated independently, as needed, to satisfy acceptance criteria.

1. Concurrent cell growth assay

- When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration.

2. Transformation assay

1) Initiation assay and promotion assay

- If the cells are killed and/or not confluent at the end of transformation assay, the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and "toxicity" is written in the data sheet..
- When contamination or technical problems are observed, if the number of damaged wells are 7 or more the concentration is not valid and excluded from focus-counting, statistical analysis and judgment, and "contamination", "accident", "technical error" and so on are written in the data sheet.

2) Initiation assay

- Negative control: The number of wells having transformed foci should be 15 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 40 wells/plate or more.
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: at least one concentration near the NOEL (80 – 120 %) and three concentrations in the range between the NOEL and IC90 in the concurrent cell growth assay.

3) Promotion assay

- Negative control: The number of wells having transformed foci should be 20 wells/plate or less.
- Positive control: The number of wells having transformed foci should be 40 wells/plate or more.
- Concentrations: Four valid concentrations are necessary at the minimum to accept the transformation assay for evaluating a chemical: in the concurrent assay, at least one concentration near the NOEL and two concentrations in the range of growth enhancement, when the chemical enhances cell growth (increase cell density). The durations of chemical exposure to the cells are different between the cell growth assay and the transformation assay. Then chemical toxicity is sometimes accumulated during 10 days in the promotion assay and plates may be lost because of chemical toxicity. In such cases four valid plates are also requested in the concentration range where cells are confluent and not killed at the end of transformation assay. It may be required to repeat the assay in a lower concentration range.

(The parts underlined were tentative in the protocol for the pre-validation phase and have been shifted with change in the batches of FBS from Moregate, Lot no. 7825120 to GIBCO, Lot no. 1391481, based on the results of the pre-validation study.)

G. Three independent control data

To evaluate interlaboratory and intralaboratory variability, three independent data, at least, of positive and negative controls are needed in each laboratory. Independent data mean data obtained from the cells thawed on different days.

An example of three independent control data by plating cells on different days

Lab	Chemicals for each lab	Chemicals evaluated at the same time	Thawing frozen cells
Lab-A	a, b, c, d, e, f	a, b	1 Dec 2009
		c, d	8 Dec 2009
		e, f	15 Dec 2009
Lab-B	a, b, c, d, e, f	a	8 Dec 2009
		b, c	25 Dec 2009
		d, e, f	5 Jan 2010

Lab-C	a, b, c, d, e, f	a, b	4 Dec 2009
		c, d, e	18 Dec 2009
		f, (repeated a)	8 Jan 2010

H. Statistical analysis

The proportion of wells with transformed foci in the plate treated with a test chemical at each concentration is compared with that in the solvent/vehicle control plate by chi-square test with Bonferroni adjustment (p-value<0.05, upper-sided)[8]. For multiplicity, the number of concentrations that satisfy "TEST acceptance criteria" (**II.F**) is considered by this methodology.

I. Judgment

The result is considered positive if significant increases in the proportion of wells with transformed foci are observed at two serial concentrations when analyzed by chi-square test (p-value<0.05, upper-sided, multiple comparison analysis). If the increase is significant at only one or non-serial concentrations, the result should be judged as "equivocal" when the assay is not repeated, and when the assay is repeated in a narrower concentration range the judgment should be based on the repeated assay as follows.

The judgment of results

Results		Judgment
1st experiment	Repeated experiment	
Significant at two and more serial concentrations	-	Positive
Significant at one or non-serial concentrations	-	equivocal
	No significant concentration	Negative
	Significant at one or more concentrations	Positive
No significant concentration	-	Negative

The final judgment is based on a comprehensive evaluation that also takes into account the biological significance.

All results are entered in the data sheet given and sent to both Shoko Arai (arai.s@fdsc.or.jp, Hadano Research Institute) and Masaya Suzuki (masaya@anpyo.or.jp, Biosafety Research Center, Foods, Drugs and Pesticides).
Transformation plates must be stored and archived in each lab until the termination of validation study.

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(1) K. Sasaki, H. Mizusawa and M. Ishidate, Isolation and characterization of ras-transfected BALB/3T3 clone showing morphplogical transformation by 12-*O*-tetradecanoyo-phorbol-13-acetate, Jpn. J. Cancer Res. 79 (1988) 921-930.

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Phorbor 12-myristate 13-acetate (TPA)	SIGMA	P-1585-1MG	038K1501
Culture reagents			
Minimum Essential Medium	GIBCO	11095-080(500mL) (Japan) 11095-098(500mLx10) 31095-029(500mL) (Europe) 31095-052(500mLx10)(Europe)	----
Fetal bovine serum	GIBCO		1391481
Dulbecco's modified Eagle's medium/F12	GIBCO	11330-032(500mL) (Japan) 11330-057(500mLx10) (Japan) 31330-038(500mL) (Europe) 31330-095(500mLx10)(Europe)	----
Penicillin(10000units/mL)-Streptomycin (10 mg/mL)	GIBCO	15140-122	----
Trypsin, 0.25%	GIBCO	15050-065	
EDTA(0.02%)-PBS(-)	----		
Other reagent			
DMSO	SIGMA	D8418	----
Fixation and staining solutions			
Methanol	----	----	----
Giemsa solution *	MERK	1.09204	----
Giemsa solution (0.4%)*	SIGMA	GS 500	----
Formalin (37% formaldehyde)	SIGMA	F8775	----
Crystal violet	SIGMA	C3886	----
Ethanol	----	----	----
HCl	----	----	----
Culture vessels			
96-well microplates	CORNING (COSTAR) (Do not use FALCON)	3598	----
100-mm tissue culture dish	CORNING	430167	

*: Giemsa solution purchased from Merck is used after ×20 dilution. Either Giemsa solution can be used.

Annex 9

Results Submitted from Laboratories in the Pre-validation Phase of Validation Study of 96-Well Method

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	MCA
Institution:	Lab 1
Test Number	

Cell Growth Assay

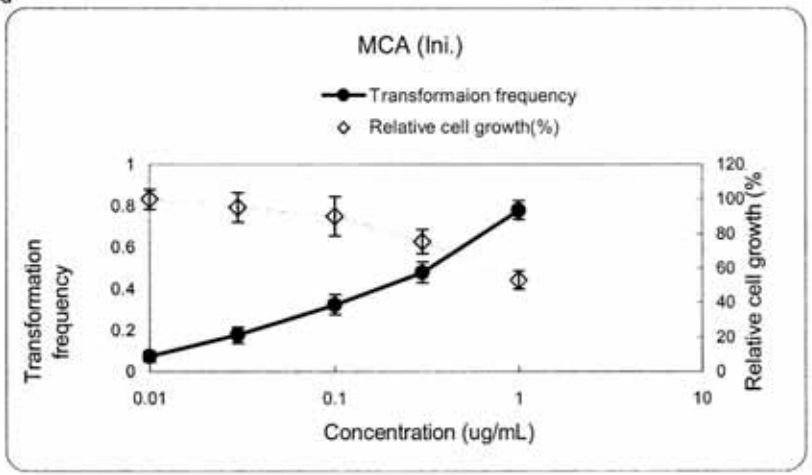
Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.239	1.566	1.555	1.526	1.366	0.921					
2	0.211	1.463	1.666	1.630	1.298	1.081					
3	0.217	1.591	1.481	1.608	1.278	0.883					
4	0.218	1.496	1.518	1.350	1.179	0.877					
5	0.191	1.475	1.538	1.320	1.081	0.919					
6	0.199	1.532	1.307	1.374	1.207	0.868					
7	0.198	1.682	1.352	1.365	1.261	0.962					
8	0.199	1.626	1.499	1.187	1.116	0.883					
Average	0.209	1.554	1.490	1.420	1.223	0.924	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.016	0.077	0.114	0.154	0.096	0.071	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.345	1.281	1.211	1.014	0.715	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	95.2	90.0	75.4	53.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		5.7	8.5	11.4	7.1	5.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	5					
No. of wells counted	96	96	96	96	91	96	96	96	96	96
No. of wells having foci	7	17	31	46	71					
Transformation frequency*	0.07291667	0.17708333	0.32291667	0.47916667	0.78021978	0	0	0	0	0
SE	0.02653610	0.03896110	0.04772332	0.05098672	0.04340923	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted

* * *



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	MCA
Institution:	Lab 2
Test Number	

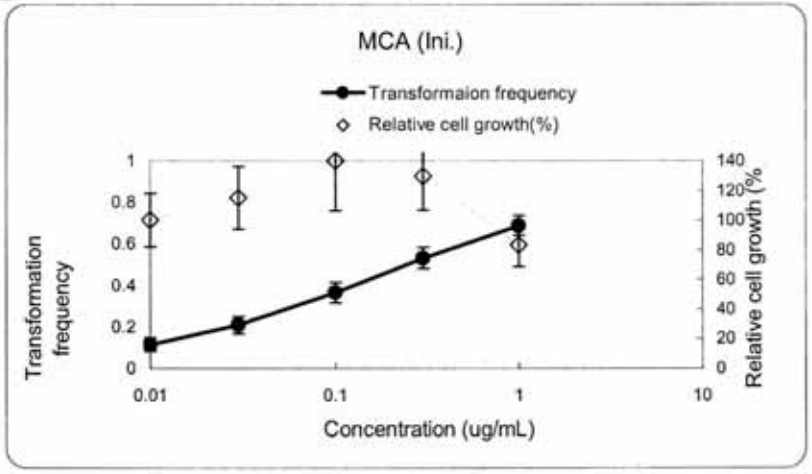
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.072	0.376	0.491	0.449	0.692	0.376					
2	0.080	0.424	0.458	0.480	0.677	0.462					
3	0.077	0.427	0.460	0.569	0.603	0.531					
4	0.082	0.494	0.592	0.677	0.677	0.444					
5	0.077	0.540	0.537	0.779	0.423	0.386					
6	0.081	0.603	0.689	0.831	0.665	0.377					
7	0.076	0.531	0.511	0.689	0.557	0.395					
8	0.070	0.489	0.643	0.716	0.558	0.361					
Average	0.077	0.486	0.548	0.649	0.607	0.417	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.004	0.074	0.086	0.137	0.092	0.058	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.409	0.471	0.572	0.530	0.340	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	115.2	140.0	129.6	83.1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		18.1	21.0	33.6	22.5	14.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	20	35	51	66					
Transformation frequency*	0.11458333	0.20833333	0.36458333	0.53125	0.6875	0	0	0	0	0
SE	0.03250865	0.04144908	0.04912382	0.05093127	0.04730704	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	MCA
Institution:	Lab 3
Test Number	

Cell Growth Assay

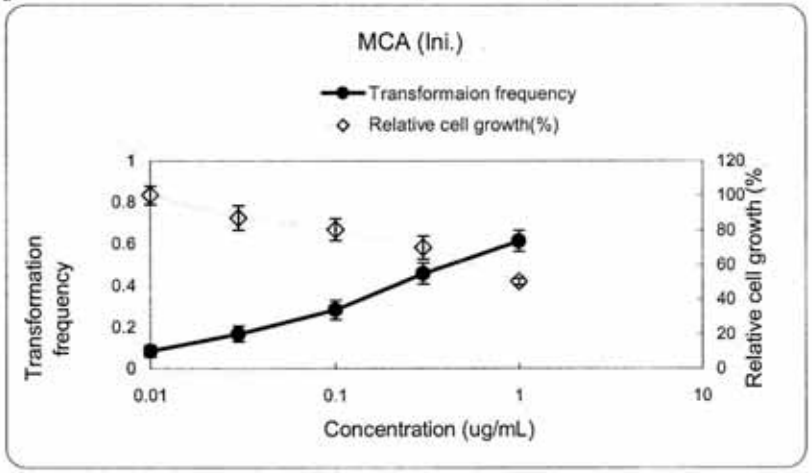
Crystal Violet Staining OD540nm	Blank	0.1% DMSO	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.154	1.198	1.015	0.963	0.938	0.705					
2	0.176	1.177	1.158	1.078	0.943	0.735					
3	0.166	1.321	1.156	0.985	0.864	0.729					
4	0.199	1.216	1.040	1.085	0.884	0.705					
5	0.221	1.323	1.134	1.045	0.902	0.742					
6	0.190	1.240	1.148	0.969	0.968	0.747					
7	0.195	1.311	1.047	1.149	0.891	0.715					
8	0.250	1.246	1.231	1.072	1.084	0.752					
Average	0.194	1.254	1.116	1.043	0.934	0.729	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.031	0.058	0.074	0.066	0.070	0.019	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.060	0.922	0.849	0.740	0.535	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	87.0	80.1	69.8	50.5	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		5.4	7.0	6.2	6.6	1.7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	0.1% DMSO	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	16	27	44	59					
Transformation frequency*	0.08333333	0.16666667	0.28125	0.45833333	0.61458333	0	0	0	0	0
SE	0.02820847	0.03803629	0.04588805	0.05085354	0.04967296	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted

* * *



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	MCA
Institution:	Lab 4
Test Number	

Cell Growth Assay

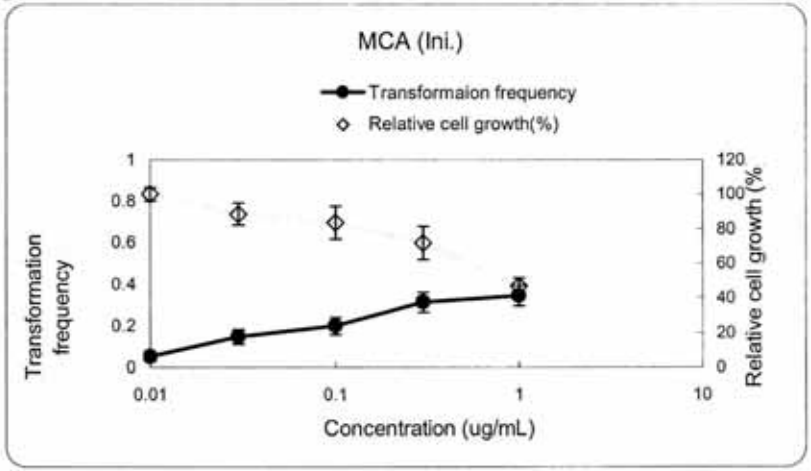
Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.065	0.300	0.284	0.267	0.230	0.175					
2	0.065	0.303	0.283	0.270	0.254	0.177					
3	0.065	0.295	0.279	0.241	0.218	0.182					
4	0.066	0.294	0.257	0.239	0.254	0.161					
5	0.066	0.303	0.275	0.251	0.249	0.155					
6	0.067	0.314	0.242	0.250	0.207	0.192					
7	0.065	0.294	0.286	0.308	0.199	0.177					
8	0.067	0.284	0.269	0.255	0.251	0.175					
Average	0.066	0.298	0.272	0.260	0.233	0.174	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.001	0.009	0.015	0.022	0.023	0.012	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.233	0.206	0.194	0.167	0.109	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	88.5	83.5	71.8	46.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		3.9	6.5	9.6	9.7	5.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	14	19	30	33					
Transformation frequency*	0.05208333	0.14583333	0.19791667	0.3125	0.34375	0	0	0	0	0
SE	0.02267769	0.03602169	0.04066449	0.04730704	0.04847529	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted

* * *



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	MCA
Institution:	Lab 1
Test Number	

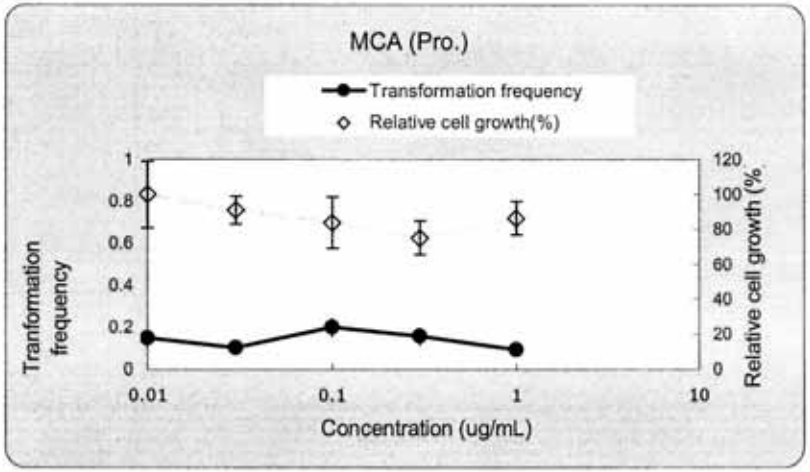
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent 0.01	Concentrations (ug/ml) 0.03	0.1	0.3	1					
1	0.241	1.674	1.399	1.271	1.157	1.237					
2	0.245	1.439	1.411	1.534	0.987	1.195					
3	0.230	1.617	1.592	1.266	1.304	1.375					
4	0.250	1.062	1.472	1.221	1.249	1.364					
5	0.280	1.397	1.583	1.409	1.262	1.579					
6	0.285	1.651	1.325	0.999	1.403	1.501					
7	0.219	1.837	1.320	1.474	1.170	1.401					
8	0.268	1.754	1.401	1.574	1.311	1.359					
Average	0.252	1.554	1.438	1.344	1.230	1.376	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.024	0.247	0.104	0.191	0.126	0.125	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.302	1.186	1.091	0.978	1.124	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	91.1	83.8	75.1	86.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		19.0	8.0	14.7	9.7	9.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent 0.01	Concentrations (ug/ml) 0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	2	0	0	0	0					
No. of wells counted	94	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	10	19	15	9					
Transformation frequency*	0.14893617	0.10416667	0.19791667	0.15625	0.09375	0	0	0	0	0
SE	0.03672123	0.03117758	0.04066449	0.03705794	0.02974911	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	MCA
Institution:	Lab 2
Test Number	

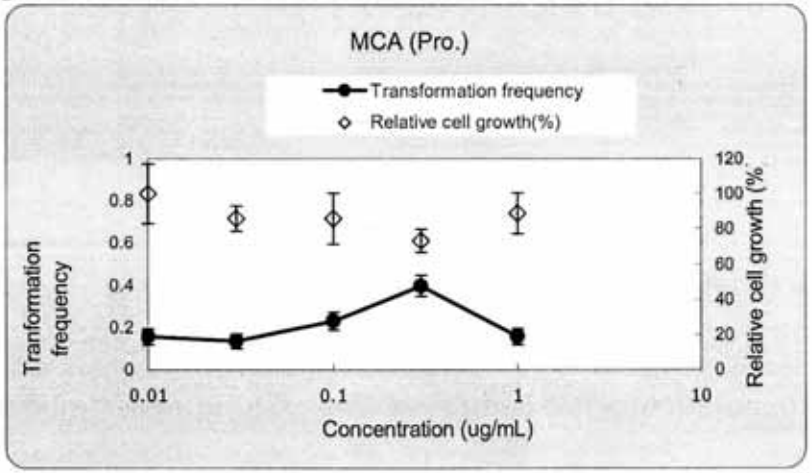
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.072	0.221	0.260	0.222	0.217	0.228					
2	0.080	0.255	0.252	0.268	0.239	0.293					
3	0.077	0.289	0.265	0.298	0.247	0.266					
4	0.082	0.296	0.258	0.264	0.244	0.281					
5	0.077	0.287	0.287	0.256	0.232	0.285					
6	0.081	0.327	0.244	0.305	0.221	0.285					
7	0.076	0.315	0.239	0.241	0.214	0.251					
8	0.070	0.325	0.272	0.223	0.247	0.237					
Average	0.077	0.289	0.260	0.260	0.233	0.266	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.004	0.036	0.015	0.031	0.014	0.024	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.213	0.183	0.183	0.156	0.189	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	86.0	86.0	73.3	88.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		17.1	7.3	14.6	6.4	11.5	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	15	13	22	38	15					
Transformation frequency*	0.15625	0.13541667	0.22916667	0.39583333	0.15625	0	0	0	0	0
SE	0.03705794	0.03492238	0.04289635	0.04991131	0.03705794	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	MCA
Institution:	Lab 3
Test Number	

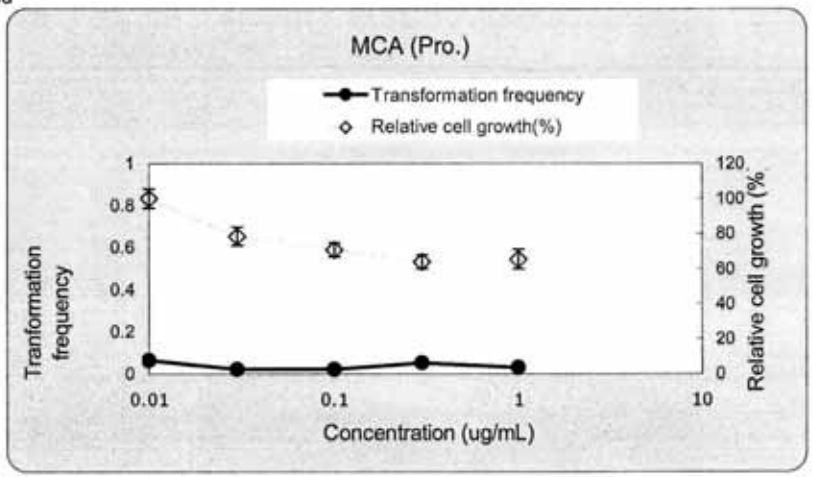
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	0.1% DMSO	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.154	1.166	0.904	0.864	0.765	0.772					
2	0.183	1.190	0.942	0.869	0.798	0.829					
3	0.182	1.163	0.988	0.924	0.869	0.834					
4	0.185	1.239	1.066	0.913	0.884	0.827					
5	0.234	1.240	1.022	0.909	0.853	0.926					
6	0.193	1.192	1.031	0.903	0.855	0.937					
7	0.210	1.181	0.961	0.950	0.875	0.907					
8	0.210	1.329	1.021	0.985	0.856	0.850					
Average	0.194	1.213	0.992	0.915	0.844	0.860	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.024	0.056	0.053	0.040	0.041	0.057	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.019	0.798	0.721	0.651	0.666	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	78.3	70.8	63.9	65.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		5.4	5.2	3.9	4.0	5.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	0.1% DMSO	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	2	2	5	3					
Transformation frequency*	0.0625	0.02083333	0.02083333	0.05208333	0.03125	0	0	0	0	0
SE	0.02470529	0.01457713	0.01457713	0.02267769	0.01775805	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	MCA
Institution:	Lab 4
Test Number	

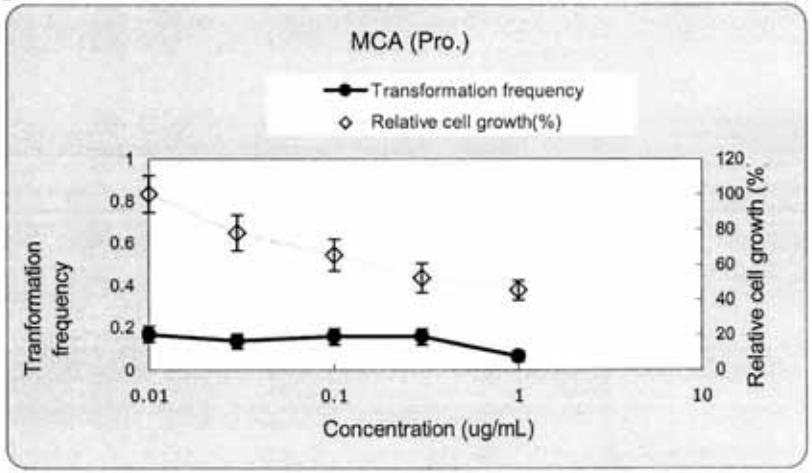
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.03	0.1	0.3	1					
1	0.065	0.303	0.269	0.232	0.215	0.186					
2	0.065	0.294	0.223	0.239	0.183	0.173					
3	0.065	0.282	0.244	0.194	0.162	0.157					
4	0.066	0.280	0.226	0.207	0.172	0.170					
5	0.066	0.259	0.243	0.189	0.178	0.162					
6	0.067	0.311	0.246	0.200	0.187	0.150					
7	0.065	0.276	0.218	0.204	0.166	0.151					
8	0.067	0.242	0.197	0.185	0.162	0.155					
Average	0.066	0.281	0.233	0.206	0.178	0.163	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.001	0.023	0.022	0.020	0.018	0.012	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.215	0.168	0.141	0.112	0.097	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	77.9	65.3	52.3	45.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		10.5	10.1	9.1	8.2	5.7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.03	0.1	0.3	1	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	16	13	15	15	6					
Transformation frequency*	0.16666667	0.13541667	0.15625	0.15625	0.0625	0	0	0	0	0
SE	0.03803629	0.03492238	0.03705794	0.03705794	0.02470529	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	TPA
Institution:	Lab 1
Test Number	

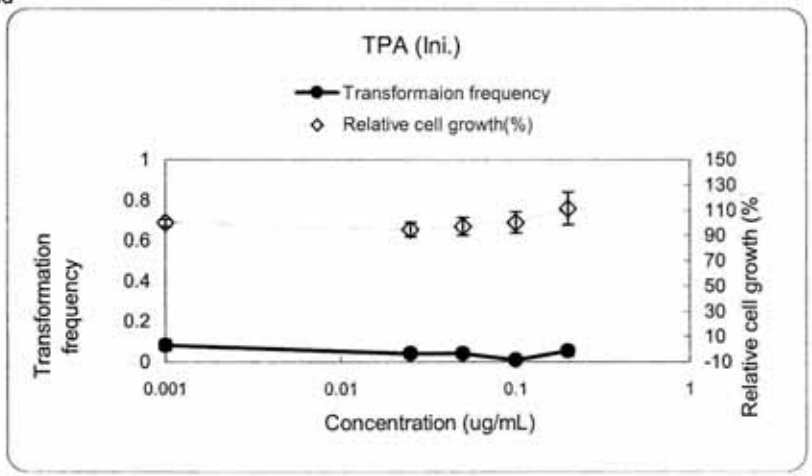
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.001	0.025	0.05	0.1	0.2					
1	0.210	1.732	1.598	1.889	1.925	2.426					
2	0.316	1.751	1.878	1.805	1.775	1.987					
3	0.212	1.788	1.747	1.603	1.600	1.836					
4	0.202	1.848	1.669	1.605	1.936	1.842					
5	0.201	1.805	1.633	1.824	1.640	1.949					
6	0.218	1.835	1.761	1.809	1.768	1.942					
7	0.214	1.817	1.699	1.653	1.780	1.776					
8	0.233	1.751	1.700	1.796	1.939	1.993					
Average	0.226	1.791	1.711	1.748	1.795	1.969	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.038	0.043	0.087	0.110	0.131	0.201	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.565	1.485	1.522	1.570	1.743	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	94.9	97.3	100.3	111.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		2.7	5.5	7.1	8.4	12.8	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.001	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	4	4	1	5					
Transformation frequency*	0.08333333	0.04166667	0.04166667	0.01041667	0.05208333	0	0	0	0	0
SE	0.02820847	0.02039469	0.02039469	0.01036227	0.02267769	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	TPA
Institution:	Lab 2
Test Number	

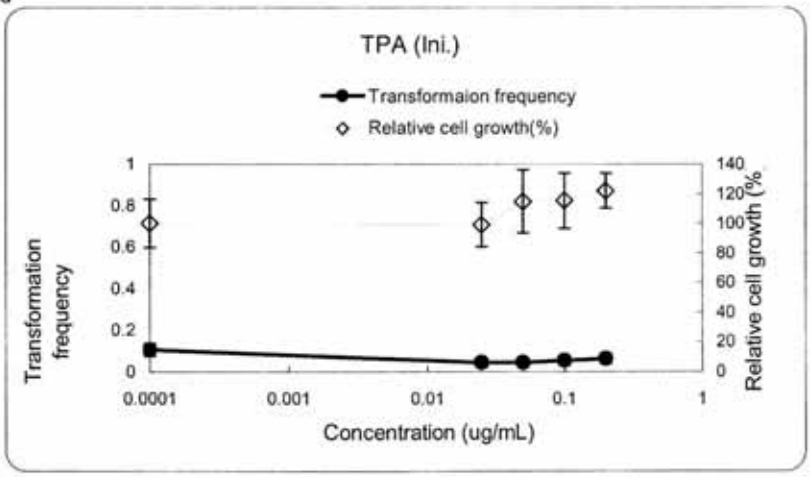
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.0001	0.025	0.05	0.1	0.2					
1	0.072	0.291	0.351	0.322	0.391	0.430					
2	0.080	0.362	0.334	0.421	0.408	0.517					
3	0.077	0.366	0.332	0.352	0.371	0.454					
4	0.082	0.419	0.332	0.425	0.354	0.457					
5	0.077	0.456	0.423	0.453	0.440	0.469					
6	0.081	0.388	0.399	0.511	0.456	0.435					
7	0.076	0.407	0.418	0.494	0.524	0.397					
8	0.070	0.359	0.439	0.431	0.477	0.426					
Average	0.077	0.381	0.379	0.426	0.428	0.448	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.004	0.049	0.046	0.064	0.057	0.036	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.304	0.302	0.349	0.351	0.371	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	99.2	114.8	115.3	122.1	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		16.2	15.1	21.1	18.9	11.7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.0001	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	4	4	5	6					
Transformation frequency*	0.10416667	0.04166667	0.04166667	0.05208333	0.0625	0	0	0	0	0
SE	0.03117758	0.02039469	0.02039469	0.02267769	0.02470529	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	TPA
Institution:	Lab 3
Test Number	

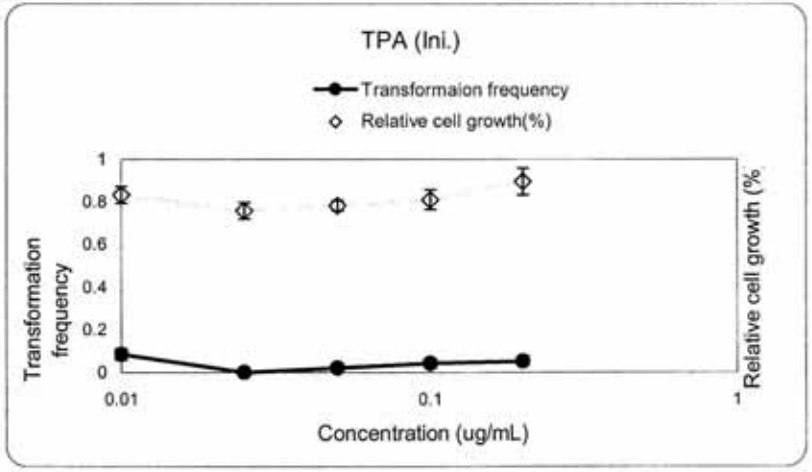
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	0.1% DMSO	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.154	1.171	1.073	1.138	1.181	1.182					
2	0.176	1.158	1.059	1.090	1.071	1.161					
3	0.166	1.120	1.125	1.114	1.162	1.227					
4	0.199	1.184	1.043	1.124	1.117	1.300					
5	0.221	1.276	1.095	1.121	1.214	1.282					
6	0.190	1.225	1.158	1.152	1.145	1.299					
7	0.195	1.219	1.140	1.152	1.206	1.315					
8	0.250	1.210	1.160	1.185	1.241	1.393					
Average	0.194	1.195	1.107	1.135	1.167	1.270	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.031	0.048	0.046	0.029	0.056	0.076	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.002	0.913	0.941	0.973	1.076	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	91.1	93.9	97.2	107.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		4.8	4.5	2.9	5.6	7.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	0.1% DMSO	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	0	2	4	5					
Transformation frequency*	0.08333333	0	0.02083333	0.04166667	0.05208333	0	0	0	0	0
SE	0.02820847	0.00000000	0.01457713	0.02039469	0.02267769	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical:	TPA
Institution:	Lab 4
Test Number	

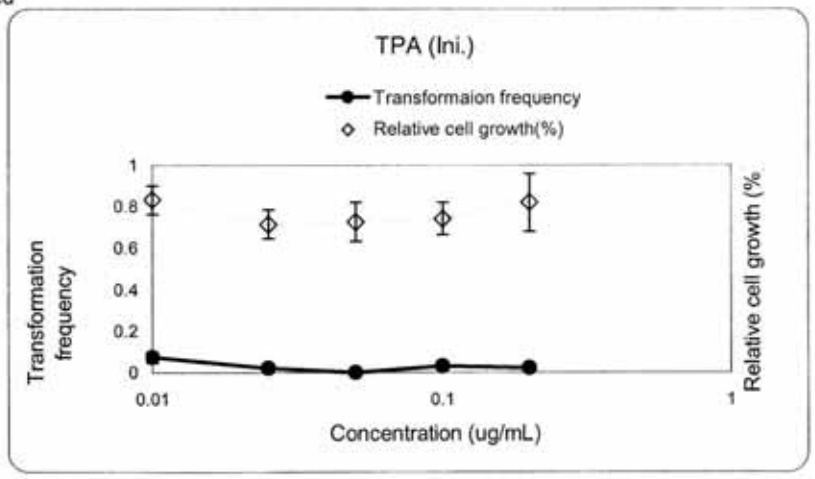
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.065	0.352	0.307	0.339	0.331	0.392					
2	0.065	0.305	0.301	0.284	0.309	0.339					
3	0.065	0.352	0.278	0.256	0.282	0.288					
4	0.066	0.317	0.295	0.307	0.259	0.361					
5	0.066	0.329	0.289	0.305	0.266	0.281					
6	0.067	0.328	0.309	0.255	0.300	0.309					
7	0.065	0.301	0.265	0.274	0.306	0.265					
8	0.067	0.299	0.248	0.303	0.307	0.316					
Average	0.066	0.323	0.287	0.290	0.295	0.319	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.001	0.021	0.022	0.029	0.024	0.043	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.257	0.221	0.225	0.229	0.253	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	85.9	87.4	89.1	98.5	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		8.3	8.4	11.1	9.4	16.7	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	2	0	3	2					
Transformation frequency*	0.07291667	0.02083333	0	0.03125	0.02083333	0	0	0	0	0
SE	0.02653610	0.01457713	0.00000000	0.01775805	0.01457713	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	TPA
Institution:	Lab 1
Test Number	

Cell Growth Assay

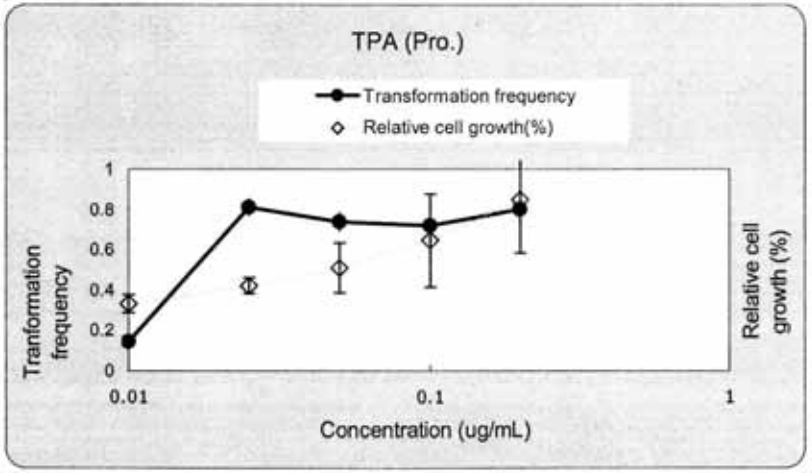
Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.047	0.164	0.202	0.207	0.239	0.565					
2	0.051	0.160	0.211	0.217	0.249	0.389					
3	0.043	0.177	0.235	0.266	0.439	0.437					
4	0.047	0.167	0.197	0.197	0.408	0.363					
5	0.043	0.155	0.218	0.203	0.214	0.259					
6	0.047	0.192	0.197	0.215	0.306	0.344					
7	0.049	0.163	0.193	0.321	0.220	0.268					
8	0.048	0.202	0.197	0.289	0.251	0.309					
Average	0.047	0.173	0.206	0.239	0.291	0.367	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.003	0.017	0.014	0.046	0.087	0.100	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.126	0.159	0.193	0.244	0.320	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	126.9	153.2	194.1	254.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		13.2	11.4	37.0	69.2	79.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	78	71	69	77					
Transformation frequency*	0.14583333	0.8125	0.73958333	0.71875	0.80208333	0	0	0	0	0
SE	0.03602169	0.03983609	0.04479116	0.04588805	0.04066449	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted

* * * *



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	TPA
Institution:	Lab 2
Test Number	

Cell Growth Assay

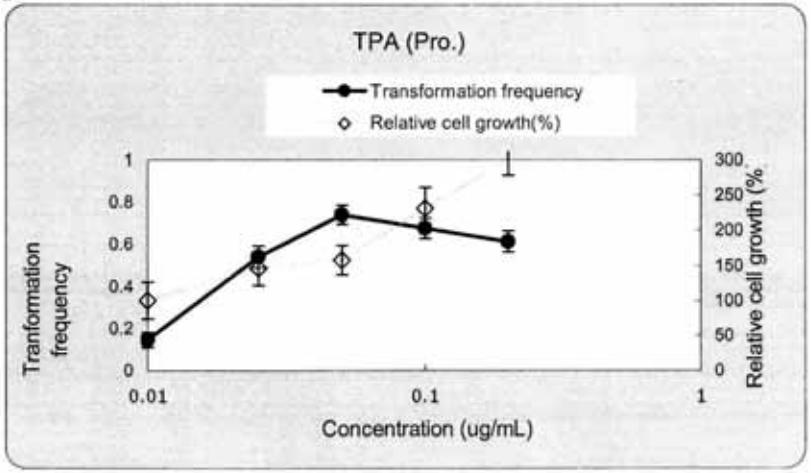
Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.072	0.225	0.356	0.365	0.465	0.695					
2	0.080	0.322	0.409	0.473	0.597	0.745					
3	0.077	0.341	0.508	0.430	0.580	0.750					
4	0.082	0.325	0.385	0.435	0.633	0.811					
5	0.077	0.360	0.385	0.499	0.670	0.805					
6	0.081	0.390	0.484	0.478	0.664	0.764					
7	0.076	0.245	0.406	0.453	0.578	0.724					
8	0.070	0.246	0.361	0.377	0.675	0.849					
Average	0.077	0.307	0.412	0.439	0.608	0.768	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.004	0.061	0.056	0.048	0.070	0.051	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.230	0.335	0.362	0.531	0.691	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	145.7	157.4	230.9	300.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		26.4	24.2	20.7	30.5	22.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	52	71	65	59					
Transformation frequency*	0.14583333	0.54166667	0.73958333	0.67708333	0.61458333	0	0	0	0	0
SE	0.03602169	0.05085354	0.04479116	0.04772332	0.04967296	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted

* * * *



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	TPA
Institution:	Lab 3
Test Number	

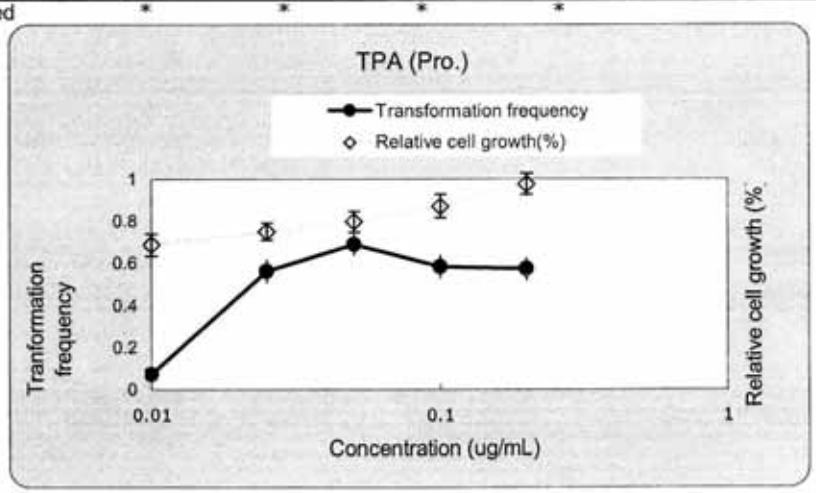
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	0.1% DMSO	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.154	1.067	1.242	1.253	1.424	1.744					
2	0.183	1.308	1.300	1.395	1.558	1.673					
3	0.182	1.239	1.342	1.444	1.449	1.660					
4	0.185	1.206	1.360	1.527	1.519	1.757					
5	0.234	1.334	1.463	1.432	1.649	1.635					
6	0.193	1.351	1.422	1.505	1.695	1.757					
7	0.210	1.281	1.394	1.451	1.591	1.838					
8	0.210	1.231	1.345	1.482	1.622	1.869					
Average	0.194	1.252	1.359	1.436	1.563	1.742	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.024	0.091	0.069	0.085	0.095	0.083	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	1.058	1.165	1.242	1.370	1.548	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	110.1	117.4	129.4	146.3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		8.6	6.6	8.0	9.0	7.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	0.1% DMSO	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	54	66	56	55					
Transformation frequency*	0.07291667	0.5625	0.6875	0.58333333	0.57291667	0	0	0	0	0
SE	0.02653610	0.05063079	0.04730704	0.05031728	0.05048547	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical:	TPA
Institution:	Lab 4
Test Number	

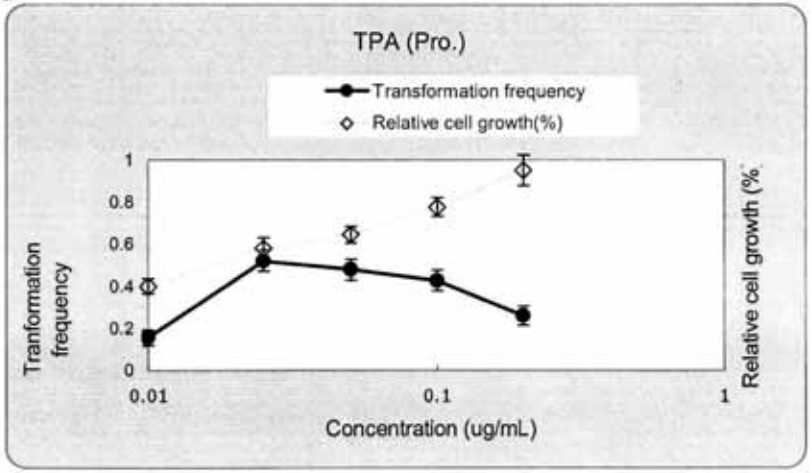
Cell Growth Assay

Crystal Violet Staining OD540nm	Blank	Solvent	Concentrations (ug/ml)								
		0.01	0.025	0.05	0.1	0.2					
1	0.065	0.310	0.419	0.440	0.490	0.615					
2	0.065	0.286	0.374	0.398	0.520	0.552					
3	0.065	0.284	0.389	0.375	0.472	0.634					
4	0.066	0.271	0.369	0.402	0.480	0.577					
5	0.066	0.284	0.381	0.434	0.486	0.554					
6	0.067	0.272	0.371	0.408	0.445	0.529					
7	0.065	0.248	0.340	0.403	0.463	0.527					
8	0.067	0.262	0.332	0.395	0.460	0.562					
Average	0.066	0.277	0.372	0.407	0.477	0.569	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD	0.001	0.019	0.027	0.021	0.023	0.038	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Average - Blank	0.000	0.211	0.306	0.341	0.411	0.503	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Relative cell growth (%)		100.0	144.9	161.4	194.4	237.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
SD of relative cell growth		8.8	12.9	10.0	10.8	18.2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

Transformation Assay

Giemsa Staining	Solvent	Concentrations (ug/ml)								
	0.01	0.025	0.05	0.1	0.2	0	0	0	0	0
No. of wells excluded	0	0	0	0	0					
No. of wells counted	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	15	50	46	41	25					
Transformation frequency*	0.15625	0.52083333	0.47916667	0.42708333	0.26041667	0	0	0	0	0
SE	0.03705794	0.05098672	0.05098672	0.05048547	0.04479116	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000

* No. of wells having foci/ No. of wells counted



Annex 10

Results Submitted from Laboratories in the Phase I of Validation Study of 96-Well Method

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	2AAF
Institution:	Lab 1
Test Number	

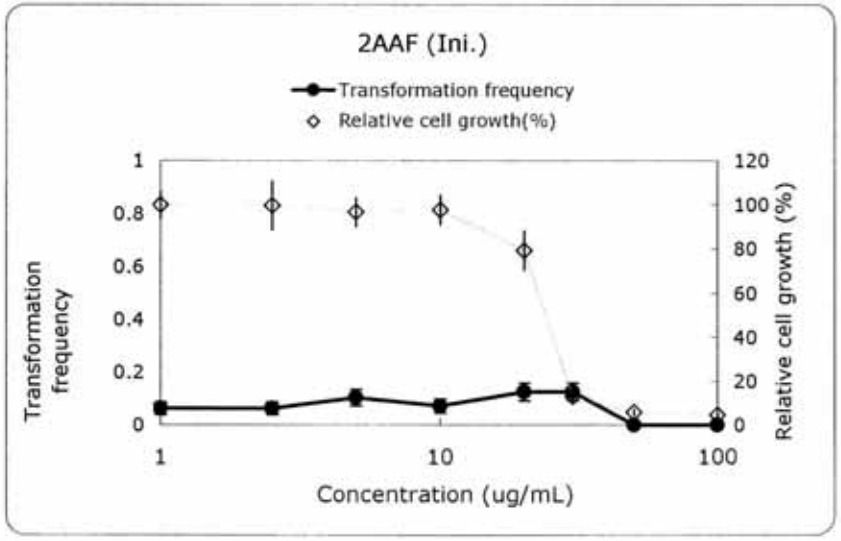
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.5	5	10	20	30	50	100			0.1% DMSO	MCA (1ug/mL)
1	0.118	0.678	0.720	0.618	0.659	0.530	0.209	0.161	0.148			0.678	0.224
2	0.124	0.701	0.711	0.631	0.625	0.538	0.191	0.148	0.139			0.701	0.275
3	0.128	0.696	0.676	0.677	0.621	0.634	0.188	0.145	0.141			0.696	0.306
4	0.114	0.661	0.676	0.663	0.648	0.511	0.164	0.147	0.138			0.661	0.288
5	0.104	0.609	0.733	0.729	0.730	0.526	0.166	0.150	0.138			0.609	0.258
6	0.123	0.697	0.534	0.634	0.701	0.590	0.202	0.138	0.146			0.697	0.287
7	0.096	0.708	0.678	0.634	0.661	0.519	0.188	0.135	0.124			0.708	0.236
8	0.102	0.651	0.663	0.668	0.654	0.623	0.185	0.140	0.137			0.651	0.220
Average	0.114	0.675	0.674	0.657	0.662	0.559	0.187	0.146	0.139	#DIV/0!	#DIV/0!	0.675	0.262
SD	0.012	0.033	0.062	0.036	0.037	0.049	0.016	0.008	0.007	#DIV/0!	#DIV/0!	0.033	0.032
Average - Blank	0.000	0.562	0.560	0.543	0.549	0.445	0.073	0.032	0.025	#DIV/0!	#DIV/0!	0.562	0.148
Relative Cell Growth (%)		100.0	99.8	96.7	97.7	79.3	13.0	5.7	4.5	#DIV/0!	#DIV/0!	100.0	26.4
SD of Relative Cell Growth		6.0	11.0	6.4	6.5	8.8	2.8	1.5	1.3	#DIV/0!	#DIV/0!	6.0	5.8

Transformation Assay

Giemsa Staining	1	2.5	5	10	20	30	50	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	96	96			0	0
No. of wells counted	96	96	96	96	96	96	0	0	96	96	96	96
No. of wells having foci	6	6	10	7	12	12	TOX	TOX			6	44
Transformation frequency*	0.0625	0.0625	0.104166667	0.072916667	0.125	0.125	#VALUE!	#VALUE!	0	0	0.0625	0.458333333
SE	0.02470529	0.02470529	0.03117758	0.02653610	0.03375386	0.03375386	#VALUE!	#VALUE!	0.00000000	0.00000000	0.02470529	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	2AAF
Institution:	Lab 2
Test Number	

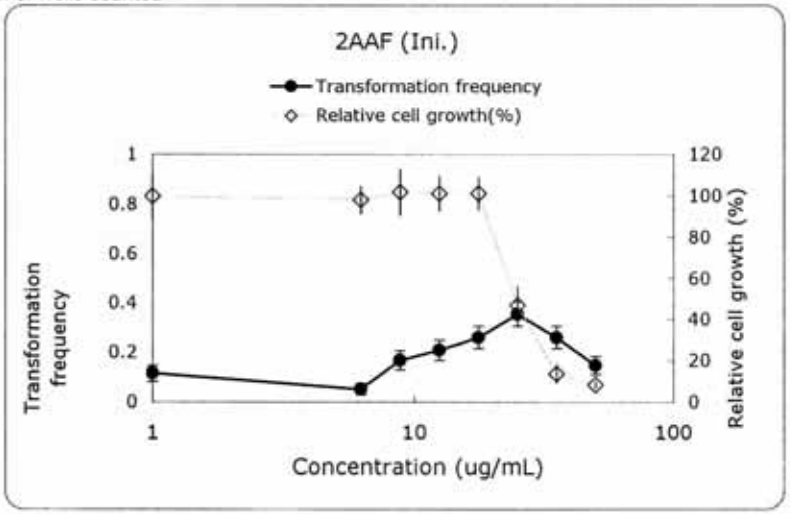
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	6.25	8.84	12.5	17.7	25	35.4	50			0.1% DMSO	MCA (1ug/mL)
1	0.018	0.246	0.282	0.301	0.288	0.295	0.182	0.064	0.041			0.246	0.146
2	0.023	0.341	0.322	0.385	0.327	0.328	0.194	0.060	0.051			0.341	0.186
3	0.021	0.316	0.299	0.279	0.322	0.358	0.135	0.060	0.046			0.316	0.189
4	0.020	0.341	0.345	0.338	0.345	0.339	0.168	0.053	0.051			0.341	0.189
5	0.019	0.318	0.327	0.321	0.356	0.305	0.119	0.045	0.039			0.318	0.134
6	0.018	0.339	0.315	0.350	0.296	0.315	0.146	0.047	0.043			0.339	0.152
7	0.016	0.317	0.295	0.300	0.341	0.339	0.188	0.064	0.039			0.317	0.155
8	0.017	0.327	0.312	0.314	0.298	0.292	0.142	0.087	0.043			0.327	0.156
Average	0.019	0.318	0.312	0.324	0.322	0.321	0.159	0.060	0.044	#DIV/0!	#DIV/0!	0.318	0.163
SD	0.002	0.031	0.020	0.033	0.025	0.024	0.028	0.013	0.005	#DIV/0!	#DIV/0!	0.031	0.022
Average - Blank	0.000	0.299	0.293	0.305	0.303	0.302	0.140	0.041	0.025	#DIV/0!	#DIV/0!	0.299	0.144
Relative Cell Growth (%)		100.0	98.0	101.8	101.2	101.1	46.9	13.7	8.4	#DIV/0!	#DIV/0!	100.0	48.3
SD of Relative Cell Growth		10.4	6.7	11.2	8.5	7.9	9.2	4.4	1.6	#DIV/0!	#DIV/0!	10.4	7.2

Transformation Assay

Giemsa Staining	1	6.25	8.84	12.5	17.7	25	35.4	50	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	5	16	20	25	34	25	14			11	68
Transformation frequency*	0.11458333	0.05208333	0.16666667	0.20833333	0.26041667	0.35416667	0.26041667	0.14583333	0	0	0.114583333	0.708333333
SE	0.03250865	0.02267769	0.03803629	0.04144908	0.04479116	0.04881221	0.04479116	0.03602169	0.00000000	0.00000000	0.03250865	0.04639024

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	2AAF
Institution:	Lab 3
Test Number	

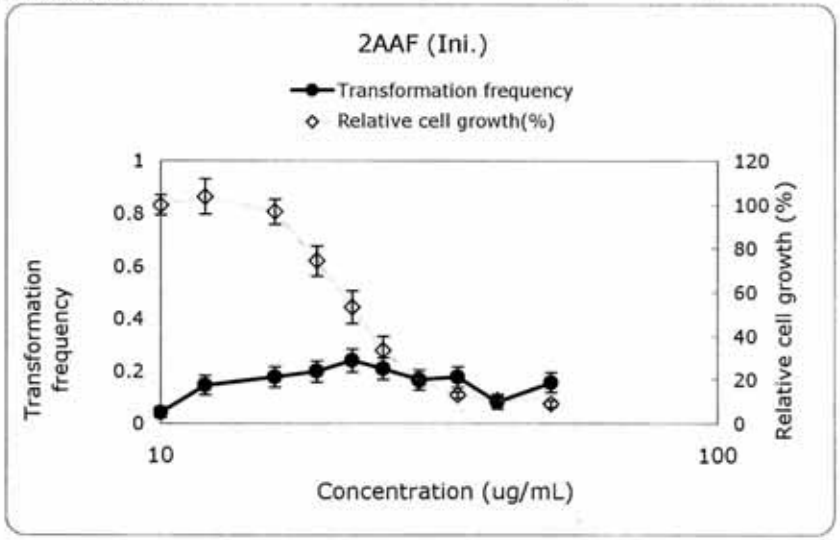
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)								Negative Control	Positive Control
		10	12	16	19	22	25	29	34	40	50	0.1% DMSO	MCA (1ug/mL)
1	0.101	0.961	0.992	0.917	0.812	0.616	0.299	0.252	0.233	0.191	0.190	0.961	0.495
2	0.111	0.885	0.973	0.880	0.724	0.528	0.390	0.257	0.209	0.188	0.197	0.885	0.520
3	0.111	0.957	0.839	0.863	0.688	0.476	0.387	0.266	0.229	0.225	0.186	0.957	0.532
4	0.112	0.897	0.904	0.934	0.721	0.495	0.414	0.287	0.217	0.212	0.193	0.897	0.523
5	0.111	0.866	1.036	0.802	0.660	0.552	0.436	0.257	0.205	0.206	0.184	0.866	0.457
6	0.122	0.875	0.879	0.867	0.638	0.630	0.431	0.299	0.210	0.194	0.177	0.875	0.535
7	0.112	0.911	0.925	0.864	0.667	0.506	0.331	0.263	0.217	0.189	0.173	0.911	0.483
8	0.113	0.906	0.954	0.934	0.727	0.483	0.332	0.255	0.199	0.181	0.159	0.906	0.475
Average	0.112	0.907	0.938	0.883	0.705	0.536	0.378	0.267	0.215	0.198	0.182	0.907	0.503
SD	0.006	0.035	0.064	0.045	0.055	0.059	0.051	0.017	0.012	0.015	0.012	0.035	0.029
Average - Blank	0.000	0.796	0.826	0.771	0.593	0.424	0.266	0.155	0.103	0.087	0.071	0.796	0.391
Relative Cell Growth (%)		100.0	103.8	96.9	74.5	53.3	33.4	19.5	13.0	10.9	8.9	100.0	49.1
SD of Relative Cell Growth		4.4	8.0	5.6	6.9	7.4	6.4	2.1	1.5	1.9	1.5	4.4	3.7

Transformation Assay

Giemsa Staining	10	12	16	19	22	25	29	34	40	50	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	4	14	17	19	23	20	16	17	8	15	4	83
Transformation frequency*	0.041666667	0.145833333	0.177083333	0.197916667	0.239583333	0.208333333	0.166666667	0.177083333	0.083333333	0.15625	0.041666667	0.864583333
SE	0.02039469	0.03602169	0.03896110	0.04066449	0.04356307	0.04144908	0.03803629	0.03896110	0.02820847	0.03705794	0.02039469	0.03492238
		*	*	*	*	*	*		*			

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	2AAF
Institution:	Lab 4
Test Number	

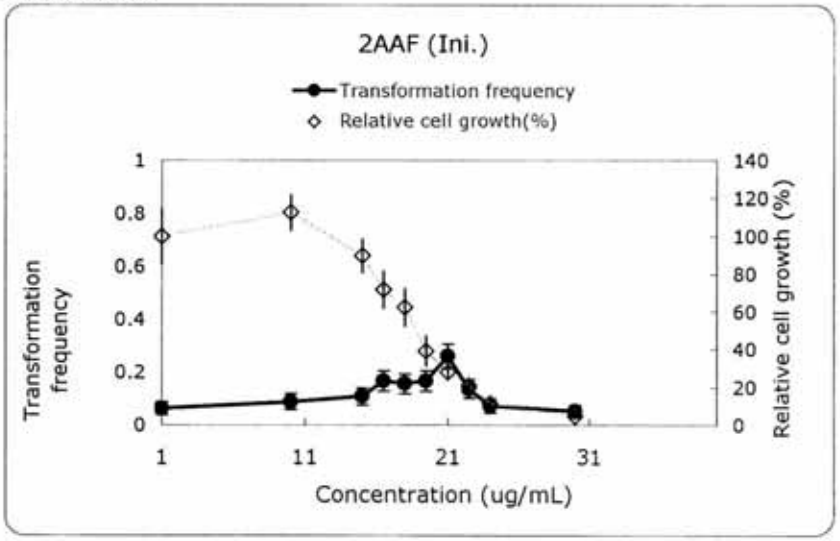
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	10	15	16.5	18	19.5	21	22.5	24	30	0.1% DMSO	MCA (1ug/mL)
1	0.072	0.268	0.341	0.263	0.242	0.210	0.156	0.128	0.124	0.108	0.085	0.268	0.221
2	0.071	0.281	0.291	0.280	0.244	0.200	0.147	0.136	0.109	0.100	0.086	0.281	0.192
3	0.074	0.271	0.285	0.292	0.234	0.254	0.175	0.130	0.124	0.099	0.086	0.271	0.184
4	0.075	0.306	0.293	0.261	0.227	0.202	0.152	0.128	0.114	0.097	0.083	0.306	0.205
5	0.073	0.345	0.314	0.258	0.222	0.206	0.190	0.135	0.122	0.097	0.087	0.345	0.192
6	0.074	0.297	0.327	0.248	0.214	0.184	0.150	0.150	0.119	0.091	0.081	0.297	0.173
7	0.075	0.255	0.321	0.232	0.238	0.197	0.145	0.128	0.114	0.096	0.079	0.255	0.180
8	0.076	0.255	0.318	0.270	0.181	0.191	0.139	0.128	0.116	0.097	0.081	0.255	0.183
Average	0.074	0.285	0.311	0.263	0.225	0.206	0.157	0.133	0.118	0.098	0.084	0.285	0.191
SD	0.001	0.030	0.020	0.018	0.021	0.021	0.017	0.008	0.005	0.005	0.003	0.030	0.015
Average - Blank	0.000	0.211	0.237	0.189	0.152	0.132	0.083	0.059	0.044	0.024	0.010	0.211	0.117
Relative Cell Growth (%)		100.0	112.6	89.7	71.9	62.5	39.3	28.0	20.9	11.5	4.6	100.0	55.6
SD of Relative Cell Growth		14.4	9.3	8.7	9.7	10.0	8.0	3.7	2.5	2.3	1.3	14.4	7.3

Transformation Assay

Giemsa Staining	1	10	15	16.5	18	19.5	21	22.5	24	30	Negative Control	Positive Control
No. of wells excluded	0	5	3	0	0	0	0	0	0	0	0	0
No. of wells counted	96	91	93	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	8	10	16	15	16	25	13	7	5	6	41
Transformation frequency*	0.0625	0.087912088	0.107526882	0.166666667	0.15625	0.166666667	0.260416667	0.135416667	0.072916667	0.052083333	0.0625	0.427083333
SE	0.02470529	0.02968397	0.03212289	0.03803629	0.03705794	0.03803629	0.04479116	0.03492238	0.02653610	0.02267769	0.02470529	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	2AAF
Institution:	Lab 4
Test Number	

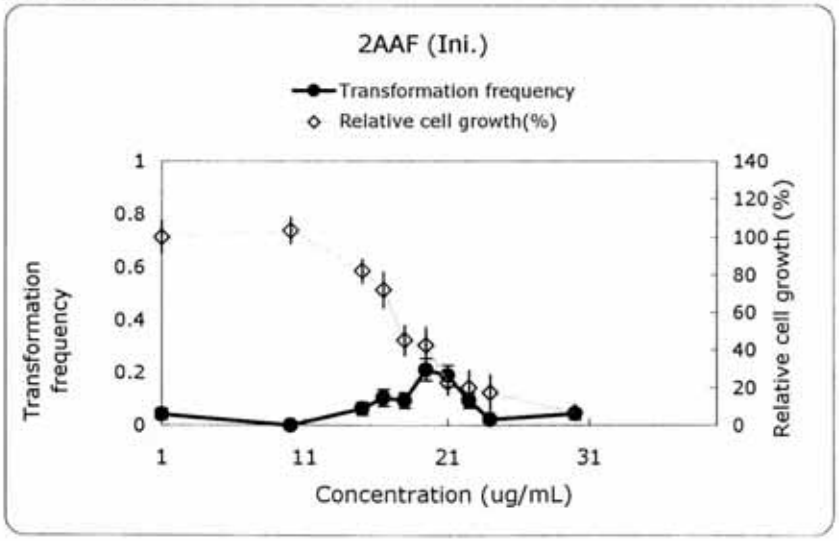
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	10	15	16.5	18	19.5	21	22.5	24	30	0.1% DMSO	MCA (1ug/mL)
1	0.062	0.320	0.334	0.264	0.287	0.232	0.208	0.163	0.160	0.167	0.084	0.320	0.165
2	0.065	0.318	0.318	0.281	0.289	0.202	0.199	0.143	0.151	0.133	0.082	0.318	0.196
3	0.065	0.361	0.343	0.282	0.288	0.172	0.188	0.116	0.128	0.097	0.081	0.361	0.201
4	0.063	0.321	0.349	0.303	0.274	0.200	0.208	0.119	0.112	0.101	0.087	0.321	0.203
5	0.066	0.364	0.344	0.318	0.243	0.184	0.192	0.116	0.103	0.097	0.083	0.364	0.212
6	0.071	0.374	0.357	0.292	0.227	0.170	0.141	0.124	0.101	0.093	0.081	0.374	0.203
7	0.063	0.335	0.379	0.277	0.234	0.180	0.152	0.105	0.097	0.097	0.082	0.335	0.178
8	0.061	0.321	0.364	0.298	0.251	0.166	0.160	0.130	0.105	0.108	0.081	0.321	0.185
Average	0.065	0.339	0.349	0.289	0.262	0.188	0.181	0.127	0.120	0.112	0.083	0.339	0.193
SD	0.003	0.023	0.019	0.017	0.026	0.022	0.026	0.018	0.024	0.026	0.002	0.023	0.016
Average - Blank	0.000	0.275	0.284	0.225	0.197	0.124	0.117	0.063	0.055	0.047	0.018	0.275	0.128
Relative Cell Growth (%)		100.0	103.4	81.8	71.7	45.0	42.4	22.7	20.1	17.2	6.6	100.0	46.7
SD of Relative Cell Growth		8.5	6.8	6.2	9.4	8.0	9.6	6.7	8.8	9.4	0.8	8.5	5.7

Transformation Assay

Giemsa Staining	1	10	15	16.5	18	19.5	21	22.5	24	30	Negative Control	Positive Control
No. of wells excluded	0	96	0	0	0	1	0	0	0	2	0	0
No. of wells counted	96	0	96	96	96	95	96	96	96	94	96	96
No. of wells having foci	4		6	10	9	20	18	9	2	4	4	52
Transformation frequency*	0.041666667	#DIV/0!	0.0625	0.104166667	0.09375	0.210526316	0.1875	0.09375	0.020833333	0.042553191	0.041666667	0.541666667
SE	0.02039469	#DIV/0!	0.02470529	0.03117758	0.02974911	0.04182734	0.03983609	0.02974911	0.01457713	0.02081898	0.02039469	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	2AAF
Institution:	Lab 1
Test Number	

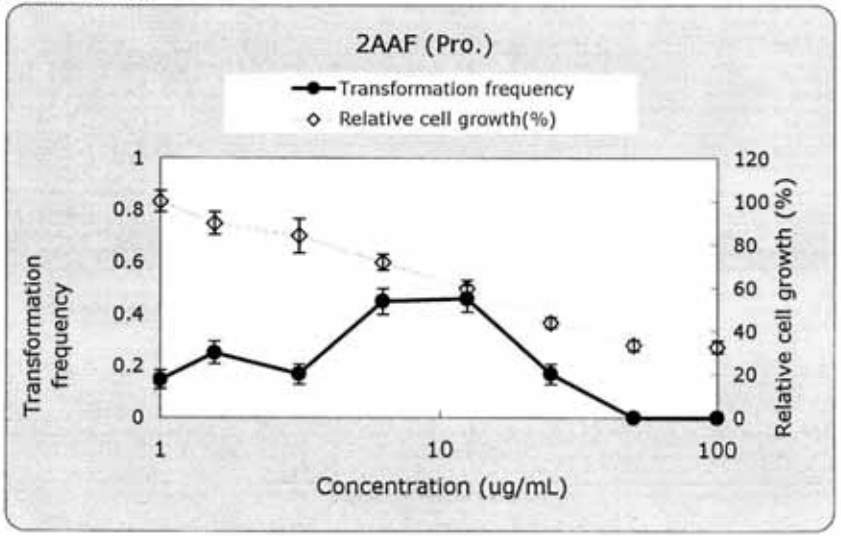
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	1.56	3.13	6.25	12.5	25	50	100			0.1% DMSO	TPA (50ng/mL)
1	0.112	0.905	0.757	0.688	0.666	0.551	0.456	0.372	0.352			0.905	0.940
2	0.121	0.882	0.807	0.725	0.684	0.569	0.464	0.369	0.359			0.882	0.971
3	0.120	0.870	0.881	0.758	0.682	0.576	0.441	0.378	0.379			0.870	0.980
4	0.128	0.981	0.849	0.767	0.689	0.589	0.452	0.374	0.362			0.981	1.053
5	0.136	0.919	0.797	0.800	0.644	0.575	0.467	0.375	0.393			0.919	0.992
6	0.134	0.896	0.852	0.791	0.696	0.624	0.502	0.408	0.410			0.896	0.967
7	0.135	0.862	0.843	0.889	0.742	0.634	0.477	0.416	0.405			0.862	1.017
8	0.153	0.897	0.808	0.816	0.673	0.613	0.486	0.407	0.387			0.897	0.985
Average	0.130	0.902	0.824	0.779	0.685	0.591	0.468	0.387	0.381	#DIV/0!	#DIV/0!	0.902	0.988
SD	0.013	0.037	0.039	0.061	0.028	0.029	0.020	0.019	0.022	#DIV/0!	#DIV/0!	0.037	0.034
Average - Blank	0.000	0.772	0.694	0.649	0.555	0.462	0.338	0.258	0.251	#DIV/0!	#DIV/0!	0.772	0.858
Relative Cell Growth (%)		100.0	90.0	84.2	71.9	59.8	43.8	33.4	32.5	#DIV/0!	#DIV/0!	100.0	111.2
SD of Relative Cell Growth		4.8	5.1	7.9	3.7	3.8	2.5	2.5	2.8	#DIV/0!	#DIV/0!	4.8	4.4

Transformation Assay

Giemsa Staining	1	1.56	3.13	6.25	12.5	25	50	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	96			0	0
No. of wells counted	96	96	96	96	96	96	96	0	96	96	96	96
No. of wells having foci	14	24	16	43	44	16	0	TOX			14	76
Transformation frequency*	0.145833333	0.25	0.166666667	0.447916667	0.458333333	0.166666667	0	#VALUE!	0	0	0.145833333	0.791666667
SE	0.03602169	0.04419417	0.03803629	0.05075342	0.05085354	0.03803629	0.00000000	#VALUE!	0.00000000	0.00000000	0.03602169	0.04144908

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	2AAF
Institution:	Lab 2
Test Number	

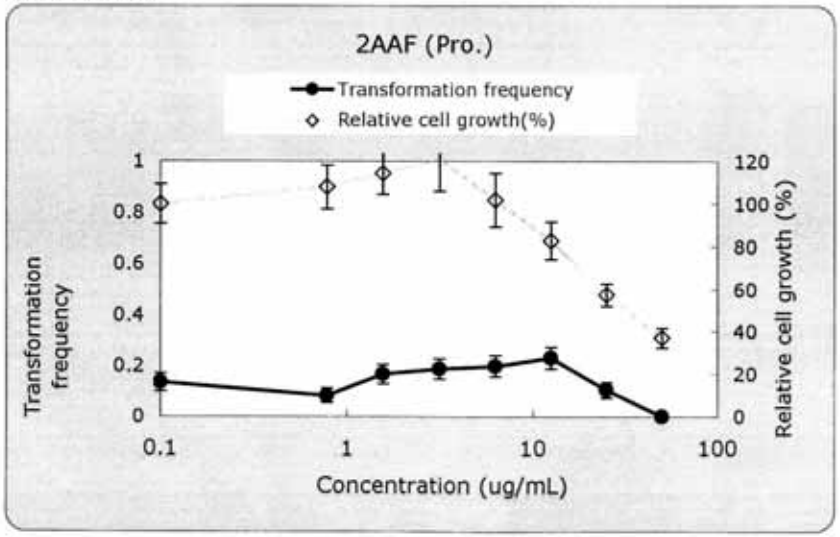
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)								Negative Control	Positive Control
		0.1	0.78	1.56	3.13	6.25	12.5	25	50		0.1% DMSO	TPA (50ng/mL)
1	0.027	0.312	0.351	0.377	0.350	0.346	0.265	0.184	0.132		0.312	0.432
2	0.027	0.369	0.362	0.336	0.450	0.363	0.313	0.195	0.157		0.369	0.525
3	0.023	0.289	0.328	0.388	0.320	0.268	0.281	0.187	0.136		0.289	0.385
4	0.027	0.280	0.352	0.351	0.392	0.355	0.253	0.226	0.126		0.280	0.439
5	0.032	0.327	0.372	0.385	0.421	0.329	0.247	0.190	0.135		0.327	0.444
6	0.024	0.317	0.309	0.371	0.350	0.306	0.242	0.174	0.136		0.317	0.368
7	0.023	0.306	0.343	0.323	0.366	0.269	0.277	0.183	0.110		0.306	0.447
8	0.022	0.315	0.282	0.315	0.347	0.319	0.236	0.190	0.127		0.315	0.330
Average	0.026	0.314	0.337	0.356	0.375	0.319	0.264	0.191	0.132	#DIV/0!	0.314	0.421
SD	0.003	0.027	0.030	0.029	0.043	0.037	0.025	0.015	0.013	#DIV/0!	0.027	0.060
Average - Blank	0.000	0.289	0.312	0.330	0.349	0.294	0.239	0.166	0.107	#DIV/0!	0.289	0.396
Relative Cell Growth (%)		100.0	108.0	114.3	120.8	101.7	82.6	57.3	37.0	#DIV/0!	100.0	137.0
SD of Relative Cell Growth		9.3	10.3	9.9	15.0	12.6	8.8	5.3	4.6	#DIV/0!	9.3	20.6

Transformation Assay

Giemsa Staining	0.1	0.78	1.56	3.13	6.25	12.5	25	50	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	8	16	18	19	22	10	0			13	46
Transformation frequency*	0.135416667	0.083333333	0.166666667	0.1875	0.197916667	0.229166667	0.104166667	0	0	0	0.135416667	0.479166667
SE	0.03492238	0.02820847	0.03803629	0.03983609	0.04066449	0.04289635	0.03117758	0.00000000	0.00000000	0.00000000	0.03492238	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	2AAF
Institution:	Lab 3
Test Number	

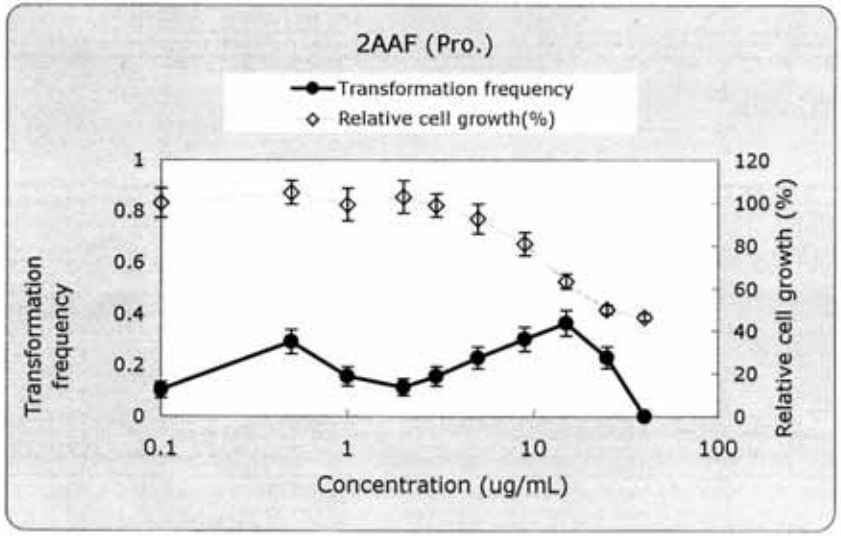
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	2	3	5	9	15	25	40	0.1% DMSO	TPA (50ng/mL)
1	0.101	0.945	0.902	0.951	1.033	0.894	0.793	0.761	0.589	0.485	0.471	0.945	1.280
2	0.111	0.875	1.030	0.910	0.975	0.909	0.963	0.838	0.650	0.523	0.476	0.875	1.208
3	0.111	1.024	0.974	0.964	0.857	0.902	0.854	0.722	0.616	0.521	0.484	1.024	1.382
4	0.112	0.861	0.963	0.833	0.977	0.923	0.859	0.750	0.629	0.530	0.488	0.861	1.252
5	0.111	0.851	0.899	1.002	0.888	0.953	0.890	0.778	0.653	0.515	0.469	0.851	1.346
6	0.122	0.925	0.976	0.879	0.966	0.947	0.832	0.774	0.628	0.496	0.480	0.925	1.209
7	0.112	0.910	0.927	0.870	0.905	0.881	0.813	0.760	0.602	0.494	0.511	0.910	1.302
8	0.113	0.910	0.942	0.839	0.876	0.813	0.807	0.688	0.581	0.526	0.494	0.910	1.199
Average	0.112	0.913	0.952	0.906	0.935	0.903	0.851	0.759	0.619	0.511	0.484	0.913	1.272
SD	0.006	0.055	0.044	0.061	0.062	0.044	0.055	0.044	0.027	0.017	0.014	0.055	0.068
Average - Blank	0.000	0.801	0.840	0.794	0.823	0.791	0.740	0.647	0.507	0.400	0.373	0.801	1.161
Relative Cell Growth (%)		100.0	104.9	99.2	102.7	98.8	92.4	80.8	63.3	49.9	46.5	100.0	144.9
SD of Relative Cell Growth		6.9	5.4	7.7	7.7	5.5	6.9	5.4	3.3	2.1	1.7	6.9	8.5

Transformation Assay

Giemsa Staining	0.1	0.5	1	2	3	5	9	15	25	40	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	28	15	11	15	22	29	35	22	tox	10	88
Transformation frequency*	0.104166667	0.291666667	0.15625	0.114583333	0.15625	0.229166667	0.302083333	0.364583333	0.229166667	#VALUE!	0.104166667	0.916666667
SE	0.03117758	0.04639024	0.03705794	0.03250865	0.03705794	0.04289635	0.04686294	0.04912382	0.04289635	#VALUE!	0.03117758	0.02820847

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	2AAF
Institution:	Lab 4
Test Number	

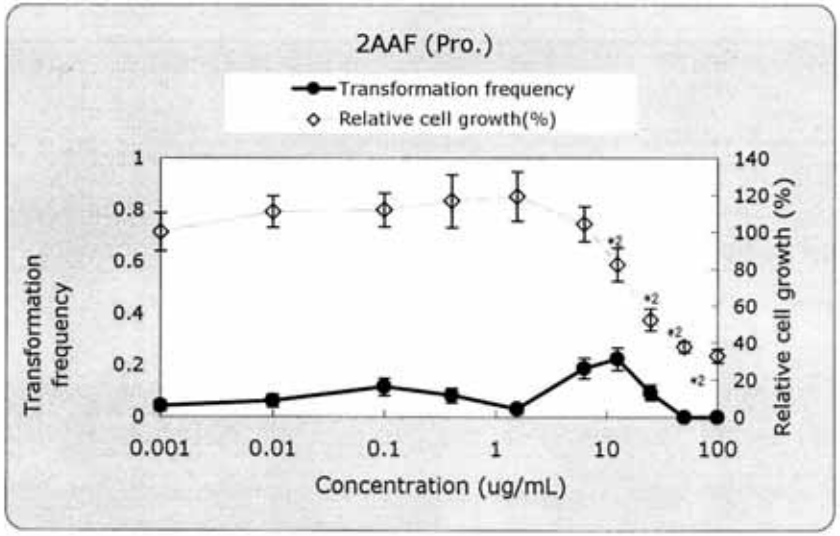
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.01	0.1	0.4	1.56	6.25	12.5	25	50	100	0.1% DMSO	TPA (50ng/mL)
1	0.072	0.245	0.264	0.278	0.252	0.267	0.250	0.210	0.150	0.133	0.126	0.245	0.319
2	0.073	0.256	0.250	0.257	0.286	0.290	0.250	0.219	0.167	0.142	0.128	0.256	0.367
3	0.073	0.244	0.266	0.278	0.321	0.289	0.260	0.249	0.179	0.139	0.124	0.244	0.376
4	0.074	0.275	0.297	0.276	0.286	0.330	0.247	0.208	0.166	0.138	0.130	0.275	0.354
5	0.074	0.248	0.264	0.298	0.289	0.285	0.247	0.234	0.180	0.144	0.144	0.248	0.354
6	0.072	0.248	0.253	0.250	0.280	0.271	0.285	0.209	0.163	0.146	0.136	0.248	0.338
7	0.071	0.259	0.282	0.263	0.269	0.274	0.274	0.203	0.161	0.140	0.131	0.259	0.324
8	0.074	0.210	0.267	0.254	0.239	0.249	0.234	0.209	0.153	0.131	0.129	0.210	0.320
Average	0.073	0.248	0.268	0.269	0.278	0.282	0.256	0.218	0.165	0.139	0.131	0.248	0.344
SD	0.001	0.018	0.015	0.016	0.025	0.024	0.016	0.016	0.011	0.005	0.006	0.018	0.022
Average - Blank	0.000	0.176	0.195	0.197	0.205	0.209	0.183	0.145	0.092	0.066	0.058	0.176	0.271
Relative Cell Growth (%)		100.0	111.0	112.0	116.7	119.1	104.4	82.5	52.6	37.8	33.1	100.0	154.6
SD of Relative Cell Growth		10.5	8.7	9.2	14.3	13.4	9.4	8.9	6.1	2.9	3.6	10.5	12.5

Transformation Assay

Giemsa Staining	0.001	0.01	0.1	0.4	1.56	6.25	12.5	25	50	100	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	2	0	0	96	0	0
No. of wells counted	96	96	96	96	96	96	94	96	96	0	96	96
No. of wells having foci	4	6	11	8	3	18	21	9	0		4	43
Transformation frequency*	0.041666667	0.0625	0.114583333	0.083333333	0.03125	0.1875	0.223404255	0.09375	0	#DIV/0!	0.041666667	0.447916667
SE	0.02039469	0.02470529	0.03250865	0.02820847	0.01775805	0.03983609	0.04296149	0.02974911	0.00000000	#DIV/0!	0.02039469	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 1
Test Number	

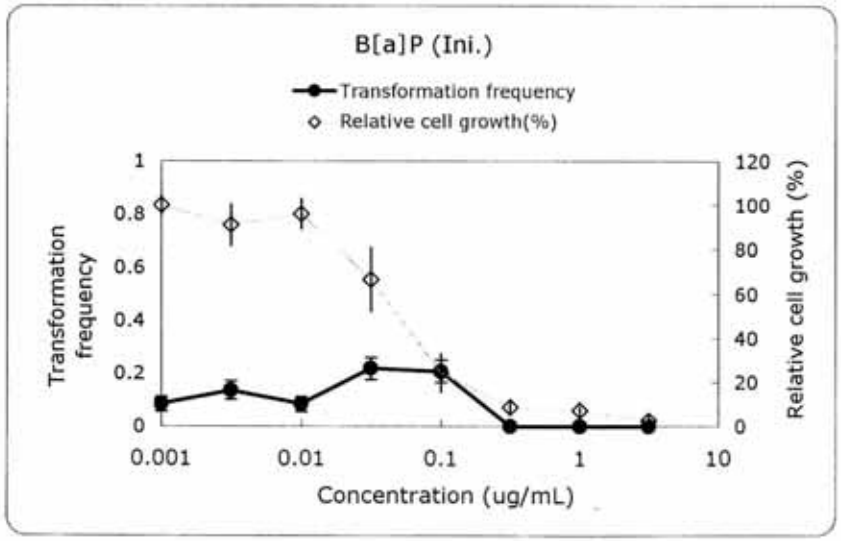
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.00316	0.01	0.0316	0.1	0.316	1	3.16			0.1% DMSO	MCA (1ug/mL)
1	0.089	0.619	0.588	0.581	0.394	0.221	0.123	0.130	0.094			0.619	0.200
2	0.095	0.651	0.624	0.607	0.472	0.229	0.167	0.126	0.122			0.651	0.256
3	0.085	0.644	0.596	0.584	0.506	0.268	0.152	0.139	0.102			0.644	0.262
4	0.098	0.597	0.551	0.545	0.516	0.253	0.143	0.138	0.102			0.597	0.245
5	0.092	0.669	0.500	0.642	0.410	0.251	0.139	0.124	0.112			0.669	0.285
6	0.089	0.628	0.541	0.650	0.548	0.240	0.126	0.144	0.119			0.628	0.249
7	0.083	0.621	0.656	0.628	0.446	0.121	0.130	0.115	0.097			0.621	0.242
8	0.095	0.614	0.601	0.637	0.308	0.190	0.120	0.119	0.087			0.614	0.182
Average	0.091	0.630	0.582	0.609	0.450	0.222	0.138	0.129	0.104	#DIV/0!	#DIV/0!	0.630	0.240
SD	0.005	0.023	0.050	0.037	0.078	0.047	0.016	0.010	0.012	#DIV/0!	#DIV/0!	0.023	0.033
Average - Blank	0.000	0.540	0.491	0.519	0.359	0.131	0.047	0.039	0.014	#DIV/0!	#DIV/0!	0.540	0.149
Relative Cell Growth (%)		100.0	91.1	96.1	66.6	24.3	8.7	7.2	2.5	#DIV/0!	#DIV/0!	100.0	27.7
SD of Relative Cell Growth		4.3	9.2	6.8	14.4	8.7	3.0	1.9	2.3	#DIV/0!	#DIV/0!	4.3	6.2

Transformation Assay

Giemsa Staining	0.001	0.00316	0.01	0.0316	0.1	0.316	1	3.16	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	33	76	96			0	0
No. of wells counted	96	96	96	96	96	63	20	0	96	96	96	96
No. of wells having foci	8	13	8	21	20	TOX	TOX	TOX			8	41
Transformation frequency*	0.083333333	0.135416667	0.083333333	0.21875	0.208333333	#VALUE!	#VALUE!	#VALUE!	0	0	0.083333333	0.427083333
SE	0.02820847	0.03492238	0.02820847	0.04219232	0.04144908	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.00000000	0.02820847	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 2
Test Number	

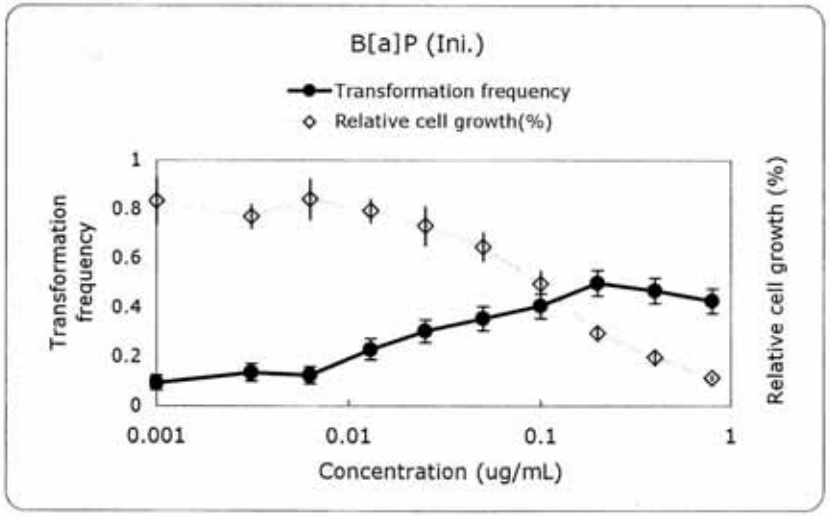
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	0.8	0.1% DMSO	MCA (1ug/mL)
1	0.049	0.496	0.502	0.494	0.486	0.491	0.417	0.260	0.177	0.138		0.496	0.228
2	0.050	0.579	0.517	0.581	0.499	0.527	0.478	0.328	0.254	0.172	0.133	0.579	0.273
3	0.049	0.594	0.484	0.532	0.522	0.391	0.395	0.357	0.231	0.180	0.133	0.594	0.210
4	0.052	0.634	0.519	0.627	0.545	0.523	0.449	0.364	0.211	0.201	0.126	0.634	0.218
5	0.070	0.527	0.482	0.565	0.510	0.487	0.410	0.321	0.232	0.173	0.130	0.527	0.238
6	0.080	0.514	0.561	0.518	0.567	0.462	0.438	0.375	0.225	0.161	0.129	0.514	0.244
7	0.080	0.501	0.476	0.561	0.523	0.464	0.409	0.303	0.225	0.180	0.123	0.501	0.201
8	0.074	0.481	0.498	0.488	0.495	0.520	0.480	0.396	0.224	0.167	0.120	0.481	0.224
Average	0.063	0.541	0.505	0.546	0.518	0.483	0.435	0.348	0.233	0.176	0.129	0.541	0.230
SD	0.014	0.055	0.028	0.047	0.027	0.045	0.032	0.031	0.016	0.012	0.006	0.055	0.022
Average - Blank	0.000	0.478	0.442	0.483	0.455	0.420	0.372	0.285	0.170	0.113	0.066	0.478	0.167
Relative Cell Growth (%)		100.0	92.5	101.0	95.3	87.9	77.8	59.5	35.5	23.7	13.8	100.0	34.9
SD of Relative Cell Growth		11.5	5.8	9.8	5.7	9.4	6.8	6.5	3.4	2.5	1.2	11.5	4.7

Transformation Assay

Giemsa Staining	0.001	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	0.8	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	13	12	22	29	34	39	48	45	41	9	70
Transformation frequency*	0.09375	0.135416667	0.125	0.229166667	0.302083333	0.354166667	0.40625	0.5	0.46875	0.427083333	0.09375	0.729166667
SE	0.02974911	0.03492238	0.03375386	0.04289635	0.04686294	0.04881221	0.05012598	0.05103104	0.05093127	0.05048547	0.02974911	0.04535538
		*	*	*	*	*	*	*	*	*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 3
Test Number	

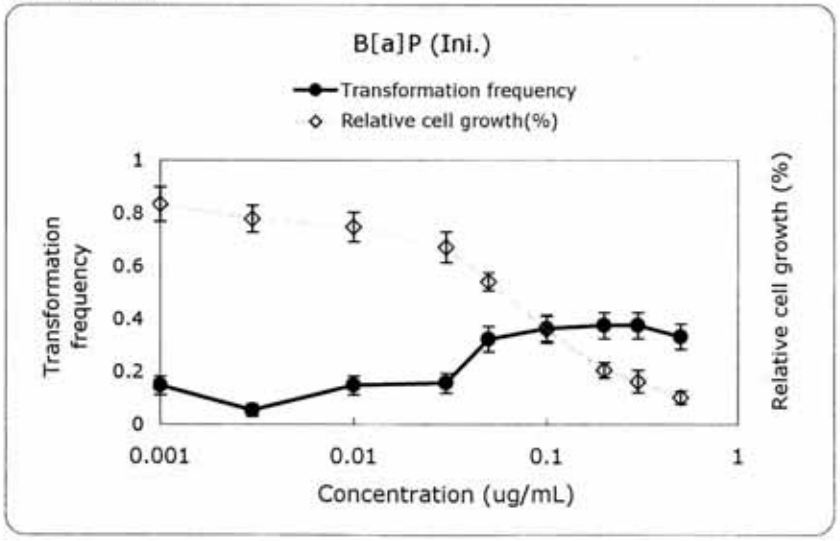
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.003	0.01	0.03	0.05	0.1	0.2	0.3	0.5		0.1% DMSO	MCA (1ug/mL)
1	0.096	1.094	0.954	1.043	1.022	0.838	0.632	0.374	0.357	0.238		1.094	0.551
2	0.111	1.238	1.167	1.040	0.942	0.836	0.644	0.408	0.310	0.260		1.238	0.541
3	0.106	1.119	1.086	1.017	0.936	0.766	0.550	0.379	0.284	0.222		1.119	0.619
4	0.106	1.059	1.078	1.003	0.844	0.746	0.484	0.377	0.296	0.270		1.059	0.620
5	0.099	1.144	1.069	0.969	0.874	0.764	0.550	0.344	0.407	0.227		1.144	0.586
6	0.097	1.314	1.125	1.010	1.061	0.726	0.470	0.371	0.301	0.220		1.314	0.536
7	0.097	1.135	1.138	1.081	0.924	0.767	0.523	0.331	0.233	0.236		1.135	0.551
8	0.099	1.136	1.064	1.197	0.981	0.822	0.587	0.291	0.266	0.169		1.136	0.432
Average	0.101	1.155	1.085	1.045	0.948	0.783	0.555	0.359	0.307	0.230	#DIV/0!	1.155	0.555
SD	0.006	0.082	0.064	0.070	0.072	0.043	0.063	0.036	0.054	0.030	#DIV/0!	0.082	0.060
Average - Blank	0.000	1.054	0.984	0.944	0.847	0.682	0.454	0.258	0.205	0.129	#DIV/0!	1.054	0.453
Relative Cell Growth (%)		100.0	93.4	89.6	80.4	64.7	43.1	24.5	19.5	12.2	#DIV/0!	100.0	43.0
SD of Relative Cell Growth		7.8	6.1	6.6	6.8	4.1	6.0	3.4	5.1	2.9	#DIV/0!	7.8	5.7

Transformation Assay

Giemsa Staining	0.001	0.003	0.01	0.03	0.05	0.1	0.2	0.3	0.5	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	5	14	15	31	35	36	36	32		14	73
Transformation frequency*	0.145833333	0.052083333	0.145833333	0.15625	0.322916667	0.364583333	0.375	0.375	0.333333333	0	0.145833333	0.760416667
SE	0.03602169	0.02267769	0.03602169	0.03705794	0.04772332	0.04912382	0.04941059	0.04941059	0.04811252	0.00000000	0.03602169	0.04356307
					*	*	*	*	*			

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 4
Test Number	

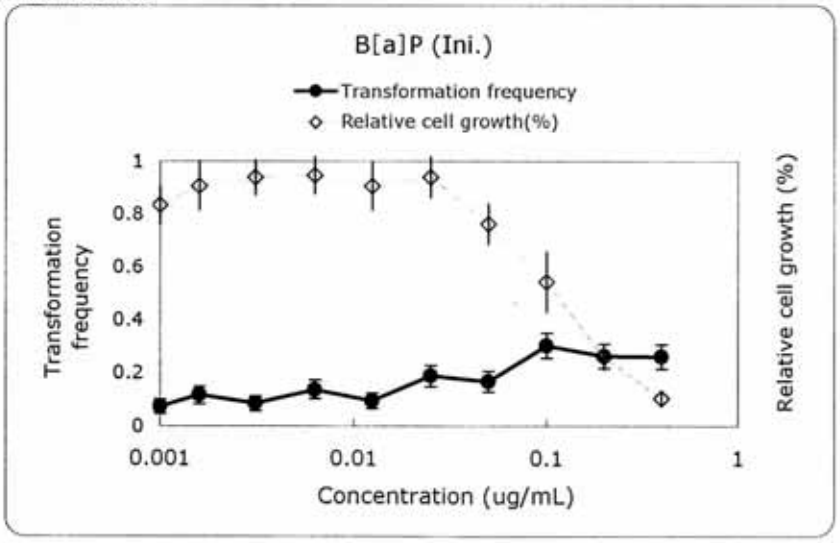
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.0016	0.0031	0.0063	0.0125	0.025	0.05	0.1	0.2	0.4	0.1% DMSO	MCA (1ug/mL)
1	0.078	0.270	0.266	0.294	0.286	0.299	0.303	0.278	0.212	0.134	0.104	0.270	0.203
2	0.074	0.267	0.285	0.340	0.328	0.333	0.305	0.286	0.204	0.154	0.102	0.267	0.173
3	0.073	0.279	0.291	0.300	0.304	0.306	0.313	0.279	0.202	0.144	0.115	0.279	0.182
4	0.076	0.267	0.323	0.320	0.338	0.308	0.318	0.275	0.279	0.150	0.102	0.267	0.215
5	0.072	0.288	0.306	0.315	0.302	0.299	0.334	0.259	0.204	0.141	0.098	0.288	0.170
6	0.074	0.301	0.302	0.330	0.330	0.336	0.336	0.276	0.200	0.147	0.098	0.301	0.181
7	0.074	0.320	0.339	0.296	0.320	0.267	0.318	0.262	0.214	0.120	0.095	0.320	0.172
8	0.079	0.293	0.322	0.303	0.305	0.283	0.274	0.225	0.182	0.129	0.096	0.293	0.163
Average	0.075	0.286	0.304	0.312	0.314	0.304	0.313	0.267	0.212	0.140	0.101	0.286	0.182
SD	0.002	0.019	0.024	0.017	0.018	0.023	0.019	0.019	0.029	0.012	0.006	0.019	0.018
Average - Blank	0.000	0.211	0.229	0.237	0.239	0.229	0.238	0.192	0.137	0.065	0.026	0.211	0.107
Relative Cell Growth (%)		100.0	108.8	112.7	113.6	108.7	112.9	91.4	65.0	30.9	12.4	100.0	51.0
SD of Relative Cell Growth		9.0	11.2	7.9	8.4	10.9	9.2	9.2	13.7	5.5	3.0	9.0	8.4

Transformation Assay

Giemsa Staining	0.001	0.0016	0.0031	0.0063	0.0125	0.025	0.05	0.1	0.2	0.4	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	1	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	95	96	96	96
No. of wells having foci	7	11	8	13	9	18	16	29	25	25	7	54
Transformation frequency*	0.072916667	0.114583333	0.083333333	0.135416667	0.09375	0.1875	0.166666667	0.302083333	0.263157895	0.260416667	0.072916667	0.5625
SE	0.02653610	0.03250865	0.02820847	0.03492238	0.02974911	0.03983609	0.03803629	0.04686294	0.04517869	0.04479116	0.02653610	0.05063079
								*	*	*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 1
Test Number	

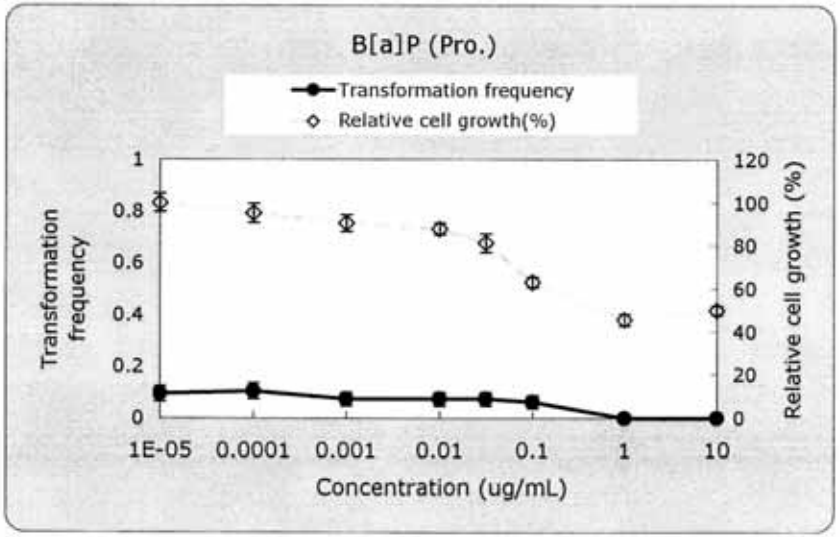
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.001	0.01	0.0316	0.1	1	10			0.1% DMSO	TPA (50ng/mL)
1	0.124	0.630	0.640	0.580	0.560	0.513	0.461	0.374	0.395			0.630	1.042
2	0.125	0.632	0.608	0.592	0.568	0.551	0.456	0.337	0.378			0.632	1.035
3	0.116	0.670	0.592	0.610	0.585	0.545	0.448	0.363	0.359			0.670	0.956
4	0.117	0.611	0.591	0.578	0.579	0.524	0.452	0.334	0.391			0.611	0.883
5	0.106	0.674	0.632	0.594	0.598	0.585	0.461	0.369	0.372			0.674	1.023
6	0.122	0.659	0.644	0.576	0.566	0.533	0.435	0.358	0.379			0.659	0.929
7	0.107	0.627	0.597	0.635	0.596	0.563	0.426	0.350	0.377			0.627	0.950
8	0.112	0.641	0.640	0.582	0.575	0.540	0.449	0.360	0.385			0.641	1.011
Average	0.116	0.643	0.618	0.593	0.578	0.544	0.449	0.356	0.380	#DIV/0!	#DIV/0!	0.643	0.979
SD	0.007	0.022	0.023	0.020	0.014	0.023	0.012	0.014	0.011	#DIV/0!	#DIV/0!	0.022	0.058
Average - Blank	0.000	0.527	0.502	0.477	0.462	0.428	0.332	0.240	0.263	#DIV/0!	#DIV/0!	0.527	0.863
Relative Cell Growth (%)		100.0	95.3	90.6	87.7	81.3	63.1	45.5	50.0	#DIV/0!	#DIV/0!	100.0	163.7
SD of Relative Cell Growth		4.3	4.4	3.8	2.6	4.3	2.3	2.7	2.1	#DIV/0!	#DIV/0!	4.3	10.9

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.001	0.01	0.0316	0.1	1	10	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	96	96			0	0
No. of wells counted	96	96	96	96	96	96	0	0	96	96	96	96
No. of wells having foci	9	10	7	7	7	6	TOX	TOX			9	54
Transformation frequency*	0.09375	0.104166667	0.072916667	0.072916667	0.072916667	0.0625	#VALUE!	#VALUE!	0	0	0.09375	0.5625
SE	0.02974911	0.03117758	0.02653610	0.02653610	0.02653610	0.02470529	#VALUE!	#VALUE!	0.00000000	0.00000000	0.02974911	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 2
Test Number	

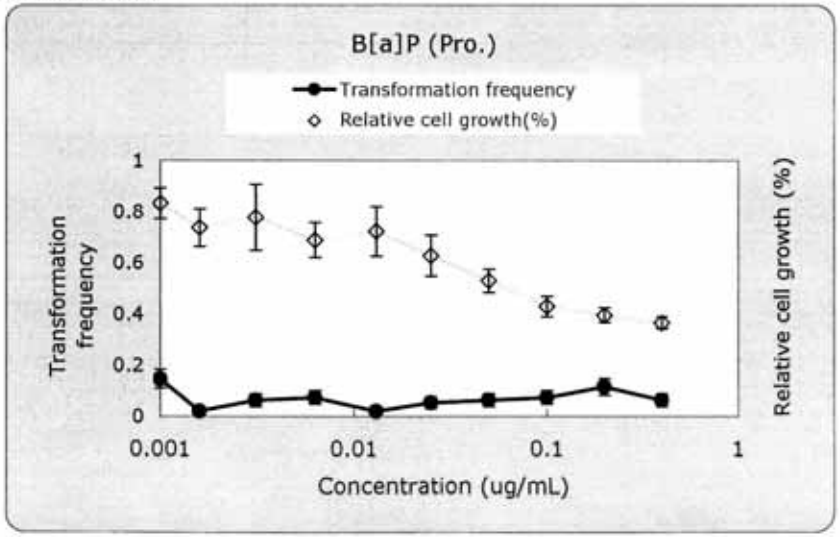
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.0016	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	0.1% DMSO	TPA (50ng/mL)
1	0.048	0.547	0.505	0.457	0.457	0.439	0.368	0.333	0.282	0.263	0.246	0.547	0.522
2	0.052	0.573	0.478	0.506	0.490	0.493	0.393	0.353	0.286	0.272	0.273	0.573	0.556
3	0.051	0.522	0.480	0.478	0.402	0.417	0.424	0.336	0.291	0.307	0.280	0.522	0.610
4	0.052	0.585	0.498	0.684	0.508	0.482	0.465	0.389	0.314	0.302	0.271	0.585	0.581
5	0.067	0.491	0.433	0.522	0.503	0.549	0.425	0.404	0.326	0.289	0.275	0.491	0.626
6	0.080	0.567	0.576	0.537	0.477	0.576	0.423	0.385	0.333	0.304	0.293	0.567	0.602
7	0.079	0.573	0.496	0.476	0.451	0.464	0.516	0.376	0.347	0.302	0.277	0.573	0.564
8	0.078	0.507	0.453	0.451	0.411	0.435	0.403	0.389	0.312	0.297	0.282	0.507	0.580
Average	0.063	0.546	0.490	0.514	0.462	0.482	0.427	0.371	0.311	0.292	0.275	0.546	0.580
SD	0.014	0.035	0.042	0.075	0.040	0.056	0.046	0.027	0.024	0.016	0.013	0.035	0.033
Average - Blank	0.000	0.482	0.427	0.451	0.399	0.419	0.364	0.307	0.248	0.229	0.211	0.482	0.517
Relative Cell Growth (%)		100.0	88.4	93.4	82.7	86.8	75.4	63.7	51.4	47.4	43.8	100.0	107.2
SD of Relative Cell Growth		7.2	8.8	15.6	8.3	11.6	9.5	5.5	4.9	3.4	2.8	7.2	6.9

Transformation Assay

Giemsa Staining	0.001	0.0016	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	2	6	7	2	5	6	7	11	6	14	55
Transformation frequency*	0.145833333	0.020833333	0.0625	0.072916667	0.020833333	0.052083333	0.0625	0.072916667	0.114583333	0.0625	0.145833333	0.572916667
SE	0.03602169	0.01457713	0.02470529	0.02653610	0.01457713	0.02267769	0.02470529	0.02653610	0.03250865	0.02470529	0.03602169	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 3
Test Number	

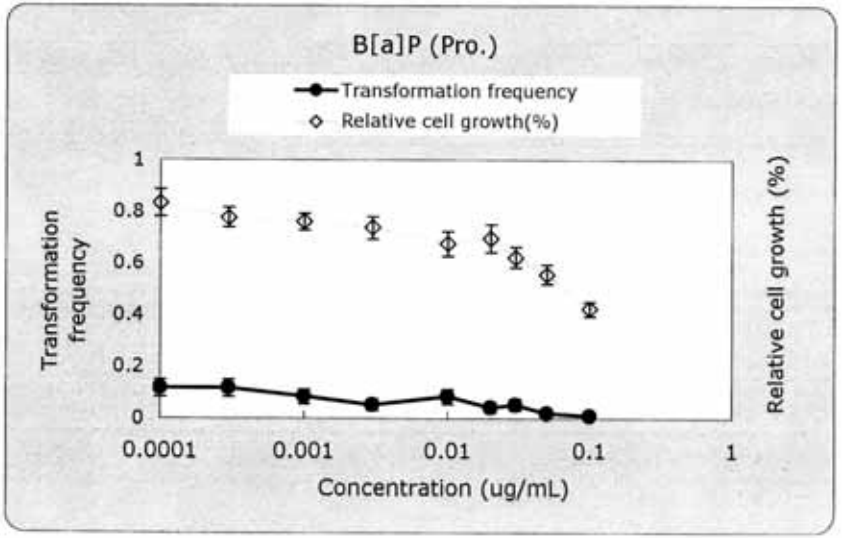
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.0003	0.001	0.003	0.01	0.02	0.03	0.05	0.1		0.1% DMSO	TPA (50ng/mL)
1	0.091	1.044	0.914	0.985	0.873	0.863	0.927	0.800	0.761	0.548		1.044	1.245
2	0.092	1.017	1.020	0.983	0.913	0.898	0.930	0.825	0.763	0.599		1.017	1.580
3	0.122	1.195	1.004	0.952	0.975	0.912	1.021	0.913	0.755	0.636		1.195	1.469
4	0.099	1.092	1.011	0.960	0.932	0.785	0.872	0.762	0.777	0.568		1.092	1.385
5	0.080	1.036	1.018	1.071	1.024	0.891	0.846	0.809	0.649	0.557		1.036	1.473
6	0.095	1.064	0.986	0.981	1.000	0.936	0.979	0.871	0.809	0.601		1.064	1.409
7	0.107	1.019	1.070	0.977	0.987	0.971	0.838	0.844	0.744	0.622		1.019	1.496
8	0.120	1.123	1.031	1.015	0.994	0.870	0.901	0.811	0.759	0.622		1.123	1.331
Average	0.101	1.074	1.007	0.991	0.962	0.891	0.914	0.829	0.752	0.594	#DIV/0!	1.074	1.424
SD	0.015	0.061	0.045	0.038	0.051	0.055	0.064	0.046	0.046	0.033	#DIV/0!	0.061	0.104
Average - Blank	0.000	0.973	0.906	0.890	0.862	0.790	0.814	0.729	0.651	0.493	#DIV/0!	0.973	1.323
Relative Cell Growth (%)		100.0	93.1	91.4	88.5	81.2	83.6	74.9	66.9	50.7	#DIV/0!	100.0	135.9
SD of Relative Cell Growth		6.3	4.6	3.9	5.3	5.7	6.5	4.8	4.7	3.4	#DIV/0!	6.3	10.7

Transformation Assay

Giemsa Staining	0.0001	0.0003	0.001	0.003	0.01	0.02	0.03	0.05	0.1	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	11	8	5	8	4	5	2	1		11	80
Transformation frequency*	0.114583333	0.114583333	0.083333333	0.052083333	0.083333333	0.041666667	0.052083333	0.020833333	0.010416667	0	0.114583333	0.833333333
SE	0.03250865	0.03250865	0.02820847	0.02267769	0.02820847	0.02039469	0.02267769	0.01457713	0.01036227	0.00000000	0.03250865	0.03803629

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 4
Test Number	

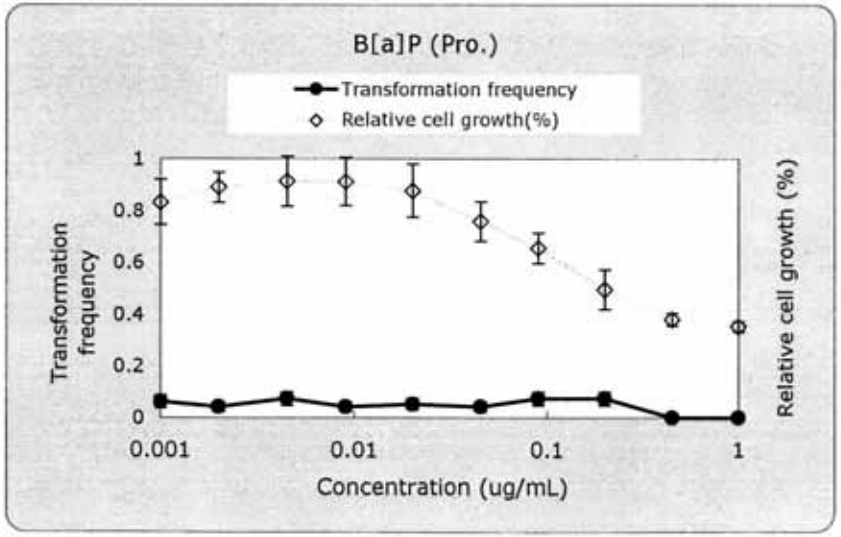
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.002	0.0045	0.009	0.02	0.045	0.09	0.2	0.45	1	0.1% DMSO	TPA (50ng/mL)
1	0.078	0.207	0.241	0.225	0.229	0.209	0.188	0.182	0.147	0.141	0.140	0.207	0.285
2	0.074	0.215	0.250	0.263	0.249	0.219	0.226	0.193	0.152	0.144	0.141	0.215	0.325
3	0.073	0.243	0.239	0.254	0.257	0.257	0.221	0.208	0.165	0.147	0.148	0.243	0.337
4	0.076	0.251	0.226	0.273	0.250	0.259	0.221	0.191	0.179	0.151	0.147	0.251	0.365
5	0.072	0.244	0.246	0.263	0.256	0.252	0.238	0.210	0.182	0.144	0.144	0.244	0.330
6	0.074	0.250	0.263	0.243	0.232	0.262	0.228	0.214	0.190	0.152	0.139	0.250	0.318
7	0.074	0.238	0.241	0.251	0.283	0.245	0.223	0.209	0.175	0.154	0.139	0.238	0.357
8	0.079	0.223	0.252	0.223	0.236	0.237	0.213	0.194	0.167	0.146	0.139	0.223	0.335
Average	0.075	0.234	0.245	0.249	0.249	0.242	0.220	0.200	0.169	0.147	0.142	0.234	0.332
SD	0.002	0.017	0.011	0.018	0.017	0.020	0.015	0.011	0.015	0.005	0.004	0.017	0.025
Average - Blank	0.000	0.159	0.170	0.174	0.174	0.167	0.145	0.125	0.094	0.072	0.067	0.159	0.257
Relative Cell Growth (%)		100.0	106.9	109.7	109.4	105.3	91.1	78.6	59.4	45.6	42.3	100.0	161.5
SD of Relative Cell Growth		10.5	6.9	11.4	11.0	12.3	9.2	7.2	9.2	2.9	2.4	10.5	15.5

Transformation Assay

Giemsa Staining	0.001	0.002	0.0045	0.009	0.02	0.045	0.09	0.2	0.45	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	96	96	0	0
No. of wells counted	96	96	96	96	96	96	96	96	0	0	96	96
No. of wells having foci	6	4	7	4	5	4	7	7			6	43
Transformation frequency*	0.0625	0.041666667	0.072916667	0.041666667	0.052083333	0.041666667	0.072916667	0.072916667	#DIV/0!	#DIV/0!	0.0625	0.447916667
SE	0.02470529	0.02039469	0.02653610	0.02039469	0.02267769	0.02039469	0.02653610	0.02653610	#DIV/0!	#DIV/0!	0.02470529	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MCA
Institution:	Lab 1
Test Number	

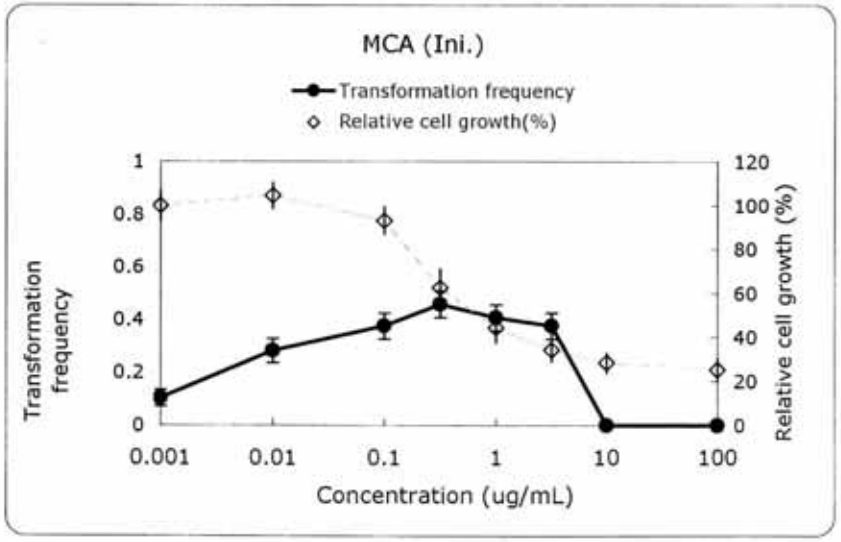
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.01	0.1	0.316	1	3.16	10	100			0.1% DMSO	MCA (1ug/mL)
1	0.100	0.583	0.662	0.604	0.387	0.387	0.261	0.223	0.227			0.633	0.229
2	0.106	0.649	0.674	0.668	0.493	0.356	0.311	0.288	0.257			0.630	0.298
3	0.106	0.642	0.686	0.638	0.418	0.367	0.333	0.280	0.226			0.633	0.304
4	0.111	0.630	0.644	0.620	0.466	0.377	0.314	0.270	0.280			0.642	0.312
5	0.110	0.696	0.709	0.558	0.490	0.355	0.273	0.282	0.236			0.583	0.289
6	0.107	0.650	0.693	0.586	0.473	0.337	0.306	0.243	0.275			0.614	0.300
7	0.107	0.621	0.685	0.601	0.453	0.314	0.265	0.246	0.238			0.598	0.280
8	0.107	0.696	0.610	0.598	0.367	0.270	0.263	0.239	0.196			0.555	0.217
Average	0.107	0.646	0.670	0.609	0.443	0.345	0.291	0.259	0.242	#DIV/0!	#DIV/0!	0.611	0.279
SD	0.003	0.038	0.031	0.033	0.047	0.038	0.028	0.024	0.028	#DIV/0!	#DIV/0!	0.030	0.036
Average - Blank	0.000	0.539	0.564	0.502	0.337	0.239	0.184	0.152	0.135	#DIV/0!	#DIV/0!	0.504	0.172
Relative Cell Growth (%)		100.0	104.5	93.2	62.4	44.3	34.1	28.2	25.1	#DIV/0!	#DIV/0!	100.0	34.1
SD of Relative Cell Growth		7.0	5.8	6.2	8.8	7.1	5.2	4.5	5.2	#DIV/0!	#DIV/0!	5.6	6.6

Transformation Assay

Giemsa Staining	0.001	0.01	0.1	0.316	1	3.16	10	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	52	96			0	0
No. of wells counted	96	96	96	96	96	96	44	0	96	96	96	96
No. of wells having foci	10	27	36	44	39	36	TOX	TOX			6	43
Transformation frequency*	0.104166667	0.28125	0.375	0.458333333	0.40625	0.375	#VALUE!	#VALUE!	0	0	0.0625	0.447916667
SE	0.03117758	0.04588805	0.04941059	0.05085354	0.05012598	0.04941059	#VALUE!	#VALUE!	0.00000000	0.00000000	0.02470529	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<15 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MCA
Institution:	Lab 2
Test Number	

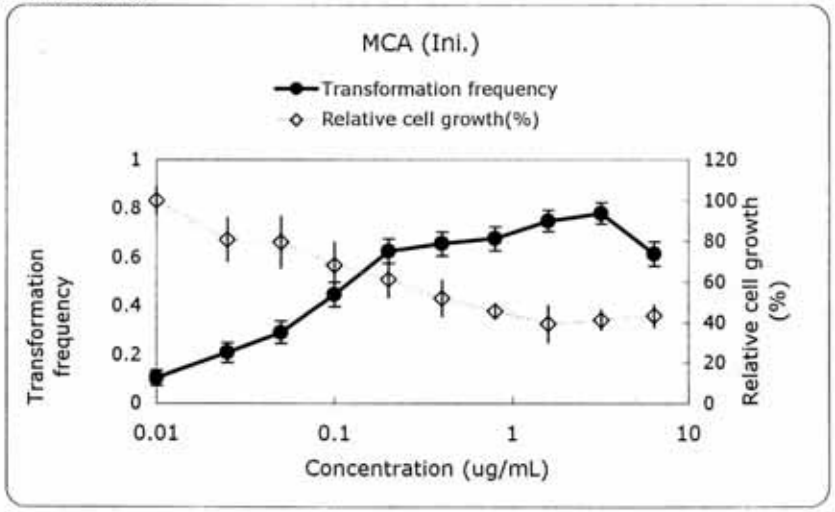
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.025	0.05	0.1	0.2	0.4	0.8	1.6	3.2	6.4	0.1% DMSO	MCA (1ug/mL)
1	0.042	0.498	0.505	0.523	0.407	0.366	0.325	0.260	0.261	0.246	0.229	0.498	0.269
2	0.046	0.524	0.439	0.420	0.432	0.347	0.308	0.270	0.220	0.223	0.245	0.524	0.299
3	0.050	0.464	0.416	0.382	0.359	0.286	0.311	0.282	0.175	0.242	0.236	0.464	0.231
4	0.041	0.519	0.441	0.409	0.273	0.384	0.250	0.256	0.302	0.247	0.245	0.519	0.294
5	0.041	0.573	0.343	0.355	0.344	0.271	0.268	0.264	0.193	0.201	0.237	0.573	0.289
6	0.048	0.532	0.385	0.368	0.334	0.339	0.219	0.240	0.196	0.278	0.244	0.532	0.301
7	0.051	0.512	0.425	0.434	0.393	0.373	0.314	0.264	0.268	0.232	0.259	0.512	0.264
8	0.046	0.538	0.475	0.495	0.415	0.319	0.342	0.258	0.241	0.259	0.310	0.538	0.291
Average	0.046	0.520	0.429	0.423	0.370	0.336	0.292	0.262	0.232	0.241	0.251	0.520	0.280
SD	0.004	0.032	0.050	0.060	0.052	0.041	0.042	0.012	0.044	0.023	0.026	0.032	0.024
Average - Blank	0.000	0.474	0.383	0.378	0.324	0.290	0.247	0.216	0.186	0.195	0.205	0.474	0.234
Relative Cell Growth (%)		100.0	80.7	79.6	68.3	61.1	52.0	45.6	39.3	41.2	43.2	100.0	49.4
SD of Relative Cell Growth		6.7	10.6	12.6	11.1	8.6	8.9	2.5	9.2	4.9	5.4	6.7	5.0

Transformation Assay

Giemsa Staining	0.01	0.025	0.05	0.1	0.2	0.4	0.8	1.6	3.2	6.4	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	20	28	43	60	63	65	72	75	59	10	65
Transformation frequency*	0.104166667	0.208333333	0.291666667	0.447916667	0.625	0.65625	0.677083333	0.75	0.78125	0.614583333	0.104166667	0.677083333
SE	0.03117758	0.04144908	0.04639024	0.05075342	0.04941059	0.04847529	0.04772332	0.04419417	0.04219232	0.04967296	0.03117758	0.04772332

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MCA
Institution:	Lab 3
Test Number	

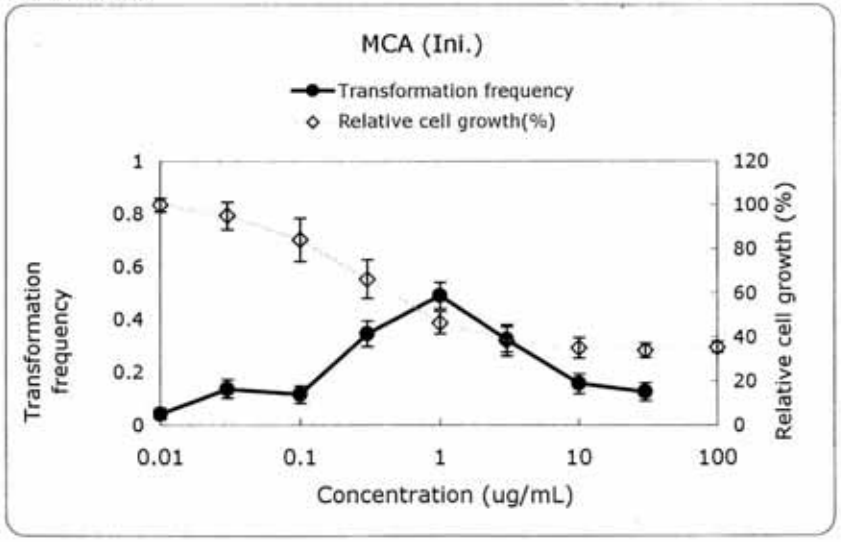
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.03	0.1	0.3	1	3	10	30	100		0.1% DMSO	MCA (1ug/mL)
1	0.120	1.384	1.234	1.273	1.146	0.673	0.676	0.557	0.539	0.543		1.185	0.602
2	0.123	1.342	1.257	1.040	0.922	0.753	0.526	0.484	0.526	0.562		1.320	0.610
3	0.119	1.338	1.374	0.982	0.929	0.604	0.514	0.536	0.517	0.535		1.262	0.648
4	0.118	1.288	1.253	0.990	0.788	0.638	0.510	0.602	0.582	0.561		1.382	0.570
5	0.115	1.289	1.139	1.204	0.825	0.737	0.627	0.547	0.544	0.580		1.411	0.585
6	0.115	1.390	1.290	1.204	0.889	0.619	0.738	0.642	0.526	0.595		1.282	0.627
7	0.119	1.336	1.348	1.235	0.971	0.761	0.548	0.474	0.561	0.502		1.222	0.615
8	0.111	1.341	1.324	1.236	0.944	0.688	0.538	0.511	0.446	0.519		1.326	0.655
Average	0.118	1.339	1.277	1.146	0.927	0.684	0.585	0.544	0.530	0.550	#DIV/0!	1.299	0.614
SD	0.004	0.037	0.074	0.120	0.108	0.061	0.086	0.057	0.040	0.031	#DIV/0!	0.077	0.029
Average - Blank	0.000	1.221	1.160	1.028	0.809	0.567	0.467	0.427	0.413	0.432	#DIV/0!	1.181	0.497
Relative Cell Growth (%)		100.0	95.0	84.2	66.3	46.4	38.3	34.9	33.8	35.4	#DIV/0!	100.0	42.0
SD of Relative Cell Growth		3.1	6.1	9.9	8.8	5.0	7.0	4.7	3.3	2.5	#DIV/0!	6.3	2.4

Transformation Assay

Giemsa Staining	0.01	0.03	0.1	0.3	1	3	10	30	100	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	4	13	11	33	47	31	15	12	tox		2	41
Transformation frequency*	0.041666667	0.135416667	0.114583333	0.34375	0.489583333	0.322916667	0.15625	0.125	#VALUE!	0	0.020833333	0.427083333
SE	0.02039469	0.03492238	0.03250865	0.04847529	0.05101996	0.04772332	0.03705794	0.03375386	#VALUE!	0.00000000	0.01457713	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MCA
Institution:	Lab 4
Test Number	

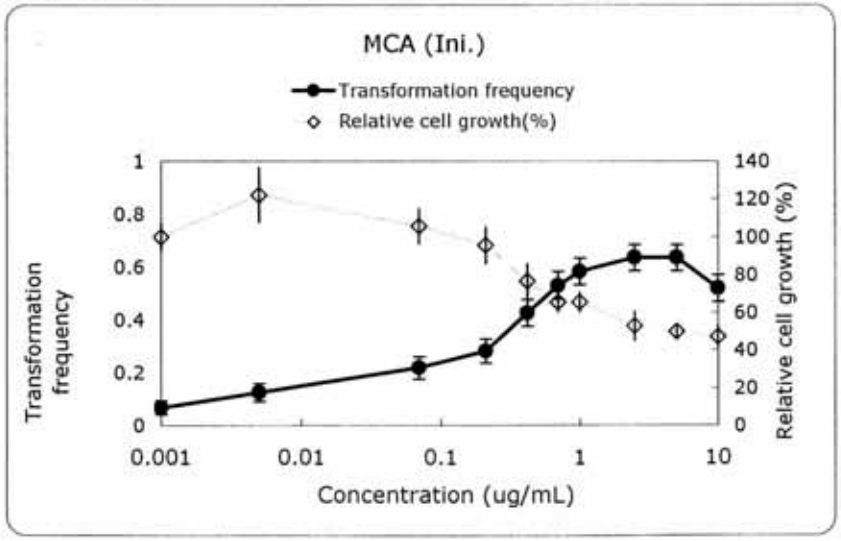
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.005	0.07	0.21	0.42	0.7	1	2.5	5	10	0.1% DMSO	MCA (1ug/mL)
1	0.072	0.265	0.272	0.259	0.270	0.236	0.207	0.206	0.180	0.174	0.187	0.293	0.203
2	0.071	0.262	0.278	0.272	0.286	0.247	0.210	0.208	0.184	0.180	0.164	0.331	0.199
3	0.074	0.279	0.324	0.252	0.283	0.206	0.203	0.206	0.193	0.167	0.156	0.333	0.201
4	0.075	0.283	0.335	0.288	0.258	0.231	0.183	0.181	0.167	0.170	0.173	0.300	0.183
5	0.073	0.273	0.315	0.276	0.248	0.235	0.209	0.205	0.186	0.167	0.163	0.310	0.206
6	0.074	0.272	0.288	0.303	0.250	0.218	0.207	0.207	0.173	0.171	0.148	0.287	0.190
7	0.075	0.237	0.346	0.297	0.236	0.200	0.195	0.201	0.181	0.165	0.169	0.269	0.178
8	0.076	0.272	0.329	0.283	0.242	0.205	0.191	0.190	0.146	0.171	0.160	0.293	0.170
Average	0.074	0.268	0.311	0.279	0.259	0.222	0.201	0.201	0.176	0.171	0.165	0.302	0.191
SD	0.001	0.014	0.028	0.018	0.019	0.018	0.010	0.010	0.015	0.005	0.012	0.022	0.013
Average - Blank	0.000	0.194	0.237	0.205	0.185	0.148	0.127	0.127	0.102	0.097	0.091	0.228	0.117
Relative Cell Growth (%)		100.0	122.2	105.6	95.5	76.5	65.4	65.3	52.8	50.0	47.1	100.0	51.4
SD of Relative Cell Growth		7.4	14.4	9.2	9.6	9.0	5.1	5.0	7.5	2.5	6.1	11.2	6.8

Transformation Assay

Giemsa Staining	0.001	0.005	0.07	0.21	0.42	0.7	1	2.5	5	10	Negative Control	Positive Control
No. of wells excluded	6	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	90	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	12	21	27	41	51	56	61	61	50	13	48
Transformation frequency*	0.066666667	0.125	0.21875	0.28125	0.427083333	0.53125	0.583333333	0.635416667	0.635416667	0.520833333	0.135416667	0.5
SE	0.02629369	0.03375386	0.04219232	0.04588805	0.05048547	0.05093127	0.05031728	0.04912382	0.04912382	0.05098672	0.03492238	0.05103104
		*	*	*	*	*	*	*	*	*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MCA
Institution:	Lab 1
Test Number	

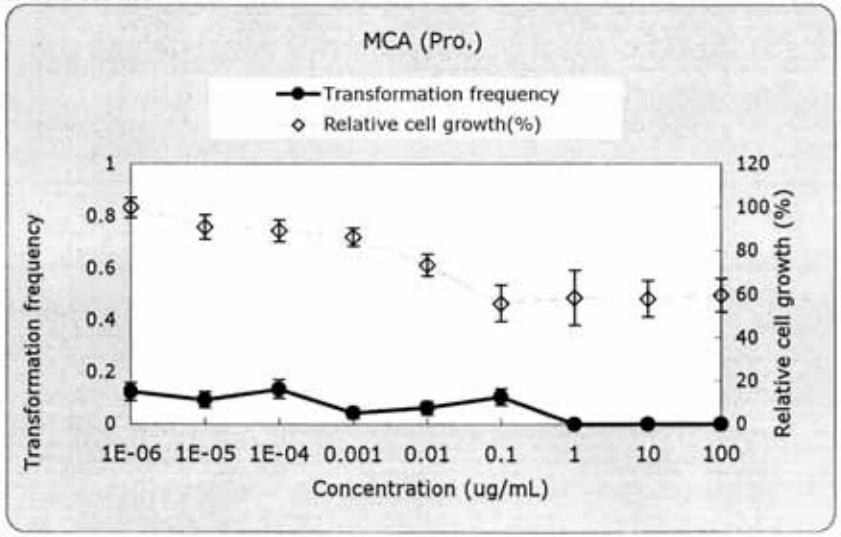
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.000001	0.00001	0.0001	0.001	0.01	0.1	1	10	100		0.1% DMSO	TPA (50ng/mL)
1	0.122	0.624	0.591	0.547	0.577	0.514	0.302	0.560	0.508	0.502		0.675	0.991
2	0.130	0.648	0.628	0.619	0.574	0.512	0.421	0.400	0.406	0.411		0.721	0.978
3	0.121	0.587	0.568	0.546	0.513	0.483	0.413	0.390	0.379	0.404		0.660	0.982
4	0.129	0.602	0.546	0.567	0.548	0.452	0.419	0.421	0.393	0.436		0.605	0.986
5	0.127	0.586	0.553	0.565	0.541	0.479	0.385	0.371	0.403	0.391		0.643	0.986
6	0.130	0.637	0.547	0.549	0.542	0.449	0.425	0.389	0.400	0.389		0.590	0.897
7	0.124	0.620	0.557	0.555	0.544	0.499	0.420	0.373	0.399	0.392		0.644	0.908
8	0.123	0.620	0.579	0.551	0.545	0.487	0.410	0.387	0.384	0.417		0.638	0.954
Average	0.126	0.616	0.571	0.562	0.548	0.484	0.399	0.411	0.409	0.418	#DIV/0!	0.647	0.960
SD	0.004	0.022	0.028	0.024	0.020	0.024	0.041	0.062	0.041	0.038	#DIV/0!	0.041	0.037
Average - Blank	0.000	0.490	0.445	0.437	0.422	0.359	0.274	0.286	0.283	0.292	#DIV/0!	0.521	0.835
Relative Cell Growth (%)		100.0	90.9	89.2	86.2	73.2	55.9	58.3	57.8	59.6	#DIV/0!	100.0	160.1
SD of Relative Cell Growth		4.6	5.7	4.9	4.1	5.0	8.4	12.7	8.4	7.7	#DIV/0!	8.3	7.7

Transformation Assay

Giemsa Staining	0.000001	0.00001	0.0001	0.001	0.01	0.1	1	10	100	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	3	0	1	96	96	96		0	0
No. of wells counted	96	96	96	93	96	95	0	0	0	96	96	96
No. of wells having foci	12	9	13	4	6	10	TOX	TOX	TOX		11	56
Transformation frequency*	0.125	0.09375	0.135416667	0.043010753	0.0625	0.105263158	#VALUE!	#VALUE!	#VALUE!	0	0.114583333	0.583333333
SE	0.03375386	0.02974911	0.03492238	0.02103781	0.02470529	0.03148648	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.03250865	0.05031728

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MCA
Institution:	Lab 2
Test Number	

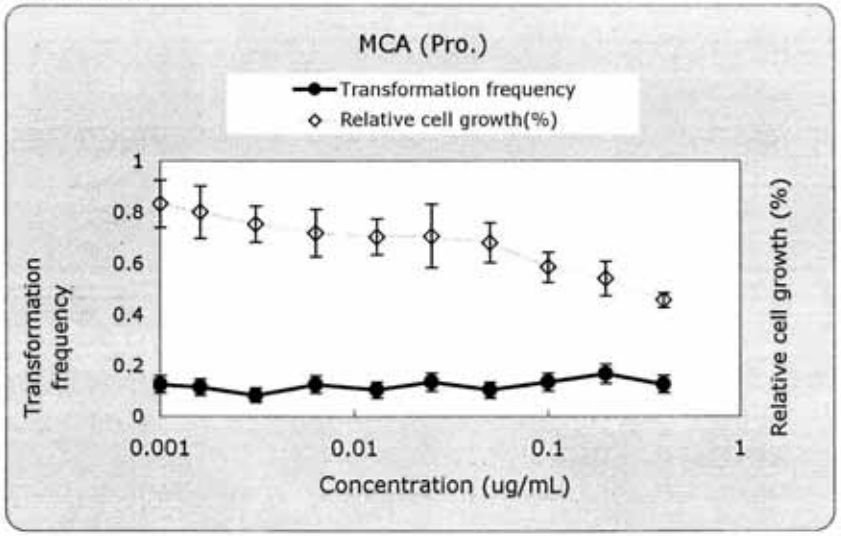
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.0016	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	0.1% DMSO	TPA (50ng/mL)
1	0.035	0.456	0.413	0.462	0.391	0.410	0.392	0.371	0.320	0.328	0.280	0.456	0.567
2	0.045	0.513	0.508	0.491	0.476	0.504	0.386	0.418	0.427	0.348	0.310	0.513	0.674
3	0.040	0.536	0.532	0.516	0.557	0.440	0.495	0.445	0.396	0.385	0.292	0.536	0.698
4	0.039	0.547	0.528	0.487	0.465	0.479	0.555	0.471	0.380	0.397	0.311	0.547	0.632
5	0.041	0.587	0.530	0.495	0.420	0.464	0.450	0.472	0.378	0.382	0.320	0.587	0.646
6	0.038	0.542	0.565	0.470	0.423	0.403	0.495	0.468	0.385	0.341	0.310	0.542	0.630
7	0.044	0.513	0.481	0.438	0.452	0.436	0.423	0.418	0.363	0.324	0.301	0.513	0.614
8	0.042	0.423	0.406	0.391	0.407	0.391	0.343	0.362	0.339	0.282	0.275	0.423	0.632
Average	0.041	0.515	0.495	0.469	0.449	0.441	0.442	0.428	0.374	0.348	0.300	0.515	0.637
SD	0.003	0.053	0.058	0.039	0.053	0.039	0.070	0.044	0.033	0.038	0.016	0.053	0.039
Average - Blank	0.000	0.474	0.455	0.428	0.408	0.400	0.402	0.388	0.333	0.308	0.259	0.474	0.596
Relative Cell Growth (%)		100.0	95.9	90.3	86.1	84.4	84.8	81.8	70.2	64.9	54.7	100.0	125.7
SD of Relative Cell Growth		11.1	12.2	8.3	11.1	8.3	14.8	9.3	7.0	8.1	3.4	11.1	8.2

Transformation Assay

Giemsa Staining	0.001	0.0016	0.0031	0.0063	0.013	0.025	0.05	0.1	0.2	0.4	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	11	8	12	10	13	10	13	16	12	12	60
Transformation frequency*	0.125	0.114583333	0.083333333	0.125	0.104166667	0.135416667	0.104166667	0.135416667	0.166666667	0.125	0.125	0.625
SE	0.03375386	0.03250865	0.02820847	0.03375386	0.03117758	0.03492238	0.03117758	0.03492238	0.03803629	0.03375386	0.03375386	0.04941059

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MCA
Institution:	Lab 3
Test Number	

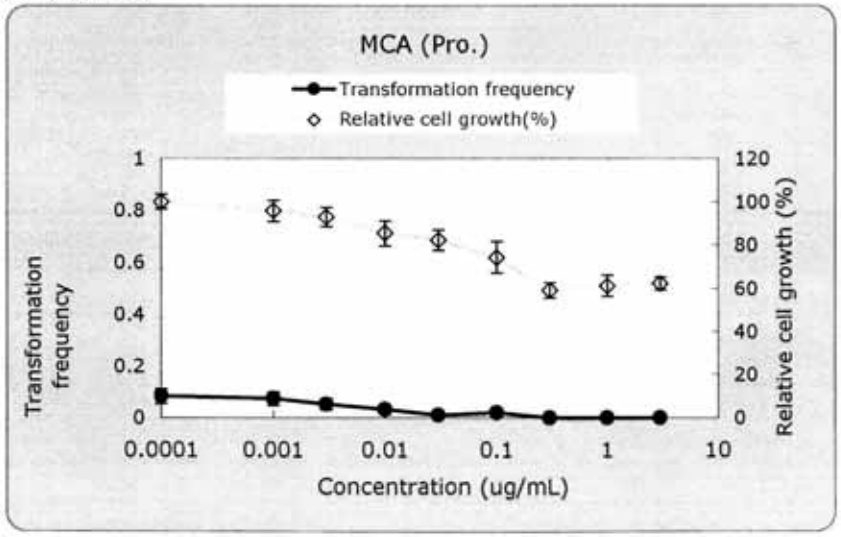
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.001	0.003	0.01	0.03	0.1	0.3	1	3		0.1% DMSO	TPA (50ng/mL)
1	0.110	1.064	0.989	1.005	1.006	0.845	0.816	0.689	0.622	0.668		1.196	1.852
2	0.104	1.034	1.039	0.980	0.898	0.865	0.869	0.667	0.668	0.723		1.329	1.772
3	0.110	1.058	1.046	0.917	0.850	0.879	0.736	0.675	0.677	0.683		1.341	1.709
4	0.128	1.083	0.932	1.069	0.907	0.853	0.839	0.722	0.707	0.698		1.219	1.925
5	0.123	1.072	1.052	0.993	0.882	0.911	0.791	0.638	0.711	0.724		1.234	1.779
6	0.124	1.012	1.048	1.006	0.997	0.960	0.862	0.728	0.713	0.745		1.239	1.809
7	0.146	1.102	1.074	1.016	0.942	0.910	0.936	0.692	0.778	0.742		1.240	1.807
8	0.141	1.105	1.023	0.997	0.945	0.961	0.739	0.638	0.735	0.705		1.358	1.798
Average	0.123	1.066	1.025	0.998	0.928	0.898	0.824	0.681	0.701	0.711	#DIV/0!	1.270	1.806
SD	0.015	0.032	0.045	0.042	0.055	0.045	0.068	0.034	0.047	0.027	#DIV/0!	0.063	0.063
Average - Blank	0.000	0.943	0.902	0.875	0.805	0.775	0.700	0.558	0.578	0.588	#DIV/0!	1.146	1.683
Relative Cell Growth (%)		100.0	95.7	92.7	85.4	82.2	74.3	59.2	61.3	62.3	#DIV/0!	100.0	146.8
SD of Relative Cell Growth		3.4	4.8	4.5	5.8	4.8	7.2	3.6	5.0	2.9	#DIV/0!	6.6	6.7

Transformation Assay

Giemsa Staining	0.0001	0.001	0.003	0.01	0.03	0.1	0.3	1	3	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	7	5	3	1	2	0	0	0		6	59
Transformation frequency*	0.083333333	0.072916667	0.052083333	0.03125	0.010416667	0.020833333	0	0	0	0	0.0625	0.614583333
SE	0.02820847	0.02653610	0.02267769	0.01775805	0.01036227	0.01457713	0.00000000	0.00000000	0.00000000	0.00000000	0.02470529	0.04967296

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MCA
Institution:	Lab 4
Test Number	

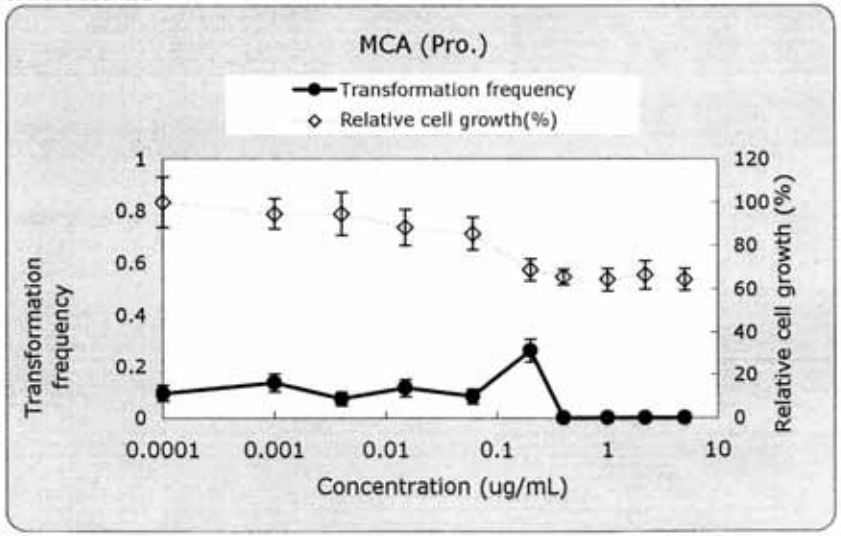
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.001	0.004	0.015	0.06	0.2	0.4	1	2.2	5	0.1% DMSO	TPA (50ng/mL)
1	0.062	0.262	0.251	0.244	0.219	0.228	0.204	0.194	0.185	0.184	0.182	0.281	0.423
2	0.065	0.262	0.267	0.242	0.257	0.248	0.214	0.215	0.202	0.208	0.209	0.300	0.469
3	0.065	0.260	0.271	0.294	0.262	0.247	0.210	0.210	0.204	0.228	0.209	0.318	0.520
4	0.063	0.272	0.262	0.265	0.267	0.243	0.219	0.214	0.208	0.228	0.208	0.324	0.498
5	0.066	0.325	0.281	0.293	0.265	0.260	0.223	0.205	0.223	0.208	0.211	0.318	0.477
6	0.071	0.305	0.287	0.288	0.272	0.272	0.216	0.221	0.210	0.221	0.211	0.333	0.483
7	0.063	0.301	0.288	0.292	0.280	0.278	0.242	0.212	0.216	0.207	0.221	0.311	0.474
8	0.061	0.312	0.297	0.285	0.271	0.265	0.216	0.214	0.215	0.216	0.212	0.310	0.466
Average	0.065	0.287	0.276	0.275	0.262	0.255	0.218	0.211	0.208	0.213	0.208	0.312	0.476
SD	0.003	0.026	0.015	0.022	0.019	0.017	0.011	0.008	0.011	0.014	0.011	0.016	0.028
Average - Blank	0.000	0.223	0.211	0.211	0.197	0.191	0.154	0.146	0.143	0.148	0.143	0.247	0.412
Relative Cell Growth (%)		100.0	94.7	94.6	88.4	85.5	68.9	65.6	64.3	66.4	64.3	100.0	166.4
SD of Relative Cell Growth		11.7	6.9	9.9	8.3	7.4	5.0	3.6	5.2	6.5	5.0	7.1	12.5

Transformation Assay

Giemsa Staining	0.0001	0.001	0.004	0.015	0.06	0.2	0.4	1	2.2	5	Negative Control	Positive Control
No. of wells excluded	1	0	0	0	0	0	96	96	96	96	0	0
No. of wells counted	95	96	96	96	96	96	0	0	0	0	96	96
No. of wells having foci	9	13	7	11	8	25					8	52
Transformation frequency*	0.094736842	0.135416667	0.072916667	0.114583333	0.083333333	0.260416667	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.083333333	0.541666667
SE	0.03004589	0.03492238	0.02653610	0.03250865	0.02820847	0.04479116	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.02820847	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	o-Toluidine
Institution:	Lab 1
Test Number	

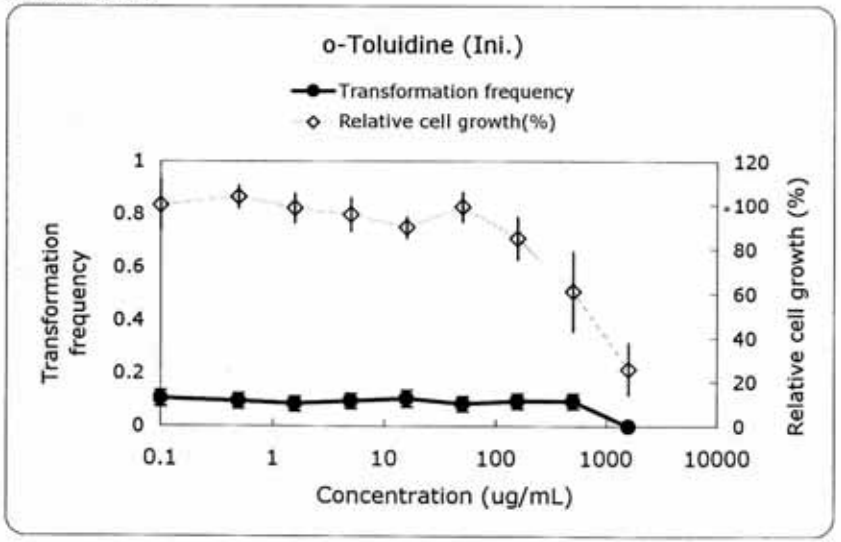
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1.58	5	15.8	50	158	500	1580		0.1% DMSO	MCA (1ug/mL)
1	0.104	0.705	0.774	0.737	0.754	0.688	0.713	0.692	0.541	0.315		0.739	0.267
2	0.115	0.872	0.785	0.792	0.718	0.674	0.745	0.660	0.499	0.255		0.701	0.297
3	0.117	0.787	0.702	0.747	0.652	0.670	0.688	0.616	0.401	0.232		0.549	0.263
4	0.114	0.709	0.738	0.685	0.679	0.632	0.659	0.677	0.401	0.272		0.628	0.284
5	0.134	0.692	0.733	0.672	0.662	0.671	0.719	0.570	0.413	0.235		0.708	0.296
6	0.141	0.652	0.718	0.690	0.716	0.618	0.780	0.654	0.394	0.235		0.606	0.295
7	0.139	0.689	0.758	0.696	0.660	0.667	0.701	0.537	0.624	0.263		0.721	0.309
8	0.144	0.706	0.785	0.720	0.770	0.707	0.765	0.685	0.661	0.443		0.781	0.323
Average	0.126	0.727	0.749	0.717	0.701	0.666	0.721	0.636	0.492	0.281	#DIV/0!	0.679	0.292
SD	0.015	0.070	0.031	0.040	0.045	0.029	0.040	0.057	0.107	0.071	#DIV/0!	0.077	0.020
Average - Blank	0.000	0.601	0.623	0.591	0.575	0.540	0.595	0.510	0.366	0.155	#DIV/0!	0.553	0.166
Relative Cell Growth (%)		100.0	103.8	98.5	95.8	89.9	99.1	85.0	60.9	25.9	#DIV/0!	100.0	30.0
SD of Relative Cell Growth		11.6	5.2	6.6	7.5	4.8	6.7	9.5	17.9	11.8	#DIV/0!	12.9	3.3

Transformation Assay

Giemsa Staining	0.1	0.5	1.58	5	15.8	50	158	500	1580	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	42		0	0
No. of wells counted	96	96	96	96	96	96	96	96	54	96	96	96
No. of wells having foci	10	9	8	9	10	8	9	9	TOX		8	44
Transformation frequency*	0.104166667	0.09375	0.083333333	0.09375	0.104166667	0.083333333	0.09375	0.09375	#VALUE!	0	0.083333333	0.458333333
SE	0.03117758	0.02974911	0.02820847	0.02974911	0.03117758	0.02820847	0.02974911	0.02974911	#VALUE!	0.00000000	0.02820847	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<15 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	o-Toluidine
Institution:	Lab 2
Test Number	

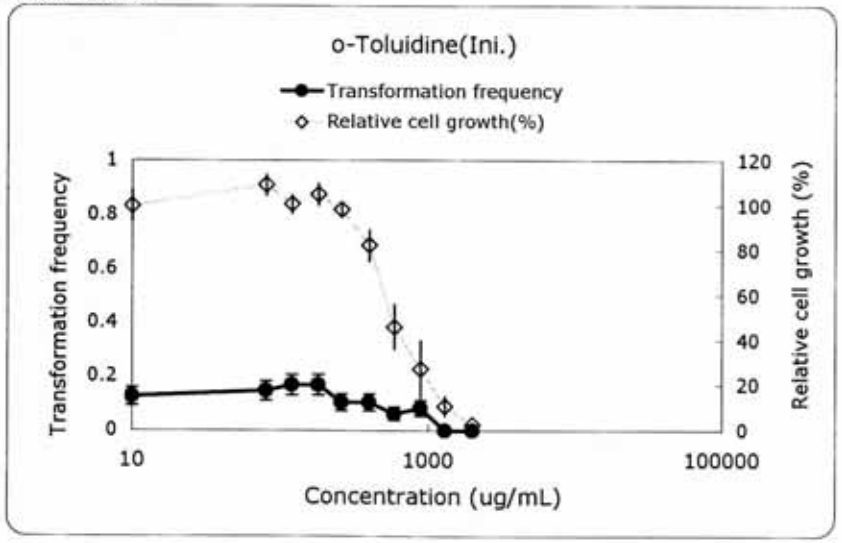
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	80	120	180	260	400	590	890	1300	2000	0.1% DMSO	MCA (1ug/mL)
1	0.054	0.510	0.539	0.530	0.572	0.511	0.521	0.380	0.217	0.142	0.074	0.543	0.328
2	0.056	0.500	0.593	0.551	0.583	0.548	0.472	0.293	0.164	0.105	0.067	0.574	0.320
3	0.053	0.515	0.592	0.572	0.568	0.546	0.448	0.272	0.144	0.091	0.063	0.596	0.372
4	0.052	0.576	0.609	0.573	0.587	0.545	0.433	0.244	0.142	0.089	0.065	0.571	0.356
5	0.052	0.552	0.603	0.561	0.591	0.541	0.432	0.237	0.142	0.088	0.061	0.597	0.396
6	0.052	0.565	0.597	0.521	0.589	0.540	0.426	0.237	0.157	0.096	0.063	0.565	0.371
7	0.053	0.596	0.608	0.549	0.541	0.542	0.437	0.253	0.196	0.104	0.061	0.575	0.291
8	0.054	0.543	0.575	0.528	0.527	0.514	0.489	0.313	0.328	0.135	0.069	0.544	0.367
Average	0.053	0.545	0.590	0.548	0.570	0.536	0.457	0.279	0.186	0.106	0.065	0.571	0.350
SD	0.001	0.034	0.023	0.020	0.024	0.015	0.034	0.049	0.063	0.021	0.004	0.020	0.034
Average - Blank	0.000	0.491	0.536	0.495	0.517	0.483	0.404	0.225	0.133	0.053	0.012	0.517	0.297
Relative Cell Growth (%)		100.0	109.1	100.7	105.1	98.2	82.2	45.9	27.1	10.8	2.5	100.0	57.4
SD of Relative Cell Growth		7.0	4.7	4.1	4.8	3.0	6.9	10.0	12.9	4.3	0.9	4.1	7.0

Transformation Assay

Giemsa Staining	10	80	120	180	260	400	590	890	1300	2000	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	14	16	16	10	10	6	8	0	0	10	68
Transformation frequency*	0.125	0.145833333	0.166666667	0.166666667	0.104166667	0.104166667	0.0625	0.083333333	0	0	0.104166667	0.708333333
SE	0.03375386	0.03602169	0.03803629	0.03803629	0.03117758	0.03117758	0.02470529	0.02820847	0.00000000	0.00000000	0.03117758	0.04639024

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	o-Toluidine
Institution:	Lab 3
Test Number	

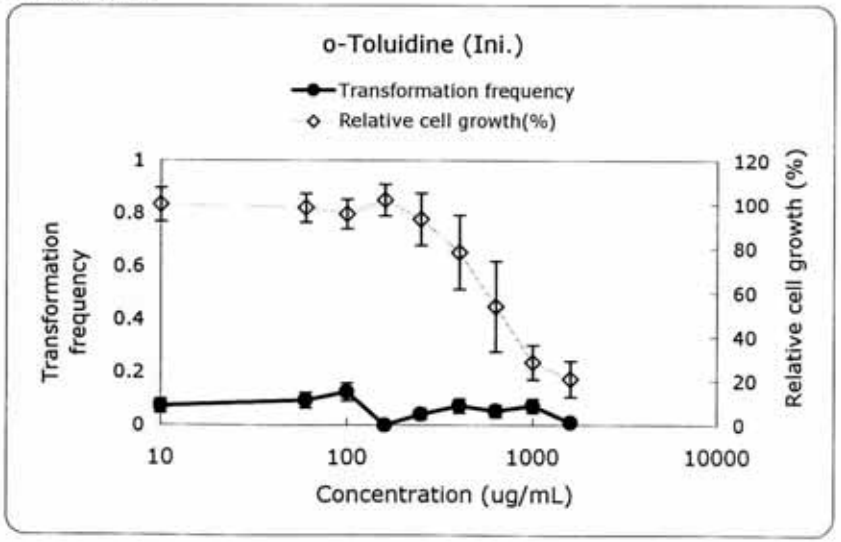
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	60	100	160	250	400	630	1000	1600		0.1% DMSO	MCA (1ug/mL)
1	0.106	1.205	1.256	1.174	1.198	1.328	1.217	1.005	0.478	0.383		0.918	0.379
2	0.102	1.058	1.230	1.091	1.219	1.172	1.115	0.698	0.436	0.271		0.660	0.363
3	0.107	1.071	1.139	1.128	1.131	1.088	0.780	0.662	0.320	0.287		0.774	0.294
4	0.106	1.116	1.044	0.972	1.055	1.013	0.820	0.472	0.364	0.247		0.556	0.293
5	0.105	1.153	1.108	1.179	1.190	0.911	0.758	0.406	0.335	0.233		0.536	0.329
6	0.099	1.151	1.121	1.075	1.134	1.035	0.789	0.536	0.339	0.324		0.673	0.339
7	0.108	1.299	1.144	1.112	1.203	1.039	0.903	0.628	0.394	0.371		0.884	0.398
8	0.108	1.227	1.116	1.180	1.316	1.142	1.070	0.967	0.556	0.486		1.067	0.442
Average	0.105	1.160	1.145	1.114	1.181	1.091	0.932	0.672	0.403	0.325	#DIV/0!	0.759	0.355
SD	0.003	0.081	0.068	0.070	0.077	0.125	0.178	0.217	0.082	0.085	#DIV/0!	0.187	0.051
Average - Blank	0.000	1.055	1.040	1.009	1.076	0.986	0.826	0.567	0.298	0.220	#DIV/0!	0.653	0.250
Relative Cell Growth (%)		100.0	98.6	95.6	102.0	93.5	78.3	53.7	28.2	20.9	#DIV/0!	100.0	38.2
SD of Relative Cell Growth		7.7	6.5	6.7	7.3	11.9	16.8	20.6	7.8	8.0	#DIV/0!	17.7	4.9

Transformation Assay

Giemsa Staining	10	60	100	160	250	400	630	1000	1600	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	9	12	0	4	7	5	7	1		5	66
Transformation frequency*	0.072916667	0.09375	0.125	0	0.041666667	0.072916667	0.052083333	0.072916667	0.010416667	0	0.052083333	0.6875
SE	0.02653610	0.02974911	0.03375386	0.00000000	0.02039469	0.02653610	0.02267769	0.02653610	0.01036227	0.00000000	0.02267769	0.04730704

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	o-Toluidine
Institution:	Lab 4
Test Number	

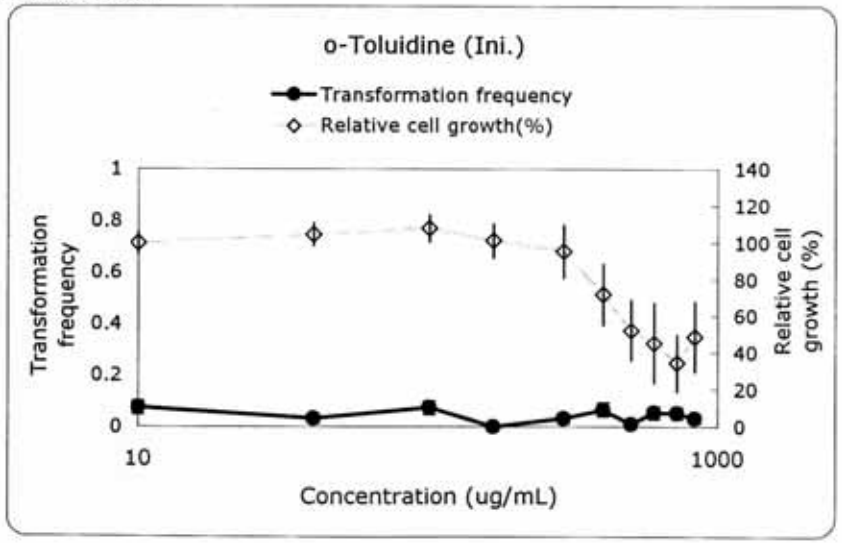
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	40	100	166.67	291.67	400	500	600	720	833.33	0.1% DMSO	MCA (1ug/mL)
1	0.062	0.275	0.295	0.304	0.323	0.285	0.269	0.236	0.232	0.182	0.256	0.275	0.139
2	0.065	0.280	0.301	0.296	0.312	0.291	0.230	0.163	0.164	0.132	0.172	0.280	0.110
3	0.065	0.282	0.320	0.292	0.289	0.273	0.182	0.144	0.131	0.121	0.179	0.282	0.123
4	0.063	0.301	0.296	0.310	0.272	0.246	0.198	0.158	0.124	0.114	0.128	0.301	0.116
5	0.066	0.310	0.317	0.325	0.269	0.251	0.196	0.159	0.133	0.118	0.136	0.310	0.122
6	0.071	0.300	0.297	0.303	0.320	0.269	0.226	0.172	0.134	0.118	0.159	0.300	0.117
7	0.063	0.304	0.286	0.324	0.303	0.302	0.239	0.201	0.177	0.154	0.155	0.304	0.132
8	0.061	0.300	0.323	0.342	0.285	0.350	0.294	0.243	0.254	0.210	0.224	0.300	0.146
Average	0.065	0.294	0.304	0.312	0.297	0.283	0.229	0.185	0.169	0.144	0.176	0.294	0.126
SD	0.003	0.013	0.014	0.017	0.021	0.033	0.038	0.038	0.050	0.036	0.044	0.013	0.012
Average - Blank	0.000	0.230	0.240	0.248	0.232	0.219	0.165	0.120	0.104	0.079	0.112	0.230	0.061
Relative Cell Growth (%)		100.0	104.5	107.8	101.1	95.4	71.8	52.3	45.4	34.5	48.6	100.0	26.6
SD of Relative Cell Growth		5.7	6.0	7.4	9.1	14.4	16.6	16.4	21.6	15.5	19.0	5.7	5.4

Transformation Assay

Giemsa Staining	10	40	100	166.67	291.67	400	500	600	720	833.33	Negative Control	Positive Control
No. of wells excluded	0	0	0	96	0	4	0	3	0	0	0	0
No. of wells counted	96	96	96	0	96	92	96	93	96	96	96	96
No. of wells having foci	7	3	7		3	6	1	5	5	3	7	50
Transformation frequency*	0.072916667	0.03125	0.072916667	#DIV/0!	0.03125	0.065217391	0.010416667	0.053763441	0.052083333	0.03125	0.072916667	0.520833333
SE	0.02653610	0.01775805	0.02653610	#DIV/0!	0.01775805	0.02574205	0.01036227	0.02338848	0.02267769	0.01775805	0.02653610	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	o-Toluidine
Institution:	Lab 1
Test Number	

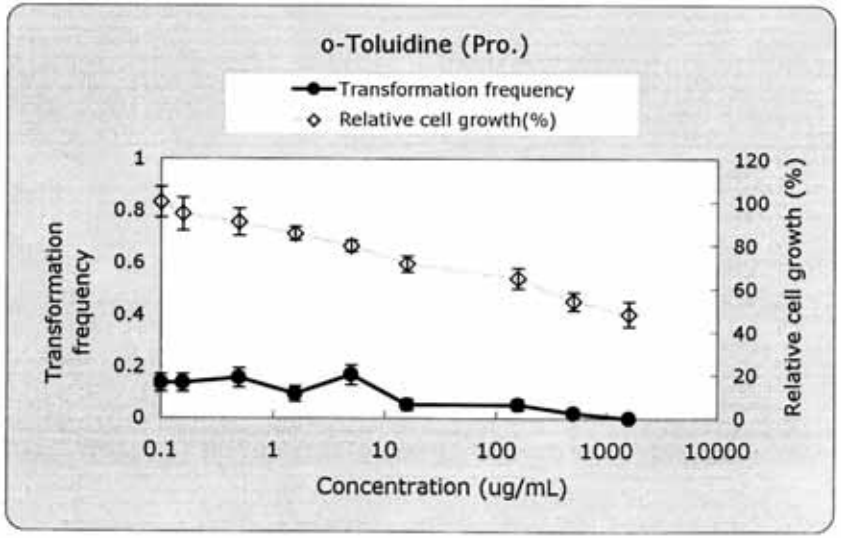
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.158	0.5	1.58	5	15.8	158	500	1580		0.1% DMSO	TPA (50ng/mL)
1	0.121	0.508	0.486	0.493	0.428	0.408	0.362	0.334	0.314	0.300		0.363	0.378
2	0.128	0.505	0.485	0.429	0.421	0.399	0.355	0.342	0.304	0.279		0.358	0.382
3	0.120	0.506	0.475	0.421	0.421	0.390	0.364	0.325	0.292	0.273		0.352	0.383
4	0.117	0.494	0.478	0.441	0.430	0.400	0.376	0.354	0.301	0.283		0.350	0.383
5	0.109	0.455	0.443	0.442	0.420	0.403	0.389	0.364	0.316	0.279		0.341	0.374
6	0.109	0.452	0.432	0.431	0.439	0.414	0.378	0.361	0.318	0.285		0.353	0.368
7	0.106	0.454	0.411	0.450	0.410	0.409	0.384	0.349	0.311	0.290		0.357	0.364
8	0.111	0.461	0.463	0.461	0.445	0.421	0.393	0.379	0.341	0.340		0.363	0.394
Average	0.115	0.479	0.459	0.446	0.427	0.406	0.375	0.351	0.312	0.291	#DIV/0!	0.355	0.378
SD	0.008	0.026	0.028	0.023	0.011	0.010	0.014	0.017	0.015	0.021	#DIV/0!	0.007	0.010
Average - Blank	0.000	0.364	0.344	0.331	0.312	0.290	0.260	0.236	0.197	0.176	#DIV/0!	0.240	0.263
Relative Cell Growth (%)		100.0	94.4	90.8	85.6	79.7	71.4	64.8	54.1	48.3	#DIV/0!	100.0	109.9
SD of Relative Cell Growth		7.1	7.6	6.3	3.1	2.6	3.7	4.8	4.0	5.9	#DIV/0!	2.0	2.6

Transformation Assay

Giemsa Staining	0.1	0.158	0.5	1.58	5	15.8	158	500	1580	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	53		0	0
No. of wells counted	96	96	96	96	96	96	96	96	43	96	96	96
No. of wells having foci	13	13	15	9	16	5	5	2	TOX		9	49
Transformation frequency*	0.135416667	0.135416667	0.15625	0.09375	0.166666667	0.052083333	0.052083333	0.020833333	#VALUE!	0	0.09375	0.510416667
SE	0.03492238	0.03492238	0.03705794	0.02974911	0.03803629	0.02267769	0.02267769	0.01457713	#VALUE!	0.00000000	0.02974911	0.05101996

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	o-Toluidine
Institution:	Lab 2
Test Number	

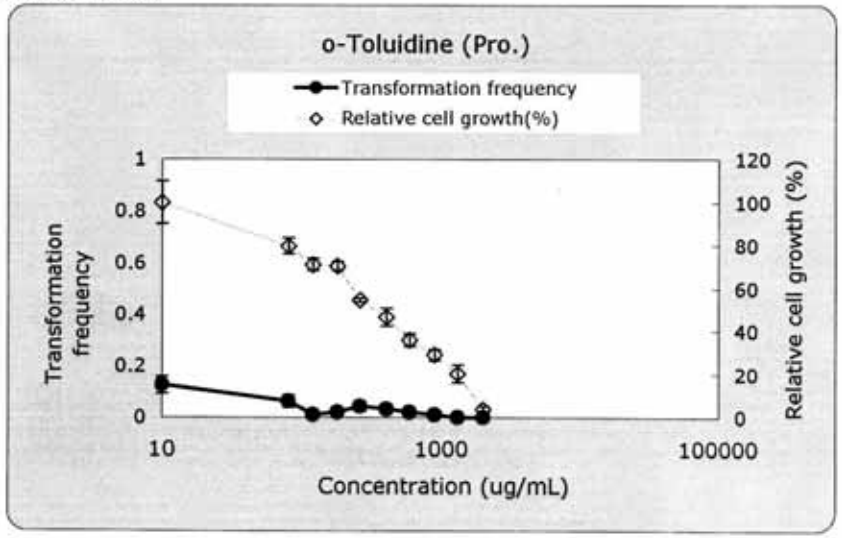
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	80	120	180	260	400	590	890	1300	2000	0.1% DMSO	TPA (50ng/mL)
1	0.054	0.404	0.367	0.330	0.354	0.279	0.263	0.220	0.187	0.162	0.080	0.450	0.609
2	0.056	0.471	0.397	0.354	0.352	0.283	0.257	0.207	0.173	0.142	0.068	0.547	0.728
3	0.053	0.455	0.405	0.357	0.352	0.278	0.265	0.193	0.164	0.125	0.063	0.556	0.829
4	0.052	0.497	0.398	0.363	0.344	0.287	0.256	0.191	0.165	0.121	0.066	0.560	0.794
5	0.052	0.532	0.390	0.356	0.350	0.277	0.250	0.187	0.160	0.118	0.064	0.654	0.765
6	0.052	0.502	0.384	0.354	0.352	0.280	0.230	0.199	0.170	0.129	0.065	0.599	0.818
7	0.053	0.459	0.375	0.339	0.338	0.276	0.222	0.200	0.179	0.136	0.064	0.530	0.703
8	0.054	0.429	0.362	0.337	0.330	0.281	0.229	0.219	0.189	0.161	0.073	0.514	0.640
Average	0.053	0.469	0.385	0.349	0.347	0.280	0.247	0.202	0.173	0.137	0.068	0.551	0.736
SD	0.001	0.041	0.016	0.012	0.009	0.004	0.017	0.012	0.011	0.017	0.006	0.060	0.081
Average - Blank	0.000	0.415	0.332	0.296	0.293	0.227	0.193	0.149	0.120	0.084	0.015	0.498	0.683
Relative Cell Growth (%)		100.0	79.8	71.1	70.6	54.6	46.5	35.8	28.9	20.1	3.5	100.0	137.0
SD of Relative Cell Growth		10.0	3.7	2.8	2.1	0.9	4.1	3.0	2.6	4.1	1.4	14.4	19.5

Transformation Assay

Giemsa Staining	10	80	120	180	260	400	590	890	1300	2000	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	6	1	2	4	3	2	1	0	0	8	36
Transformation frequency*	0.125	0.0625	0.010416667	0.020833333	0.041666667	0.03125	0.020833333	0.010416667	0	0	0.083333333	0.375
SE	0.03375386	0.02470529	0.01036227	0.01457713	0.02039469	0.01775805	0.01457713	0.01036227	0.00000000	0.00000000	0.02820847	0.04941059

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	o-Toluidine
Institution:	Lab 3
Test Number	

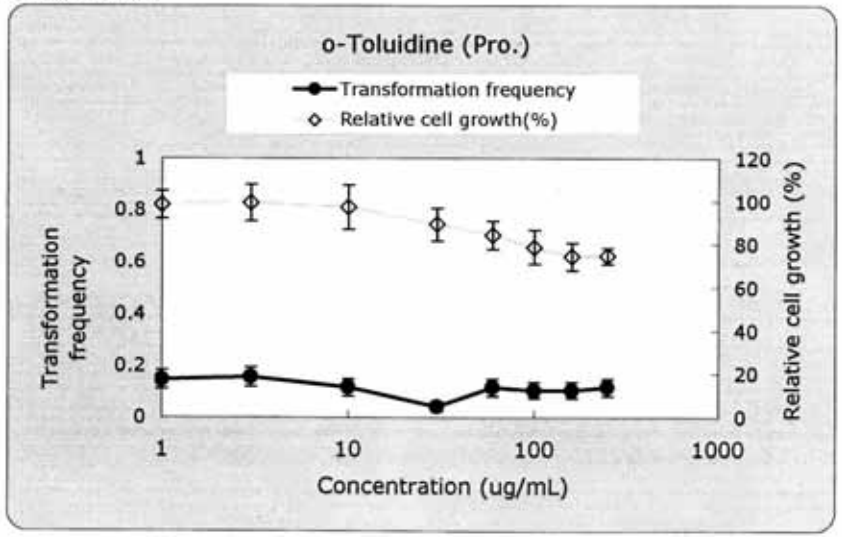
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	3	10	30	60	100	160	250		0.1% DMSO	TPA (50ng/mL)
1	0.106	0.891	0.917	0.996	0.956	0.842	0.770	0.703	0.704	0.678		0.764	1.310
2	0.102	0.906	0.896	0.897	0.836	0.828	0.772	0.692	0.671	0.696		0.780	1.221
3	0.107	0.881	0.877	0.859	0.838	0.787	0.730	0.709	0.662	0.690		0.718	1.158
4	0.106	0.886	0.807	0.812	0.728	0.692	0.677	0.643	0.643	0.631		0.668	1.053
5	0.105	0.870	0.801	0.816	0.759	0.734	0.718	0.651	0.645	0.631		0.691	1.181
6	0.099	0.830	0.782	0.789	0.826	0.744	0.700	0.678	0.623	0.687		0.736	1.163
7	0.108	0.787	0.877	0.865	0.901	0.786	0.767	0.722	0.645	0.672		0.785	1.242
8	0.108	0.877	0.873	0.849	0.924	0.856	0.833	0.834	0.776	0.704		0.809	1.232
Average	0.105	0.866	0.854	0.860	0.846	0.784	0.746	0.704	0.671	0.674	#DIV/0!	0.744	1.195
SD	0.003	0.039	0.050	0.065	0.079	0.057	0.050	0.059	0.049	0.028	#DIV/0!	0.049	0.076
Average - Blank	0.000	0.761	0.749	0.755	0.741	0.679	0.641	0.599	0.566	0.569	#DIV/0!	0.639	1.090
Relative Cell Growth (%)		100.0	98.4	99.3	97.4	89.2	84.2	78.7	74.4	74.7	#DIV/0!	100.0	170.6
SD of Relative Cell Growth		5.1	6.5	8.5	10.3	7.5	6.5	7.8	6.4	3.7	#DIV/0!	6.5	10.0

Transformation Assay

Giemsa Staining	0.1	1	3	10	30	60	100	160	250	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	14	15	11	4	11	10	10	11		5	46
Transformation frequency*	0.135416667	0.145833333	0.15625	0.114583333	0.041666667	0.114583333	0.104166667	0.104166667	0.114583333	0	0.052083333	0.479166667
SE	0.03492238	0.03602169	0.03705794	0.03250865	0.02039469	0.03250865	0.03117758	0.03117758	0.03250865	0.00000000	0.02267769	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	o-Toluidine
Institution:	Lab 4
Test Number	

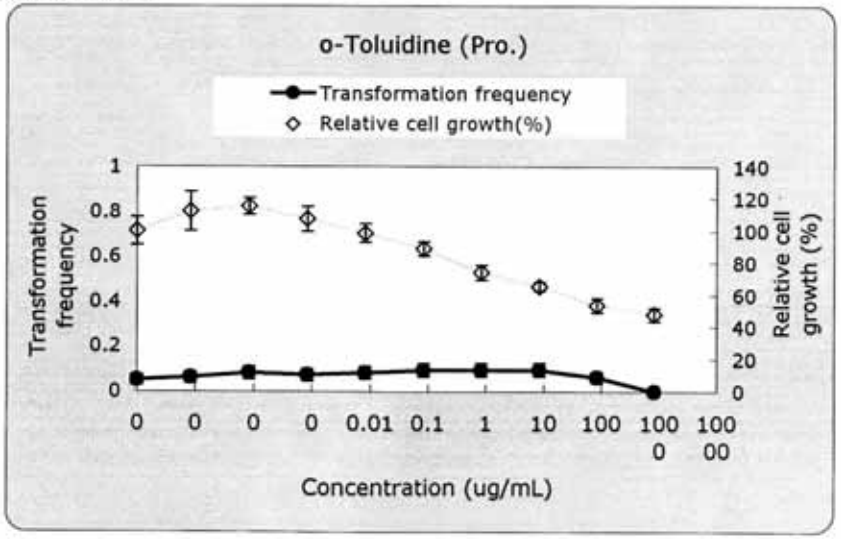
Cell Growth Assay

Crystal Violet Staining	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
OD_{540nm}		0.000001	8.33E-06	0.0000833	0.000833	0.00833	0.0833	0.833	8.33	83.33	833.33	0.1% DMSO	TPA (50ng/mL)
1	0.078	0.225	0.243	0.269	0.240	0.244	0.223	0.211	0.190	0.184	0.164	0.225	0.223
2	0.074	0.242	0.244	0.276	0.250	0.261	0.229	0.199	0.183	0.161	0.160	0.242	0.244
3	0.073	0.241	0.268	0.271	0.260	0.244	0.234	0.207	0.185	0.171	0.159	0.241	0.228
4	0.076	0.237	0.270	0.275	0.269	0.254	0.235	0.198	0.194	0.167	0.151	0.237	0.221
5	0.072	0.247	0.285	0.278	0.285	0.238	0.222	0.215	0.182	0.168	0.150	0.247	0.223
6	0.074	0.263	0.307	0.294	0.264	0.254	0.216	0.203	0.186	0.158	0.148	0.263	0.226
7	0.074	0.272	0.277	0.272	0.263	0.234	0.237	0.189	0.192	0.169	0.168	0.272	0.234
8	0.079	0.257	0.260	0.263	0.257	0.235	0.234	0.203	0.193	0.165	0.163	0.257	0.233
Average	0.075	0.248	0.269	0.275	0.261	0.245	0.229	0.203	0.188	0.168	0.158	0.248	0.229
SD	0.002	0.015	0.021	0.009	0.013	0.010	0.008	0.008	0.005	0.008	0.007	0.015	0.008
Average - Blank	0.000	0.173	0.194	0.200	0.186	0.170	0.154	0.128	0.113	0.093	0.083	0.173	0.154
Relative Cell Growth (%)		100.0	112.3	115.4	107.3	98.5	88.9	74.0	65.3	53.6	47.8	100.0	89.0
SD of Relative Cell Growth		8.7	12.3	5.2	7.7	5.7	4.4	4.7	2.8	4.4	4.3	8.7	4.4

Transformation Assay

Giemsa Staining	0.000001	0.00000833	0.0000833	0.000833	0.00833	0.0833	0.833	8.33	83.33	833.33	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	6	8	7	8	9	9	9	6	0	5	50
Transformation frequency*	0.052083333	0.0625	0.083333333	0.072916667	0.083333333	0.09375	0.09375	0.09375	0.0625	0	0.052083333	0.520833333
SE	0.02267769	0.02470529	0.02820847	0.02653610	0.02820847	0.02974911	0.02974911	0.02974911	0.02470529	0.00000000	0.02267769	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	TPA
Institution:	Lab 1
Test Number	

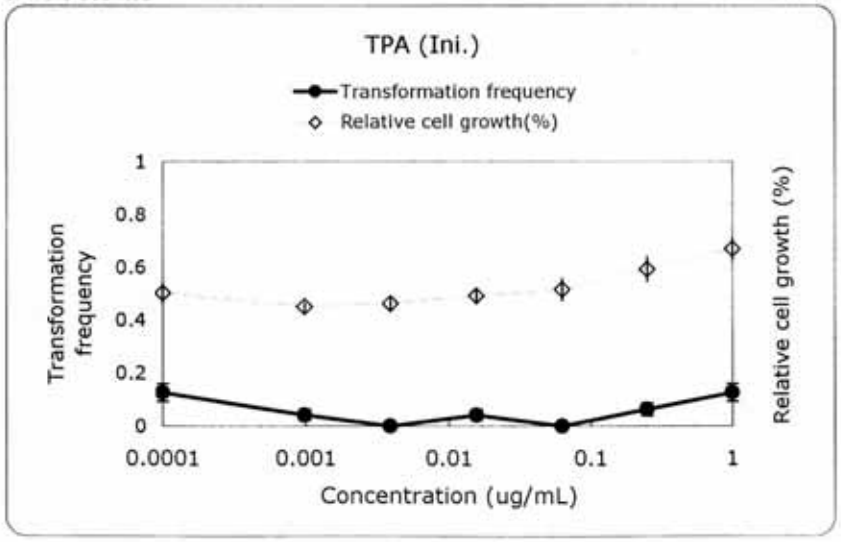
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.000977	0.00391	0.0156	0.0625	0.25	1				0.1% DMSO	MCA (1ug/mL)
1	0.095	0.765	0.719	0.709	0.720	0.789	0.958	1.088				0.765	0.409
2	0.097	0.722	0.660	0.683	0.747	0.807	0.869	0.975				0.722	0.519
3	0.095	0.838	0.705	0.654	0.783	0.774	0.976	0.937				0.838	0.451
4	0.091	0.746	0.679	0.708	0.729	0.761	0.887	1.025				0.746	0.449
5	0.083	0.773	0.684	0.752	0.720	0.762	0.892	1.036				0.773	0.343
6	0.088	0.745	0.692	0.740	0.770	0.681	0.848	0.993				0.745	0.395
7	0.087	0.827	0.724	0.713	0.734	0.870	0.775	0.950				0.827	0.347
8	0.094	0.688	0.698	0.731	0.802	0.829	0.908	0.928				0.688	0.317
Average	0.091	0.763	0.695	0.711	0.751	0.784	0.889	0.992	#DIV/0!	#DIV/0!	#DIV/0!	0.763	0.404
SD	0.005	0.050	0.021	0.032	0.031	0.056	0.063	0.055	#DIV/0!	#DIV/0!	#DIV/0!	0.050	0.068
Average - Blank	0.000	0.672	0.604	0.620	0.659	0.693	0.798	0.900	#DIV/0!	#DIV/0!	#DIV/0!	0.672	0.313
Relative Cell Growth (%)		100.0	89.9	92.3	98.2	103.1	118.8	134.0	#DIV/0!	#DIV/0!	#DIV/0!	100.0	46.5
SD of Relative Cell Growth		7.5	3.2	4.7	4.6	8.3	9.4	8.2	#DIV/0!	#DIV/0!	#DIV/0!	7.5	10.1

Transformation Assay

Giemsa Staining	0.0001	0.000977	0.00391	0.0156	0.0625	0.25	1	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	4	0	4	0	6	12				12	54
Transformation frequency*	0.125	0.041666667	0	0.041666667	0	0.0625	0.125	0	0	0	0.125	0.5625
SE	0.03375386	0.02039469	0.00000000	0.02039469	0.00000000	0.02470529	0.03375386	0.00000000	0.00000000	0.00000000	0.03375386	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	TPA
Institution:	Lab 2
Test Number	

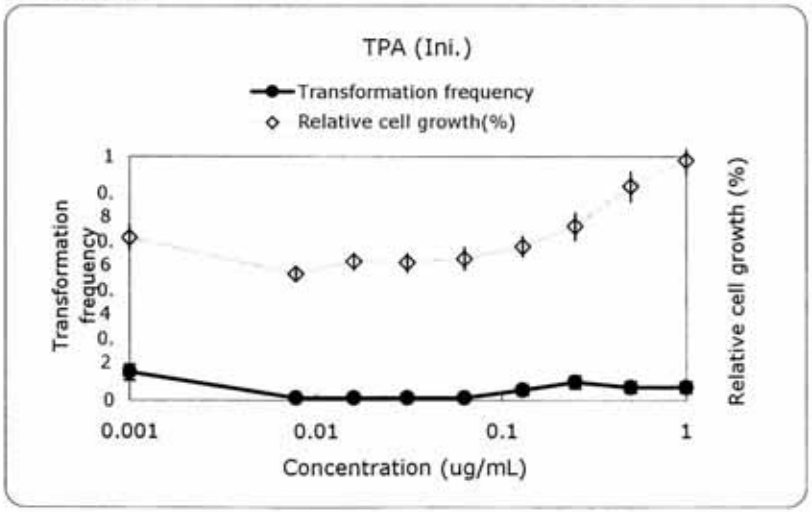
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.0078	0.016	0.031	0.063	0.13	0.25	0.5	1		0.1% DMSO	MCA (1ug/mL)
1	0.049	0.532	0.447	0.433	0.436	0.457	0.482	0.533	0.657	0.684		0.532	0.315
2	0.052	0.581	0.430	0.441	0.469	0.450	0.488	0.531	0.648	0.717		0.581	0.303
3	0.050	0.537	0.410	0.452	0.466	0.424	0.544	0.590	0.752	0.772		0.537	0.273
4	0.059	0.498	0.408	0.481	0.444	0.518	0.547	0.525	0.643	0.774		0.498	0.301
5	0.068	0.517	0.420	0.450	0.483	0.505	0.519	0.532	0.637	0.786		0.517	0.314
6	0.080	0.552	0.466	0.486	0.479	0.486	0.490	0.619	0.689	0.780		0.552	0.261
7	0.082	0.554	0.421	0.498	0.476	0.465	0.486	0.613	0.726	0.774		0.554	0.295
8	0.080	0.466	0.404	0.450	0.405	0.453	0.476	0.545	0.656	0.714		0.466	0.325
Average	0.065	0.530	0.426	0.461	0.457	0.470	0.504	0.561	0.676	0.750	#DIV/0!	0.530	0.298
SD	0.014	0.036	0.021	0.024	0.027	0.031	0.029	0.040	0.042	0.039	#DIV/0!	0.036	0.022
Average - Blank	0.000	0.465	0.361	0.396	0.392	0.405	0.439	0.496	0.611	0.685	#DIV/0!	0.465	0.233
Relative Cell Growth (%)		100.0	77.6	85.3	84.4	87.1	94.5	106.8	131.5	147.5	#DIV/0!	100.0	50.2
SD of Relative Cell Growth		7.7	4.6	5.1	5.8	6.7	6.2	8.5	9.1	8.4	#DIV/0!	7.7	4.7

Transformation Assay

Giemsa Staining	0.001	0.0078	0.016	0.031	0.063	0.13	0.25	0.5	1	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	1	1	1	1	4	7	5	5		11	66
Transformation frequency*	0.114583333	0.010416667	0.010416667	0.010416667	0.010416667	0.041666667	0.072916667	0.052083333	0.052083333	0	0.114583333	0.6875
SE	0.03250865	0.01036227	0.01036227	0.01036227	0.01036227	0.02039469	0.02653610	0.02267769	0.02267769	0.00000000	0.03250865	0.04730704

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	TPA
Institution:	Lab 3
Test Number	

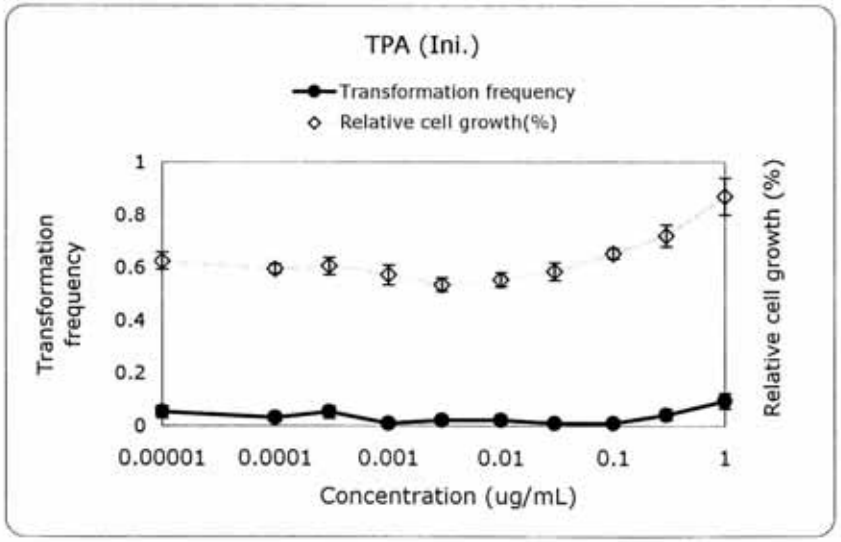
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	0.1% DMSO	MCA (1ug/mL)
1	0.078	0.934	0.913	0.964	0.885	0.872	0.892	0.942	0.973	1.113	1.423	0.934	0.507
2	0.060	0.862	0.861	0.880	0.851	0.838	0.836	0.911	0.995	1.054	1.369	0.862	0.469
3	0.048	0.950	0.914	0.838	0.877	0.787	0.774	0.879	0.988	0.984	1.139	0.950	0.480
4	0.053	0.885	0.911	0.905	0.866	0.803	0.823	0.833	0.933	1.007	1.251	0.885	0.432
5	0.051	0.978	0.856	0.851	0.823	0.768	0.827	0.818	0.961	1.149	1.233	0.978	0.446
6	0.055	0.954	0.900	0.901	0.933	0.813	0.793	0.813	0.926	1.050	1.198	0.954	0.477
7	0.064	0.982	0.893	0.957	0.751	0.766	0.842	0.888	0.995	1.022	1.205	0.982	0.499
8	0.073	0.887	0.859	0.927	0.865	0.794	0.862	0.900	0.973	1.104	1.344	0.887	0.552
Average	0.060	0.929	0.888	0.903	0.856	0.805	0.831	0.873	0.968	1.060	1.270	0.929	0.483
SD	0.011	0.045	0.026	0.046	0.053	0.036	0.037	0.047	0.027	0.057	0.098	0.045	0.037
Average - Blank	0.000	0.869	0.828	0.843	0.796	0.745	0.771	0.813	0.908	1.000	1.210	0.869	0.423
Relative Cell Growth (%)		100.0	95.3	97.0	91.6	85.7	88.7	93.6	104.5	115.1	139.3	100.0	48.6
SD of Relative Cell Growth		5.2	2.9	5.3	6.1	4.1	4.3	5.4	3.1	6.6	11.3	5.2	4.3

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	3	5	1	2	2	1	1	4	9	5	53
Transformation frequency*	0.052083333	0.03125	0.052083333	0.010416667	0.020833333	0.020833333	0.010416667	0.010416667	0.041666667	0.09375	0.052083333	0.552083333
SE	0.02267769	0.01775805	0.02267769	0.01036227	0.01457713	0.01457713	0.01036227	0.01036227	0.02039469	0.02974911	0.02267769	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	TPA
Institution:	Lab 4
Test Number	

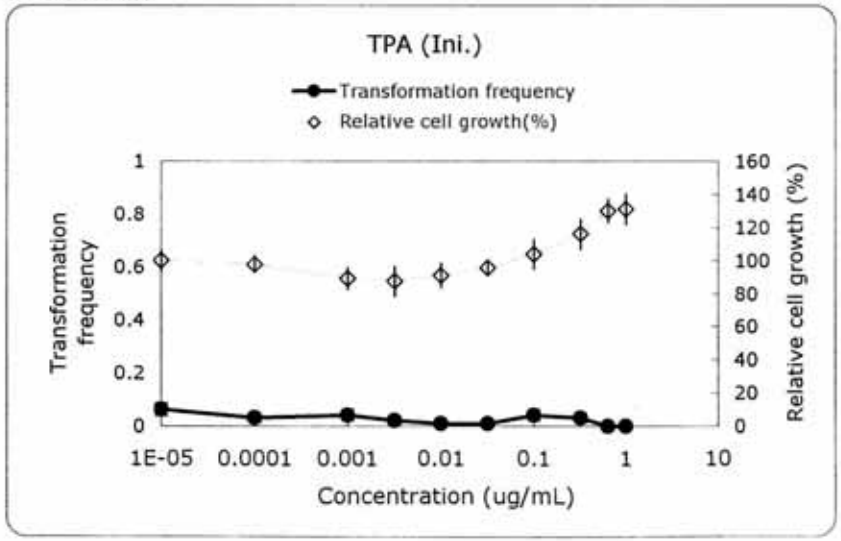
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.001	0.0032	0.01	0.032	0.1	0.32	0.64	1	0.1% DMSO	MCA (1ug/mL)
1	0.078	0.259	0.276	0.250	0.277	0.279	0.266	0.291	0.354	0.348	0.345	0.259	0.137
2	0.074	0.290	0.270	0.281	0.268	0.262	0.275	0.276	0.326	0.367	0.358	0.290	0.175
3	0.073	0.300	0.290	0.268	0.254	0.270	0.280	0.311	0.309	0.345	0.329	0.300	0.166
4	0.076	0.298	0.284	0.282	0.246	0.250	0.289	0.283	0.318	0.374	0.356	0.298	0.177
5	0.072	0.290	0.294	0.279	0.277	0.295	0.285	0.293	0.314	0.340	0.392	0.290	0.163
6	0.074	0.285	0.294	0.246	0.249	0.277	0.268	0.278	0.292	0.366	0.348	0.285	0.164
7	0.074	0.298	0.293	0.260	0.290	0.250	0.285	0.325	0.343	0.330	0.375	0.298	0.177
8	0.079	0.292	0.272	0.260	0.234	0.277	0.292	0.318	0.327	0.356	0.341	0.292	0.144
Average	0.075	0.289	0.284	0.266	0.262	0.270	0.280	0.297	0.323	0.353	0.356	0.289	0.163
SD	0.002	0.013	0.010	0.014	0.019	0.016	0.009	0.019	0.019	0.015	0.020	0.013	0.015
Average - Blank	0.000	0.214	0.209	0.191	0.187	0.195	0.205	0.222	0.248	0.278	0.281	0.214	0.088
Relative Cell Growth (%)		100.0	97.7	89.1	87.4	91.1	95.7	103.8	115.9	130.0	131.1	100.0	41.0
SD of Relative Cell Growth		6.2	4.7	6.6	8.9	7.3	4.4	8.8	9.1	7.1	9.4	6.2	7.0

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.001	0.0032	0.01	0.032	0.1	0.32	0.64	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	96	96	0	0
No. of wells counted	96	96	96	96	96	96	96	96	0	0	96	96
No. of wells having foci	6	3	4	2	1	1	4	3			6	43
Transformation frequency*	0.0625	0.03125	0.041666667	0.020833333	0.010416667	0.010416667	0.041666667	0.03125	#DIV/0!	#DIV/0!	0.0625	0.447916667
SE	0.02470529	0.01775805	0.02039469	0.01457713	0.01036227	0.01036227	0.02039469	0.01775805	#DIV/0!	#DIV/0!	0.02470529	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	TPA
Institution:	Lab 1
Test Number	

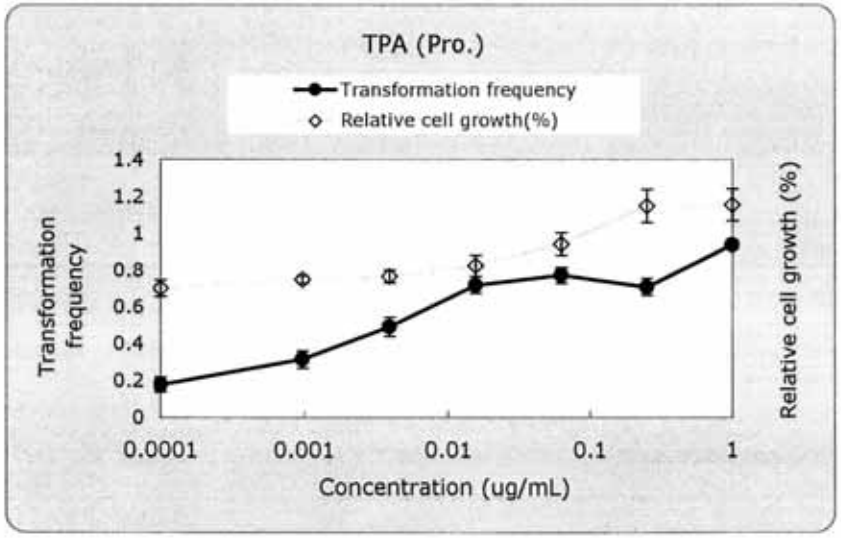
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.000977	0.00391	0.0156	0.0625	0.25	1				0.1% DMSO	TPA (50ng/mL)
1	0.101	0.821	0.828	0.825	0.877	1.007	1.185	1.136				0.821	0.921
2	0.112	0.837	0.845	0.911	0.954	1.111	1.186	1.203				0.837	0.964
3	0.103	0.882	0.823	0.858	0.897	0.942	1.148	1.215				0.882	0.934
4	0.104	0.791	0.862	0.860	0.975	1.101	1.190	1.277				0.791	1.058
5	0.101	0.747	0.863	0.915	0.864	1.021	1.356	1.258				0.747	1.012
6	0.100	0.823	0.875	0.901	0.888	1.011	1.383	1.317				0.823	1.101
7	0.099	0.809	0.882	0.835	0.975	1.104	1.299	1.311				0.809	1.065
8	0.095	0.750	0.861	0.885	1.024	1.097	1.328	1.422				0.750	1.026
Average	0.102	0.808	0.855	0.874	0.932	1.049	1.259	1.267	#DIV/0!	#DIV/0!	#DIV/0!	0.808	1.010
SD	0.005	0.045	0.021	0.034	0.058	0.062	0.092	0.087	#DIV/0!	#DIV/0!	#DIV/0!	0.045	0.065
Average - Blank	0.000	0.706	0.753	0.772	0.830	0.947	1.158	1.166	#DIV/0!	#DIV/0!	#DIV/0!	0.706	0.908
Relative Cell Growth (%)		100.0	106.7	109.4	117.6	134.3	164.0	165.2	#DIV/0!	#DIV/0!	#DIV/0!	100.0	128.7
SD of Relative Cell Growth		6.4	3.0	4.9	8.2	8.9	13.0	12.3	#DIV/0!	#DIV/0!	#DIV/0!	6.4	9.2

Transformation Assay

Giemsa Staining	0.0001	0.000977	0.00391	0.0156	0.0625	0.25	1	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	17	30	47	69	74	68	90				17	73
Transformation frequency*	0.177083333	0.3125	0.489583333	0.71875	0.770833333	0.708333333	0.9375	0	0	0	0.177083333	0.760416667
SE	0.03896110	0.04730704	0.05101996	0.04588805	0.04289635	0.04639024	0.02470529	0.00000000	0.00000000	0.00000000	0.03896110	0.04356307

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	TPA
Institution:	Lab 2
Test Number	

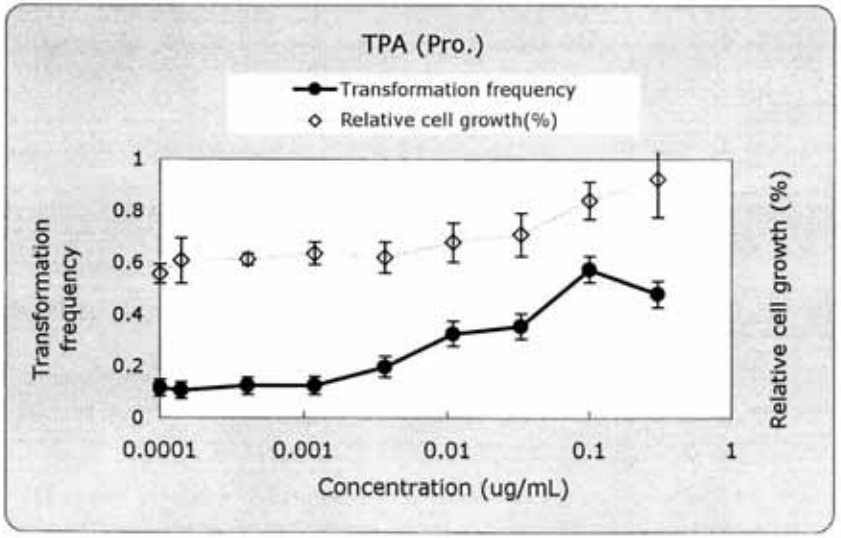
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.00014	0.00041	0.0012	0.0037	0.011	0.033	0.1	0.3		0.1% DMSO	TPA (50ng/mL)
1	0.029	0.325	0.321	0.337	0.365	0.346	0.376	0.356	0.514	0.570		0.325	0.370
2	0.027	0.330	0.360	0.357	0.389	0.395	0.370	0.418	0.480	0.602		0.330	0.396
3	0.030	0.317	0.299	0.360	0.352	0.328	0.418	0.436	0.493	0.585		0.317	0.358
4	0.031	0.286	0.442	0.359	0.388	0.393	0.388	0.423	0.456	0.443		0.286	0.329
5	0.030	0.300	0.316	0.352	0.360	0.353	0.453	0.456	0.466	0.468		0.300	0.339
6	0.028	0.334	0.372	0.364	0.382	0.382	0.399	0.413	0.497	0.586		0.334	0.331
7	0.034	0.338	0.363	0.354	0.322	0.331	0.362	0.385	0.468	0.443		0.338	0.347
8	0.033	0.341	0.315	0.331	0.350	0.317	0.322	0.325	0.391	0.421		0.341	0.356
Average	0.030	0.321	0.349	0.352	0.364	0.356	0.386	0.402	0.471	0.515	#DIV/0!	0.321	0.353
SD	0.002	0.019	0.046	0.012	0.023	0.031	0.039	0.043	0.037	0.077	#DIV/0!	0.019	0.022
Average - Blank	0.000	0.291	0.318	0.322	0.333	0.325	0.356	0.371	0.440	0.485	#DIV/0!	0.291	0.323
Relative Cell Growth (%)		100.0	109.3	110.4	114.5	111.8	122.2	127.5	151.3	166.4	#DIV/0!	100.0	110.9
SD of Relative Cell Growth		6.7	15.9	4.0	7.8	10.6	13.4	14.9	12.8	26.6	#DIV/0!	6.7	7.6

Transformation Assay

Giemsa Staining	0.0001	0.00014	0.00041	0.0012	0.0037	0.011	0.033	0.1	0.3	0	Negative Control	Positive Control
No. of wells excluded	1	1	0	1	0	1	0	2	0		1	0
No. of wells counted	95	95	96	95	96	95	96	94	96	96	95	96
No. of wells having foci	11	10	12	12	19	31	34	54	46		11	51
Transformation frequency*	0.115789474	0.105263158	0.125	0.126315789	0.197916667	0.326315789	0.354166667	0.574468085	0.479166667	0	0.115789474	0.53125
SE	0.03282847	0.03148648	0.03375386	0.03408351	0.04066449	0.04810447	0.04881221	0.05099588	0.05098672	0.00000000	0.03282847	0.05093127
						*	*	*	*			

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	TPA
Institution:	Lab 3
Test Number	

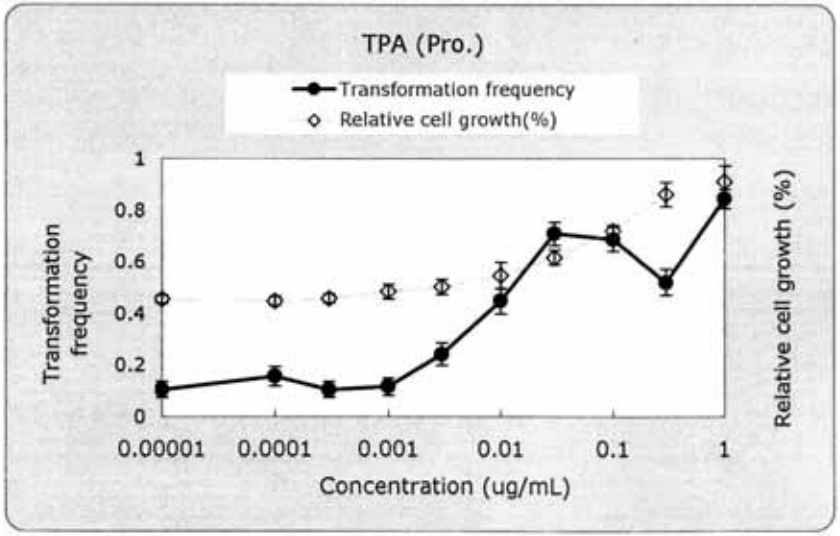
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	0.1% DMSO	TPA (50ng/mL)
1	0.095	0.998	0.962	0.959	0.992	1.100	1.211	1.259	1.445	1.859	1.944	0.998	1.358
2	0.098	0.985	0.925	0.942	1.055	1.096	1.283	1.218	1.442	1.819	1.971	0.985	1.248
3	0.101	0.943	0.960	1.018	1.002	1.031	1.094	1.299	1.509	1.768	1.854	0.943	1.421
4	0.104	0.971	0.913	0.969	1.028	1.055	1.205	1.319	1.428	1.678	1.788	0.971	1.332
5	0.106	0.932	0.993	0.972	1.068	1.068	1.099	1.209	1.472	1.609	1.658	0.932	1.319
6	0.104	0.902	0.945	0.910	0.958	1.071	1.096	1.330	1.439	1.679	1.795	0.902	1.288
7	0.106	0.939	0.943	0.934	1.058	0.956	1.020	1.189	1.481	1.657	1.805	0.939	1.429
8	0.108	0.957	0.895	0.972	0.915	0.966	1.005	1.227	1.364	1.668	1.647	0.957	1.290
Average	0.103	0.953	0.942	0.960	1.010	1.043	1.127	1.256	1.448	1.717	1.808	0.953	1.336
SD	0.004	0.031	0.031	0.032	0.054	0.055	0.098	0.054	0.043	0.088	0.117	0.031	0.064
Average - Blank	0.000	0.851	0.839	0.857	0.907	0.940	1.024	1.154	1.345	1.614	1.705	0.851	1.233
Relative Cell Growth (%)		100.0	98.7	100.7	106.6	110.5	120.4	135.6	158.1	189.8	200.4	100.0	144.9
SD of Relative Cell Growth		3.6	3.6	3.8	6.3	6.5	11.5	6.3	5.1	10.3	13.8	3.6	7.5

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	15	10	11	23	43	68	66	50	81	10	75
Transformation frequency*	0.104166667	0.15625	0.104166667	0.114583333	0.239583333	0.447916667	0.708333333	0.6875	0.520833333	0.84375	0.104166667	0.78125
SE	0.03117758	0.03705794	0.03117758	0.03250865	0.04356307	0.05075342	0.04639024	0.04730704	0.05098672	0.03705794	0.03117758	0.04219232
						*	*	*	*	*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	TPA
Institution:	Lab 4
Test Number	

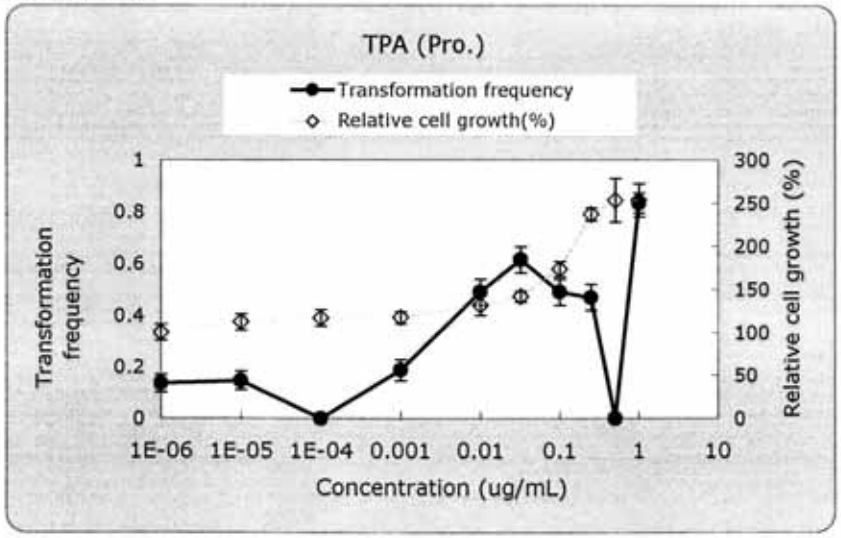
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.000001	0.00001	0.0001	0.001	0.01	0.032	0.1	0.25	0.5	1	0.1% DMSO	TPA (50ng/mL)
1	0.077	0.275	0.344	0.333	0.312	0.362	0.382	0.456	0.603	0.595	0.645	0.275	0.359
2	0.096	0.283	0.300	0.326	0.337	0.328	0.365	0.458	0.598	0.623	0.620	0.283	0.374
3	0.088	0.299	0.309	0.338	0.334	0.367	0.389	0.449	0.573	0.572	0.559	0.299	0.404
4	0.085	0.326	0.342	0.366	0.320	0.353	0.396	0.495	0.607	0.615	0.691	0.326	0.380
5	0.086	0.308	0.319	0.305	0.350	0.398	0.373	0.449	0.587	0.556	0.604	0.308	0.412
6	0.078	0.324	0.347	0.350	0.334	0.340	0.407	0.441	0.564	0.633	0.603	0.324	0.416
7	0.082	0.288	0.326	0.345	0.359	0.374	0.389	0.460	0.586	0.700	0.661	0.288	0.420
8	0.097	0.283	0.302	0.309	0.335	0.398	0.395	0.428	0.602	0.698	0.608	0.283	0.351
Average	0.086	0.298	0.324	0.334	0.335	0.365	0.387	0.454	0.590	0.624	0.624	0.298	0.389
SD	0.007	0.020	0.019	0.021	0.015	0.025	0.013	0.019	0.015	0.053	0.041	0.020	0.027
Average - Blank	0.000	0.212	0.238	0.248	0.249	0.279	0.301	0.368	0.504	0.538	0.538	0.212	0.303
Relative Cell Growth (%)		100.0	112.0	116.9	117.4	131.5	141.8	173.7	237.4	253.5	253.5	100.0	143.0
SD of Relative Cell Growth		9.2	9.0	9.7	7.1	11.8	6.3	9.2	7.3	24.9	19.2	9.2	12.8

Transformation Assay

Giemsa Staining	0.000001	0.00001	0.0001	0.001	0.01	0.032	0.1	0.25	0.5	1	Negative Control	Positive Control
No. of wells excluded	0	0	96	5	0	0	0	0	96	0	0	0
No. of wells counted	96	96	0	91	96	96	96	96	0	96	96	96
No. of wells having foci	13	14		17	47	59	47	45		80	13	54
Transformation frequency*	0.135416667	0.145833333	#DIV/0!	0.186813187	0.489583333	0.614583333	0.489583333	0.46875	#DIV/0!	0.833333333	0.135416667	0.5625
SE	0.03492238	0.03602169	#DIV/0!	0.04085811	0.05101996	0.04967296	0.05101996	0.05093127	#DIV/0!	0.03803629	0.03492238	0.05063079
					*	*	*	*		*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Anthracene
Institution:	Lab 1
Test Number	

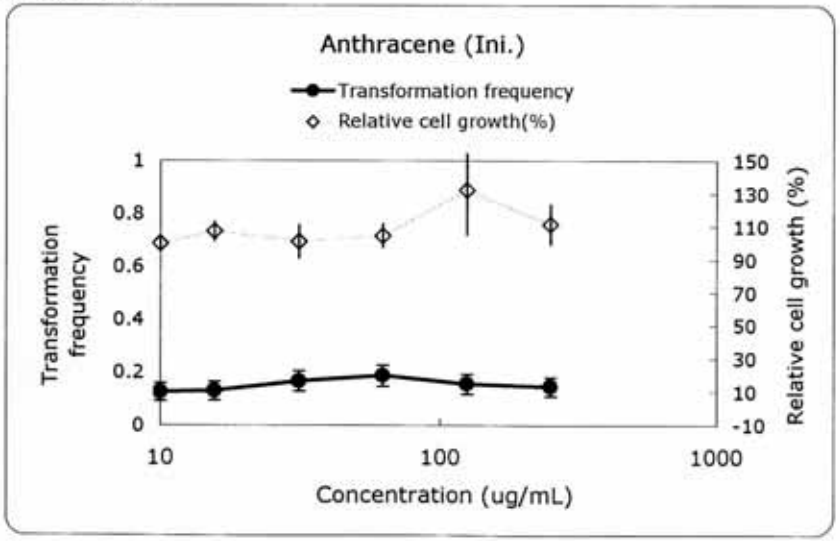
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	15.6	31.3	62.5	125	250					0.1% DMSO	MCA (1ug/mL)
1	0.099	0.686	0.698	0.652	0.660	0.624	0.700					0.631	0.213
2	0.105	0.639	0.688	0.716	0.774	1.065	0.803					0.684	0.285
3	0.096	0.665	0.728	0.669	0.671	0.982	0.720					0.742	0.311
4	0.100	0.710	0.783	0.618	0.681	0.906	0.847					0.653	0.309
5	0.102	0.674	0.704	0.754	0.746	0.910	0.714					0.609	0.346
6	0.098	0.717	0.762	0.768	0.757	0.867	0.788					0.718	0.353
7	0.099	0.690	0.750	0.729	0.710	0.983	0.639					0.687	0.285
8	0.100	0.702	0.711	0.627	0.703	0.656	0.812					0.616	0.173
Average	0.100	0.685	0.728	0.692	0.713	0.874	0.753	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.668	0.284
SD	0.003	0.026	0.034	0.058	0.042	0.157	0.070	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.048	0.062
Average - Blank	0.000	0.586	0.628	0.592	0.613	0.774	0.653	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.568	0.185
Relative Cell Growth (%)		100.0	107.3	101.1	104.7	132.2	111.5	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	100.0	32.5
SD of Relative Cell Growth		4.4	5.8	9.9	7.2	26.8	12.0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	8.3	10.7

Transformation Assay

Giemsa Staining	10	15.6	31.3	62.5	125	250	0	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	3	0	0	0	0					0	0
No. of wells counted	96	93	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	12	16	18	15	14					8	47
Transformation frequency*	0.125	0.129032258	0.166666667	0.1875	0.15625	0.145833333	0	0	0	0	0.083333333	0.489583333
SE	0.03375386	0.03476232	0.03803629	0.03983609	0.03705794	0.03602169	0.00000000	0.00000000	0.00000000	0.00000000	0.02820847	0.05101996

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Anthracene
Institution:	Lab 2
Test Number	

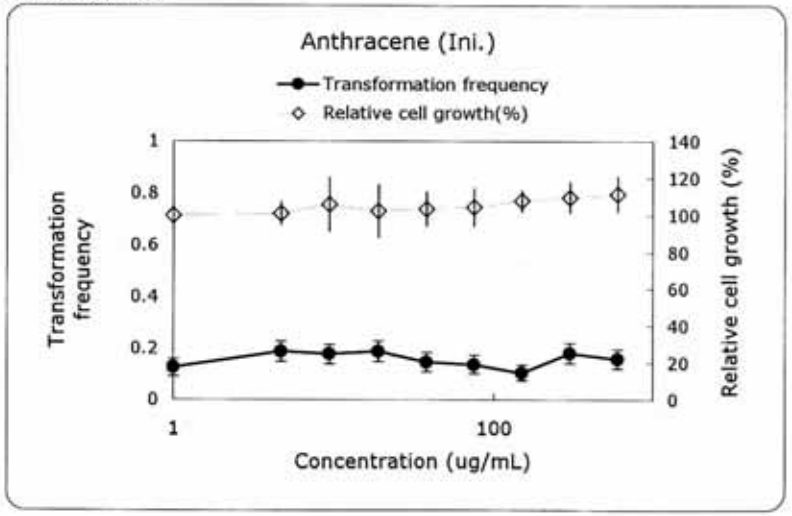
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	4.7	9.4	19	38	75	150	300	600		0.1% DMSO	MCA (1ug/mL)
1	0.028	0.316	0.316	0.346	0.373	0.331	0.294	0.334	0.325	0.343		0.271	0.177
2	0.030	0.304	0.287	0.389	0.355	0.343	0.359	0.333	0.356	0.362		0.294	0.174
3	0.026	0.297	0.307	0.326	0.284	0.302	0.311	0.299	0.299	0.345		0.272	0.180
4	0.025	0.328	0.309	0.341	0.332	0.340	0.311	0.343	0.307	0.309		0.293	0.157
5	0.032	0.291	0.305	0.280	0.288	0.282	0.284	0.309	0.363	0.304		0.302	0.174
6	0.027	0.318	0.317	0.299	0.265	0.278	0.291	0.337	0.320	0.321		0.282	0.184
7	0.040	0.311	0.330	0.304	0.280	0.309	0.351	0.314	0.337	0.318		0.307	0.212
8	0.033	0.262	0.278	0.271	0.301	0.319	0.324	0.329	0.331	0.373		0.239	0.164
Average	0.030	0.303	0.306	0.320	0.310	0.313	0.316	0.325	0.330	0.334	#DIV/0!	0.283	0.178
SD	0.005	0.020	0.017	0.039	0.039	0.025	0.028	0.015	0.022	0.025	#DIV/0!	0.022	0.016
Average - Blank	0.000	0.273	0.276	0.289	0.280	0.283	0.286	0.295	0.300	0.304	#DIV/0!	0.252	0.148
Relative Cell Growth (%)		100.0	101.0	105.9	102.3	103.5	104.5	107.8	109.6	111.3	#DIV/0!	100.0	58.5
SD of Relative Cell Growth		7.5	6.1	14.2	14.3	9.1	10.1	5.7	8.1	9.2	#DIV/0!	8.0	6.0

Transformation Assay

Giemsa Staining	1	4.7	9.4	19	38	75	150	300	600	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	1	0	2	1		0	0
No. of wells counted	96	96	96	96	96	95	96	94	95	96	96	96
No. of wells having foci	12	18	17	18	14	13	10	17	15		10	58
Transformation frequency*	0.125	0.1875	0.17708333	0.1875	0.14583333	0.13684211	0.10416667	0.18085106	0.15789474	0	0.104166667	0.604166667
SE	0.03375386	0.03983609	0.03896110	0.03983609	0.03602169	0.03526088	0.03117758	0.03969886	0.03741151	0.00000000	0.03117758	0.04991131

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass or Failure

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass or Failure

This Data is ...

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Anthracene
Institution:	Lab 3
Test Number	

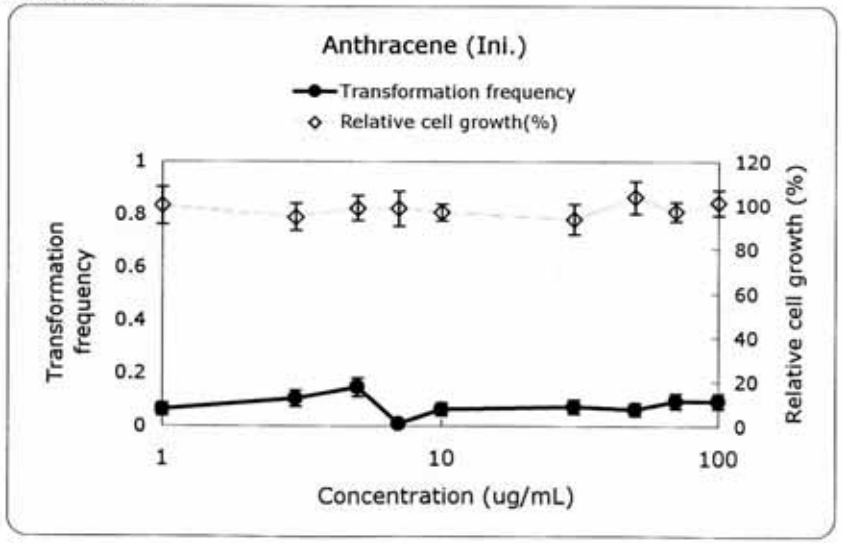
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	3	5	7	10	30	50	70	100		0.1% DMSO	MCA (1ug/mL)
1	0.094	1.324	1.147	1.220	1.270	1.153	1.199	1.290	1.116	1.257		1.144	0.547
2	0.087	1.375	1.168	1.148	1.042	1.258	1.165	1.186	1.227	1.188		1.275	0.562
3	0.086	1.189	1.197	1.262	1.284	1.172	1.230	1.266	1.186	1.249		1.147	0.530
4	0.089	1.089	1.189	1.151	1.191	1.181	1.042	1.421	1.168	1.163		1.253	0.533
5	0.094	1.115	1.119	1.163	1.126	1.195	1.141	1.194	1.230	1.188		1.104	0.564
6	0.100	1.225	1.032	1.316	1.246	1.132	1.202	1.228	1.215	1.372		1.160	0.515
7	0.107	1.258	1.262	1.267	1.222	1.211	1.040	1.354	1.270	1.265		1.039	0.472
8	0.112	1.247	1.226	1.179	1.306	1.236	1.237	1.217	1.157	1.239		1.170	0.477
Average	0.096	1.228	1.168	1.213	1.211	1.192	1.157	1.270	1.196	1.240	#DIV/0!	1.162	0.525
SD	0.009	0.097	0.071	0.063	0.089	0.042	0.078	0.083	0.049	0.065	#DIV/0!	0.076	0.035
Average - Blank	0.000	1.132	1.071	1.117	1.115	1.096	1.061	1.173	1.100	1.144	#DIV/0!	1.065	0.429
Relative Cell Growth (%)		100.0	94.7	98.7	98.5	96.9	93.7	103.7	97.2	101.1	#DIV/0!	100.0	40.3
SD of Relative Cell Growth		8.6	6.2	5.6	7.9	3.7	6.9	7.3	4.3	5.8	#DIV/0!	6.7	3.1

Transformation Assay

Giemsa Staining	1	3	5	7	10	30	50	70	100	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	10	14	1	6	7	6	9	9		1	44
Transformation frequency*	0.0625	0.104166667	0.145833333	0.010416667	0.0625	0.072916667	0.0625	0.09375	0.09375	0	0.010416667	0.458333333
SE	0.02470529	0.03117758	0.03602169	0.01036227	0.02470529	0.02653610	0.02470529	0.02974911	0.02974911	0.00000000	0.01036227	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Anthracene
Institution:	Lab 4
Test Number	

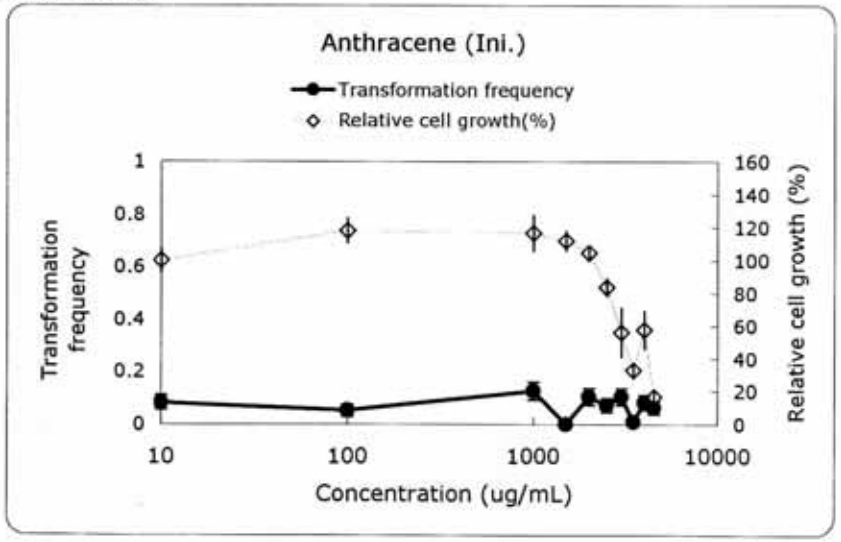
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	100	1000	1500	2000	2500	3000	3500	4000	4500	0.1% DMSO	MCA (1ug/mL)
1	0.062	0.335	0.357	0.349	0.383	0.364	0.310	0.285	0.181	0.242	0.106	0.307	0.177
2	0.065	0.337	0.423	0.381	0.388	0.355	0.292	0.199	0.155	0.225	0.125	0.329	0.192
3	0.065	0.336	0.423	0.384	0.386	0.362	0.302	0.205	0.153	0.206	0.106	0.335	0.169
4	0.063	0.373	0.400	0.390	0.383	0.371	0.279	0.183	0.145	0.225	0.107	0.338	0.183
5	0.066	0.382	0.416	0.451	0.357	0.343	0.296	0.201	0.168	0.193	0.104	0.331	0.176
6	0.071	0.327	0.399	0.390	0.368	0.360	0.306	0.199	0.150	0.221	0.117	0.346	0.170
7	0.063	0.341	0.407	0.423	0.412	0.374	0.318	0.229	0.161	0.219	0.111	0.358	0.187
8	0.061	0.369	0.393	0.410	0.397	0.380	0.327	0.297	0.154	0.303	0.124	0.347	0.180
Average	0.065	0.350	0.402	0.397	0.384	0.364	0.304	0.225	0.158	0.229	0.113	0.336	0.179
SD	0.003	0.021	0.021	0.031	0.017	0.012	0.015	0.043	0.011	0.033	0.008	0.015	0.008
Average - Blank	0.000	0.286	0.338	0.333	0.320	0.299	0.239	0.160	0.094	0.165	0.048	0.272	0.115
Relative Cell Growth (%)		100.0	118.3	116.5	112.0	104.8	83.8	56.1	32.9	57.7	16.8	100.0	42.2
SD of Relative Cell Growth		7.4	7.5	10.7	5.9	4.1	5.3	15.0	4.0	11.6	3.0	5.3	2.8

Transformation Assay

Giemsa Staining	10	100	1000	1500	2000	2500	3000	3500	4000	4500	Negative Control	Positive Control
No. of wells excluded	0	0	0	96	0	0	0	0	0	0	3	0
No. of wells counted	96	96	96	0	96	96	96	96	96	96	93	96
No. of wells having foci	8	5	12		10	7	10	1	8	6	5	52
Transformation frequency*	0.083333333	0.052083333	0.125	#DIV/0!	0.104166667	0.072916667	0.104166667	0.010416667	0.083333333	0.0625	0.053763441	0.541666667
SE	0.02820847	0.02267769	0.03375386	#DIV/0!	0.03117758	0.02653610	0.03117758	0.01036227	0.02820847	0.02470529	0.02338848	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Anthracene
Institution:	Lab 1
Test Number	

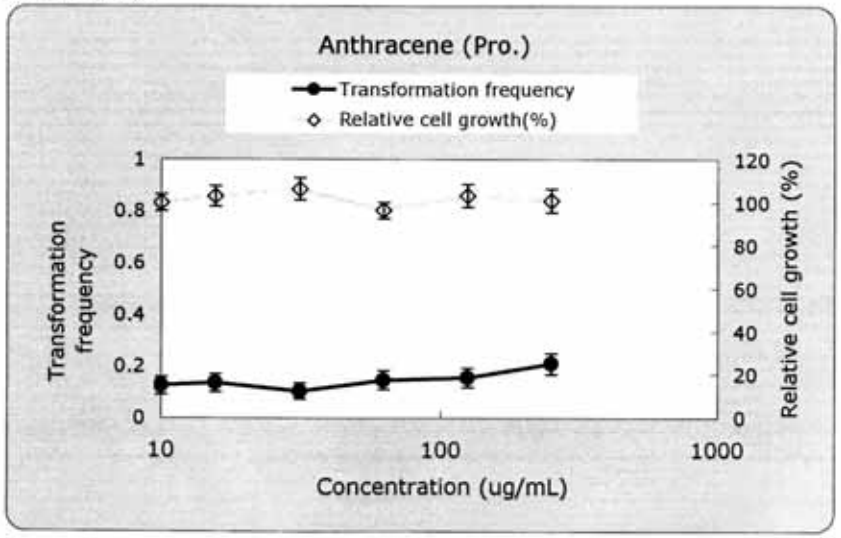
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	15.6	31.3	62.5	125	250					0.1% DMSO	TPA (50ng/mL)
1	0.098	0.507	0.495	0.499	0.487	0.498	0.492					0.478	0.813
2	0.096	0.495	0.530	0.540	0.499	0.493	0.488					0.579	0.857
3	0.107	0.464	0.519	0.476	0.465	0.489	0.537					0.590	0.839
4	0.103	0.504	0.511	0.534	0.475	0.526	0.491					0.587	0.866
5	0.098	0.503	0.481	0.518	0.473	0.475	0.501					0.553	0.821
6	0.103	0.493	0.516	0.519	0.494	0.518	0.498					0.601	0.862
7	0.105	0.494	0.488	0.518	0.467	0.498	0.457					0.575	0.801
8	0.104	0.475	0.483	0.522	0.458	0.535	0.500					0.579	0.846
Average	0.102	0.492	0.503	0.516	0.477	0.504	0.496	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.568	0.838
SD	0.004	0.015	0.018	0.020	0.015	0.020	0.022	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.039	0.024
Average - Blank	0.000	0.390	0.401	0.414	0.376	0.402	0.394	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0.466	0.736
Relative Cell Growth (%)		100.0	102.8	106.1	96.3	103.1	100.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	100.0	158.0
SD of Relative Cell Growth		3.8	4.7	5.2	3.7	5.2	5.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	10.0	6.2

Transformation Assay

Giemsa Staining	10	15.6	31.3	62.5	125	250	0	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0					0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	13	10	14	15	20					10	66
Transformation frequency*	0.125	0.135416667	0.104166667	0.145833333	0.15625	0.208333333	0	0	0	0	0.104166667	0.6875
SE	0.03375386	0.03492238	0.03117758	0.03602169	0.03705794	0.04144908	0.00000000	0.00000000	0.00000000	0.00000000	0.03117758	0.04730704

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Anthracene
Institution:	Lab 2
Test Number	

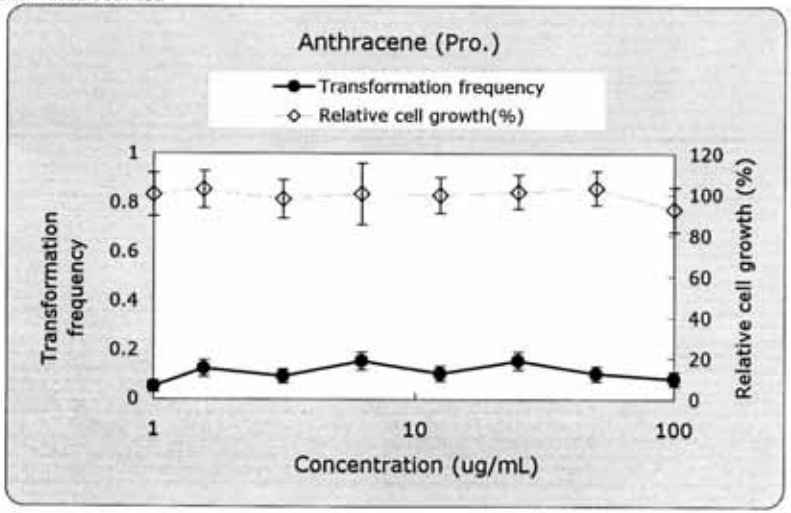
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	1.56	3.13	6.25	12.5	25	50	100			0.1% DMSO	TPA (50ng/mL)
1	0.054	0.316	0.361	0.327	0.301	0.306	0.324	0.328	0.301			0.414	0.442
2	0.050	0.337	0.361	0.317	0.371	0.344	0.382	0.382	0.353			0.389	0.491
3	0.047	0.350	0.361	0.383	0.436	0.375	0.357	0.387	0.301			0.442	0.531
4	0.047	0.404	0.390	0.340	0.325	0.363	0.372	0.361	0.334			0.359	0.527
5	0.045	0.368	0.351	0.325	0.345	0.347	0.356	0.369	0.393			0.420	0.550
6	0.048	0.389	0.383	0.390	0.362	0.338	0.395	0.397	0.348			0.470	0.574
7	0.051	0.347	0.379	0.361	0.388	0.346	0.344	0.341	0.325			0.455	0.567
8	0.056	0.314	0.302	0.329	0.303	0.395	0.329	0.338	0.296			0.388	0.472
Average	0.050	0.353	0.361	0.347	0.354	0.352	0.357	0.363	0.331	#DIV/0!	#DIV/0!	0.417	0.519
SD	0.004	0.032	0.027	0.028	0.046	0.027	0.025	0.025	0.033	#DIV/0!	#DIV/0!	0.038	0.047
Average - Blank	0.000	0.303	0.311	0.297	0.304	0.302	0.308	0.313	0.282	#DIV/0!	#DIV/0!	0.367	0.470
Relative Cell Growth (%)		100.0	102.6	97.8	100.2	99.5	101.4	103.2	92.8	#DIV/0!	#DIV/0!	100.0	127.8
SD of Relative Cell Growth		10.7	9.0	9.2	15.0	8.8	8.2	8.3	10.9	#DIV/0!	#DIV/0!	12.4	15.5

Transformation Assay

Giemsa Staining	1	1.56	3.13	6.25	12.5	25	50	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	12	9	15	10	15	10	8			10	41
Transformation frequency*	0.05208333	0.125	0.09375	0.15625	0.10416667	0.15625	0.10416667	0.08333333	0	0	0.104166667	0.427083333
SE	0.02267769	0.03375386	0.02974911	0.03705794	0.03117758	0.03705794	0.03117758	0.02820847	0.00000000	0.00000000	0.03117758	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Anthracene
Institution:	Lab 3
Test Number	

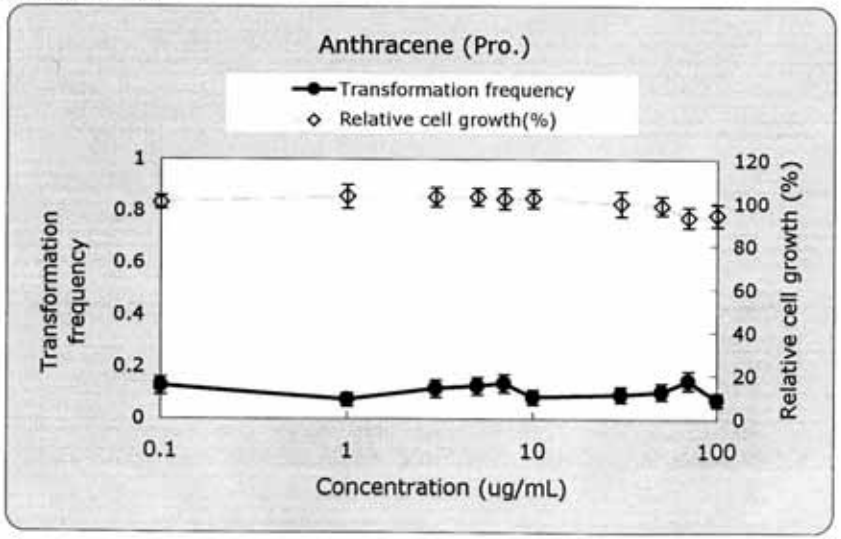
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	3	5	7	10	30	50	70	100	0.1% DMSO	TPA (50ng/mL)
1	0.098	1.096	1.045	1.080	1.073	1.063	1.005	1.005	1.010	0.973	0.989	1.193	1.367
2	0.103	1.102	1.040	1.110	1.160	1.062	1.085	1.074	1.037	0.962	0.957	1.163	1.379
3	0.109	1.040	1.115	1.018	1.082	1.052	1.057	1.118	1.024	0.991	0.987	1.192	1.387
4	0.111	1.042	1.029	1.142	1.054	1.042	1.071	1.092	1.119	1.068	1.095	1.241	1.540
5	0.112	1.051	1.076	1.115	1.099	1.048	1.147	1.104	1.091	0.929	0.952	1.231	1.525
6	0.117	1.017	1.180	1.096	1.100	1.049	1.062	1.080	1.007	1.043	0.975	1.275	1.407
7	0.122	1.080	1.124	1.037	1.086	1.155	1.086	1.002	1.056	0.997	1.038	1.156	1.419
8	0.125	1.057	1.084	1.081	1.025	1.152	1.112	0.970	1.032	0.997	1.048	1.220	1.436
Average	0.112	1.061	1.087	1.085	1.085	1.078	1.078	1.056	1.047	0.995	1.005	1.209	1.433
SD	0.009	0.030	0.051	0.041	0.039	0.047	0.042	0.055	0.040	0.044	0.050	0.040	0.066
Average - Blank	0.000	0.949	0.975	0.973	0.973	0.966	0.966	0.944	0.935	0.883	0.893	1.097	1.320
Relative Cell Growth (%)		100.0	102.7	102.6	102.6	101.8	101.8	99.5	98.6	93.1	94.1	100.0	120.4
SD of Relative Cell Growth		3.1	5.4	4.3	4.1	5.0	4.4	5.8	4.2	4.6	5.3	4.3	6.9

Transformation Assay

Giemsa Staining	0.1	1	3	5	7	10	30	50	70	100	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	7	11	12	13	8	9	10	14	7	11	45
Transformation frequency*	0.125	0.072916667	0.114583333	0.125	0.135416667	0.083333333	0.09375	0.104166667	0.145833333	0.072916667	0.114583333	0.46875
SE	0.03375386	0.02653610	0.03250865	0.03375386	0.03492238	0.02820847	0.02974911	0.03117758	0.03602169	0.02653610	0.03250865	0.05093127

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Anthracene
Institution:	Lab 4
Test Number	

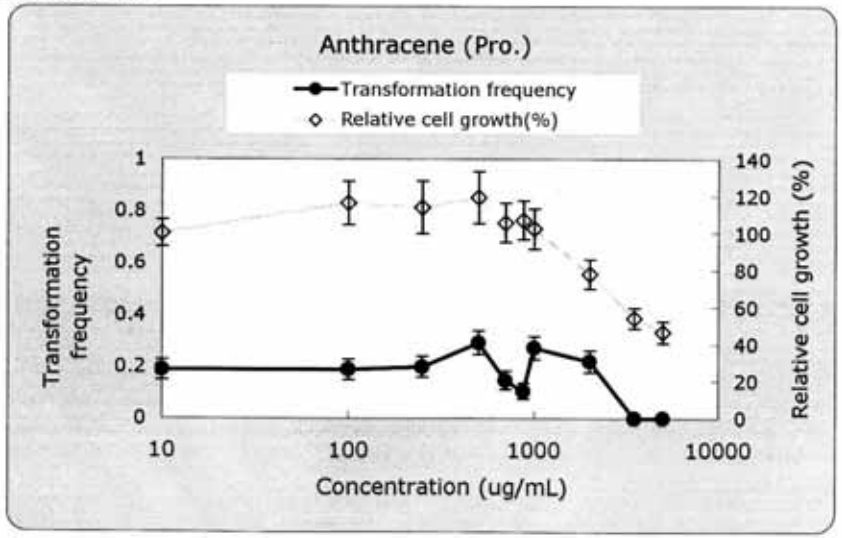
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	100	250	500	700	875	1000	2000	3500	5000	0.1% DMSO	TPA (50ng/mL)
1	0.077	0.258	0.285	0.272	0.285	0.259	0.275	0.245	0.216	0.174	0.175	0.374	0.399
2	0.096	0.281	0.321	0.330	0.309	0.304	0.303	0.309	0.228	0.190	0.163	0.398	0.403
3	0.088	0.297	0.298	0.314	0.321	0.282	0.338	0.306	0.256	0.188	0.173	0.355	0.462
4	0.085	0.286	0.321	0.358	0.371	0.313	0.274	0.308	0.229	0.188	0.168	0.354	0.422
5	0.086	0.299	0.331	0.322	0.347	0.306	0.298	0.282	0.253	0.208	0.191	0.366	0.422
6	0.078	0.280	0.322	0.326	0.329	0.302	0.288	0.280	0.257	0.203	0.186	0.368	0.418
7	0.082	0.302	0.360	0.302	0.332	0.324	0.313	0.301	0.251	0.202	0.179	0.362	0.435
8	0.097	0.277	0.301	0.277	0.294	0.281	0.302	0.286	0.239	0.197	0.196	0.344	0.423
Average	0.086	0.285	0.317	0.313	0.323	0.296	0.299	0.290	0.241	0.194	0.179	0.365	0.423
SD	0.007	0.015	0.023	0.028	0.028	0.021	0.021	0.022	0.016	0.011	0.011	0.016	0.020
Average - Blank	0.000	0.199	0.231	0.227	0.237	0.210	0.213	0.203	0.155	0.108	0.093	0.279	0.337
Relative Cell Growth (%)		100.0	116.2	113.9	119.3	105.7	107.0	102.3	77.9	54.1	46.6	100.0	120.7
SD of Relative Cell Growth		7.4	11.7	14.3	14.1	10.4	10.5	10.9	7.8	5.5	5.8	8.3	9.8

Transformation Assay

Giemsa Staining	10	100	250	500	700	875	1000	2000	3500	5000	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	96	96	0	0
No. of wells counted	96	96	96	96	96	96	96	96	0	0	96	96
No. of wells having foci	18	18	19	28	14	10	26	21			12	56
Transformation frequency*	0.1875	0.1875	0.197916667	0.291666667	0.145833333	0.104166667	0.270833333	0.21875	#DIV/0!	#DIV/0!	0.125	0.583333333
SE	0.03983609	0.03983609	0.04066449	0.04639024	0.03602169	0.03117758	0.04535538	0.04219232	#DIV/0!	#DIV/0!	0.03375386	0.05031728

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phenanthrene
Institution:	Lab 1
Test Number	

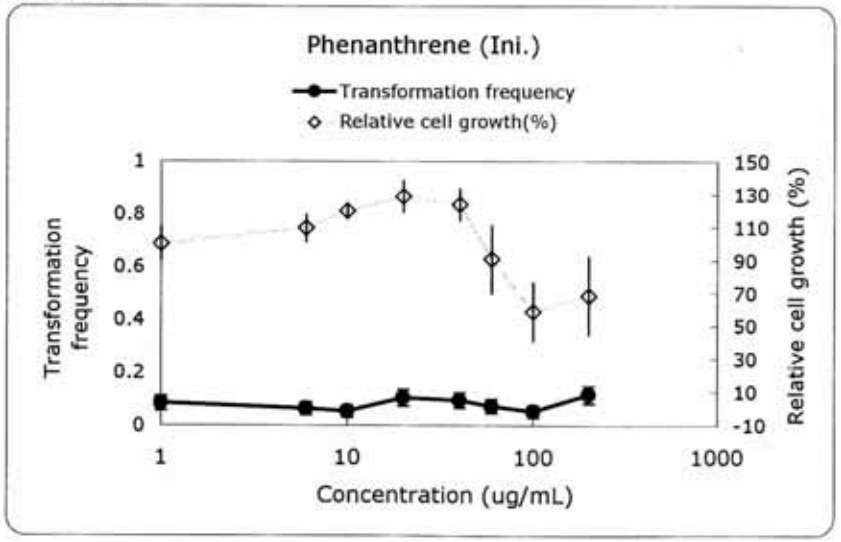
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	6	10	20	40	60	100	200			0.1% DMSO	MCA (1ug/mL)
1	0.093	0.434	0.464	0.563	0.547	0.610	0.561	0.450	0.529			0.434	0.268
2	0.100	0.449	0.541	0.542	0.586	0.583	0.421	0.363	0.372			0.449	0.237
3	0.096	0.464	0.488	0.551	0.615	0.559	0.331	0.285	0.335			0.464	0.255
4	0.100	0.448	0.488	0.541	0.643	0.545	0.346	0.293	0.270			0.448	0.246
5	0.101	0.476	0.494	0.548	0.566	0.509	0.429	0.261	0.272			0.476	0.293
6	0.103	0.517	0.528	0.574	0.578	0.552	0.477	0.248	0.297			0.517	0.228
7	0.104	0.464	0.524	0.533	0.530	0.540	0.445	0.309	0.343			0.464	0.243
8	0.115	0.545	0.553	0.543	0.595	0.613	0.505	0.352	0.435			0.545	0.233
Average	0.102	0.475	0.510	0.549	0.583	0.564	0.439	0.320	0.357	#DIV/0!	#DIV/0!	0.475	0.250
SD	0.007	0.038	0.031	0.013	0.036	0.036	0.077	0.066	0.089	#DIV/0!	#DIV/0!	0.038	0.021
Average - Blank	0.000	0.373	0.409	0.448	0.481	0.462	0.338	0.219	0.255	#DIV/0!	#DIV/0!	0.373	0.149
Relative Cell Growth (%)		100.0	109.5	120.0	128.9	123.9	90.6	58.6	68.4	#DIV/0!	#DIV/0!	100.0	39.9
SD of Relative Cell Growth		10.1	8.3	3.6	9.7	9.6	20.6	17.7	23.7	#DIV/0!	#DIV/0!	10.1	5.7

Transformation Assay

Giemsa Staining	1	6	10	20	40	60	100	200	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	6	5	10	9	7	5	11			8	42
Transformation frequency*	0.083333333	0.0625	0.052083333	0.104166667	0.09375	0.072916667	0.052083333	0.114583333	0	0	0.083333333	0.4375
SE	0.02820847	0.02470529	0.02267769	0.03117758	0.02974911	0.02653610	0.02267769	0.03250865	0.00000000	0.00000000	0.02820847	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phenanthrene
Institution:	Lab 2
Test Number	

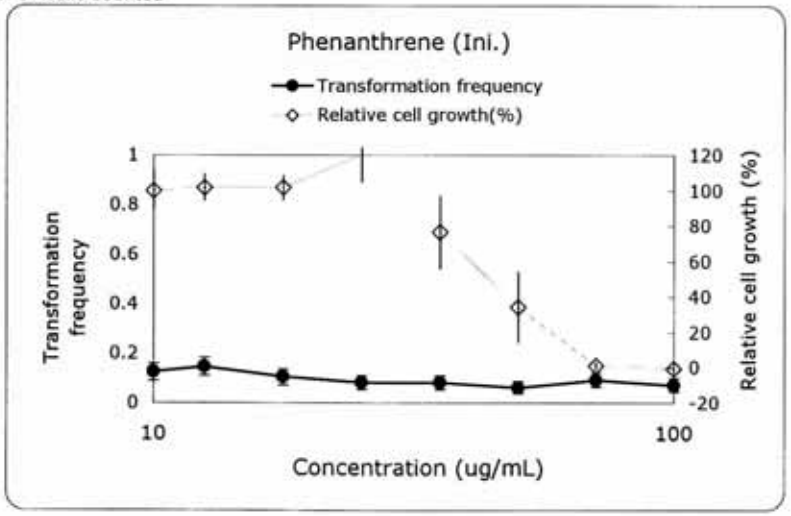
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	12.5	17.7	25	35.4	50	70.7	100			0.1% DMSO	MCA (1ug/mL)
1	0.074	0.412	0.443	0.442	0.453	0.493	0.377	0.084	0.078			0.475	0.313
2	0.070	0.441	0.467	0.510	0.619	0.470	0.214	0.074	0.072			0.478	0.313
3	0.075	0.462	0.509	0.476	0.519	0.286	0.170	0.067	0.072			0.551	0.279
4	0.079	0.523	0.470	0.518	0.618	0.400	0.181	0.077	0.063			0.482	0.312
5	0.072	0.530	0.508	0.464	0.596	0.268	0.195	0.073	0.059			0.473	0.325
6	0.075	0.527	0.537	0.486	0.619	0.446	0.143	0.083	0.067			0.483	0.298
7	0.070	0.493	0.494	0.523	0.577	0.332	0.142	0.074	0.075			0.526	0.336
8	0.074	0.455	0.471	0.478	0.506	0.384	0.285	0.095	0.079			0.459	0.257
Average	0.074	0.480	0.487	0.487	0.563	0.385	0.213	0.078	0.071	#DIV/0!	#DIV/0!	0.491	0.304
SD	0.003	0.044	0.030	0.028	0.063	0.084	0.080	0.009	0.007	#DIV/0!	#DIV/0!	0.031	0.026
Average - Blank	0.000	0.407	0.414	0.414	0.490	0.311	0.140	0.005	-0.003	#DIV/0!	#DIV/0!	0.417	0.231
Relative Cell Growth (%)		100.0	101.7	101.7	120.4	76.5	34.4	1.2	-0.7	#DIV/0!	#DIV/0!	100.0	55.2
SD of Relative Cell Growth		10.9	7.4	6.9	15.5	20.6	19.8	2.1	1.7	#DIV/0!	#DIV/0!	7.6	6.3

Transformation Assay

Giemsa Staining	10	12.5	17.7	25	35.4	50	70.7	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	14	10	8	8	6	9	7			11	69
Transformation frequency*	0.125	0.14583333	0.10416667	0.08333333	0.08333333	0.0625	0.09375	0.07291667	0	0	0.114583333	0.71875
SE	0.03375386	0.03602169	0.03117758	0.02820847	0.02820847	0.02470529	0.02974911	0.02653610	0.00000000	0.00000000	0.03250865	0.04588805

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<15 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phenanthrene
Institution:	Lab 3
Test Number	

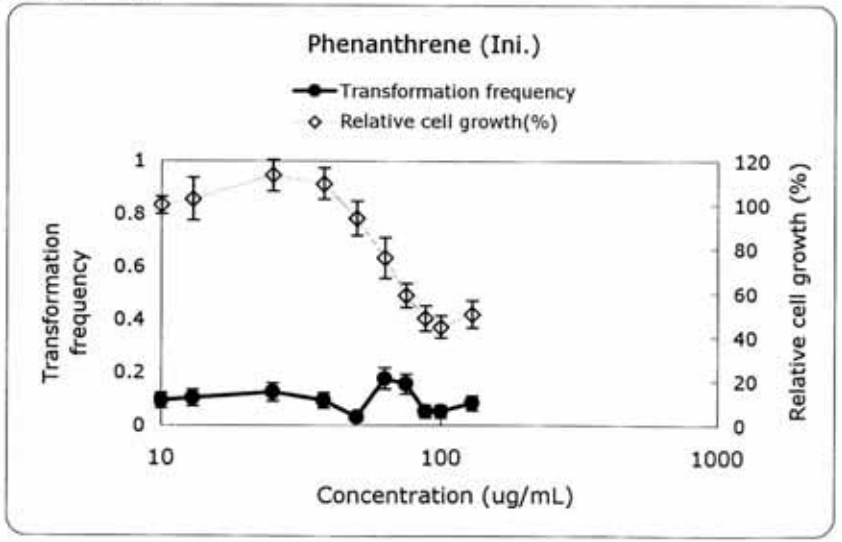
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	13	25	38	50	63	75	88	100	130	0.1% DMSO	MCA (1ug/mL)
1	0.102	1.079	1.068	1.101	1.102	0.981	0.974	0.646	0.644	0.543	0.555	1.079	0.408
2	0.109	1.005	1.018	1.159	1.183	0.961	0.778	0.618	0.488	0.505	0.608	1.005	0.412
3	0.113	0.970	1.221	1.206	1.199	1.052	0.779	0.628	0.508	0.467	0.499	0.970	0.452
4	0.116	1.010	1.012	1.151	1.096	0.946	0.766	0.650	0.544	0.495	0.509	1.010	0.398
5	0.118	1.028	1.047	1.224	1.029	0.833	0.689	0.600	0.591	0.533	0.597	1.028	0.413
6	0.124	1.034	0.993	1.135	1.098	0.932	0.766	0.715	0.521	0.463	0.576	1.034	0.424
7	0.127	0.972	1.057	1.021	1.020	0.934	0.789	0.592	0.537	0.531	0.542	0.972	0.422
8	0.119	1.029	0.912	1.111	1.099	1.052	0.859	0.722	0.595	0.602	0.668	1.029	0.447
Average	0.116	1.016	1.041	1.139	1.103	0.961	0.800	0.646	0.554	0.517	0.569	1.016	0.422
SD	0.008	0.035	0.088	0.064	0.063	0.071	0.084	0.049	0.052	0.045	0.056	0.035	0.019
Average - Blank	0.000	0.900	0.925	1.023	0.987	0.845	0.684	0.530	0.438	0.401	0.453	0.900	0.306
Relative Cell Growth (%)		100.0	102.8	113.6	109.7	93.9	76.0	58.9	48.6	44.6	50.4	100.0	34.0
SD of Relative Cell Growth		3.9	9.7	7.1	7.0	7.9	9.3	5.4	5.8	5.0	6.2	3.9	2.1

Transformation Assay

Giemsa Staining	10	13	25	38	50	63	75	88	100	130	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	10	12	9	3	17	15	5	5	8	9	42
Transformation frequency*	0.09375	0.104166667	0.125	0.09375	0.03125	0.177083333	0.15625	0.052083333	0.052083333	0.083333333	0.09375	0.4375
SE	0.02974911	0.03117758	0.03375386	0.02974911	0.01775805	0.03896110	0.03705794	0.02267769	0.02267769	0.02820847	0.02974911	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phenanthrene
Institution:	Lab 4
Test Number	

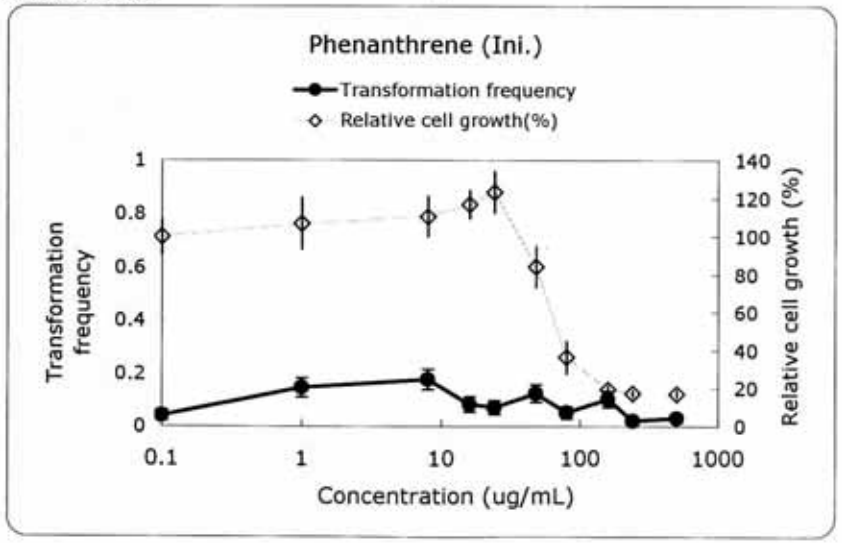
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	8	16	24	48	80	160	240	500	0.1% DMSO	MCA (1ug/mL)
1	0.072	0.292	0.286	0.323	0.338	0.364	0.288	0.199	0.124	0.115	0.116	0.309	0.156
2	0.071	0.295	0.318	0.350	0.342	0.398	0.290	0.148	0.122	0.111	0.115	0.293	0.143
3	0.074	0.320	0.364	0.333	0.361	0.367	0.296	0.145	0.115	0.120	0.115	0.334	0.132
4	0.075	0.296	0.307	0.365	0.356	0.344	0.276	0.151	0.113	0.114	0.116	0.316	0.142
5	0.073	0.328	0.332	0.349	0.328	0.385	0.251	0.141	0.123	0.117	0.113	0.321	0.156
6	0.074	0.329	0.371	0.313	0.367	0.329	0.224	0.149	0.122	0.117	0.117	0.286	0.143
7	0.075	0.319	0.302	0.325	0.352	0.369	0.255	0.157	0.116	0.112	0.114	0.291	0.139
8	0.076	0.269	0.295	0.286	0.318	0.326	0.277	0.179	0.117	0.107	0.104	0.263	0.140
Average	0.074	0.306	0.322	0.331	0.345	0.360	0.269	0.159	0.119	0.114	0.114	0.302	0.144
SD	0.001	0.021	0.031	0.025	0.017	0.025	0.025	0.020	0.004	0.004	0.004	0.023	0.008
Average - Blank	0.000	0.232	0.248	0.257	0.272	0.286	0.196	0.085	0.045	0.040	0.040	0.228	0.070
Relative Cell Growth (%)		100.0	106.8	110.6	116.9	123.3	84.3	36.5	19.5	17.4	17.2	100.0	30.8
SD of Relative Cell Growth		9.2	13.6	10.6	7.3	10.9	10.6	8.6	1.8	1.7	1.9	9.8	3.6

Transformation Assay

Giemsa Staining	0.1	1	8	16	24	48	80	160	240	500	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	2	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	94	96
No. of wells having foci	4	14	17	8	7	12	5	10	2	3	9	41
Transformation frequency*	0.041666667	0.145833333	0.177083333	0.083333333	0.072916667	0.125	0.052083333	0.104166667	0.020833333	0.03125	0.095744681	0.427083333
SE	0.02039469	0.03602169	0.03896110	0.02820847	0.02653610	0.03375386	0.02267769	0.03117758	0.01457713	0.01775805	0.03034862	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phenanthrene
Institution:	Lab 4
Test Number	

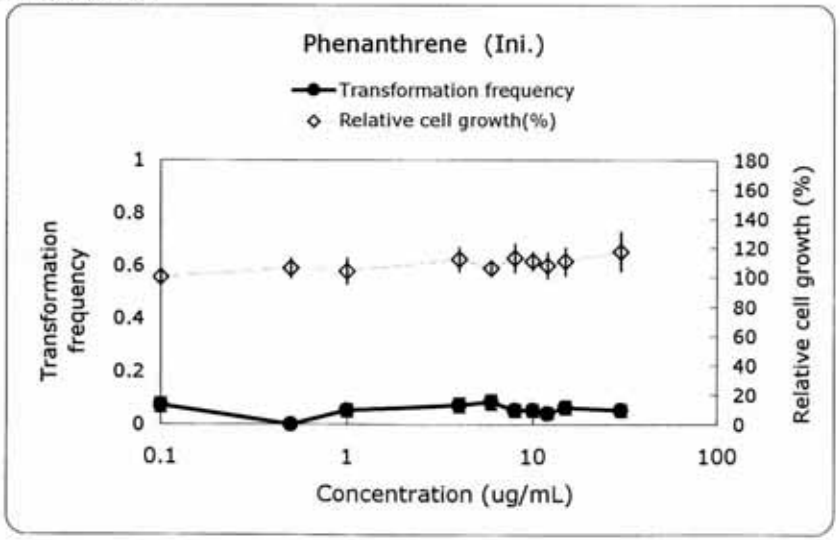
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	4	6	8	10	12	15	30	0.1% DMSO	MCA (1ug/mL)
1	0.062	0.303	0.318	0.298	0.325	0.320	0.306	0.316	0.303	0.336	0.296	0.254	0.174
2	0.065	0.305	0.324	0.291	0.330	0.312	0.353	0.346	0.348	0.337	0.342	0.288	0.164
3	0.065	0.321	0.316	0.331	0.346	0.326	0.348	0.351	0.365	0.337	0.367	0.291	0.178
4	0.063	0.332	0.334	0.335	0.350	0.349	0.345	0.363	0.335	0.319	0.370	0.311	0.172
5	0.066	0.319	0.350	0.328	0.338	0.331	0.337	0.329	0.339	0.334	0.380	0.286	0.174
6	0.071	0.312	0.316	0.341	0.368	0.341	0.378	0.356	0.360	0.362	0.388	0.303	0.185
7	0.063	0.322	0.335	0.356	0.384	0.345	0.384	0.352	0.310	0.392	0.400	0.312	0.170
8	0.061	0.315	0.360	0.330	0.333	0.335	0.344	0.341	0.330	0.330	0.341	0.271	0.160
Average	0.065	0.316	0.332	0.326	0.347	0.332	0.349	0.344	0.336	0.343	0.361	0.290	0.172
SD	0.003	0.010	0.016	0.022	0.020	0.013	0.024	0.015	0.022	0.023	0.033	0.020	0.008
Average - Blank	0.000	0.252	0.267	0.262	0.282	0.268	0.285	0.280	0.272	0.279	0.296	0.225	0.108
Relative Cell Growth (%)		100.0	106.2	104.0	112.2	106.5	113.2	111.2	108.0	110.8	117.6	100.0	47.8
SD of Relative Cell Growth		3.8	6.5	8.6	8.0	5.0	9.6	6.1	8.7	9.1	13.2	7.9	3.1

Transformation Assay

Giemsa Staining	0.1	0.5	1	4	6	8	10	12	15	30	Negative Control	Positive Control
No. of wells excluded	0	96	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	0	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7		5	7	8	5	5	4	6	5	1	45
Transformation frequency*	0.072916667	#DIV/0!	0.052083333	0.072916667	0.083333333	0.052083333	0.052083333	0.041666667	0.0625	0.052083333	0.010416667	0.46875
SE	0.02653610	#DIV/0!	0.02267769	0.02653610	0.02820847	0.02267769	0.02267769	0.02039469	0.02470529	0.02267769	0.01036227	0.05093127

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phenanthrene
Institution:	Lab 1
Test Number	

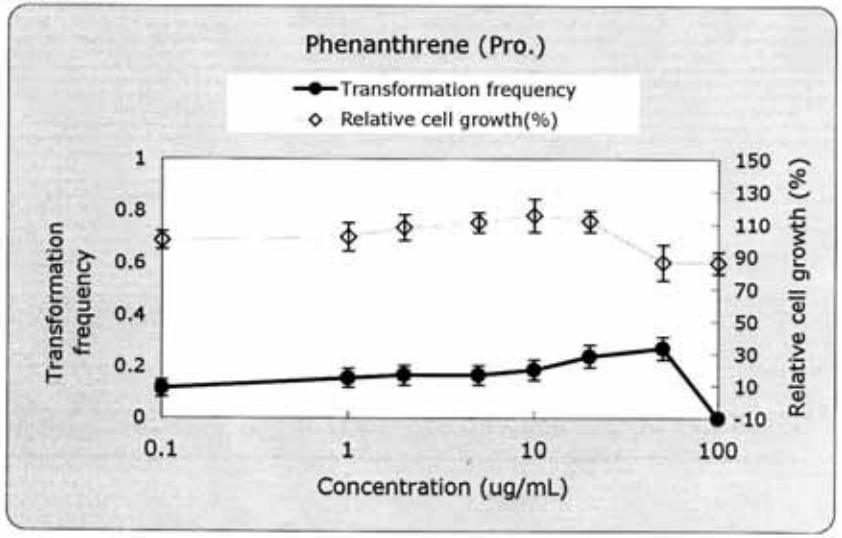
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	2	5	10	20	50	100			0.1% DMSO	TPA (50ng/mL)
1	0.099	0.477	0.491	0.474	0.510	0.504	0.549	0.486	0.484			0.477	0.839
2	0.108	0.474	0.477	0.488	0.515	0.547	0.540	0.449	0.441			0.474	0.809
3	0.111	0.498	0.438	0.511	0.510	0.529	0.522	0.386	0.416			0.498	0.817
4	0.104	0.481	0.510	0.527	0.546	0.526	0.499	0.407	0.444			0.481	0.850
5	0.106	0.509	0.492	0.531	0.537	0.519	0.561	0.397	0.416			0.509	0.798
6	0.111	0.501	0.524	0.525	0.573	0.594	0.555	0.467	0.423			0.501	0.822
7	0.109	0.468	0.515	0.564	0.526	0.614	0.504	0.431	0.420			0.468	0.812
8	0.112	0.530	0.549	0.554	0.558	0.577	0.565	0.497	0.471			0.530	0.803
Average	0.108	0.492	0.500	0.522	0.534	0.551	0.537	0.440	0.439	#DIV/0!	#DIV/0!	0.492	0.819
SD	0.004	0.021	0.033	0.030	0.023	0.039	0.026	0.042	0.026	#DIV/0!	#DIV/0!	0.021	0.018
Average - Blank	0.000	0.385	0.392	0.414	0.427	0.444	0.429	0.333	0.332	#DIV/0!	#DIV/0!	0.385	0.711
Relative Cell Growth (%)		100.0	101.9	107.7	110.9	115.3	111.6	86.4	86.3	#DIV/0!	#DIV/0!	100.0	184.9
SD of Relative Cell Growth		5.5	8.7	7.9	6.1	10.2	6.7	10.8	6.8	#DIV/0!	#DIV/0!	5.5	4.6

Transformation Assay

Giemsa Staining	0.1	1	2	5	10	20	50	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	53			0	0
No. of wells counted	96	96	96	96	96	96	96	43	96	96	96	96
No. of wells having foci	11	15	16	16	18	23	26	TOX			11	53
Transformation frequency*	0.114583333	0.15625	0.166666667	0.166666667	0.1875	0.239583333	0.270833333	#VALUE!	0	0	0.114583333	0.552083333
SE	0.03250865	0.03705794	0.03803629	0.03803629	0.03983609	0.04356307	0.04535538	#VALUE!	0.00000000	0.00000000	0.03250865	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phenanthrene
Institution:	Lab 1
Test Number	

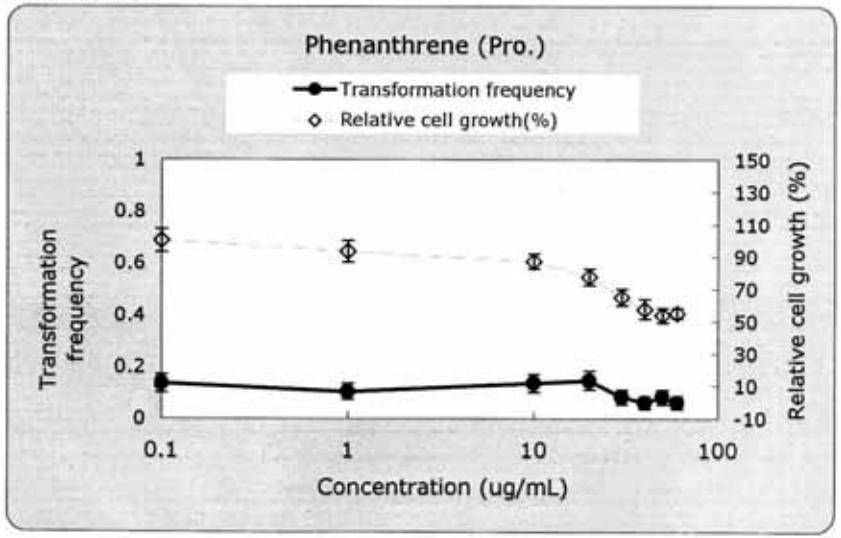
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	10	20	30	40	50	60			0.1% DMSO	TPA (50ng/mL)
1	0.117	0.779	0.794	0.729	0.666	0.647	0.536	0.564	0.538			0.779	1.166
2	0.118	0.801	0.855	0.805	0.718	0.565	0.539	0.464	0.484			0.801	1.047
3	0.108	0.824	0.806	0.713	0.622	0.546	0.522	0.503	0.479			0.824	1.099
4	0.108	0.851	0.803	0.699	0.733	0.554	0.478	0.512	0.514			0.851	1.046
5	0.125	0.868	0.778	0.758	0.672	0.568	0.514	0.463	0.497			0.868	1.039
6	0.128	0.939	0.687	0.753	0.651	0.609	0.581	0.521	0.540			0.939	1.026
7	0.104	0.810	0.812	0.743	0.653	0.553	0.467	0.489	0.515			0.810	1.084
8	0.111	0.807	0.756	0.730	0.661	0.614	0.593	0.502	0.518			0.807	1.112
Average	0.115	0.835	0.786	0.741	0.672	0.582	0.529	0.502	0.511	#DIV/0!	#DIV/0!	0.835	1.077
SD	0.009	0.051	0.049	0.032	0.036	0.037	0.044	0.033	0.023	#DIV/0!	#DIV/0!	0.051	0.047
Average - Blank	0.000	0.720	0.672	0.626	0.557	0.467	0.414	0.387	0.396	#DIV/0!	#DIV/0!	0.720	0.963
Relative Cell Growth (%)		100.0	93.3	87.0	77.4	64.9	57.5	53.8	55.0	#DIV/0!	#DIV/0!	100.0	133.7
SD of Relative Cell Growth		7.0	6.8	4.5	5.1	5.1	6.1	4.5	3.1	#DIV/0!	#DIV/0!	7.0	6.6

Transformation Assay

Giemsa Staining	0.1	1	10	20	30	40	50	60	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	10	13	14	8	6	8	6			13	63
Transformation frequency*	0.135416667	0.104166667	0.135416667	0.145833333	0.083333333	0.0625	0.083333333	0.0625	0	0	0.135416667	0.65625
SE	0.03492238	0.03117758	0.03492238	0.03602169	0.02820847	0.02470529	0.02820847	0.02470529	0.00000000	0.00000000	0.03492238	0.04847529

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phenanthrene
Institution:	Lab 2
Test Number	

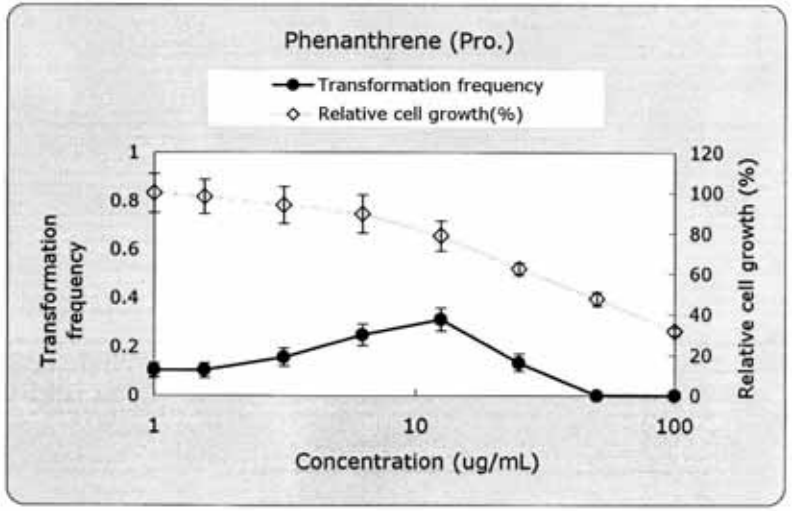
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	1.56	3.13	6.25	12.5	25	50	100			0.1% DMSO	TPA (50ng/mL)
1	0.068	0.359	0.345	0.357	0.320	0.271	0.281	0.220	0.173			0.472	0.452
2	0.065	0.392	0.372	0.350	0.360	0.331	0.274	0.208	0.183			0.457	0.520
3	0.063	0.441	0.434	0.420	0.357	0.321	0.277	0.216	0.167			0.441	0.538
4	0.067	0.404	0.403	0.372	0.372	0.333	0.269	0.228	0.176			0.450	0.519
5	0.062	0.407	0.404	0.365	0.411	0.352	0.281	0.218	0.163			0.423	0.500
6	0.064	0.427	0.411	0.404	0.391	0.341	0.261	0.222	0.173			0.441	0.512
7	0.059	0.410	0.403	0.416	0.378	0.342	0.256	0.228	0.163			0.455	0.439
8	0.065	0.347	0.368	0.345	0.321	0.330	0.284	0.244	0.166			0.399	0.416
Average	0.064	0.398	0.393	0.379	0.364	0.328	0.273	0.223	0.171	#DIV/0!	#DIV/0!	0.442	0.487
SD	0.003	0.032	0.029	0.030	0.032	0.025	0.010	0.011	0.007	#DIV/0!	#DIV/0!	0.023	0.045
Average - Blank	0.000	0.334	0.328	0.315	0.300	0.264	0.209	0.159	0.106	#DIV/0!	#DIV/0!	0.378	0.423
Relative Cell Growth (%)		100.0	98.2	94.1	89.6	78.8	62.5	47.5	31.8	#DIV/0!	#DIV/0!	100.0	111.8
SD of Relative Cell Growth		9.5	8.5	9.0	9.5	7.4	3.0	3.2	2.1	#DIV/0!	#DIV/0!	6.8	13.4

Transformation Assay

Giemsa Staining	1	1.56	3.13	6.25	12.5	25	50	100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	1	0	0	0	0	0			0	0
No. of wells counted	96	96	95	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	10	15	24	30	13	0	0			13	69
Transformation frequency*	0.10416667	0.10416667	0.15789474	0.25	0.3125	0.13541667	0	0	0	0	0.135416667	0.71875
SE	0.03117758	0.03117758	0.03741151	0.04419417	0.04730704	0.03492238	0.00000000	0.00000000	0.00000000	0.00000000	0.03492238	0.04588805

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phenanthrene
Institution:	Lab 3
Test Number	

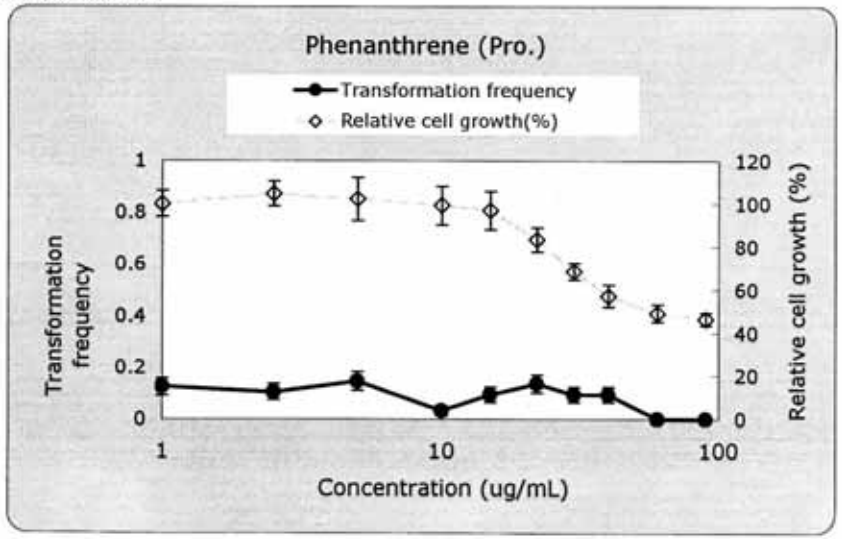
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.5	5	10	15	22	30	40	60	90	0.1% DMSO	TPA (50ng/mL)
1	0.386	0.975	1.029	0.882	0.913	0.921	0.870	0.777	0.729	0.677	0.673	0.975	1.285
2	0.358	1.090	1.050	0.990	1.088	1.005	0.915	0.818	0.727	0.682	0.629	1.090	1.325
3	0.340	1.003	0.975	1.101	1.012	0.999	0.873	0.792	0.689	0.699	0.656	1.003	1.312
4	0.317	0.985	1.025	1.020	1.061	0.895	0.912	0.737	0.683	0.652	0.660	0.985	1.318
5	0.330	0.984	1.014	1.073	0.989	0.996	0.937	0.813	0.715	0.636	0.625	0.984	1.316
6	0.291	0.963	1.087	1.046	0.977	1.006	0.919	0.794	0.698	0.673	0.615	0.963	1.209
7	0.329	0.978	1.074	1.028	0.993	1.078	0.851	0.798	0.789	0.620	0.649	0.978	1.257
8	0.344	1.020	0.994	0.978	0.923	0.936	0.833	0.798	0.696	0.664	0.647	1.020	1.290
Average	0.337	1.000	1.031	1.015	0.995	0.980	0.889	0.791	0.716	0.663	0.644	1.000	1.289
SD	0.028	0.040	0.038	0.067	0.060	0.059	0.037	0.025	0.034	0.026	0.020	0.040	0.039
Average - Blank	0.000	0.663	0.694	0.678	0.658	0.643	0.552	0.454	0.379	0.326	0.307	0.663	0.952
Relative Cell Growth (%)		100.0	104.7	102.3	99.2	96.9	83.3	68.5	57.2	49.2	46.4	100.0	143.6
SD of Relative Cell Growth		6.1	5.8	10.1	9.1	8.9	5.6	3.8	5.2	3.9	3.0	6.1	5.9

Transformation Assay

Giemsa Staining	1	2.5	5	10	15	22	30	40	60	90	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	10	14	3	9	13	9	9	0	0	12	70
Transformation frequency*	0.125	0.104166667	0.145833333	0.03125	0.09375	0.135416667	0.09375	0.09375	0	0	0.125	0.729166667
SE	0.03375386	0.03117758	0.03602169	0.01775805	0.02974911	0.03492238	0.02974911	0.02974911	0.00000000	0.00000000	0.03375386	0.04535538

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phenanthrene
Institution:	Lab 4
Test Number	

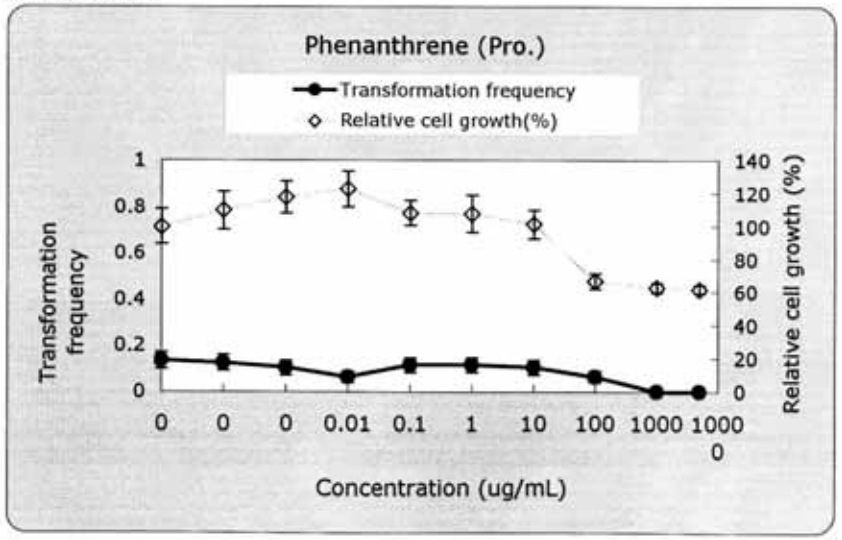
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.001	0.01	0.1	1	10	100	1000	5000	0.1% DMSO	TPA (50ng/mL)
1	0.077	0.284	0.283	0.304	0.336	0.303	0.288	0.280	0.217	0.206	0.219	0.317	0.385
2	0.096	0.299	0.341	0.331	0.335	0.297	0.318	0.308	0.219	0.226	0.212	0.326	0.387
3	0.088	0.287	0.325	0.370	0.364	0.313	0.305	0.293	0.241	0.218	0.224	0.350	0.396
4	0.085	0.306	0.327	0.342	0.361	0.329	0.313	0.331	0.221	0.224	0.225	0.332	0.364
5	0.086	0.308	0.332	0.346	0.365	0.347	0.333	0.329	0.231	0.223	0.221	0.390	0.383
6	0.078	0.306	0.358	0.352	0.359	0.322	0.348	0.289	0.233	0.225	0.211	0.383	0.397
7	0.082	0.344	0.304	0.340	0.370	0.323	0.343	0.305	0.244	0.224	0.217	0.348	0.340
8	0.097	0.268	0.301	0.318	0.301	0.309	0.284	0.290	0.226	0.219	0.219	0.311	0.392
Average	0.086	0.300	0.321	0.338	0.349	0.318	0.317	0.303	0.229	0.220	0.219	0.345	0.381
SD	0.007	0.023	0.024	0.021	0.024	0.016	0.024	0.019	0.010	0.007	0.005	0.029	0.019
Average - Blank	0.000	0.214	0.235	0.252	0.263	0.232	0.230	0.217	0.143	0.134	0.132	0.258	0.294
Relative Cell Growth (%)		100.0	109.8	117.5	122.7	108.1	107.6	101.2	66.7	62.7	61.8	100.0	113.9
SD of Relative Cell Growth		10.5	11.4	9.6	11.0	7.5	11.1	8.8	4.7	3.1	2.5	13.7	9.0

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.001	0.01	0.1	1	10	100	1000	5000	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	96	96	0	0
No. of wells counted	96	96	96	96	96	96	96	96	0	0	96	96
No. of wells having foci	13	12	10	6	11	11	10	6			15	67
Transformation frequency*	0.135416667	0.125	0.104166667	0.0625	0.114583333	0.114583333	0.104166667	0.0625	#DIV/0!	#DIV/0!	0.15625	0.697916667
SE	0.03492238	0.03375386	0.03117758	0.02470529	0.03250865	0.03250865	0.03117758	0.02470529	#DIV/0!	#DIV/0!	0.03705794	0.04686294

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Annex 11

Results Submitted from Laboratories in the Phase II of Validation Study of 96-Well Method

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 1
Test Number	

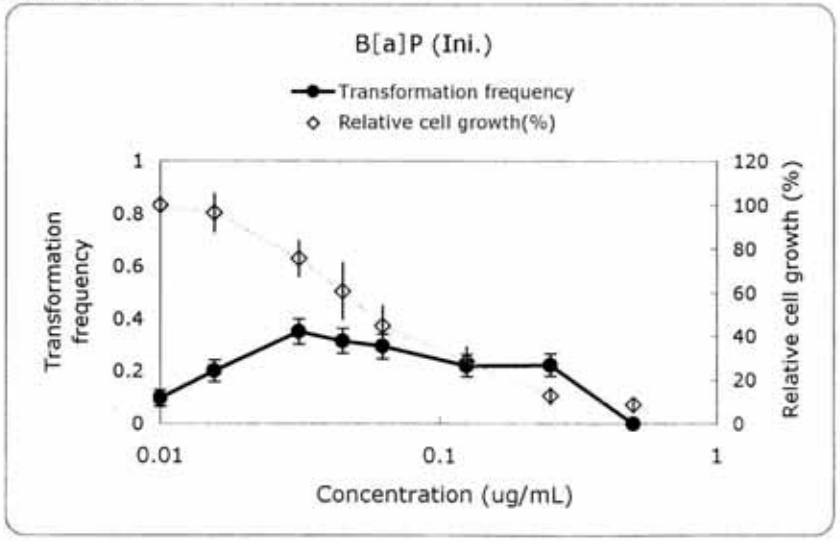
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.0156	0.0313	0.045	0.0625	0.125	0.25	0.5			0.1% DMSO	MCA (1ug/mL)
1	0.072	0.665	0.691	0.462	0.298	0.248	0.186	0.102	0.094			0.665	0.254
2	0.073	0.680	0.702	0.550	0.562	0.367	0.295	0.172	0.153			0.680	0.445
3	0.072	0.716	0.675	0.568	0.465	0.395	0.261	0.153	0.130			0.716	0.377
4	0.075	0.688	0.635	0.492	0.433	0.416	0.205	0.149	0.143			0.688	0.300
5	0.079	0.739	0.619	0.570	0.420	0.387	0.243	0.177	0.123			0.739	0.358
6	0.080	0.694	0.610	0.528	0.502	0.379	0.296	0.156	0.142			0.694	0.329
7	0.082	0.696	0.773	0.568	0.430	0.354	0.286	0.174	0.140			0.696	0.353
8	0.078	0.703	0.698	0.626	0.521	0.290	0.257	0.164	0.115			0.703	0.410
Average	0.076	0.698	0.675	0.546	0.454	0.355	0.254	0.156	0.130	#DIV/0!	#DIV/0!	0.698	0.353
SD	0.004	0.023	0.054	0.051	0.080	0.057	0.041	0.024	0.019	#DIV/0!	#DIV/0!	0.023	0.060
Average - Blank	0.000	0.621	0.599	0.469	0.378	0.278	0.177	0.080	0.054	#DIV/0!	#DIV/0!	0.621	0.277
Relative Cell Growth (%)		100.0	96.4	75.5	60.8	44.8	28.5	12.8	8.6	#DIV/0!	#DIV/0!	100.0	44.6
SD of Relative Cell Growth		3.6	8.6	8.2	12.9	9.2	6.6	3.9	3.0	#DIV/0!	#DIV/0!	3.6	9.7

Transformation Assay

Giemsa Staining	0.01	0.0156	0.0313	0.045	0.0625	0.125	0.25	0.5	0	0	Negative Control	Positive Control
No. of wells excluded	3	1	2	1	1	1	2	8			3	0
No. of wells counted	93	95	94	95	95	95	94	88	96	96	93	96
No. of wells having foci	9	19	33	30	28	21	21	TOX			9	50
Transformation frequency*	0.096774194	0.2	0.35106383	0.315789474	0.294736842	0.221052632	0.223404255	#VALUE!	0	0	0.096774194	0.520833333
SE	0.03065748	0.04103913	0.04923003	0.04769050	0.04677685	0.04257357	0.04296149	#VALUE!	0.00000000	0.00000000	0.03065748	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	B[a]P
Institution:	Lab 3
Test Number	

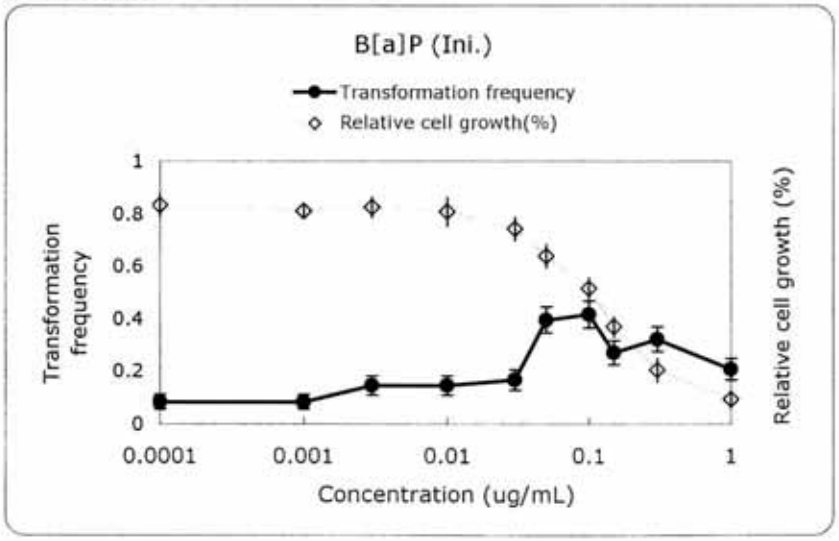
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.001	0.003	0.01	0.03	0.05	0.1	0.15	0.3	1	0.1% DMSO	MCA (1ug/mL)
1	0.128	1.319	1.393	1.368	1.246	1.160	1.168	1.022	0.622	0.337	0.224	1.319	0.817
2	0.135	1.389	1.345	1.333	1.410	1.324	1.065	0.939	0.674	0.471	0.299	1.389	0.844
3	0.135	1.385	1.384	1.456	1.499	1.288	1.025	0.898	0.679	0.445	0.250	1.385	0.826
4	0.137	1.456	1.388	1.343	1.315	1.277	1.190	0.983	0.672	0.453	0.327	1.456	0.827
5	0.135	1.399	1.398	1.447	1.475	1.229	1.201	0.846	0.679	0.575	0.252	1.399	0.922
6	0.125	1.469	1.476	1.420	1.346	1.284	1.157	0.887	0.797	0.465	0.290	1.469	0.880
7	0.117	1.527	1.388	1.423	1.421	1.326	1.065	0.870	0.720	0.386	0.281	1.527	0.813
8	0.118	1.442	1.336	1.504	1.361	1.386	1.101	0.992	0.776	0.448	0.279	1.442	0.754
Average	0.129	1.423	1.389	1.412	1.384	1.284	1.122	0.930	0.702	0.448	0.275	1.423	0.835
SD	0.008	0.064	0.042	0.059	0.084	0.068	0.066	0.064	0.059	0.069	0.032	0.064	0.050
Average - Blank	0.000	1.295	1.260	1.283	1.255	1.156	0.993	0.801	0.574	0.319	0.147	1.295	0.707
Relative Cell Growth (%)		100.0	97.3	99.1	97.0	89.3	76.7	61.9	44.3	24.6	11.3	100.0	54.6
SD of Relative Cell Growth		4.9	3.3	4.6	6.5	5.2	5.1	4.9	4.5	5.3	2.5	4.9	3.8

Transformation Assay

Giemsa Staining	0.0001	0.001	0.003	0.01	0.03	0.05	0.1	0.15	0.3	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	8	8	14	14	16	38	40	26	31	20	8	54
Transformation frequency*	0.083333333	0.083333333	0.145833333	0.145833333	0.166666667	0.395833333	0.416666667	0.270833333	0.322916667	0.208333333	0.083333333	0.5625
SE	0.02820847	0.02820847	0.03602169	0.03602169	0.03803629	0.04991131	0.05031728	0.04535538	0.04772332	0.04144908	0.02820847	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 1
Test Number	

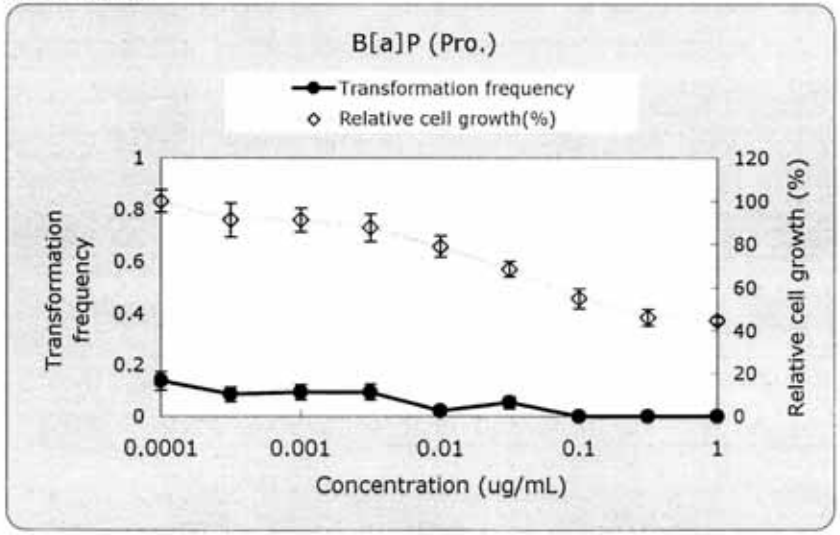
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.000316	0.001	0.00316	0.01	0.0316	0.1	0.316	1		0.1% DMSO	TPA (50ng/mL)
1	0.074	0.672	0.658	0.579	0.597	0.543	0.473	0.374	0.323	0.314		0.672	0.860
2	0.072	0.716	0.656	0.640	0.625	0.522	0.498	0.424	0.333	0.344		0.716	0.764
3	0.075	0.634	0.645	0.621	0.597	0.531	0.444	0.441	0.358	0.338		0.634	0.883
4	0.078	0.674	0.599	0.615	0.534	0.541	0.500	0.397	0.362	0.340		0.674	0.890
5	0.079	0.633	0.621	0.553	0.614	0.601	0.465	0.400	0.320	0.342		0.633	0.932
6	0.081	0.657	0.528	0.644	0.527	0.525	0.478	0.397	0.381	0.344		0.657	0.885
7	0.078	0.622	0.594	0.591	0.608	0.507	0.461	0.381	0.358	0.341		0.622	0.917
8	0.080	0.670	0.567	0.627	0.602	0.528	0.498	0.360	0.332	0.338		0.670	0.864
Average	0.077	0.660	0.609	0.609	0.588	0.537	0.477	0.397	0.346	0.338	#DIV/0!	0.660	0.874
SD	0.003	0.030	0.046	0.032	0.037	0.028	0.020	0.026	0.022	0.010	#DIV/0!	0.030	0.051
Average - Blank	0.000	0.583	0.531	0.532	0.511	0.460	0.400	0.320	0.269	0.261	#DIV/0!	0.583	0.797
Relative Cell Growth (%)		100.0	91.2	91.2	87.7	79.0	68.7	54.9	46.1	44.7	#DIV/0!	100.0	136.8
SD of Relative Cell Growth		5.2	7.9	5.4	6.3	4.8	3.5	4.5	3.7	1.7	#DIV/0!	5.2	8.7

Transformation Assay

Giemsa Staining	0.0001	0.000316	0.001	0.00316	0.01	0.0316	0.1	0.316	1	0	Negative Control	Positive Control
No. of wells excluded	1	2	0	1	1	3	8	3	96		1	0
No. of wells counted	95	94	96	95	95	93	88	93	0	96	95	96
No. of wells having foci	13	8	9	9	2	5	TOX	TOX	TOX		13	56
Transformation frequency*	0.136842105	0.085106383	0.09375	0.094736842	0.021052632	0.053763441	#VALUE!	#VALUE!	#VALUE!	0	0.136842105	0.583333333
SE	0.03526088	0.02878077	0.02974911	0.03004589	0.01472893	0.02338848	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.03526088	0.05031728

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	B[a]P
Institution:	Lab 3
Test Number	

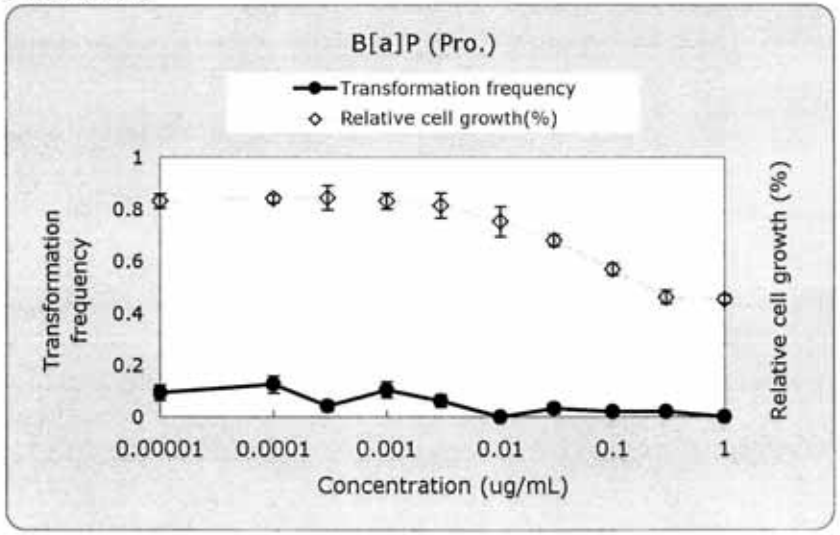
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	0.1% DMSO	TPA (50ng/mL)
1	0.128	1.250	1.286	1.212	1.245	1.267	1.081	1.026	0.863	0.714	0.736	1.250	1.552
2	0.139	1.360	1.298	1.291	1.283	1.154	1.104	1.095	0.925	0.758	0.742	1.360	1.596
3	0.138	1.270	1.331	1.337	1.336	1.255	1.339	1.105	0.957	0.757	0.789	1.270	1.633
4	0.147	1.272	1.307	1.407	1.322	1.289	1.173	1.075	0.932	0.786	0.787	1.272	1.731
5	0.128	1.296	1.319	1.380	1.342	1.238	1.205	1.070	0.906	0.805	0.741	1.296	1.708
6	0.131	1.336	1.332	1.260	1.280	1.293	1.143	1.040	0.952	0.811	0.775	1.336	1.659
7	0.123	1.266	1.283	1.280	1.293	1.394	1.213	1.112	0.943	0.819	0.774	1.266	1.764
8	0.120	1.285	1.274	1.293	1.220	1.236	1.177	1.091	0.907	0.746	0.766	1.285	1.645
Average	0.132	1.292	1.304	1.308	1.290	1.266	1.179	1.077	0.923	0.775	0.764	1.292	1.661
SD	0.009	0.038	0.022	0.064	0.043	0.068	0.079	0.031	0.031	0.037	0.021	0.038	0.071
Average - Blank	0.000	1.160	1.172	1.176	1.158	1.134	1.048	0.945	0.791	0.643	0.632	1.160	1.529
Relative Cell Growth (%)		100.0	101.0	101.3	99.8	97.7	90.3	81.5	68.2	55.4	54.5	100.0	131.8
SD of Relative Cell Growth		3.2	1.9	5.5	3.7	5.8	6.8	2.6	2.7	3.2	1.8	3.2	6.1

Transformation Assay

Giemsa Staining	0.00001	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	12	4	10	6	0	3	2	2	Tox.	9	72
Transformation frequency*	0.09375	0.125	0.041666667	0.104166667	0.0625	0	0.03125	0.020833333	0.020833333	#VALUE!	0.09375	0.75
SE	0.02974911	0.03375386	0.02039469	0.03117758	0.02470529	0.00000000	0.01775805	0.01457713	0.01457713	#VALUE!	0.02974911	0.04419417

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Cadmium chloride
Institution:	Lab 1
Test Number	

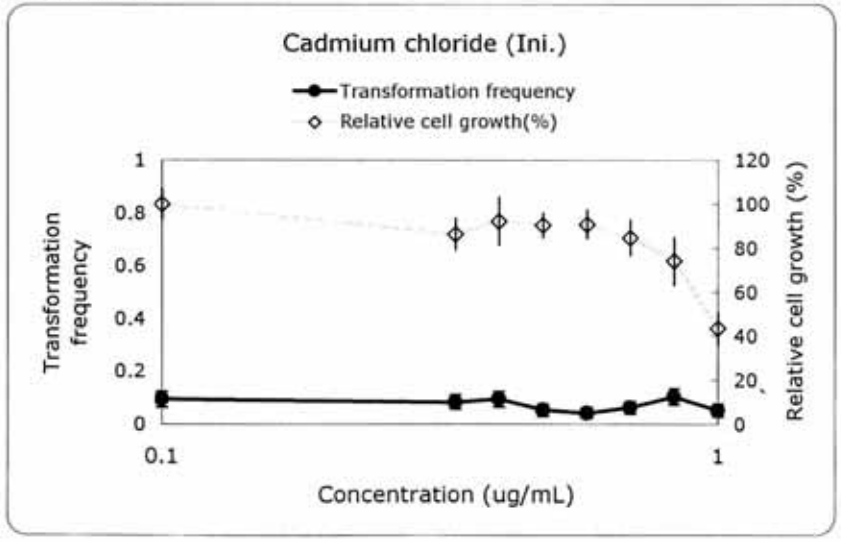
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.335	0.402	0.482	0.579	0.694	0.833	1			0.1% DMSO	MCA (1ug/mL)
1	0.094	0.809	0.632	0.785	0.701	0.704	0.638	0.480	0.329			0.817	0.320
2	0.108	0.768	0.739	0.740	0.696	0.750	0.689	0.599	0.340			0.806	0.322
3	0.142	0.713	0.635	0.763	0.783	0.691	0.615	0.727	0.446			0.760	0.330
4	0.120	0.742	0.684	0.661	0.675	0.659	0.648	0.581	0.436			0.705	0.320
5	0.107	0.723	0.675	0.638	0.678	0.647	0.658	0.572	0.433			0.837	0.318
6	0.124	0.791	0.646	0.820	0.733	0.738	0.688	0.648	0.448			0.801	0.362
7	0.110	0.778	0.687	0.733	0.686	0.756	0.785	0.636	0.371			0.754	0.358
8	0.106	0.853	0.760	0.628	0.715	0.745	0.646	0.563	0.402			0.851	0.286
Average	0.114	0.772	0.682	0.721	0.708	0.711	0.671	0.601	0.401	#DIV/0!	#DIV/0!	0.791	0.327
SD	0.015	0.046	0.047	0.071	0.036	0.043	0.052	0.072	0.048	#DIV/0!	#DIV/0!	0.048	0.024
Average - Blank	0.000	0.658	0.568	0.607	0.595	0.597	0.557	0.487	0.287	#DIV/0!	#DIV/0!	0.678	0.213
Relative Cell Growth (%)		100.0	86.3	92.2	90.3	90.8	84.6	74.0	43.6	#DIV/0!	#DIV/0!	100.0	31.5
SD of Relative Cell Growth		7.1	7.1	10.8	5.4	6.5	7.9	11.0	7.3	#DIV/0!	#DIV/0!	7.4	3.7

Transformation Assay

Giemsa Staining	0.1	0.335	0.402	0.482	0.579	0.694	0.833	1	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	1	2			0	0
No. of wells counted	96	96	96	96	96	96	95	94	96	96	96	96
No. of wells having foci	9	8	9	5	4	6	10	5			14	47
Transformation frequency*	0.09375	0.083333333	0.09375	0.052083333	0.041666667	0.0625	0.105263158	0.053191489	0	0	0.145833333	0.489583333
SE	0.02974911	0.02820847	0.02974911	0.02267769	0.02039469	0.02470529	0.03148648	0.02314665	0.00000000	0.00000000	0.03602169	0.05101996

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Cadmium chloride
Institution:	Lab 3
Test Number	

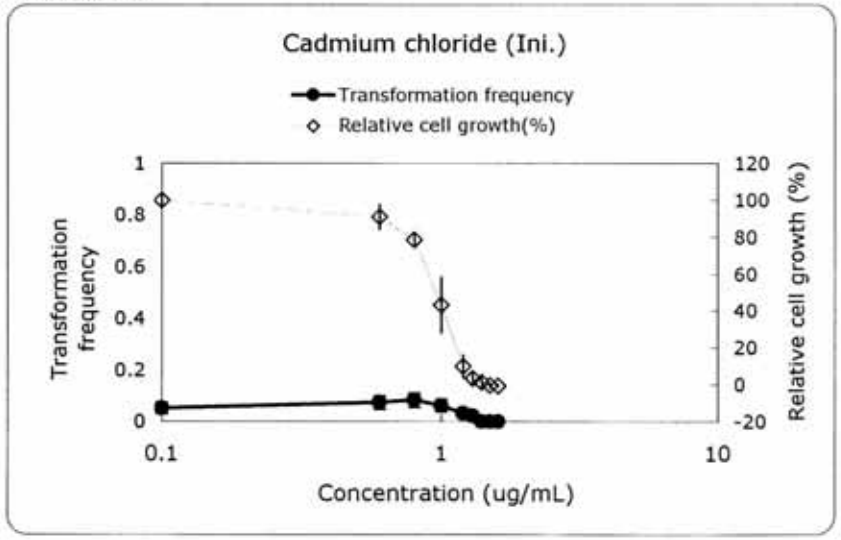
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.6	0.8	1	1.2	1.3	1.4	1.5	1.6		0.1% DMSO	MCA (1ug/mL)
1	0.107	1.314	1.090	1.075	0.570	0.169	0.116	0.097	0.092	0.094		1.154	0.517
2	0.108	1.286	1.325	1.030	0.612	0.328	0.118	0.101	0.102	0.101		1.203	0.615
3	0.109	1.246	1.216	1.062	0.933	0.226	0.131	0.128	0.099	0.100		1.309	0.608
4	0.114	1.339	1.137	1.060	0.762	0.318	0.143	0.114	0.100	0.100		1.219	0.736
5	0.119	1.326	1.192	1.079	0.746	0.147	0.148	0.132	0.107	0.107		1.347	0.665
6	0.115	1.355	1.252	0.993	0.626	0.175	0.260	0.210	0.138	0.118		1.293	0.530
7	0.113	1.275	1.146	1.152	0.500	0.316	0.145	0.132	0.117	0.120		1.435	0.596
8	0.115	1.382	1.284	0.998	0.328	0.191	0.179	0.118	0.114	0.113		1.377	0.580
Average	0.113	1.315	1.205	1.056	0.635	0.234	0.155	0.129	0.109	0.107	#DIV/0!	1.292	0.606
SD	0.004	0.045	0.080	0.051	0.183	0.075	0.047	0.035	0.014	0.009	#DIV/0!	0.095	0.071
Average - Blank	0.000	1.203	1.093	0.944	0.522	0.121	0.043	0.017	-0.004	-0.006	#DIV/0!	1.180	0.493
Relative Cell Growth (%)		100.0	90.8	78.4	43.4	10.1	3.5	1.4	-0.3	-0.5	#DIV/0!	100.0	41.8
SD of Relative Cell Growth		3.7	6.6	4.2	15.2	6.3	3.9	2.9	1.2	0.8	#DIV/0!	7.9	5.9

Transformation Assay

Giemsa Staining	0.1	0.6	0.8	1	1.2	1.3	1.4	1.5	1.6	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	2	13				0	0
No. of wells counted	96	96	96	96	96	94	83	96	96	96	96	96
No. of wells having foci	5	7	8	6	3	2	Tox.	Tox.	Tox.		7	57
Transformation frequency*	0.052083333	0.072916667	0.083333333	0.0625	0.03125	0.021276596	#VALUE!	#VALUE!	#VALUE!	0	0.072916667	0.59375
SE	0.02267769	0.02653610	0.02820847	0.02470529	0.01775805	0.01488391	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.02653610	0.05012598

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Cadmium chloride
Institution:	Lab 1
Test Number	

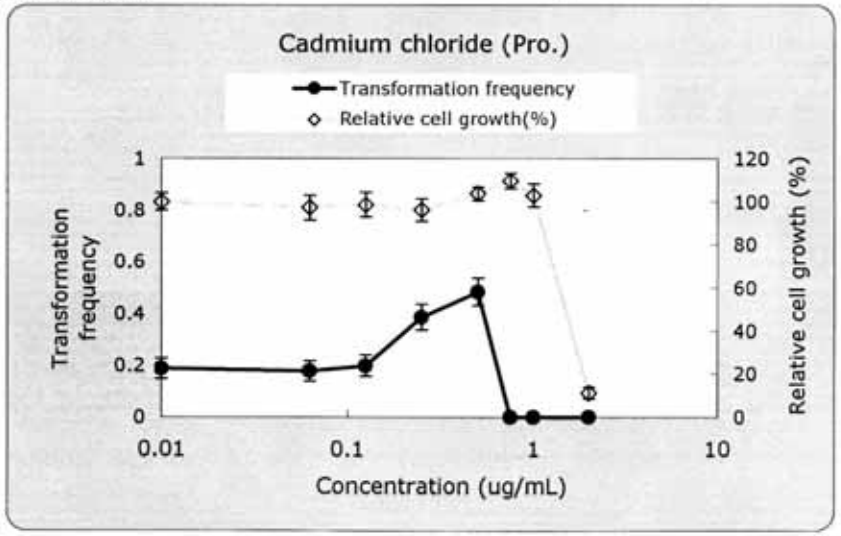
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.0625	0.125	0.25	0.5	0.75	1	2			0.1% DMSO	TPA (50ng/mL)
1	0.108	0.733	0.766	0.700	0.778	0.829	0.859	0.781	0.181			0.783	1.135
2	0.098	0.778	0.767	0.782	0.734	0.800	0.836	0.730	0.186			0.818	1.288
3	0.112	0.819	0.769	0.734	0.788	0.800	0.870	0.803	0.201			0.819	1.107
4	0.100	0.790	0.787	0.794	0.716	0.816	0.854	0.834	0.218			0.721	1.360
5	0.120	0.792	0.715	0.812	0.709	0.769	0.814	0.819	0.187			0.694	1.265
6	0.115	0.781	0.713	0.800	0.802	0.807	0.884	0.814	0.177			0.745	1.356
7	0.129	0.779	0.784	0.780	0.772	0.828	0.828	0.831	0.159			0.734	1.312
8	0.126	0.816	0.834	0.802	0.777	0.834	0.865	0.831	0.205			0.801	1.306
Average	0.114	0.786	0.767	0.776	0.760	0.810	0.851	0.805	0.189	#DIV/0!	#DIV/0!	0.764	1.266
SD	0.011	0.027	0.039	0.039	0.035	0.021	0.023	0.035	0.018	#DIV/0!	#DIV/0!	0.047	0.095
Average - Blank	0.000	0.673	0.653	0.662	0.646	0.697	0.738	0.692	0.076	#DIV/0!	#DIV/0!	0.651	1.153
Relative Cell Growth (%)		100.0	97.2	98.4	96.1	103.6	109.7	102.9	11.3	#DIV/0!	#DIV/0!	100.0	177.1
SD of Relative Cell Growth		4.0	5.8	5.7	5.2	3.2	3.5	5.2	2.7	#DIV/0!	#DIV/0!	7.0	14.2

Transformation Assay

Giemsa Staining	0.01	0.0625	0.125	0.25	0.5	0.75	1	2	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	5	25	63	96			0	0
No. of wells counted	96	96	96	96	91	71	33	0	96	96	96	96
No. of wells having foci	18	17	19	37	44	TOX	TOX	TOX			17	77
Transformation frequency*	0.1875	0.177083333	0.197916667	0.385416667	0.483516484	#VALUE!	#VALUE!	#VALUE!	0	0	0.177083333	0.802083333
SE	0.03983609	0.03896110	0.04066449	0.04967296	0.05238575	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.00000000	0.03896110	0.04066449

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Cadmium chloride
Institution:	Lab 3
Test Number	

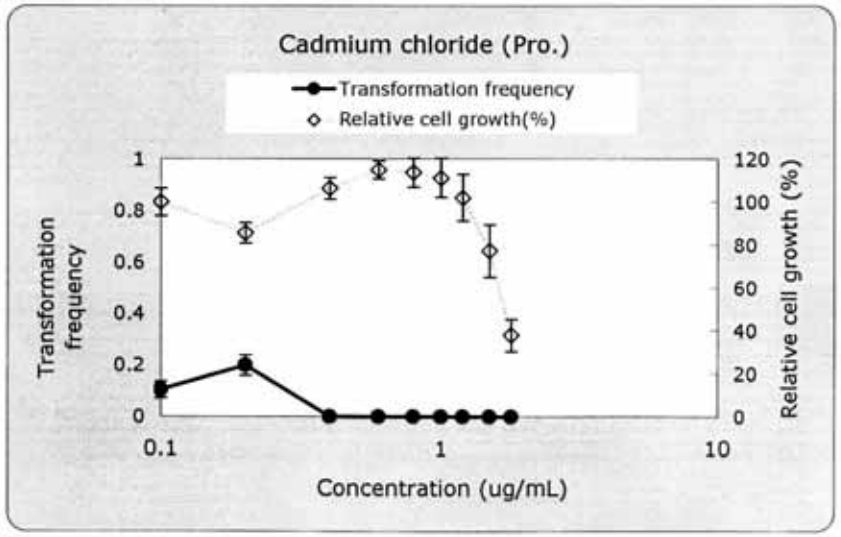
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.2	0.4	0.6	0.8	1	1.2	1.5	1.8		0.1% DMSO	TPA (50ng/mL)
1	0.108	1.216	1.058	1.415	1.409	1.410	1.370	1.264	0.996	0.483		1.284	1.780
2	0.118	1.237	1.110	1.306	1.437	1.338	1.224	1.110	0.950	0.448		1.144	2.119
3	0.118	1.310	1.097	1.323	1.483	1.374	1.504	1.254	1.049	0.679		1.281	2.028
4	0.115	1.241	1.081	1.302	1.493	1.442	1.365	1.292	0.726	0.598		1.253	1.994
5	0.118	1.296	1.111	1.425	1.429	1.506	1.438	1.359	1.161	0.675		1.298	1.813
6	0.114	1.298	1.238	1.426	1.558	1.507	1.558	1.505	1.189	0.616		1.315	2.132
7	0.118	1.461	1.159	1.332	1.464	1.501	1.518	1.477	1.062	0.504		1.345	2.016
8	0.122	1.308	1.151	1.436	1.508	1.574	1.437	1.297	1.084	0.503		1.320	2.000
Average	0.116	1.296	1.126	1.371	1.473	1.457	1.427	1.320	1.027	0.563	#DIV/0!	1.280	1.985
SD	0.004	0.076	0.056	0.060	0.048	0.079	0.107	0.127	0.145	0.090	#DIV/0!	0.062	0.128
Average - Blank	0.000	1.180	1.009	1.254	1.356	1.340	1.310	1.203	0.911	0.447	#DIV/0!	1.164	1.869
Relative Cell Growth (%)		100.0	85.6	106.3	115.0	113.6	111.1	102.0	77.2	37.9	#DIV/0!	100.0	160.6
SD of Relative Cell Growth		6.4	4.8	5.1	4.1	6.7	9.1	10.8	12.3	7.6	#DIV/0!	5.2	10.8

Transformation Assay

Giemsa Staining	0.1	0.2	0.4	0.6	0.8	1	1.2	1.5	1.8	0	Negative Control	Positive Control
No. of wells excluded	0	0	8								0	0
No. of wells counted	96	96	88	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	19	25 (Tox.)	Tox.	Tox.	Tox.	Tox.	Tox.	Tox.		13	70
Transformation frequency*	0.104166667	0.197916667	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0.135416667	0.729166667
SE	0.03117758	0.04066449	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.03492238	0.04535538

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Failure

The Progressing Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Cadmium chloride
Institution:	Lab 3
Test Number	

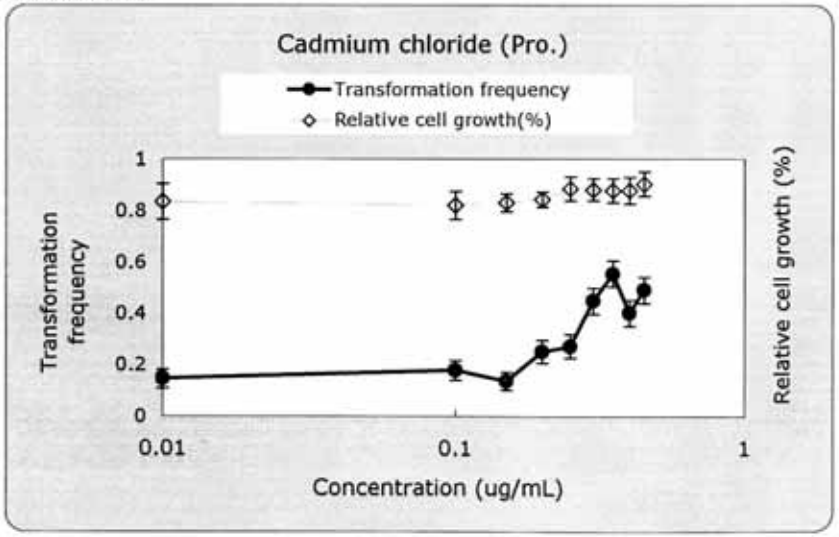
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45		0.1% DMSO	TPA (50ng/mL)
1	0.081	1.189	1.080	1.127	1.127	1.168	1.239	1.175	1.250	1.336		1.107	1.440
2	0.084	1.312	1.106	1.148	1.142	1.250	1.195	1.176	1.220	1.261		1.155	1.526
3	0.090	1.227	1.102	1.255	1.189	1.141	1.214	1.302	1.311	1.275		1.352	1.461
4	0.086	1.113	1.209	1.209	1.214	1.293	1.251	1.234	1.122	1.347		1.073	1.518
5	0.085	1.194	1.162	1.165	1.185	1.329	1.233	1.247	1.211	1.255		1.144	1.551
6	0.081	1.090	1.283	1.161	1.221	1.267	1.218	1.245	1.238	1.225		1.046	1.522
7	0.086	1.238	1.210	1.169	1.174	1.236	1.358	1.317	1.328	1.266		1.103	1.632
8	0.090	1.034	1.102	1.112	1.240	1.236	1.171	1.151	1.164	1.148		1.114	1.609
Average	0.085	1.175	1.157	1.168	1.187	1.240	1.235	1.231	1.231	1.264	#DIV/0!	1.137	1.532
SD	0.003	0.090	0.072	0.046	0.039	0.062	0.056	0.060	0.069	0.062	#DIV/0!	0.094	0.066
Average - Blank	0.000	1.089	1.071	1.083	1.101	1.155	1.150	1.146	1.145	1.179	#DIV/0!	1.051	1.447
Relative Cell Growth (%)		100.0	98.4	99.4	101.1	106.0	105.5	105.2	105.1	108.2	#DIV/0!	100.0	137.6
SD of Relative Cell Growth		8.3	6.6	4.2	3.6	5.7	5.1	5.5	6.3	5.7	#DIV/0!	8.6	6.0

Transformation Assay

Giemsa Staining	0.01	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	1	0		0	0
No. of wells counted	96	96	96	96	96	96	96	95	96	96	96	96
No. of wells having foci	14	17	13	24	26	43	53	38	47		7	60
Transformation frequency*	0.145833333	0.177083333	0.135416667	0.25	0.270833333	0.447916667	0.552083333	0.4	0.489583333	0	0.072916667	0.625
SE	0.03602169	0.03896110	0.03492238	0.04419417	0.04535538	0.05075342	0.05075342	0.05026247	0.05101996	0.00000000	0.02653610	0.04941059
						*	*	*	*			

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	DB[a,h]A
Institution:	Lab 2
Test Number	

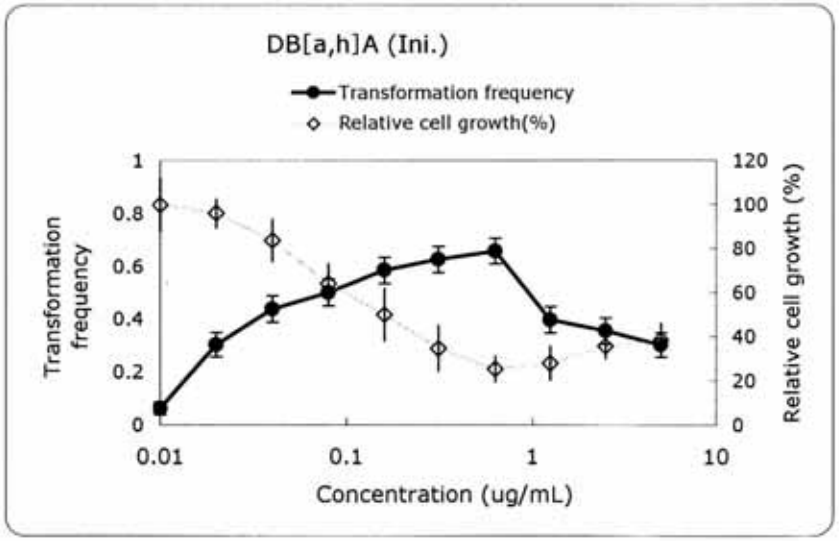
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.02	0.04	0.08	0.16	0.31	0.63	1.25	2.5	5	0.1% DMSO	MCA (1ug/mL)
1	0.105	0.215	0.211	0.229	0.193	0.188	0.152	0.149	0.156	0.159	0.159	0.215	0.174
2	0.103	0.241	0.238	0.226	0.199	0.191	0.167	0.137	0.147	0.154	0.167	0.241	0.164
3	0.097	0.240	0.235	0.206	0.190	0.173	0.137	0.137	0.129	0.149	0.152	0.240	0.171
4	0.102	0.232	0.228	0.217	0.170	0.162	0.166	0.135	0.139	0.147	0.157	0.232	0.181
5	0.102	0.249	0.220	0.202	0.174	0.161	0.136	0.128	0.131	0.141	0.145	0.249	0.159
6	0.113	0.232	0.227	0.194	0.198	0.155	0.144	0.129	0.132	0.137	0.139	0.232	0.159
7	0.099	0.244	0.231	0.202	0.173	0.150	0.145	0.126	0.130	0.148	0.141	0.244	0.154
8	0.100	0.202	0.225	0.211	0.187	0.157	0.132	0.140	0.144	0.156	0.159	0.202	0.170
Average	0.103	0.232	0.227	0.211	0.186	0.167	0.147	0.135	0.139	0.149	0.152	0.232	0.167
SD	0.005	0.016	0.009	0.012	0.012	0.015	0.013	0.008	0.010	0.007	0.010	0.016	0.009
Average - Blank	0.000	0.129	0.124	0.108	0.083	0.065	0.045	0.033	0.036	0.046	0.050	0.129	0.064
Relative Cell Growth (%)		100.0	96.1	83.8	64.1	49.9	34.6	25.1	27.8	35.8	38.5	100.0	49.4
SD of Relative Cell Growth		12.3	6.6	9.5	9.0	11.9	10.3	5.8	7.6	5.8	7.7	12.3	7.0

Transformation Assay

Giemsa Staining	0.01	0.02	0.04	0.08	0.16	0.31	0.63	1.25	2.5	5	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	29	42	48	56	60	63	38	34	29	6	75
Transformation frequency*	0.0625	0.302083333	0.4375	0.5	0.583333333	0.625	0.65625	0.395833333	0.354166667	0.302083333	0.0625	0.78125
SE	0.02470529	0.04686294	0.05063079	0.05103104	0.05031728	0.04941059	0.04847529	0.04991131	0.04881221	0.04686294	0.02470529	0.04219232

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	DB[a,h]A
Institution:	Lab 3
Test Number	

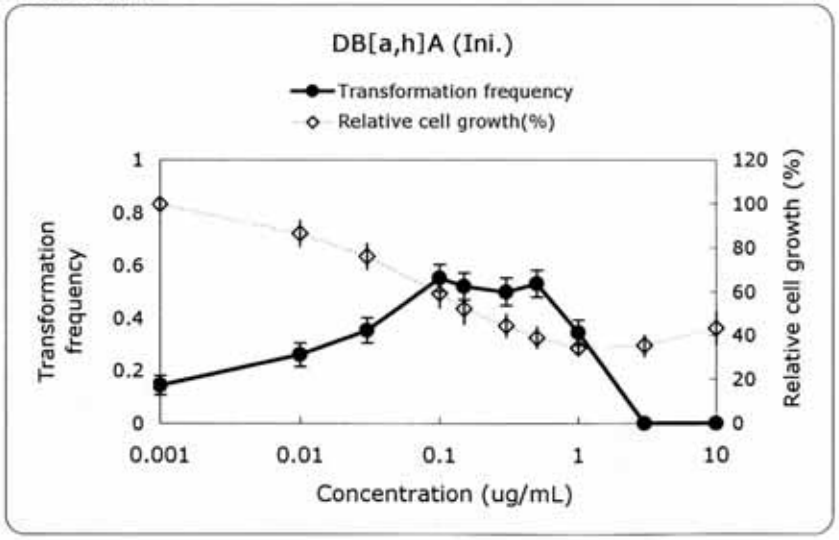
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.001	0.01	0.03	0.1	0.15	0.3	0.5	1	3	10	0.1% DMSO	MCA (1ug/mL)
1	0.119	1.390	1.215	1.083	0.825	0.887	0.722	0.573	0.511	0.604	0.622	1.390	0.785
2	0.126	1.472	1.199	1.033	0.864	0.749	0.726	0.722	0.572	0.588	0.836	1.472	0.863
3	0.127	1.359	1.282	1.263	0.916	0.873	0.611	0.582	0.572	0.520	0.687	1.359	1.040
4	0.122	1.474	1.396	1.137	0.944	0.805	0.646	0.594	0.576	0.634	0.845	1.474	0.952
5	0.123	1.462	1.269	1.143	0.887	0.651	0.779	0.626	0.541	0.575	0.643	1.462	0.870
6	0.149	1.397	1.304	1.073	0.878	0.779	0.807	0.617	0.616	0.516	0.648	1.397	0.968
7	0.128	1.474	1.251	1.181	1.079	0.801	0.723	0.651	0.617	0.571	0.609	1.474	0.924
8	0.135	1.429	1.140	1.053	0.817	0.942	0.664	0.736	0.604	0.710	0.643	1.429	0.863
Average	0.129	1.432	1.257	1.121	0.901	0.811	0.710	0.638	0.576	0.590	0.692	1.432	0.908
SD	0.010	0.045	0.077	0.076	0.083	0.091	0.066	0.062	0.037	0.063	0.095	0.045	0.079
Average - Blank	0.000	1.304	1.128	0.992	0.773	0.682	0.581	0.509	0.448	0.461	0.563	1.304	0.780
Relative Cell Growth (%)		100.0	86.6	76.1	59.3	52.3	44.6	39.0	34.3	35.4	43.2	100.0	59.8
SD of Relative Cell Growth		3.5	5.9	5.8	6.4	6.9	5.1	4.7	2.8	4.8	7.3	3.5	6.1

Transformation Assay

Giemsa Staining	0.001	0.01	0.03	0.1	0.15	0.3	0.5	1	3	10	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	25	34	53	50	48	51	33	Tox.	Tox.	14	51
Transformation frequency*	0.145833333	0.260416667	0.354166667	0.552083333	0.520833333	0.5	0.53125	0.34375	#VALUE!	#VALUE!	0.145833333	0.53125
SE	0.03602169	0.04479116	0.04881221	0.05075342	0.05098672	0.05103104	0.05093127	0.04847529	#VALUE!	#VALUE!	0.03602169	0.05093127

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	DB[a,h]A
Institution:	Lab 2
Test Number	

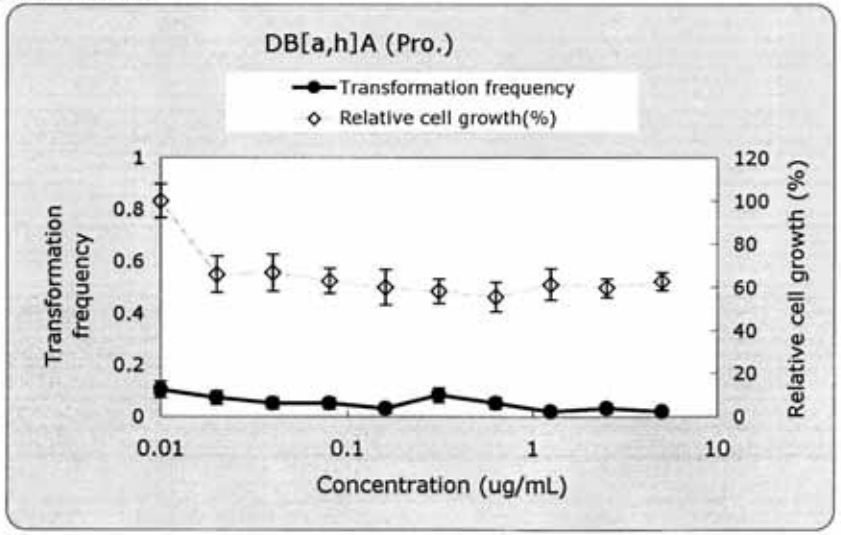
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)								Negative Control	Positive Control
		0.01	0.02	0.04	0.08	0.16	0.31	0.63	1.25	2.5	5	0.1% DMSO	TPA (50ng/mL)
1	0.105	0.286	0.218	0.241	0.214	0.210	0.198	0.190	0.204	0.205	0.212	0.286	0.332
2	0.103	0.292	0.234	0.253	0.234	0.235	0.223	0.221	0.216	0.224	0.210	0.292	0.370
3	0.097	0.313	0.250	0.233	0.227	0.215	0.228	0.209	0.218	0.215	0.217	0.313	0.377
4	0.102	0.286	0.240	0.231	0.237	0.216	0.215	0.216	0.219	0.221	0.223	0.286	0.383
5	0.102	0.287	0.231	0.214	0.213	0.203	0.203	0.208	0.227	0.214	0.229	0.287	0.347
6	0.113	0.303	0.220	0.229	0.215	0.240	0.207	0.219	0.243	0.222	0.232	0.303	0.348
7	0.099	0.283	0.210	0.210	0.217	0.199	0.213	0.187	0.206	0.206	0.219	0.283	0.350
8	0.100	0.264	0.204	0.207	0.206	0.201	0.204	0.200	0.203	0.204	0.216	0.264	0.333
Average	0.103	0.289	0.226	0.227	0.220	0.215	0.211	0.206	0.217	0.214	0.220	0.289	0.355
SD	0.005	0.014	0.016	0.016	0.011	0.015	0.010	0.013	0.013	0.008	0.008	0.014	0.019
Average - Blank	0.000	0.187	0.123	0.125	0.118	0.112	0.109	0.104	0.114	0.111	0.117	0.187	0.252
Relative Cell Growth (%)		100.0	66.0	66.8	63.1	60.1	58.3	55.5	61.3	59.6	62.8	100.0	135.2
SD of Relative Cell Growth		7.7	8.4	8.6	5.9	8.2	5.5	6.9	7.2	4.3	4.2	7.7	10.4

Transformation Assay

Giemsa Staining	0.01	0.02	0.04	0.08	0.16	0.31	0.63	1.25	2.5	5	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	7	5	5	3	8	5	2	3	2	10	50
Transformation frequency*	0.104166667	0.072916667	0.052083333	0.052083333	0.03125	0.083333333	0.052083333	0.020833333	0.03125	0.020833333	0.104166667	0.520833333
SE	0.03117758	0.02653610	0.02267769	0.02267769	0.01775805	0.02820847	0.02267769	0.01457713	0.01775805	0.01457713	0.03117758	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	DB[a,h]A
Institution:	Lab 3
Test Number	

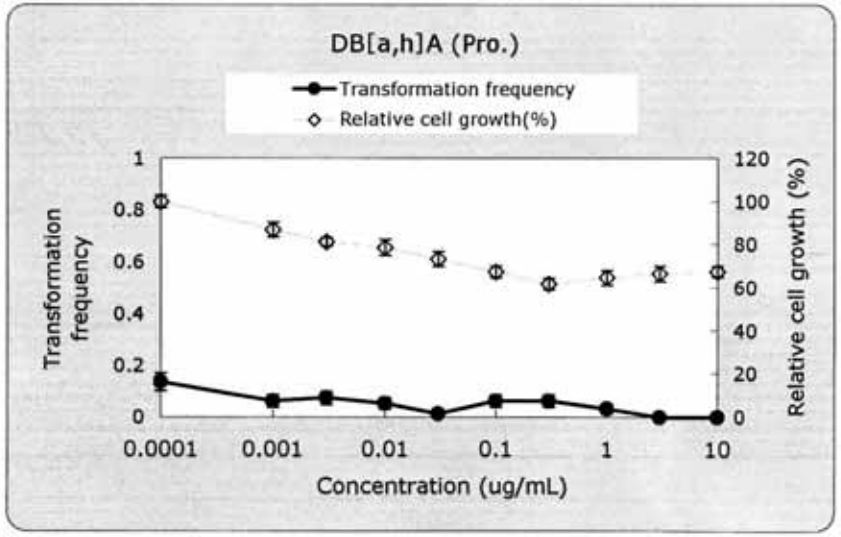
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10	0.1% DMSO	TPA (50ng/mL)
1	0.111	1.311	1.162	1.110	1.056	0.993	0.911	0.868	0.917	0.906	0.939	1.311	1.735
2	0.124	1.390	1.206	1.121	1.069	1.023	1.003	0.903	0.968	0.956	0.980	1.390	1.774
3	0.121	1.360	1.186	1.110	1.162	1.101	0.955	0.921	0.932	0.957	0.951	1.360	1.816
4	0.121	1.363	1.234	1.182	1.122	1.015	0.960	0.913	0.967	0.985	0.973	1.363	1.746
5	0.109	1.346	1.258	1.124	1.107	1.066	0.973	0.834	0.933	0.990	1.015	1.346	1.615
6	0.113	1.399	1.201	1.144	1.095	0.991	0.974	0.880	0.930	0.905	0.937	1.399	1.658
7	0.108	1.417	1.237	1.123	1.148	1.075	0.965	0.921	0.894	0.980	0.962	1.417	1.607
8	0.101	1.328	1.132	1.141	1.030	0.990	0.903	0.846	0.838	0.872	0.908	1.328	1.683
Average	0.114	1.364	1.202	1.132	1.099	1.032	0.956	0.886	0.922	0.944	0.958	1.364	1.704
SD	0.008	0.036	0.042	0.024	0.045	0.043	0.033	0.034	0.042	0.044	0.032	0.036	0.076
Average - Blank	0.000	1.251	1.089	1.018	0.985	0.918	0.842	0.772	0.809	0.830	0.845	1.251	1.591
Relative Cell Growth (%)		100.0	87.0	81.4	78.8	73.4	67.3	61.7	64.7	66.4	67.5	100.0	127.2
SD of Relative Cell Growth		2.9	3.3	1.9	3.6	3.5	2.7	2.7	3.4	3.5	2.6	2.9	6.0

Transformation Assay

Giemsa Staining	0.0001	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	9	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	87	96	96	96	96	96	96	96
No. of wells having foci	13	6	7	5	1	6	6	3	0	0	13	73
Transformation frequency*	0.135416667	0.0625	0.072916667	0.052083333	0.011494253	0.0625	0.0625	0.03125	0	0	0.135416667	0.760416667
SE	0.03492238	0.02470529	0.02653610	0.02267769	0.01142800	0.02470529	0.02470529	0.01775805	0.00000000	0.00000000	0.03492238	0.04356307

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Lithocholic acid
Institution:	Lab 2
Test Number	

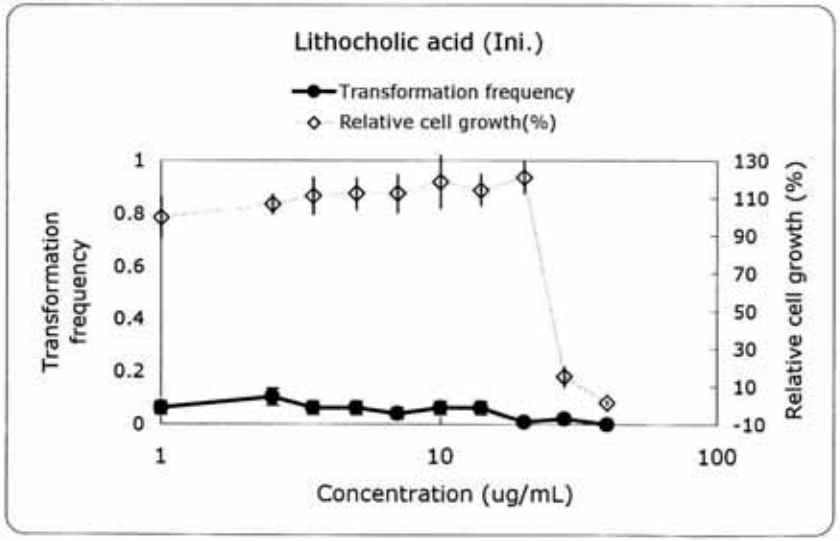
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.5	3.5	5	7	10	14	20	28	40	0.1% DMSO	MCA (1ug/mL)
1	0.052	0.236	0.269	0.261	0.292	0.300	0.305	0.284	0.316	0.086	0.060	0.236	0.190
2	0.057	0.276	0.294	0.321	0.287	0.306	0.291	0.324	0.324	0.106	0.060	0.276	0.188
3	0.060	0.275	0.279	0.296	0.320	0.302	0.332	0.295	0.325	0.085	0.060	0.275	0.168
4	0.055	0.281	0.292	0.305	0.307	0.315	0.328	0.304	0.319	0.098	0.057	0.281	0.184
5	0.055	0.280	0.285	0.283	0.295	0.289	0.318	0.307	0.312	0.083	0.055	0.280	0.184
6	0.054	0.290	0.276	0.277	0.284	0.284	0.332	0.291	0.306	0.080	0.057	0.290	0.178
7	0.052	0.247	0.272	0.297	0.279	0.285	0.268	0.287	0.294	0.086	0.060	0.247	0.208
8	0.053	0.230	0.264	0.264	0.263	0.246	0.258	0.266	0.273	0.069	0.056	0.230	0.198
Average	0.055	0.264	0.279	0.288	0.291	0.291	0.304	0.295	0.309	0.087	0.058	0.264	0.187
SD	0.003	0.023	0.011	0.021	0.017	0.021	0.029	0.017	0.018	0.011	0.002	0.023	0.012
Average - Blank	0.000	0.210	0.224	0.233	0.236	0.236	0.249	0.240	0.254	0.032	0.003	0.210	0.133
Relative Cell Growth (%)		100.0	106.9	111.3	112.6	112.6	118.9	114.5	121.1	15.2	1.6	100.0	63.2
SD of Relative Cell Growth		11.0	5.1	9.8	8.3	10.1	13.9	8.3	8.4	5.3	1.0	11.0	5.8

Transformation Assay

Giemsa Staining	1	2.5	3.5	5	7	10	14	20	28	40	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	10	6	6	4	6	6	1	2	TOX	6	52
Transformation frequency*	0.0625	0.104166667	0.0625	0.0625	0.041666667	0.0625	0.0625	0.010416667	0.020833333	#VALUE!	0.0625	0.541666667
SE	0.02470529	0.03117758	0.02470529	0.02470529	0.02039469	0.02470529	0.02470529	0.01036227	0.01457713	#VALUE!	0.02470529	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Lithocholic acid
Institution:	Lab 3
Test Number	

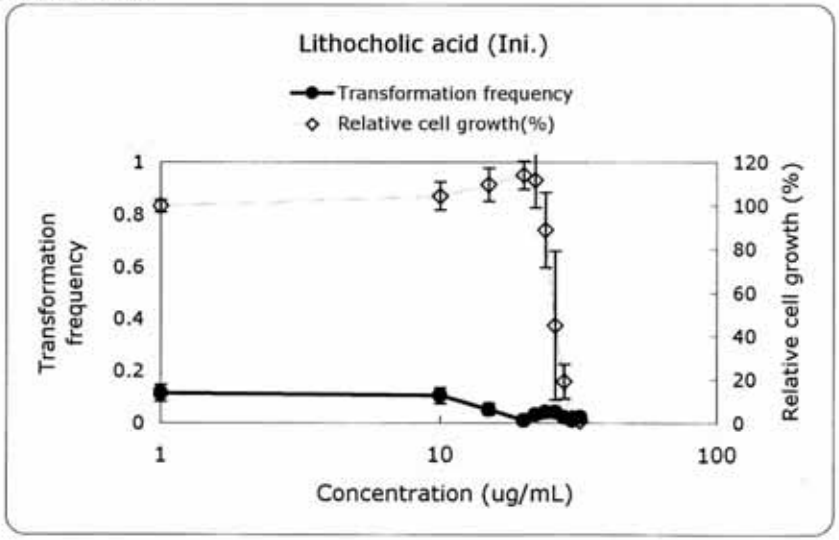
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	10	15	20	22	24	26	28	30	32	0.1% DMSO	MCA (1ug/mL)
1	0.106	1.342	1.339	1.375	1.417	1.572	1.215	1.121	0.466	0.184	0.139	1.342	0.718
2	0.113	1.364	1.390	1.354	1.575	1.611	1.437	1.165	0.358	0.164	0.128	1.364	0.838
3	0.117	1.320	1.263	1.562	1.543	1.513	1.498	0.975	0.474	0.173	0.132	1.320	0.801
4	0.116	1.297	1.361	1.593	1.609	1.522	1.332	0.885	0.374	0.132	0.126	1.297	0.734
5	0.109	1.326	1.466	1.406	1.429	1.592	1.142	0.417	0.327	0.124	0.118	1.326	0.832
6	0.112	1.383	1.484	1.358	1.576	1.167	0.914	0.357	0.300	0.133	0.115	1.383	0.775
7	0.115	1.284	1.470	1.476	1.425	1.327	1.073	0.150	0.280	0.117	0.114	1.284	0.821
8	0.119	1.352	1.327	1.502	1.481	1.501	0.971	0.211	0.186	0.115	0.116	1.352	0.713
Average	0.113	1.334	1.388	1.453	1.507	1.476	1.198	0.660	0.346	0.143	0.124	1.334	0.779
SD	0.004	0.033	0.080	0.094	0.078	0.152	0.213	0.419	0.096	0.027	0.009	0.033	0.052
Average - Blank	0.000	1.220	1.274	1.340	1.394	1.362	1.084	0.547	0.232	0.029	0.010	1.220	0.666
Relative Cell Growth (%)		100.0	104.4	109.8	114.2	111.6	88.9	44.8	19.0	2.4	0.8	100.0	54.6
SD of Relative Cell Growth		2.7	6.5	7.7	6.4	12.5	17.4	34.4	7.8	2.2	0.8	2.7	4.2

Transformation Assay

Giemsa Staining	1	10	15	20	22	24	26	28	30	32	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	1	5	7	0	0
No. of wells counted	96	96	96	96	96	96	96	95	91	89	96	96
No. of wells having foci	11	10	5	1	3	4	4	2	1	2	11	59
Transformation frequency*	0.114583333	0.104166667	0.052083333	0.010416667	0.03125	0.041666667	0.041666667	0.021052632	0.010989011	0.02247191	0.114583333	0.614583333
SE	0.03250865	0.03117758	0.02267769	0.01036227	0.01775805	0.02039469	0.02039469	0.01472893	0.01092847	0.01571049	0.03250865	0.04967296

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Lithocholic acid
Institution:	Lab 2
Test Number	

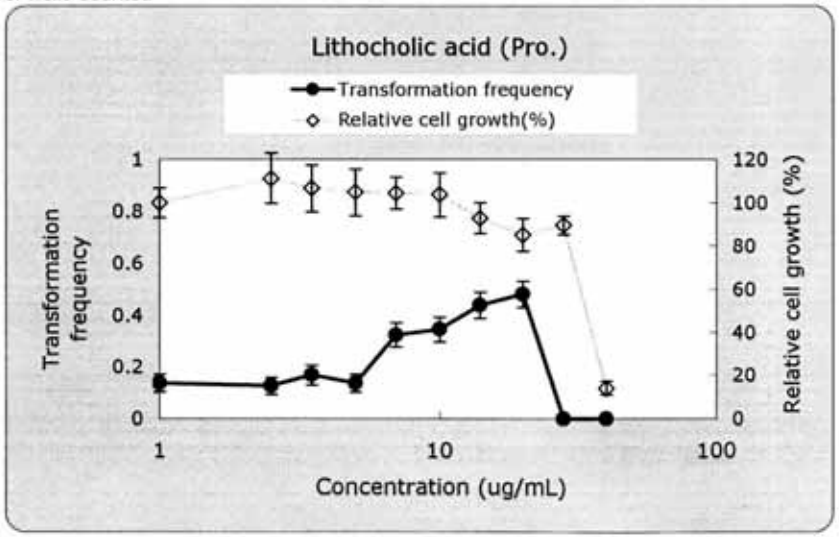
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.5	3.5	5	7	10	14	20	28	40	0.1% DMSO	TPA (50ng/mL)
1	0.052	0.306	0.280	0.267	0.268	0.295	0.293	0.268	0.249	0.293	0.081	0.306	0.392
2	0.057	0.292	0.319	0.322	0.299	0.321	0.309	0.312	0.274	0.265	0.085	0.292	0.440
3	0.060	0.304	0.346	0.329	0.335	0.323	0.344	0.293	0.249	0.294	0.089	0.304	0.417
4	0.055	0.341	0.380	0.339	0.349	0.317	0.328	0.283	0.280	0.283	0.100	0.341	0.481
5	0.055	0.317	0.333	0.355	0.338	0.334	0.345	0.299	0.303	0.266	0.095	0.317	0.482
6	0.054	0.300	0.355	0.335	0.333	0.338	0.309	0.306	0.277	0.282	0.100	0.300	0.447
7	0.052	0.306	0.321	0.333	0.325	0.324	0.326	0.283	0.265	0.276	0.091	0.306	0.457
8	0.053	0.284	0.341	0.303	0.300	0.284	0.270	0.260	0.252	0.280	0.079	0.284	0.434
Average	0.055	0.306	0.334	0.323	0.318	0.317	0.316	0.288	0.269	0.280	0.090	0.306	0.444
SD	0.003	0.017	0.029	0.027	0.027	0.019	0.026	0.018	0.019	0.011	0.008	0.017	0.031
Average - Blank	0.000	0.252	0.280	0.268	0.264	0.262	0.261	0.233	0.214	0.225	0.035	0.252	0.389
Relative Cell Growth (%)		100.0	111.2	106.6	104.8	104.3	103.7	92.7	85.0	89.5	14.0	100.0	154.7
SD of Relative Cell Growth		6.8	11.7	10.7	10.7	7.4	10.2	7.2	7.5	4.3	3.2	6.8	12.2

Transformation Assay

Giemsa Staining	1	2.5	3.5	5	7	10	14	20	28	40	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	12	16	13	31	33	42	46	0	TOX	13	48
Transformation frequency*	0.135416667	0.125	0.166666667	0.135416667	0.322916667	0.34375	0.4375	0.479166667	0	#VALUE!	0.135416667	0.5
SE	0.03492238	0.03375386	0.03803629	0.03492238	0.04772332	0.04847529	0.05063079	0.05098672	0.00000000	#VALUE!	0.03492238	0.05103104

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Lithocholic acid
Institution:	Lab 3
Test Number	

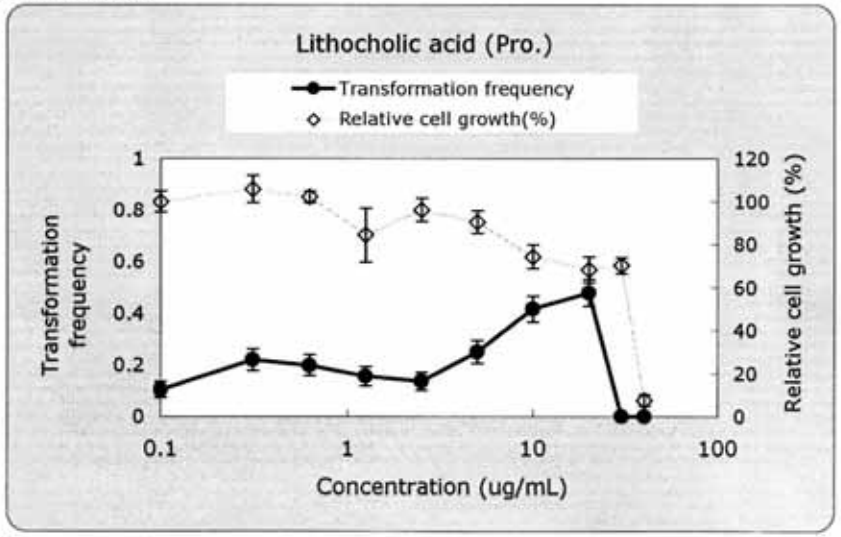
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)								Negative Control	Positive Control
		0.1	0.3125	0.625	1.25	2.5	5	10	20	30	40	0.1% DMSO	TPA (50ng/mL)
1	0.118	1.286	1.321	1.344	1.308	1.299	1.198	1.010	1.044	1.028	0.178	1.286	1.718
2	0.125	1.337	1.533	1.400	0.972	1.335	1.211	1.186	1.065	0.981	0.199	1.337	1.589
3	0.128	1.337	1.353	1.373	1.026	1.382	1.210	1.082	0.945	1.023	0.261	1.337	1.749
4	0.125	1.309	1.399	1.403	1.335	1.345	1.361	0.992	1.017	1.021	0.179	1.309	1.680
5	0.121	1.403	1.493	1.395	0.997	1.256	1.303	1.014	0.927	1.016	0.267	1.403	1.579
6	0.119	1.423	1.418	1.369	1.083	1.385	1.229	0.991	0.916	0.914	0.205	1.423	1.707
7	0.117	1.439	1.515	1.438	1.314	1.198	1.223	1.021	0.855	0.980	0.204	1.439	1.665
8	0.106	1.295	1.377	1.338	1.247	1.243	1.147	0.983	0.920	0.919	0.200	1.295	1.582
Average	0.120	1.354	1.426	1.383	1.160	1.305	1.235	1.035	0.961	0.985	0.212	1.354	1.659
SD	0.007	0.060	0.079	0.033	0.156	0.068	0.067	0.068	0.073	0.046	0.034	0.060	0.067
Average - Blank	0.000	1.234	1.306	1.263	1.040	1.186	1.115	0.915	0.841	0.865	0.092	1.234	1.539
Relative Cell Growth (%)		100.0	105.9	102.3	84.3	96.1	90.4	74.2	68.2	70.1	7.4	100.0	124.7
SD of Relative Cell Growth		4.9	6.4	2.7	12.6	5.5	5.4	5.5	5.9	3.8	2.8	4.9	5.4

Transformation Assay

Giemsa Staining	0.1	0.3125	0.625	1.25	2.5	5	10	20	30	40	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	21	19	15	13	24	40	46	0	tox	10	57
Transformation frequency*	0.104166667	0.21875	0.197916667	0.15625	0.135416667	0.25	0.416666667	0.479166667	0	#VALUE!	0.104166667	0.59375
SE	0.03117758	0.04219232	0.04066449	0.03705794	0.03492238	0.04419417	0.05031728	0.05098672	0.00000000	#VALUE!	0.03117758	0.05012598

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Methapyrilene HCl
Institution:	Lab 2
Test Number	

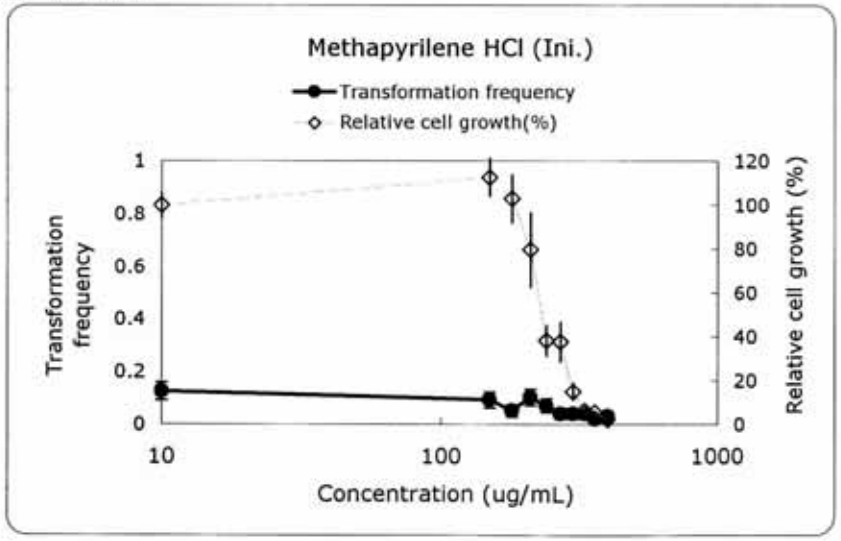
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	150	180	210	240	270	300	330	360	400	0.1% DMSO	MCA (1ug/mL)
1	0.065	0.407	0.412	0.436	0.286	0.179	0.160	0.098	0.077	0.079	0.070	0.400	0.235
2	0.062	0.409	0.480	0.398	0.340	0.197	0.190	0.116	0.089	0.080	0.071	0.346	0.286
3	0.060	0.398	0.433	0.369	0.279	0.201	0.167	0.107	0.081	0.079	0.070	0.347	0.273
4	0.059	0.432	0.471	0.402	0.469	0.181	0.173	0.097	0.080	0.079	0.069	0.400	0.259
5	0.060	0.415	0.498	0.478	0.305	0.191	0.211	0.132	0.086	0.076	0.070	0.391	0.256
6	0.060	0.409	0.458	0.480	0.355	0.203	0.196	0.127	0.089	0.081	0.070	0.396	0.258
7	0.060	0.463	0.501	0.442	0.368	0.178	0.210	0.117	0.087	0.085	0.067	0.350	0.269
8	0.065	0.421	0.459	0.428	0.367	0.254	0.261	0.132	0.084	0.087	0.067	0.347	0.249
Average	0.061	0.419	0.464	0.429	0.346	0.198	0.196	0.116	0.084	0.081	0.069	0.372	0.261
SD	0.002	0.020	0.031	0.039	0.061	0.025	0.032	0.014	0.004	0.004	0.001	0.026	0.016
Average - Blank	0.000	0.358	0.403	0.368	0.285	0.137	0.135	0.054	0.023	0.019	0.008	0.311	0.199
Relative Cell Growth (%)		100.0	112.5	102.8	79.6	38.2	37.6	15.2	6.4	5.4	2.2	100.0	64.1
SD of Relative Cell Growth		5.7	8.6	10.8	17.0	6.9	9.1	4.0	1.2	1.0	0.4	7.4	4.3

Transformation Assay

Giemsa Staining	10	150	180	210	240	270	300	330	360	400	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	9	5	10	7	4	4	4	2	3	11	68
Transformation frequency*	0.125	0.09375	0.052083333	0.104166667	0.072916667	0.041666667	0.041666667	0.041666667	0.020833333	0.03125	0.114583333	0.708333333
SE	0.03375386	0.02974911	0.02267769	0.03117758	0.02653610	0.02039469	0.02039469	0.02039469	0.01457713	0.01775805	0.03250865	0.04639024

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Methapyrilene HCl
Institution:	Lab 3
Test Number	

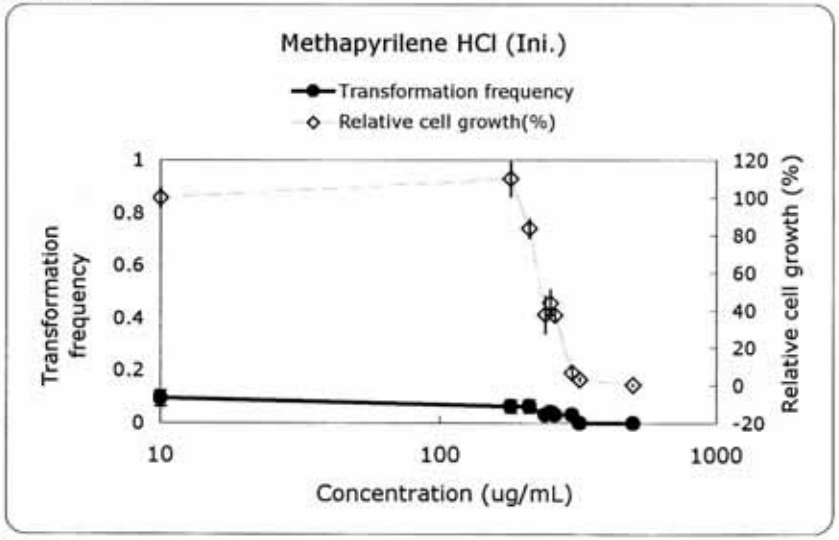
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	180	210	240	250	260	300	320	500		0.1% DMSO	MCA (1ug/mL)
1	0.122	1.247	1.553	1.054	0.528	0.600	0.523	0.223	0.152	0.126		1.276	0.705
2	0.124	1.214	1.345	1.024	0.349	0.641	0.533	0.239	0.162	0.131		1.301	0.696
3	0.123	1.231	1.253	1.087	0.454	0.487	0.586	0.197	0.158	0.128		1.308	0.739
4	0.127	1.326	1.393	1.087	0.701	0.638	0.561	0.210	0.185	0.125		1.248	0.725
5	0.123	1.389	1.464	1.139	0.568	0.667	0.520	0.227	0.164	0.123		1.320	0.833
6	0.125	1.319	1.496	1.104	0.668	0.772	0.603	0.181	0.158	0.121		1.305	0.753
7	0.118	1.276	1.264	1.026	0.600	0.598	0.549	0.170	0.138	0.117		1.274	0.803
8	0.118	1.222	1.386	1.195	0.620	0.656	0.571	0.165	0.150	0.125		1.301	0.706
Average	0.123	1.278	1.394	1.090	0.561	0.632	0.556	0.202	0.158	0.125	#DIV/0!	1.292	0.745
SD	0.003	0.062	0.107	0.058	0.116	0.080	0.030	0.028	0.014	0.004	#DIV/0!	0.024	0.049
Average - Blank	0.000	1.156	1.272	0.967	0.439	0.510	0.433	0.079	0.036	0.002	#DIV/0!	1.169	0.623
Relative Cell Growth (%)		100.0	110.1	83.7	37.9	44.1	37.5	6.8	3.1	0.2	#DIV/0!	100.0	53.2
SD of Relative Cell Growth		5.3	9.2	5.0	10.0	6.9	2.6	2.4	1.2	0.4	#DIV/0!	2.0	4.3

Transformation Assay

Giemsa Staining	10	180	210	240	250	260	300	320	500	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	1			0	0
No. of wells counted	96	96	96	96	96	96	96	95	96	96	96	96
No. of wells having foci	9	6	6	3	4	3	3	0	Tox.		12	46
Transformation frequency*	0.09375	0.0625	0.0625	0.03125	0.041666667	0.03125	0.03125	0	#VALUE!	0	0.125	0.479166667
SE	0.02974911	0.02470529	0.02470529	0.01775805	0.02039469	0.01775805	0.01775805	0.00000000	#VALUE!	0.00000000	0.03375386	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Methapyrilene HCl
Institution:	Lab 2
Test Number	

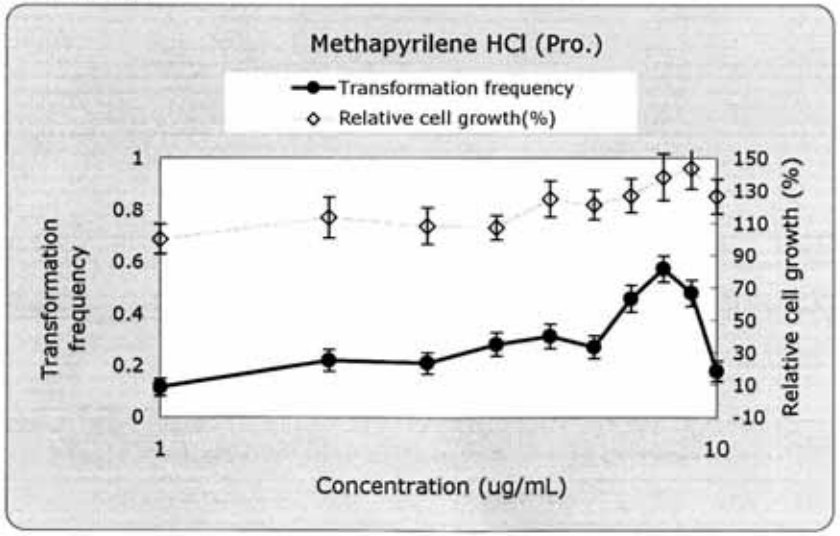
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water µg/mL	Concentrations (ug/ml) 5.9	8.8	13	20	30	44	67	100	150	Negative Control 0.1% DMSO	Positive Control TPA (50ng/mL)
1	0.104	0.342	0.356	0.365	0.365	0.424	0.405	0.395	0.503	0.467	0.481	0.319	0.420
2	0.107	0.386	0.420	0.383	0.365	0.448	0.418	0.440	0.490	0.494	0.426	0.344	0.430
3	0.104	0.395	0.401	0.416	0.388	0.404	0.442	0.441	0.514	0.513	0.437	0.361	0.448
4	0.107	0.384	0.457	0.425	0.403	0.467	0.472	0.456	0.487	0.475	0.465	0.361	0.436
5	0.097	0.389	0.432	0.416	0.406	0.460	0.402	0.481	0.403	0.464	0.446	0.350	0.447
6	0.100	0.365	0.399	0.362	0.414	0.458	0.422	0.460	0.469	0.524	0.440	0.339	0.453
7	0.095	0.331	0.392	0.397	0.381	0.411	0.428	0.414	0.458	0.510	0.406	0.333	0.436
8	0.098	0.346	0.363	0.343	0.366	0.390	0.400	0.421	0.427	0.421	0.396	0.322	0.400
Average	0.102	0.367	0.403	0.388	0.386	0.433	0.424	0.439	0.469	0.484	0.437	0.341	0.434
SD	0.005	0.025	0.034	0.030	0.020	0.029	0.024	0.028	0.038	0.034	0.028	0.016	0.017
Average - Blank	0.000	0.266	0.301	0.287	0.285	0.331	0.322	0.337	0.367	0.382	0.336	0.240	0.332
Relative Cell Growth (%)		100.0	113.3	107.9	107.1	124.6	121.2	126.8	138.2	143.7	126.3	100.0	138.7
SD of Relative Cell Growth		9.3	12.7	11.3	7.5	11.0	9.1	10.4	14.4	12.7	10.6	6.0	6.5

Transformation Assay

Giemsa Staining	µg/mL	5.9	8.8	13	20	30	44	67	100	150	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	21	20	27	30	26	44	55	46	17	12	49
Transformation frequency*	0.114583333	0.21875	0.208333333	0.28125	0.3125	0.270833333	0.458333333	0.572916667	0.479166667	0.177083333	0.125	0.510416667
SE	0.03250865	0.04219232	0.04144908	0.04588805	0.04730704	0.04535538	0.05085354	0.05048547	0.05098672	0.03896110	0.03375386	0.05101996

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Methapyrilene HCl
Institution:	Lab 3
Test Number	

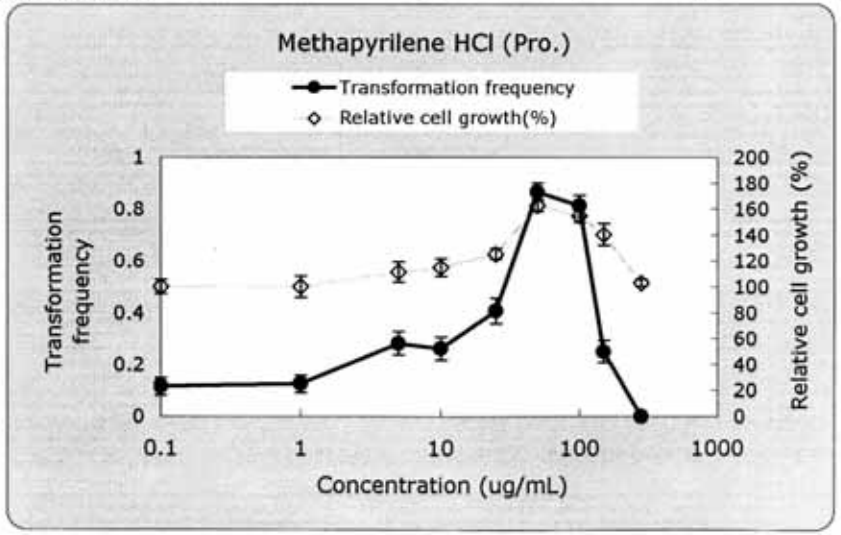
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	5	10	25	50	100	150	280		0.1% DMSO	TPA (50ng/mL)
1	0.124	1.236	1.222	1.250	1.448	1.600	2.051	1.914	1.691	1.329		1.216	1.701
2	0.134	1.266	1.301	1.467	1.372	1.540	2.023	1.958	1.757	1.349		1.331	1.521
3	0.131	1.306	1.180	1.435	1.473	1.674	2.147	1.919	1.647	1.351		1.487	1.696
4	0.129	1.303	1.299	1.499	1.475	1.651	2.053	1.881	1.807	1.344		1.419	1.801
5	0.122	1.399	1.331	1.427	1.642	1.663	2.071	1.952	1.957	1.392		1.360	1.624
6	0.122	1.288	1.304	1.568	1.432	1.545	2.022	2.020	1.845	1.286		1.334	1.678
7	0.127	1.426	1.511	1.423	1.498	1.622	2.088	2.071	1.764	1.364		1.251	1.653
8	0.113	1.278	1.359	1.498	1.564	1.606	1.968	1.990	1.873	1.390		1.396	1.691
Average	0.125	1.313	1.313	1.446	1.488	1.613	2.053	1.963	1.793	1.351	#DIV/0!	1.349	1.671
SD	0.007	0.066	0.099	0.093	0.083	0.051	0.053	0.062	0.100	0.034	#DIV/0!	0.088	0.079
Average - Blank	0.000	1.188	1.188	1.321	1.363	1.487	1.928	1.838	1.667	1.225	#DIV/0!	1.224	1.545
Relative Cell Growth (%)		100.0	100.1	111.2	114.8	125.3	162.3	154.8	140.4	103.2	#DIV/0!	100.0	126.3
SD of Relative Cell Growth		5.5	8.3	7.8	7.0	4.3	4.4	5.2	8.4	2.9	#DIV/0!	7.4	6.7

Transformation Assay

Giemsa Staining	0.1	1	5	10	25	50	100	150	280	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	12	27	25	39	83	78	24	Tox.		14	86
Transformation frequency*	0.114583333	0.125	0.28125	0.260416667	0.40625	0.864583333	0.8125	0.25	#VALUE!	0	0.145833333	0.895833333
SE	0.03250865	0.03375386	0.04588805	0.04479116	0.05012598	0.03492238	0.03983609	0.04419417	#VALUE!	0.00000000	0.03602169	0.03117758

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Mezerein
Institution:	Lab 2
Test Number	

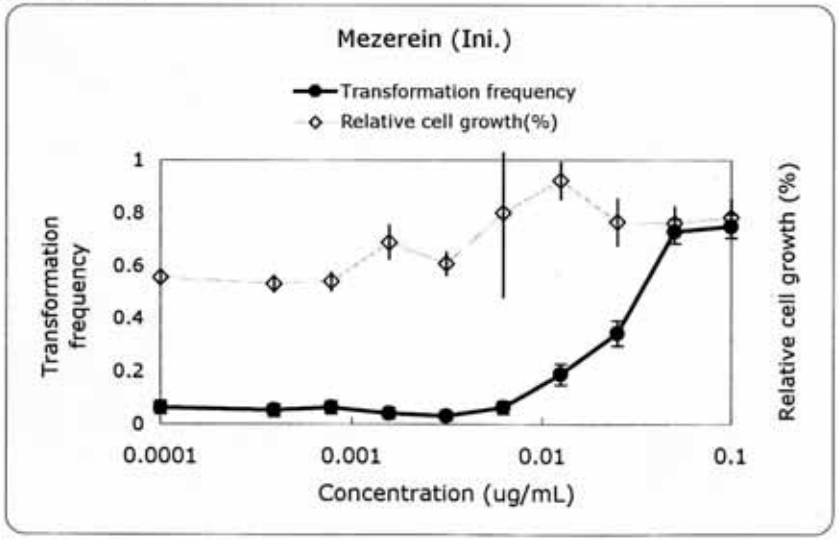
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.0001	0.000391	0.000781	0.001563	0.003125	0.00625	0.0125	0.025	0.05	0.1	0.1% DMSO	MCA (1ug/mL)
1	0.105	0.290	0.290	0.285	0.370	0.321	0.400	0.432	0.392	0.394	0.421	0.290	0.174
2	0.103	0.302	0.290	0.280	0.381	0.323	0.459	0.435	0.403	0.388	0.361	0.302	0.180
3	0.097	0.306	0.309	0.305	0.353	0.323	0.419	0.411	0.357	0.363	0.395	0.306	0.176
4	0.102	0.302	0.303	0.294	0.356	0.352	0.434	0.423	0.414	0.363	0.370	0.302	0.173
5	0.102	0.302	0.277	0.279	0.326	0.310	0.111	0.478	0.316	0.331	0.407	0.302	0.170
6	0.113	0.303	0.295	0.314	0.339	0.312	0.429	0.395	0.376	0.395	0.363	0.303	0.178
7	0.099	0.281	0.281	0.296	0.312	0.303	0.400	0.416	0.380	0.357	0.375	0.281	0.173
8	0.100	0.311	0.284	0.300	0.339	0.306	0.437	0.448	0.354	0.386	0.351	0.311	0.181
Average	0.103	0.300	0.291	0.294	0.347	0.319	0.386	0.430	0.374	0.372	0.380	0.300	0.176
SD	0.005	0.010	0.011	0.012	0.023	0.015	0.113	0.025	0.031	0.022	0.025	0.010	0.004
Average - Blank	0.000	0.197	0.189	0.192	0.244	0.216	0.284	0.327	0.271	0.270	0.278	0.197	0.073
Relative Cell Growth (%)		100.0	95.7	97.2	124.0	109.7	143.9	166.1	137.8	136.8	141.0	100.0	37.1
SD of Relative Cell Growth		4.8	5.5	6.3	11.5	7.9	57.3	12.9	15.9	11.4	12.5	4.8	1.9

Transformation Assay

Giemsa Staining	0.0001	0.000391	0.000781	0.001563	0.003125	0.00625	0.0125	0.025	0.05	0.1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	5	6	4	3	6	18	33	70	72	6	57
Transformation frequency*	0.0625	0.052083333	0.0625	0.041666667	0.03125	0.0625	0.1875	0.34375	0.729166667	0.75	0.0625	0.59375
SE	0.02470529	0.02267769	0.02470529	0.02039469	0.01775805	0.02470529	0.03983609	0.04847529	0.04535538	0.04419417	0.02470529	0.05012598

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Mezerein
Institution:	Lab 3
Test Number	

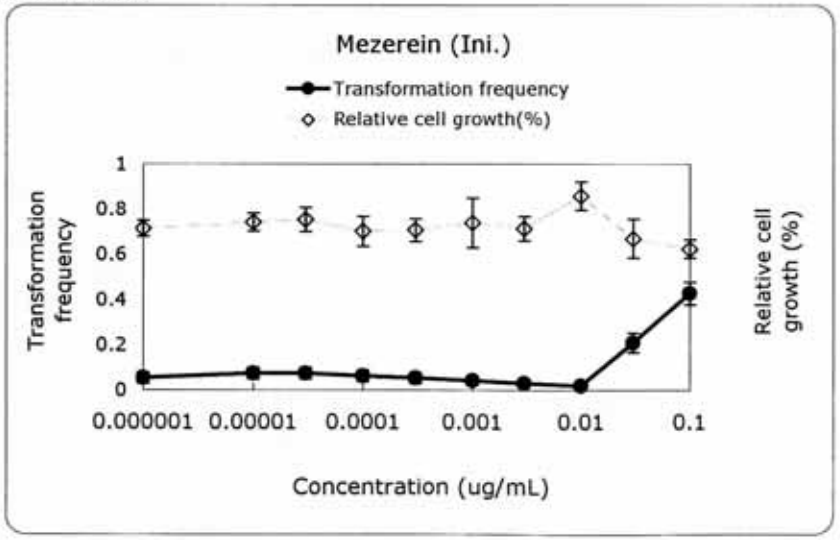
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.000001	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.1% DMSO	MCA (1ug/mL)
1	0.116	1.248	1.342	1.229	1.283	1.325	1.118	1.308	1.298	1.219	1.242	1.248	0.768
2	0.123	1.352	1.364	1.405	1.462	1.290	1.292	1.338	1.618	1.342	1.161	1.352	0.785
3	0.130	1.397	1.335	1.422	1.195	1.338	1.470	1.271	1.499	1.424	1.184	1.397	0.680
4	0.125	1.253	1.261	1.368	1.409	1.414	1.283	1.371	1.573	1.093	1.124	1.253	0.780
5	0.133	1.332	1.460	1.482	1.317	1.242	1.357	1.394	1.561	1.302	1.173	1.332	0.901
6	0.123	1.250	1.411	1.248	1.235	1.332	1.312	1.269	1.586	1.319	1.197	1.250	0.814
7	0.126	1.259	1.333	1.369	1.185	1.208	1.713	1.117	1.597	1.083	1.137	1.259	0.674
8	0.115	1.330	1.270	1.419	1.167	1.170	1.213	1.340	1.585	1.045	1.019	1.330	0.649
Average	0.124	1.303	1.347	1.368	1.282	1.290	1.345	1.301	1.540	1.228	1.155	1.303	0.756
SD	0.006	0.057	0.066	0.088	0.108	0.079	0.180	0.086	0.104	0.140	0.066	0.057	0.084
Average - Blank	0.000	1.179	1.223	1.244	1.158	1.166	1.221	1.177	1.416	1.105	1.031	1.179	0.633
Relative Cell Growth (%)		100.0	103.8	105.5	98.2	98.9	103.6	99.9	120.1	93.7	87.4	100.0	53.7
SD of Relative Cell Growth		4.9	5.6	7.4	9.2	6.7	15.3	7.3	8.8	11.9	5.6	4.9	7.2

Transformation Assay

Giemsa Staining	0.000001	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	7	7	6	5	4	3	2	20	41	5	66
Transformation frequency*	0.052083333	0.072916667	0.072916667	0.0625	0.052083333	0.041666667	0.03125	0.020833333	0.208333333	0.427083333	0.052083333	0.6875
SE	0.02267769	0.02653610	0.02653610	0.02470529	0.02267769	0.02039469	0.01775805	0.01457713	0.04144908	0.05048547	0.02267769	0.04730704

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Mezerein
Institution:	Lab 2
Test Number	

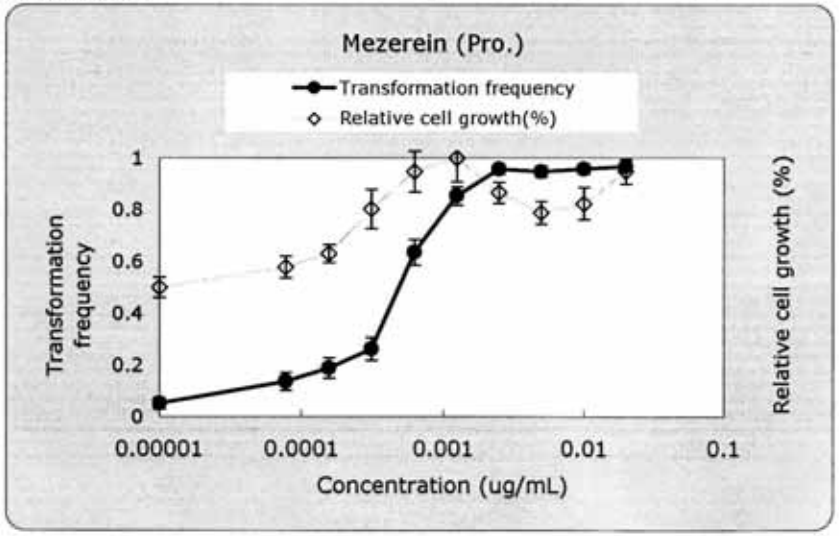
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.00001	0.000078	0.000156	0.000313	0.000625	0.00125	0.0025	0.005	0.01	0.02	0.1% DMSO	TPA (50ng/mL)
1	0.052	0.285	0.311	0.330	0.368	0.461	0.500	0.492	0.462	0.460	0.511	0.285	0.444
2	0.057	0.299	0.351	0.346	0.469	0.505	0.501	0.425	0.411	0.445	0.481	0.299	0.478
3	0.060	0.303	0.308	0.367	0.456	0.490	0.512	0.468	0.418	0.475	0.481	0.303	0.486
4	0.055	0.308	0.328	0.342	0.470	0.487	0.620	0.460	0.413	0.419	0.481	0.308	0.482
5	0.055	0.279	0.332	0.370	0.452	0.527	0.551	0.472	0.434	0.438	0.541	0.279	0.469
6	0.054	0.310	0.352	0.358	0.397	0.567	0.503	0.452	0.403	0.439	0.498	0.310	0.508
7	0.052	0.277	0.332	0.364	0.425	0.516	0.514	0.457	0.414	0.387	0.527	0.277	0.441
8	0.053	0.258	0.299	0.331	0.422	0.452	0.497	0.470	0.449	0.473	0.486	0.258	0.416
Average	0.055	0.290	0.327	0.351	0.432	0.501	0.525	0.462	0.426	0.442	0.501	0.290	0.466
SD	0.003	0.018	0.019	0.016	0.036	0.037	0.042	0.019	0.021	0.029	0.023	0.018	0.030
Average - Blank	0.000	0.235	0.272	0.296	0.378	0.446	0.470	0.407	0.371	0.387	0.446	0.235	0.411
Relative Cell Growth (%)		100.0	115.6	126.0	160.6	189.6	199.9	173.2	157.7	164.7	189.7	100.0	174.7
SD of Relative Cell Growth		7.7	8.3	6.8	15.5	15.7	17.9	8.2	8.8	12.4	9.9	7.7	12.6

Transformation Assay

Giemsa Staining	0.00001	0.000078	0.000156	0.000313	0.000625	0.00125	0.0025	0.005	0.01	0.02	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	13	18	25	61	82	92	91	92	93	5	46
Transformation frequency*	0.052083333	0.135416667	0.1875	0.260416667	0.635416667	0.854166667	0.958333333	0.947916667	0.958333333	0.96875	0.052083333	0.479166667
SE	0.02267769	0.03492238	0.03983609	0.04479116	0.04912382	0.03602169	0.02039469	0.02267769	0.02039469	0.01775805	0.02267769	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Mezerein
Institution:	Lab 3
Test Number	

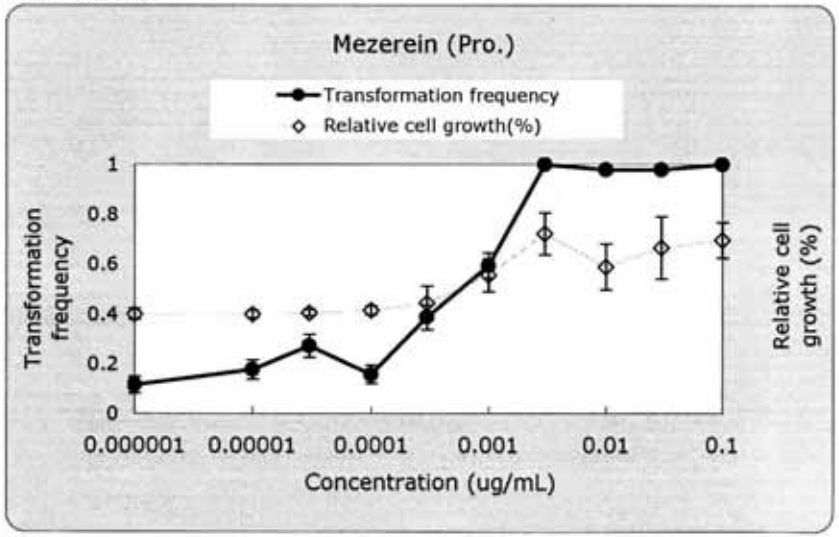
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.000001	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.1% DMSO	TPA (50ng/mL)
1	0.123	1.326	1.285	1.360	1.244	1.580	2.101	1.650	2.089	2.524	1.712	1.326	1.825
2	0.134	1.332	1.281	1.272	1.311	1.526	1.783	2.274	2.028	2.379	2.426	1.332	1.837
3	0.138	1.310	1.298	1.316	1.350	1.478	1.882	2.327	1.952	2.231	2.150	1.310	1.734
4	0.134	1.305	1.239	1.303	1.321	0.971	1.470	2.180	1.824	2.076	2.290	1.305	1.901
5	0.126	1.302	1.267	1.341	1.411	1.541	1.781	2.365	1.907	2.085	2.192	1.302	1.627
6	0.128	1.354	1.396	1.343	1.346	1.505	1.656	2.313	1.912	1.943	2.206	1.354	1.578
7	0.127	1.312	1.314	1.339	1.405	1.486	1.751	2.452	1.854	2.073	2.092	1.312	1.618
8	0.113	1.143	1.297	1.230	1.353	1.304	1.586	2.339	1.211	1.293	2.226	1.143	1.539
Average	0.128	1.298	1.297	1.313	1.343	1.424	1.751	2.238	1.847	2.076	2.162	1.298	1.707
SD	0.008	0.065	0.046	0.044	0.053	0.200	0.192	0.250	0.271	0.368	0.207	0.065	0.135
Average - Blank	0.000	1.170	1.169	1.185	1.215	1.296	1.623	2.110	1.719	1.948	2.034	1.170	1.580
Relative Cell Growth (%)		100.0	99.9	101.3	103.8	110.8	138.7	180.3	146.9	166.4	173.8	100.0	135.0
SD of Relative Cell Growth		5.5	3.9	3.7	4.6	17.1	16.4	21.3	23.2	31.4	17.7	5.5	11.6

Transformation Assay

Giemsa Staining	0.000001	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	17	26	15	37	57	96	94	94	96	11	48
Transformation frequency*	0.114583333	0.177083333	0.270833333	0.15625	0.385416667	0.59375	1	0.979166667	0.979166667	1	0.114583333	0.5
SE	0.03250865	0.03896110	0.04535538	0.03705794	0.04967296	0.05012598	0.00000000	0.01457713	0.01457713	0.00000000	0.03250865	0.05103104
					*	*	*	*	*	*		

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MNNG
Institution:	Lab 1
Test Number	

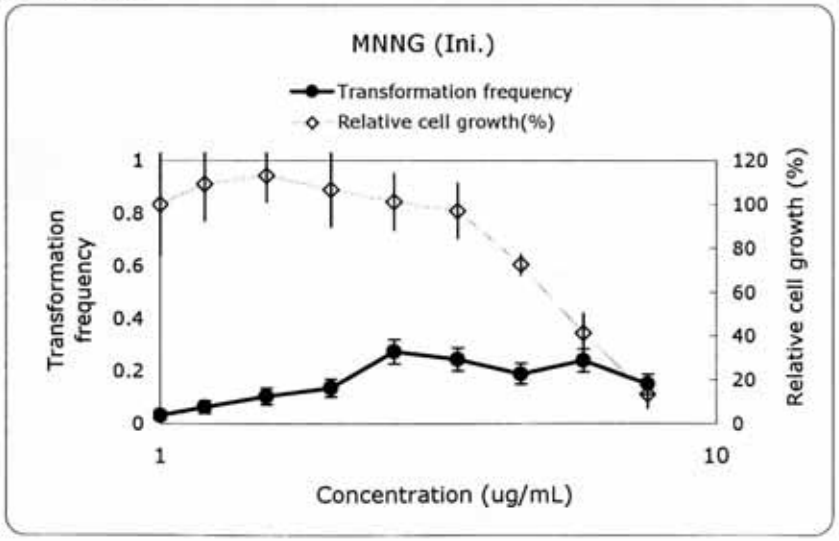
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		1	1.2	1.55	2.02	2.62	3.41	4.43	5.75	7.5		0.1% DMSO	MCA (1ug/mL)
1	0.090	0.733	0.758	0.659	0.777	0.728	0.769	0.515	0.321	0.144		0.681	0.323
2	0.096	0.638	0.814	0.731	0.795	0.756	0.685	0.509	0.332	0.154		0.740	0.341
3	0.088	0.480	0.757	0.796	0.710	0.686	0.684	0.551	0.438	0.181		0.700	0.364
4	0.090	0.607	0.578	0.776	0.627	0.695	0.609	0.463	0.263	0.172		0.789	0.340
5	0.087	0.554	0.576	0.692	0.573	0.640	0.633	0.518	0.315	0.127		0.769	0.300
6	0.089	0.610	0.663	0.695	0.594	0.517	0.597	0.486	0.331	0.150		0.772	0.350
7	0.086	0.809	0.797	0.694	0.699	0.641	0.528	0.487	0.278	0.248		0.684	0.343
8	0.089	0.877	0.790	0.867	0.832	0.696	0.663	0.519	0.326	0.159		0.776	0.298
Average	0.089	0.664	0.717	0.739	0.701	0.670	0.646	0.506	0.326	0.167	#DIV/0!	0.739	0.332
SD	0.003	0.133	0.097	0.069	0.096	0.073	0.072	0.027	0.052	0.037	#DIV/0!	0.044	0.024
Average - Blank	0.000	0.574	0.627	0.649	0.612	0.581	0.557	0.417	0.236	0.078	#DIV/0!	0.650	0.243
Relative Cell Growth (%)		100.0	109.3	113.1	106.5	101.1	97.0	72.6	41.1	13.5	#DIV/0!	100.0	37.4
SD of Relative Cell Growth		23.2	17.0	12.1	16.8	12.7	12.5	4.7	9.1	6.4	#DIV/0!	7.7	4.1

Transformation Assay

Giemsa Staining	1	1.2	1.55	2.02	2.62	3.41	4.43	5.75	7.5	0	Negative Control	Positive Control
No. of wells excluded	0	0	1	0	1	2	1	0	2		0	2
No. of wells counted	96	96	95	96	95	94	95	96	94	96	96	94
No. of wells having foci	3	6	10	13	26	23	18	23	14		9	44
Transformation frequency*	0.03125	0.0625	0.105263158	0.135416667	0.273684211	0.244680851	0.189473684	0.239583333	0.14893617	0	0.09375	0.468085106
SE	0.01775805	0.02470529	0.03148648	0.03492238	0.04574312	0.04434057	0.04020649	0.04356307	0.03672123	0.00000000	0.02974911	0.05146590

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	MNNG
Institution:	Lab 3
Test Number	

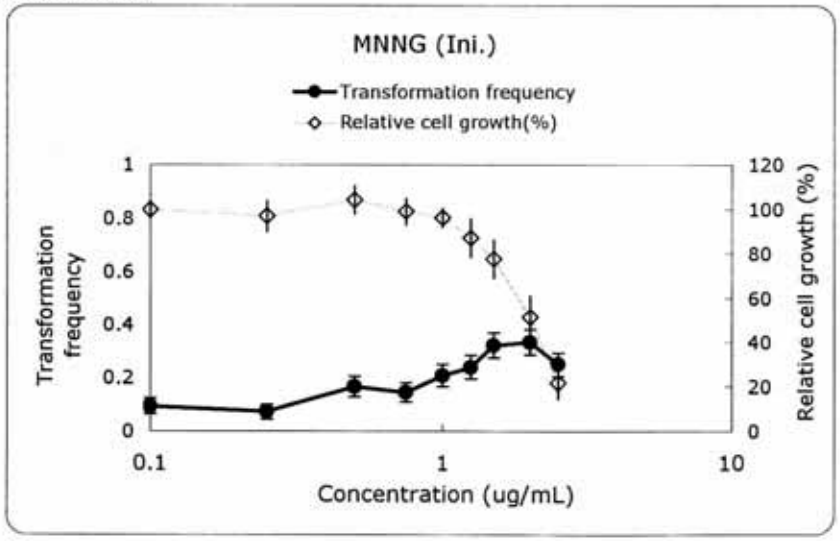
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.25	0.5	0.75	1	1.25	1.5	2	2.5		0.1% DMSO	MCA (1ug/mL)
1	0.075	1.170	1.211	1.286	1.169	1.113	1.112	0.898	0.685	0.310		1.109	0.580
2	0.079	1.249	1.040	1.245	1.129	1.145	1.025	0.738	0.575	0.389		1.094	0.551
3	0.083	1.112	1.250	1.139	1.134	1.133	0.888	0.881	0.541	0.319		1.192	0.573
4	0.085	1.188	1.170	1.225	1.223	1.194	1.167	0.999	0.557	0.319		1.181	0.606
5	0.092	1.141	1.166	1.234	1.212	1.107	1.012	0.933	0.749	0.381		1.243	0.662
6	0.091	1.172	1.078	1.098	1.044	1.159	0.935	1.027	0.777	0.153		1.095	0.559
7	0.093	1.155	1.157	1.297	1.146	1.038	1.108	0.962	0.749	0.395		1.182	0.779
8	0.094	1.197	1.052	1.222	1.243	1.156	1.023	1.000	0.549	0.327		1.108	0.612
Average	0.087	1.173	1.141	1.218	1.163	1.131	1.034	0.930	0.648	0.324	#DIV/0!	1.151	0.615
SD	0.007	0.041	0.076	0.068	0.064	0.046	0.093	0.093	0.102	0.077	#DIV/0!	0.056	0.075
Average - Blank	0.000	1.087	1.054	1.132	1.076	1.044	0.947	0.843	0.561	0.238	#DIV/0!	1.064	0.529
Relative Cell Growth (%)		100.0	97.0	104.2	99.0	96.1	87.2	77.6	51.7	21.9	#DIV/0!	100.0	49.7
SD of Relative Cell Growth		3.8	7.0	6.3	5.9	4.3	8.6	8.5	9.4	7.1	#DIV/0!	5.2	6.9

Transformation Assay

Giemsa Staining	0.1	0.25	0.5	0.75	1	1.25	1.5	2	2.5	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	7	16	14	20	23	31	32	24		8	63
Transformation frequency*	0.09375	0.072916667	0.166666667	0.145833333	0.208333333	0.239583333	0.322916667	0.333333333	0.25	0	0.083333333	0.65625
SE	0.02974911	0.02653610	0.03803629	0.03602169	0.04144908	0.04356307	0.04772332	0.04811252	0.04419417	0.00000000	0.02820847	0.04847529
						*	*	*	*			

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MNNG
Institution:	Lab 1
Test Number	

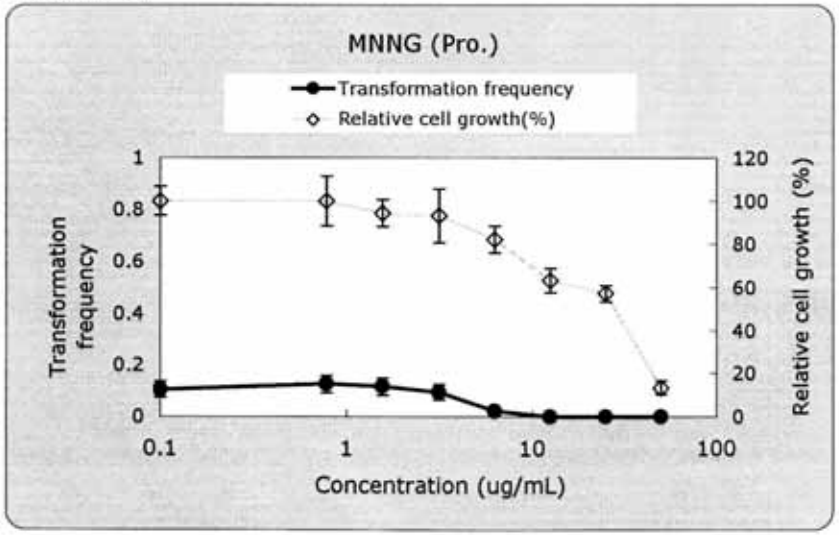
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.78	1.56	3.13	6.26	12.5	25	50			0.1% DMSO	TPA (50ng/mL)
1	0.068	0.570	0.502	0.561	0.483	0.463	0.377	0.373	0.179			0.568	0.747
2	0.073	0.630	0.595	0.552	0.622	0.530	0.403	0.359	0.155			0.624	0.801
3	0.071	0.668	0.652	0.624	0.555	0.497	0.443	0.373	0.121			0.563	0.876
4	0.077	0.629	0.636	0.650	0.597	0.569	0.465	0.404	0.149			0.620	0.859
5	0.074	0.662	0.677	0.586	0.511	0.537	0.387	0.411	0.141			0.630	0.935
6	0.077	0.669	0.719	0.570	0.640	0.538	0.436	0.390	0.136			0.607	0.880
7	0.071	0.610	0.612	0.615	0.623	0.538	0.436	0.407	0.157			0.664	0.832
8	0.074	0.590	0.632	0.614	0.687	0.561	0.450	0.412	0.143			0.696	0.948
Average	0.073	0.629	0.628	0.597	0.590	0.529	0.425	0.391	0.148	#DIV/0!	#DIV/0!	0.622	0.860
SD	0.003	0.037	0.064	0.034	0.069	0.034	0.032	0.020	0.017	#DIV/0!	#DIV/0!	0.045	0.067
Average - Blank	0.000	0.555	0.555	0.523	0.517	0.456	0.352	0.318	0.075	#DIV/0!	#DIV/0!	0.548	0.787
Relative Cell Growth (%)		100.0	99.9	94.2	93.0	82.1	63.3	57.3	13.4	#DIV/0!	#DIV/0!	100.0	143.4
SD of Relative Cell Growth		6.7	11.5	6.2	12.4	6.2	5.7	3.7	3.1	#DIV/0!	#DIV/0!	8.0	12.0

Transformation Assay

Giemsa Staining	0.1	0.78	1.56	3.13	6.26	12.5	25	50	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	1	0	0	96			1	0
No. of wells counted	96	96	96	96	95	96	96	0	96	96	95	96
No. of wells having foci	10	12	11	9	2	0	0	TOX			12	57
Transformation frequency*	0.104166667	0.125	0.114583333	0.09375	0.021052632	0	0	#VALUE!	0	0	0.126315789	0.59375
SE	0.03117758	0.03375386	0.03250865	0.02974911	0.01472893	0.00000000	0.00000000	#VALUE!	0.00000000	0.00000000	0.03408351	0.05012598

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	MNNG
Institution:	Lab 3
Test Number	

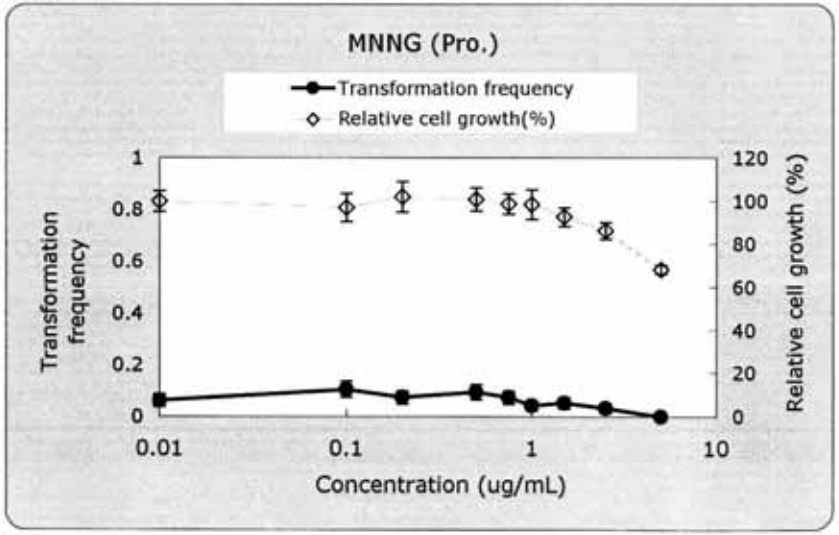
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.1	0.2	0.5	0.75	1	1.5	2.5	5		0.1% DMSO	TPA (50ng/mL)
1	0.116	1.173	1.131	1.206	1.229	1.223	1.227	1.162	1.116	0.924		1.271	1.595
2	0.122	1.271	1.288	1.314	1.290	1.261	1.099	1.117	1.046	0.927		1.260	1.530
3	0.123	1.255	1.222	1.277	1.258	1.285	1.304	1.220	1.164	0.866		1.216	1.738
4	0.128	1.354	1.293	1.249	1.196	1.181	1.215	1.275	1.086	0.893		1.287	1.752
5	0.123	1.310	1.300	1.402	1.343	1.247	1.318	1.174	1.104	0.923		1.269	1.727
6	0.124	1.267	1.328	1.213	1.281	1.265	1.263	1.170	1.164	0.916		1.365	1.870
7	0.121	1.269	1.254	1.429	1.321	1.367	1.325	1.235	1.111	0.945		1.198	1.780
8	0.117	1.329	1.136	1.320	1.381	1.286	1.331	1.205	1.178	0.898		1.507	1.801
Average	0.122	1.279	1.244	1.301	1.287	1.264	1.260	1.195	1.121	0.912	#DIV/0!	1.297	1.724
SD	0.004	0.055	0.075	0.082	0.061	0.054	0.079	0.049	0.045	0.025	#DIV/0!	0.099	0.111
Average - Blank	0.000	1.157	1.122	1.180	1.166	1.143	1.139	1.073	0.999	0.790	#DIV/0!	1.175	1.602
Relative Cell Growth (%)		100.0	97.0	102.0	100.8	98.8	98.4	92.8	86.4	68.3	#DIV/0!	100.0	136.4
SD of Relative Cell Growth		4.8	6.5	7.1	5.2	4.7	6.8	4.3	3.9	2.1	#DIV/0!	8.5	9.6

Transformation Assay

Giemsa Staining	0.01	0.1	0.2	0.5	0.75	1	1.5	2.5	5	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	10	7	9	7	4	5	3	0		7	62
Transformation frequency*	0.0625	0.104166667	0.072916667	0.09375	0.072916667	0.041666667	0.052083333	0.03125	0	0	0.072916667	0.645833333
SE	0.02470529	0.03117758	0.02653610	0.02974911	0.02653610	0.02039469	0.02267769	0.01775805	0.00000000	0.00000000	0.02653610	0.04881221

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Sodium arsenite
Institution:	Lab 1
Test Number	

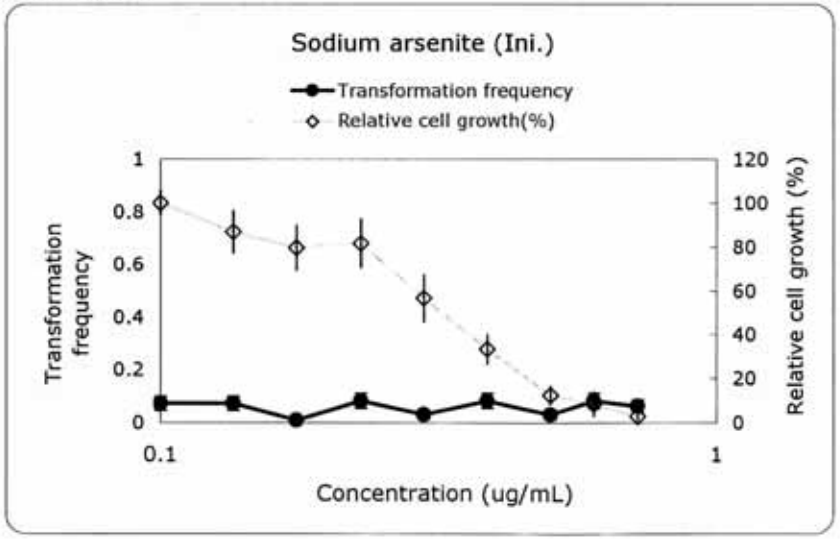
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.135	0.175	0.228	0.296	0.385	0.5	0.6	0.72		0.1% DMSO	MCA (1ug/mL)
1	0.109	0.873	0.794	0.777	0.809	0.687	0.439	0.259	0.261	0.149		0.808	0.300
2	0.119	0.882	0.774	0.752	0.844	0.566	0.421	0.222	0.198	0.144		0.912	0.391
3	0.112	0.820	0.775	0.753	0.685	0.590	0.303	0.196	0.132	0.137		0.805	0.327
4	0.109	0.889	0.851	0.723	0.630	0.498	0.375	0.228	0.183	0.154		0.851	0.350
5	0.112	0.861	0.662	0.576	0.605	0.503	0.361	0.186	0.156	0.125		0.869	0.387
6	0.110	0.844	0.805	0.713	0.721	0.455	0.321	0.177	0.160	0.136		0.827	0.368
7	0.123	0.765	0.732	0.727	0.718	0.488	0.314	0.214	0.147	0.112		1.083	0.312
8	0.123	0.882	0.650	0.593	0.721	0.465	0.356	0.171	0.185	0.133		0.963	0.277
Average	0.115	0.852	0.755	0.702	0.717	0.532	0.361	0.207	0.178	0.136	#DIV/0!	0.890	0.339
SD	0.006	0.042	0.070	0.075	0.081	0.078	0.049	0.030	0.040	0.013	#DIV/0!	0.095	0.042
Average - Blank	0.000	0.737	0.641	0.587	0.602	0.417	0.247	0.092	0.063	0.022	#DIV/0!	0.775	0.224
Relative Cell Growth (%)		100.0	86.9	79.6	81.6	56.5	33.4	12.5	8.6	2.9	#DIV/0!	100.0	28.9
SD of Relative Cell Growth		5.7	9.5	10.2	11.0	10.6	6.7	4.0	5.4	1.8	#DIV/0!	12.9	5.7

Transformation Assay

Giemsa Staining	0.1	0.135	0.175	0.228	0.296	0.385	0.5	0.6	0.72	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	1		0	0
No. of wells counted	96	96	96	96	96	96	96	96	95	96	96	96
No. of wells having foci	7	7	1	8	3	8	3	8	6		9	41
Transformation frequency*	0.072916667	0.072916667	0.010416667	0.083333333	0.03125	0.083333333	0.03125	0.083333333	0.063157895	0	0.09375	0.427083333
SE	0.02653610	0.02653610	0.01036227	0.02820847	0.01775805	0.02820847	0.01775805	0.02820847	0.02495659	0.00000000	0.02974911	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Sodium arsenite
Institution:	Lab 3
Test Number	

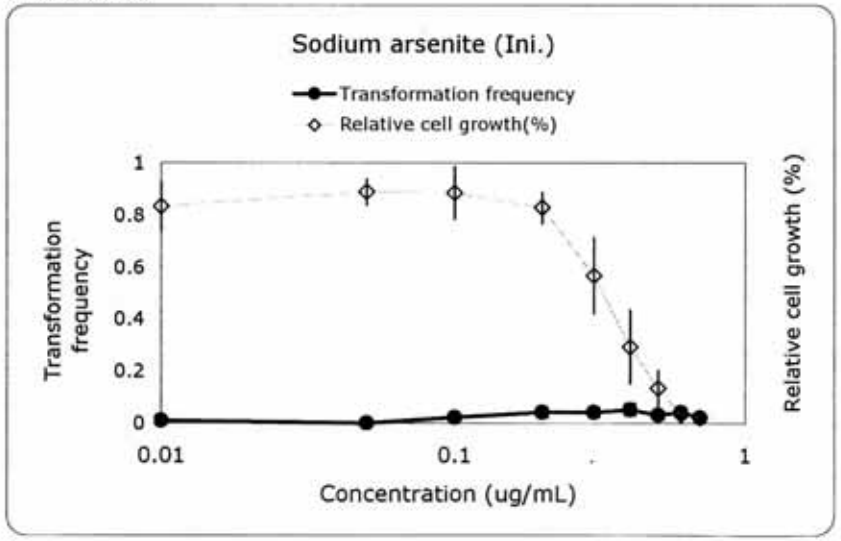
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7		0.1% DMSO	MCA (1ug/mL)
1	0.073	0.976	1.063	1.216	1.226	0.848	0.743	0.285	0.148	0.136		1.149	0.679
2	0.074	1.093	1.135	1.192	1.069	0.545	0.677	0.217	0.105	0.084		1.206	0.719
3	0.075	1.073	1.199	1.053	1.035	1.000	0.415	0.271	0.101	0.092		1.189	0.696
4	0.077	1.355	1.216	1.087	1.022	0.641	0.358	0.188	0.113	0.096		1.193	0.669
5	0.077	1.143	1.175	1.215	1.026	0.890	0.360	0.420	0.113	0.127		1.157	0.588
6	0.076	1.031	1.158	1.375	1.143	0.989	0.353	0.179	0.125	0.086		1.136	0.538
7	0.079	1.026	1.131	1.187	1.072	0.636	0.261	0.171	0.105	0.113		1.113	0.723
8	0.079	1.096	1.266	0.978	1.151	0.632	0.323	0.197	0.129	0.083		1.194	0.522
Average	0.076	1.099	1.168	1.163	1.093	0.773	0.436	0.241	0.117	0.102	#DIV/0!	1.167	0.642
SD	0.002	0.115	0.062	0.122	0.073	0.180	0.175	0.084	0.016	0.021	#DIV/0!	0.033	0.081
Average - Blank	0.000	1.023	1.092	1.087	1.017	0.696	0.360	0.165	0.041	0.026	#DIV/0!	1.091	0.566
Relative Cell Growth (%)		100.0	106.7	106.2	99.4	68.1	35.2	16.1	4.0	2.5	#DIV/0!	100.0	51.8
SD of Relative Cell Growth		11.3	6.0	11.9	7.2	17.5	17.1	8.2	1.5	2.0	#DIV/0!	3.2	7.9

Transformation Assay

Giemsa Staining	0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7		Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	1	0	2	4	4	5	3	4	2		3	52
Transformation frequency*	0.010416667	0	0.020833333	0.041666667	0.041666667	0.052083333	0.03125	0.041666667	0.020833333	0	0.03125	0.541666667
SE	0.01036227	0.00000000	0.01457713	0.02039469	0.02039469	0.02267769	0.01775805	0.02039469	0.01457713	0.00000000	0.01775805	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<15 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Sodium arsenite
Institution:	Lab 1
Test Number	

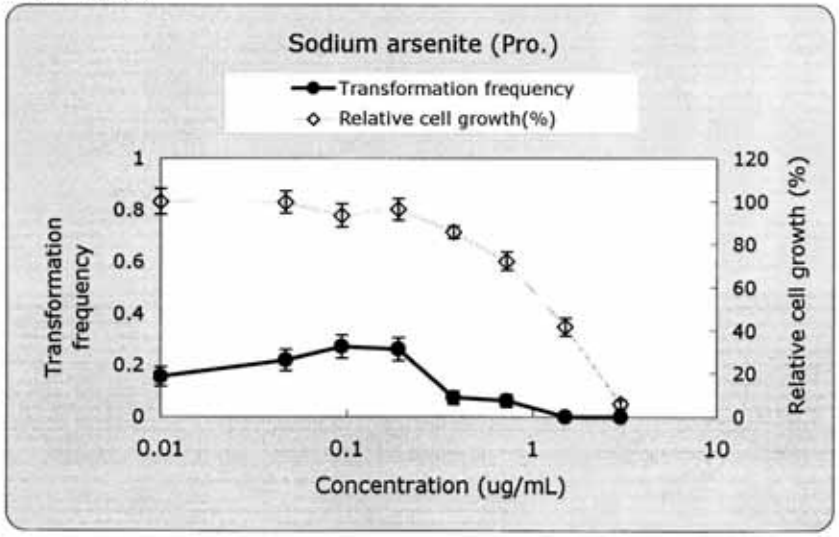
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.0469	0.0938	0.188	0.375	0.725	1.5	3			0.1% DMSO	TPA (50ng/mL)
1	0.107	0.707	0.754	0.697	0.730	0.617	0.564	0.399	0.146			0.699	1.039
2	0.114	0.736	0.702	0.638	0.630	0.618	0.542	0.327	0.148			0.804	1.183
3	0.105	0.666	0.698	0.676	0.658	0.629	0.526	0.343	0.130			0.750	1.026
4	0.110	0.738	0.692	0.626	0.685	0.614	0.520	0.336	0.135			0.688	1.272
5	0.112	0.665	0.646	0.688	0.701	0.628	0.525	0.364	0.143			0.719	1.101
6	0.119	0.713	0.706	0.636	0.671	0.595	0.498	0.364	0.166			0.745	1.127
7	0.116	0.652	0.681	0.638	0.682	0.592	0.550	0.380	0.148			0.697	1.203
8	0.112	0.736	0.720	0.706	0.686	0.641	0.577	0.343	0.151			0.702	1.163
Average	0.112	0.702	0.700	0.663	0.680	0.617	0.538	0.357	0.146	#DIV/0!	#DIV/0!	0.726	1.139
SD	0.005	0.036	0.031	0.032	0.029	0.017	0.026	0.024	0.011	#DIV/0!	#DIV/0!	0.039	0.083
Average - Blank	0.000	0.590	0.588	0.551	0.569	0.505	0.426	0.245	0.034	#DIV/0!	#DIV/0!	0.614	1.027
Relative Cell Growth (%)		100.0	99.7	93.5	96.4	85.6	72.2	41.6	5.8	#DIV/0!	#DIV/0!	100.0	167.4
SD of Relative Cell Growth		6.1	5.2	5.4	5.0	2.8	4.3	4.1	1.8	#DIV/0!	#DIV/0!	6.6	14.1

Transformation Assay

Giemsa Staining	0.01	0.0469	0.0938	0.188	0.375	0.725	1.5	3	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	43	96			0	0
No. of wells counted	96	96	96	96	96	96	53	0	96	96	96	96
No. of wells having foci	15	21	26	25	7	6	TOX	TOX			14	72
Transformation frequency*	0.15625	0.21875	0.270833333	0.260416667	0.072916667	0.0625	#VALUE!	#VALUE!	0	0	0.145833333	0.75
SE	0.03705794	0.04219232	0.04535538	0.04479116	0.02653610	0.02470529	#VALUE!	#VALUE!	0.00000000	0.00000000	0.03602169	0.04419417

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Sodium arsenite
Institution:	Lab 3
Test Number	

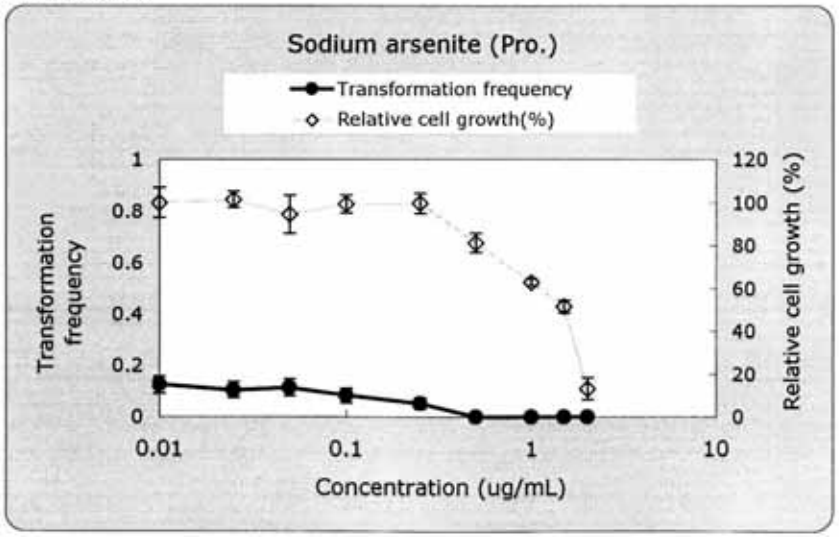
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.01	0.025	0.05	0.1	0.25	0.5	1	1.5	2		0.1% DMSO	TPA (50ng/mL)
1	0.078	1.147	1.142	0.942	1.220	1.099	1.032	0.774	0.611	0.142		0.954	1.723
2	0.078	1.034	1.194	1.166	1.107	1.132	0.971	0.775	0.610	0.151		0.979	1.599
3	0.081	1.166	1.228	0.998	1.157	1.140	1.027	0.780	0.685	0.279		1.024	1.595
4	0.083	1.275	1.125	1.165	1.209	1.176	0.937	0.762	0.613	0.288		0.994	1.538
5	0.082	1.228	1.224	1.111	1.213	1.220	0.915	0.770	0.659	0.233		1.061	1.703
6	0.081	1.231	1.175	1.174	1.119	1.206	0.931	0.778	0.664	0.215		1.039	1.642
7	0.084	1.181	1.236	1.114	1.171	1.245	1.005	0.778	0.678	0.277		1.050	1.672
8	0.081	1.117	1.182	1.229	1.126	1.122	0.913	0.726	0.624	0.204		0.968	1.612
Average	0.081	1.172	1.188	1.112	1.165	1.168	0.966	0.768	0.643	0.224	#DIV/0!	1.009	1.636
SD	0.002	0.076	0.041	0.096	0.045	0.052	0.049	0.018	0.032	0.057	#DIV/0!	0.040	0.062
Average - Blank	0.000	1.091	1.107	1.031	1.084	1.087	0.885	0.687	0.562	0.143	#DIV/0!	0.928	1.555
Relative Cell Growth (%)		100.0	101.5	94.5	99.3	99.6	81.1	62.9	51.5	13.1	#DIV/0!	100.0	167.6
SD of Relative Cell Growth		6.9	3.7	8.8	4.2	4.8	4.5	1.6	2.9	5.2	#DIV/0!	3.7	5.7

Transformation Assay

Giemsa Staining	0.01	0.025	0.05	0.1	0.25	0.5	1	1.5	2		Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0					0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	10	11	8	5	0	Tox	Tox	Tox		7	68
Transformation frequency*	0.125	0.104166667	0.114583333	0.083333333	0.052083333	0	#VALUE!	#VALUE!	#VALUE!	0	0.072916667	0.708333333
SE	0.03375386	0.03117758	0.03250865	0.02820847	0.02267769	0.00000000	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.02653610	0.04639024

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Ampicillin sodium
Institution:	Lab 1
Test Number	

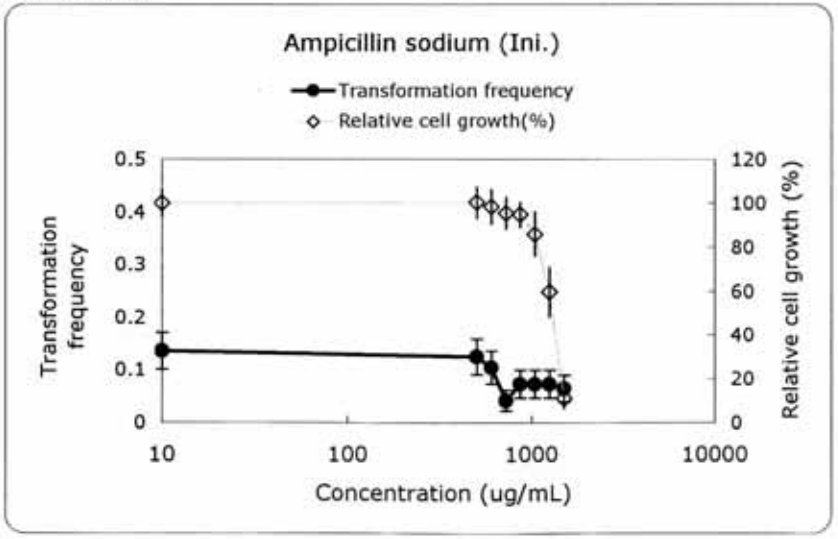
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	502	603	724	868	1040	1250	1500			0.1% DMSO	MCA (1ug/mL)
1	0.137	0.692	0.736	0.701	0.690	0.696	0.641	0.554	0.234			0.677	0.392
2	0.133	0.694	0.708	0.624	0.632	0.622	0.618	0.506	0.167			0.660	0.374
3	0.129	0.697	0.609	0.663	0.646	0.665	0.522	0.367	0.178			0.683	0.359
4	0.132	0.663	0.699	0.656	0.660	0.677	0.661	0.466	0.231			0.674	0.379
5	0.137	0.647	0.678	0.642	0.654	0.614	0.552	0.451	0.192			0.716	0.333
6	0.140	0.700	0.697	0.698	0.597	0.644	0.573	0.423	0.223			0.686	0.345
7	0.151	0.659	0.717	0.685	0.710	0.692	0.665	0.432	0.178			0.717	0.325
8	0.159	0.756	0.669	0.761	0.714	0.660	0.655	0.529	0.203			0.711	0.341
Average	0.140	0.689	0.689	0.679	0.663	0.659	0.611	0.466	0.201	#DIV/0!	#DIV/0!	0.691	0.356
SD	0.010	0.034	0.039	0.043	0.040	0.030	0.055	0.061	0.026	#DIV/0!	#DIV/0!	0.021	0.024
Average - Blank	0.000	0.549	0.549	0.539	0.523	0.519	0.471	0.326	0.061	#DIV/0!	#DIV/0!	0.551	0.216
Relative Cell Growth (%)		100.0	100.1	98.2	95.3	94.6	85.9	59.5	11.1	#DIV/0!	#DIV/0!	100.0	39.3
SD of Relative Cell Growth		6.2	7.0	7.8	7.3	5.5	10.0	11.2	4.8	#DIV/0!	#DIV/0!	3.9	4.4

Transformation Assay

Giemsa Staining	10	502	603	724	868	1040	1250	1500	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	2			0	0
No. of wells counted	96	96	96	96	96	96	96	94	96	96	96	96
No. of wells having foci	13	12	10	4	7	7	7	6			12	48
Transformation frequency*	0.135416667	0.125	0.104166667	0.041666667	0.072916667	0.072916667	0.072916667	0.063829787	0	0	0.125	0.5
SE	0.03492238	0.03375386	0.03117758	0.02039469	0.02653610	0.02653610	0.02653610	0.02521304	0.00000000	0.00000000	0.03375386	0.05103104

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Ampicillin sodium
Institution:	Lab 3
Test Number	

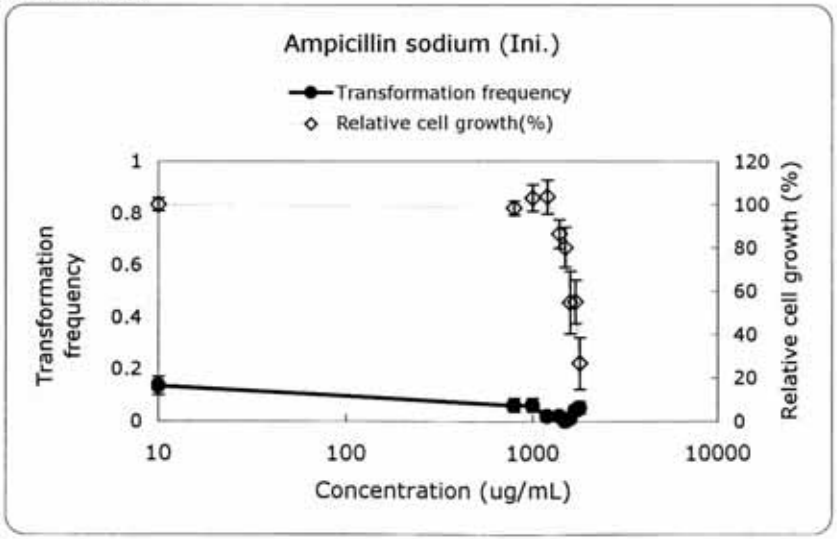
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	800	1000	1200	1400	1500	1600	1700	1800		0.1% DMSO	MCA (1ug/mL)
1	0.115	1.126	1.163	1.218	1.389	1.112	1.096	0.510	0.799	0.258		1.175	0.578
2	0.119	1.148	1.103	1.236	1.141	0.965	0.912	0.814	0.622	0.307		1.180	0.687
3	0.123	1.215	1.146	1.276	1.150	0.975	0.970	0.548	0.716	0.425		1.253	0.659
4	0.123	1.180	1.130	1.130	1.206	1.012	0.795	0.565	0.797	0.324		1.067	0.626
5	0.111	1.178	1.156	1.157	1.220	0.941	1.030	0.824	0.656	0.346		1.146	0.742
6	0.116	1.209	1.207	1.139	1.148	1.106	1.050	0.843	0.722	0.427		1.244	0.673
7	0.108	1.195	1.167	1.202	1.275	1.042	0.990	0.866	0.784	0.426		1.164	0.676
8	0.106	1.170	1.199	1.310	1.182	1.110	0.881	0.600	0.492	0.672		1.100	0.682
Average	0.115	1.178	1.159	1.209	1.214	1.033	0.966	0.696	0.699	0.398	#DIV/0!	1.166	0.665
SD	0.006	0.030	0.034	0.065	0.084	0.070	0.099	0.153	0.106	0.127	#DIV/0!	0.064	0.048
Average - Blank	0.000	1.063	1.044	1.093	1.099	0.918	0.850	0.581	0.583	0.283	#DIV/0!	1.051	0.550
Relative Cell Growth (%)		100.0	98.2	102.9	103.4	86.4	80.0	54.7	54.9	26.6	#DIV/0!	100.0	52.4
SD of Relative Cell Growth		2.8	3.2	6.1	7.9	6.6	9.3	14.4	10.0	12.0	#DIV/0!	6.0	4.5

Transformation Assay

Giemsa Staining	10	800	1000	1200	1400	1500	1600	1700	1800	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	6	6	2	2	0	1	4	5		13	54
Transformation frequency*	0.135416667	0.0625	0.0625	0.020833333	0.020833333	0	0.010416667	0.041666667	0.052083333	0	0.135416667	0.5625
SE	0.03492238	0.02470529	0.02470529	0.01457713	0.01457713	0.00000000	0.01036227	0.02039469	0.02267769	0.00000000	0.03492238	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Ampicillin sodium
Institution:	Lab 1
Test Number	

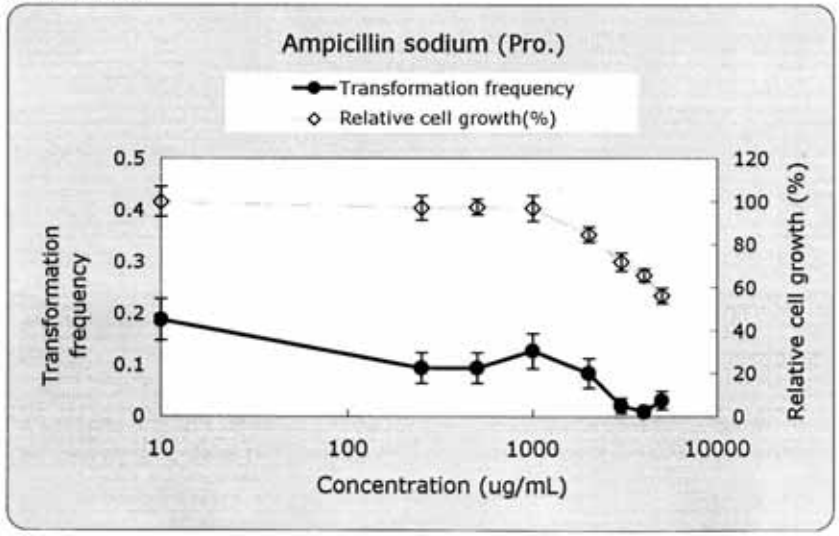
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	250	500	1000	2000	3000	4000	5000			0.1% DMSO	TPA (50ng/mL)
1	0.153	0.692	0.727	0.700	0.671	0.638	0.582	0.555	0.498			0.760	0.838
2	0.152	0.648	0.712	0.729	0.744	0.634	0.552	0.516	0.488			0.680	0.879
3	0.154	0.712	0.676	0.703	0.725	0.627	0.558	0.522	0.466			0.707	0.849
4	0.170	0.750	0.690	0.695	0.672	0.635	0.563	0.518	0.448			0.677	0.935
5	0.151	0.743	0.668	0.675	0.674	0.680	0.539	0.525	0.480			0.674	0.894
6	0.163	0.772	0.719	0.737	0.699	0.617	0.574	0.533	0.449			0.684	0.964
7	0.154	0.750	0.703	0.729	0.706	0.616	0.544	0.513	0.486			0.727	0.963
8	0.169	0.731	0.768	0.708	0.756	0.644	0.611	0.558	0.499			0.684	0.927
Average	0.158	0.725	0.708	0.710	0.706	0.636	0.565	0.530	0.477	#DIV/0!	#DIV/0!	0.699	0.906
SD	0.008	0.040	0.032	0.021	0.033	0.020	0.023	0.017	0.020	#DIV/0!	#DIV/0!	0.030	0.049
Average - Blank	0.000	0.567	0.550	0.551	0.548	0.478	0.407	0.372	0.319	#DIV/0!	#DIV/0!	0.541	0.748
Relative Cell Growth (%)		100.0	97.0	97.3	96.7	84.4	71.9	65.6	56.2	#DIV/0!	#DIV/0!	100.0	138.3
SD of Relative Cell Growth		7.0	5.6	3.7	5.9	3.6	4.1	3.1	3.6	#DIV/0!	#DIV/0!	5.4	8.6

Transformation Assay

Giemsa Staining	10	250	500	1000	2000	3000	4000	5000	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	1	0	0	0	0			0	0
No. of wells counted	96	96	96	95	96	96	96	96	96	96	96	96
No. of wells having foci	18	9	9	12	8	2	1	3			13	67
Transformation frequency*	0.1875	0.09375	0.09375	0.126315789	0.083333333	0.020833333	0.010416667	0.03125	0	0	0.135416667	0.697916667
SE	0.03983609	0.02974911	0.02974911	0.03408351	0.02820847	0.01457713	0.01036227	0.01775805	0.00000000	0.00000000	0.03492238	0.04686294

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Ampicillin sodium
Institution:	Lab 3
Test Number	

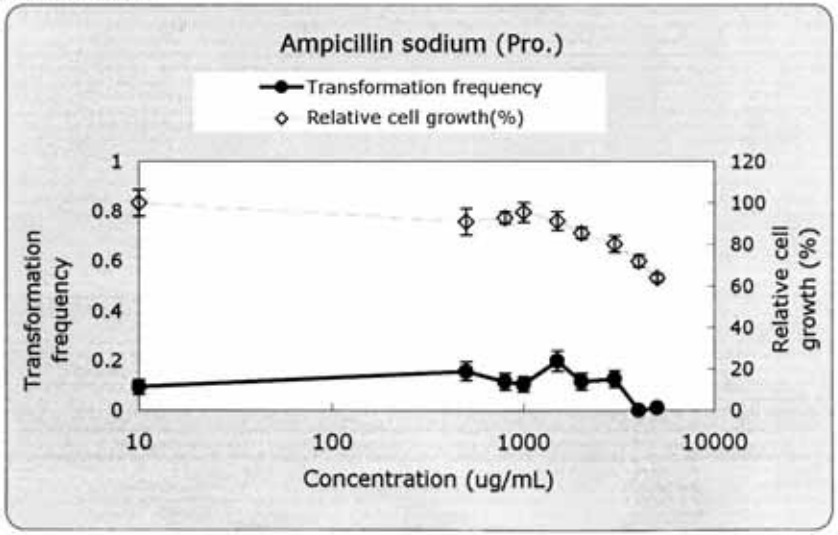
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	500	800	1000	1500	2000	3000	4000	5000		0.1% DMSO	TPA (50ng/mL)
1	0.121	1.307	1.194	1.264	1.261	1.215	1.180	1.032	0.990	0.936		1.013	1.623
2	0.130	1.318	1.315	1.289	1.346	1.237	1.222	1.188	1.017	0.921		1.277	1.770
3	0.128	1.478	1.265	1.338	1.354	1.298	1.182	1.148	1.079	0.971		1.221	1.712
4	0.127	1.394	1.321	1.363	1.377	1.398	1.266	1.203	1.034	0.895		1.171	1.703
5	0.118	1.345	1.249	1.285	1.436	1.295	1.238	1.137	0.992	0.935		1.258	1.746
6	0.119	1.403	1.449	1.344	1.307	1.235	1.167	1.136	1.055	0.929		1.282	1.565
7	0.117	1.368	1.241	1.269	1.346	1.263	1.233	1.148	1.035	0.956		1.310	1.776
8	0.112	1.546	1.199	1.276	1.253	1.313	1.188	1.149	1.091	0.928		1.197	1.695
Average	0.122	1.395	1.279	1.304	1.335	1.282	1.210	1.143	1.037	0.934	#DIV/0!	1.216	1.699
SD	0.006	0.082	0.083	0.039	0.060	0.058	0.035	0.051	0.037	0.023	#DIV/0!	0.094	0.073
Average - Blank	0.000	1.273	1.158	1.182	1.214	1.160	1.088	1.021	0.915	0.812	#DIV/0!	1.095	1.577
Relative Cell Growth (%)		100.0	90.9	92.8	95.3	91.1	85.4	80.2	71.9	63.8	#DIV/0!	100.0	144.1
SD of Relative Cell Growth		6.4	6.5	3.0	4.7	4.6	2.8	4.0	2.9	1.8	#DIV/0!	7.4	5.7

Transformation Assay

Giemsa Staining	10	500	800	1000	1500	2000	3000	4000	5000	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	15	11	10	19	11	12	0	1		14	78
Transformation frequency*	0.09375	0.15625	0.114583333	0.104166667	0.197916667	0.114583333	0.125	0	0.010416667	0	0.145833333	0.8125
SE	0.02974911	0.03705794	0.03250865	0.03117758	0.04066449	0.03250865	0.03375386	0.00000000	0.01036227	0.00000000	0.03602169	0.03983609

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

This Data is ...

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	L-Ascorbic acid
Institution:	Lab 1
Test Number	

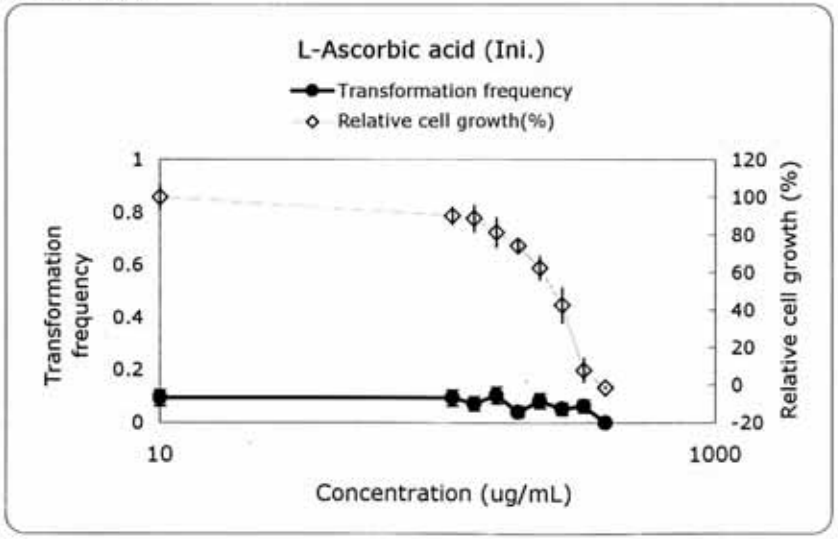
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	112	134	161	193	231	278	333	400		0.1% DMSO	MCA (1ug/mL)
1	0.093	0.855	0.817	0.759	0.735	0.694	0.677	0.433	0.137	0.113		0.851	0.315
2	0.108	0.872	0.772	0.707	0.707	0.634	0.614	0.377	0.128	0.103		0.818	0.446
3	0.112	0.814	0.812	0.813	0.666	0.666	0.625	0.395	0.186	0.111		0.786	0.450
4	0.127	0.846	0.823	0.779	0.838	0.710	0.528	0.452	0.260	0.113		0.933	0.414
5	0.126	0.908	0.795	0.821	0.812	0.689	0.576	0.429	0.200	0.110		0.823	0.388
6	0.135	0.953	0.877	0.855	0.761	0.695	0.604	0.564	0.170	0.118		0.786	0.466
7	0.137	0.916	0.827	0.875	0.681	0.706	0.550	0.387	0.141	0.110		0.798	0.398
8	0.131	0.942	0.777	0.801	0.756	0.734	0.616	0.544	0.233	0.117		0.888	0.385
Average	0.121	0.888	0.813	0.801	0.745	0.691	0.599	0.448	0.182	0.112	#DIV/0!	0.835	0.408
SD	0.015	0.049	0.033	0.053	0.060	0.030	0.047	0.071	0.047	0.005	#DIV/0!	0.052	0.048
Average - Blank	0.000	0.767	0.691	0.680	0.623	0.570	0.478	0.327	0.061	-0.009	#DIV/0!	0.714	0.287
Relative Cell Growth (%)		100.0	90.1	88.7	81.3	74.3	62.3	42.6	7.9	-1.2	#DIV/0!	100.0	40.1
SD of Relative Cell Growth		6.4	4.3	7.0	7.9	3.9	6.1	9.2	6.2	0.6	#DIV/0!	6.8	6.3

Transformation Assay

Giemsa Staining	10	112	134	161	193	231	278	333	400	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	20		0	0
No. of wells counted	96	96	96	96	96	96	96	96	76	96	96	96
No. of wells having foci	9	9	7	10	4	8	5	6	TOX		10	41
Transformation frequency*	0.09375	0.09375	0.072916667	0.104166667	0.041666667	0.083333333	0.052083333	0.0625	#VALUE!	0	0.104166667	0.427083333
SE	0.02974911	0.02974911	0.02653610	0.03117758	0.02039469	0.02820847	0.02267769	0.02470529	#VALUE!	0.00000000	0.03117758	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	L-Ascorbic acid
Institution:	Lab 3
Test Number	

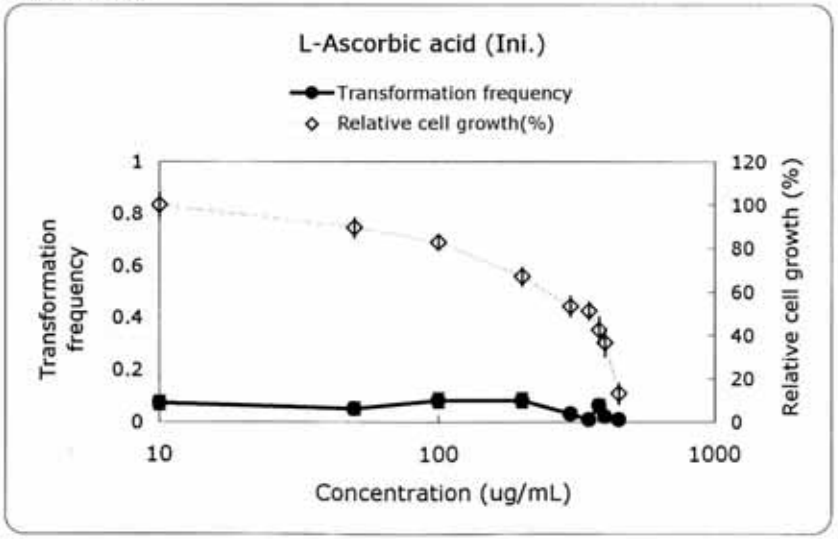
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	50	100	200	300	350	380	400	450		0.1% DMSO	MCA (1ug/mL)
1	0.128	1.448	1.292	1.250	1.047	0.904	0.867	0.807	0.605	0.366		1.424	0.802
2	0.133	1.484	1.389	1.197	1.092	0.816	0.752	0.570	0.640	0.332		1.381	0.947
3	0.131	1.297	1.267	1.245	1.033	0.855	0.784	0.669	0.546	0.333		1.481	0.868
4	0.130	1.417	1.290	1.191	0.932	0.725	0.840	0.681	0.569	0.279		1.407	0.794
5	0.130	1.432	1.245	1.185	1.012	0.780	0.751	0.627	0.716	0.313		1.363	0.768
6	0.127	1.471	1.223	1.222	0.938	0.812	0.739	0.698	0.579	0.226		1.379	0.942
7	0.132	1.544	1.375	1.232	1.004	0.813	0.872	0.639	0.473	0.194		1.353	0.774
8	0.128	1.369	1.272	1.143	0.974	0.907	0.787	0.768	0.719	0.361		1.297	0.817
Average	0.130	1.433	1.294	1.208	1.004	0.827	0.799	0.682	0.606	0.301	#DIV/0!	1.386	0.839
SD	0.002	0.075	0.059	0.036	0.055	0.061	0.054	0.076	0.084	0.063	#DIV/0!	0.054	0.072
Average - Blank	0.000	1.303	1.164	1.078	0.874	0.697	0.669	0.553	0.476	0.171	#DIV/0!	1.256	0.709
Relative Cell Growth (%)		100.0	89.4	82.8	67.1	53.5	51.4	42.4	36.5	13.1	#DIV/0!	100.0	56.5
SD of Relative Cell Growth		5.8	4.5	2.8	4.2	4.7	4.1	5.9	6.4	4.8	#DIV/0!	4.2	5.5

Transformation Assay

Giemsa Staining	10	50	100	200	300	350	380	400	450	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	5	8	8	3	1	6	2	1		7	60
Transformation frequency*	0.072916667	0.052083333	0.083333333	0.083333333	0.03125	0.010416667	0.0625	0.020833333	0.010416667	0	0.072916667	0.625
SE	0.02653610	0.02267769	0.02820847	0.02820847	0.01775805	0.01036227	0.02470529	0.01457713	0.01036227	0.00000000	0.02653610	0.04941059

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	L-Ascorbic acid
Institution:	Lab 1
Test Number	

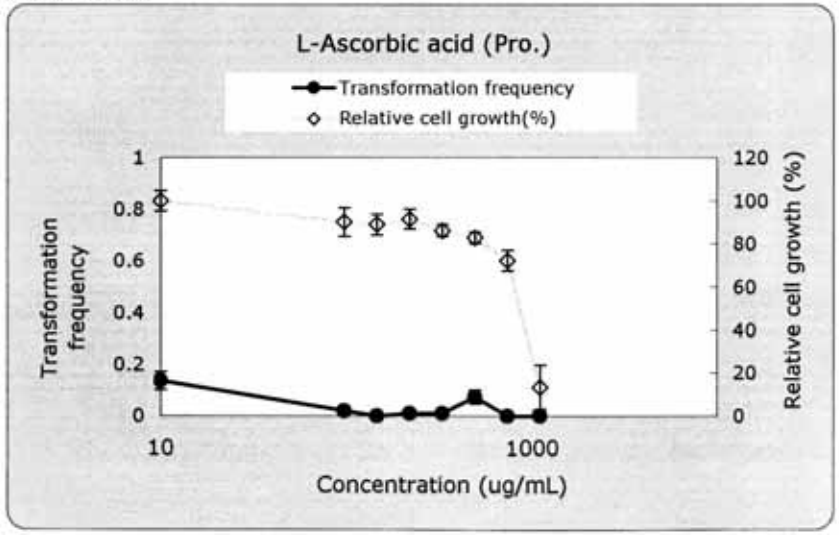
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	96.6	145	217	326	489	733	1100			0.1% DMSO	TPA (50ng/mL)
1	0.091	0.718	0.647	0.668	0.662	0.696	0.644	0.573	0.232			0.741	0.978
2	0.099	0.776	0.748	0.703	0.747	0.685	0.669	0.610	0.292			0.716	0.979
3	0.111	0.794	0.725	0.708	0.713	0.703	0.664	0.610	0.118			0.718	0.960
4	0.116	0.796	0.668	0.733	0.717	0.691	0.637	0.561	0.187			0.737	0.963
5	0.112	0.779	0.716	0.720	0.726	0.702	0.686	0.589	0.164			0.753	0.975
6	0.128	0.827	0.788	0.760	0.742	0.674	0.690	0.582	0.156			0.795	1.037
7	0.120	0.763	0.719	0.659	0.712	0.704	0.661	0.572	0.305			0.757	0.977
8	0.118	0.791	0.699	0.699	0.767	0.647	0.669	0.658	0.156			0.745	0.974
Average	0.112	0.781	0.714	0.706	0.723	0.688	0.665	0.594	0.201	#DIV/0!	#DIV/0!	0.745	0.980
SD	0.012	0.031	0.044	0.033	0.031	0.019	0.018	0.031	0.068	#DIV/0!	#DIV/0!	0.025	0.024
Average - Blank	0.000	0.669	0.602	0.594	0.611	0.576	0.553	0.483	0.089	#DIV/0!	#DIV/0!	0.633	0.869
Relative Cell Growth (%)		100.0	90.0	88.9	91.4	86.1	82.7	72.2	13.4	#DIV/0!	#DIV/0!	100.0	137.1
SD of Relative Cell Growth		4.7	6.6	4.9	4.7	2.9	2.7	4.7	10.2	#DIV/0!	#DIV/0!	3.7	3.6

Transformation Assay

Giemsa Staining	10	96.6	145	217	326	489	733	1100	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	1	94	96			0	0
No. of wells counted	96	96	96	96	96	95	2	0	96	96	96	96
No. of wells having foci	13	2	0	1	1	7	TOX	TOX			15	56
Transformation frequency*	0.135416667	0.020833333	0	0.010416667	0.010416667	0.073684211	#VALUE!	#VALUE!	0	0	0.15625	0.583333333
SE	0.03492238	0.01457713	0.00000000	0.01036227	0.01036227	0.02680433	#VALUE!	#VALUE!	0.00000000	0.00000000	0.03705794	0.05031728

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Progressing Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	L-Ascorbic acid
Institution:	Lab 3
Test Number	

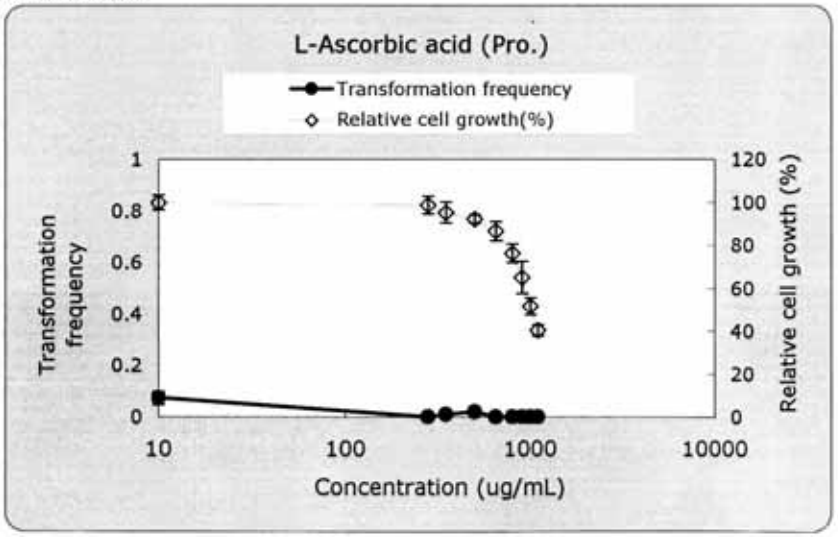
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	280	350	500	650	800	900	1000	1100		0.1% DMSO	TPA (50ng/mL)
1	0.127	1.336	1.380	1.324	1.273	1.168	1.118	1.004	0.778	0.628		1.305	1.728
2	0.133	1.405	1.377	1.357	1.272	1.231	1.124	1.004	0.705	0.634		1.371	1.740
3	0.134	1.410	1.348	1.360	1.312	1.158	1.112	1.026	0.761	0.671		1.391	1.656
4	0.128	1.425	1.331	1.195	1.311	1.177	1.060	0.778	0.768	0.594		1.428	1.806
5	0.126	1.390	1.391	1.384	1.254	1.158	0.972	0.871	0.731	0.633		1.442	1.765
6	0.127	1.382	1.447	1.300	1.288	1.251	1.049	0.857	0.868	0.590		1.357	1.718
7	0.121	1.364	1.300	1.330	1.269	1.312	1.058	1.035	0.754	0.682		1.284	1.690
8	0.118	1.290	1.294	1.270	1.253	1.203	1.133	0.927	0.794	0.615		1.341	1.596
Average	0.127	1.375	1.359	1.315	1.279	1.207	1.078	0.938	0.770	0.631	#DIV/0!	1.365	1.712
SD	0.005	0.044	0.051	0.060	0.023	0.054	0.054	0.095	0.048	0.033	#DIV/0!	0.055	0.065
Average - Blank	0.000	1.249	1.232	1.188	1.152	1.081	0.952	0.811	0.643	0.504	#DIV/0!	1.238	1.586
Relative Cell Growth (%)		100.0	98.7	95.2	92.3	86.5	76.2	65.0	51.5	40.4	#DIV/0!	100.0	128.1
SD of Relative Cell Growth		3.6	4.1	4.8	1.8	4.4	4.4	7.6	3.9	2.6	#DIV/0!	4.4	5.2

Transformation Assay

Giemsa Staining	10	280	350	500	650	800	900	1000	1100	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	0	1	2	Tox.	Tox.	Tox.	Tox.	Tox.		21	85
Transformation frequency*	0.072916667	0	0.010416667	0.020833333	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0	0.21875	0.885416667
SE	0.02653610	0.00000000	0.01036227	0.01457713	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0.00000000	0.04219232	0.03250865

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Failure

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Failure

The Progressing Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	L-Ascorbic acid
Institution:	Lab 3
Test Number	

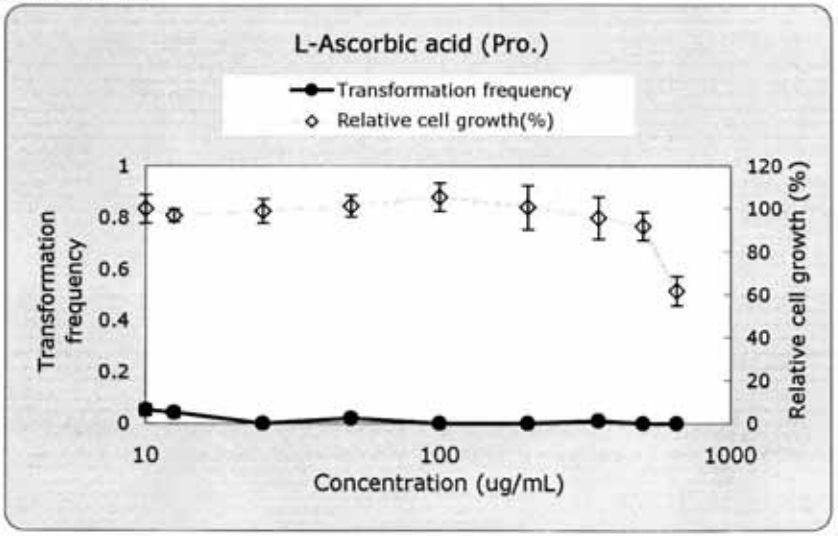
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	12.5	25	50	100	200	350	500	650		0.1% DMSO	TPA (50ng/mL)
1	0.076	0.502	0.470	0.519	0.474	0.503	0.431	0.399	0.430	0.350		0.501	0.594
2	0.079	0.485	0.494	0.494	0.526	0.511	0.519	0.497	0.483	0.388		0.507	0.669
3	0.089	0.497	0.473	0.496	0.498	0.467	0.437	0.465	0.463	0.334		0.474	0.623
4	0.079	0.509	0.500	0.514	0.485	0.547	0.514	0.488	0.476	0.319		0.580	0.707
5	0.077	0.485	0.497	0.499	0.537	0.514	0.550	0.503	0.506	0.334		0.523	0.570
6	0.078	0.470	0.487	0.443	0.512	0.533	0.514	0.492	0.449	0.298		0.584	0.619
7	0.084	0.557	0.468	0.483	0.489	0.549	0.525	0.536	0.472	0.362		0.576	0.660
8	0.083	0.474	0.484	0.497	0.503	0.537	0.508	0.455	0.425	0.321		0.513	0.532
Average	0.081	0.497	0.484	0.493	0.503	0.520	0.500	0.479	0.463	0.338	#DIV/0!	0.532	0.622
SD	0.004	0.028	0.013	0.023	0.021	0.027	0.043	0.041	0.027	0.028	#DIV/0!	0.042	0.057
Average - Blank	0.000	0.417	0.404	0.413	0.422	0.440	0.419	0.399	0.382	0.258	#DIV/0!	0.452	0.541
Relative Cell Growth (%)		100.0	96.8	99.0	101.3	105.5	100.6	95.7	91.8	61.8	#DIV/0!	100.0	119.8
SD of Relative Cell Growth		6.6	3.0	5.6	5.1	6.6	10.2	9.8	6.6	6.7	#DIV/0!	10.1	13.6

Transformation Assay

Giemsa Staining	10	12.5	25	50	100	200	350	500	650	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	4	0	2	0	0	1	Tox	Tox		8	45
Transformation frequency*	0.052083333	0.041666667	0	0.020833333	0	0	0.010416667	#VALUE!	#VALUE!	0	0.083333333	0.46875
SE	0.02267769	0.02039469	0.00000000	0.01457713	0.00000000	0.00000000	0.01036227	#VALUE!	#VALUE!	0.00000000	0.02820847	0.05093127

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Caffeine
Institution:	Lab 2
Test Number	

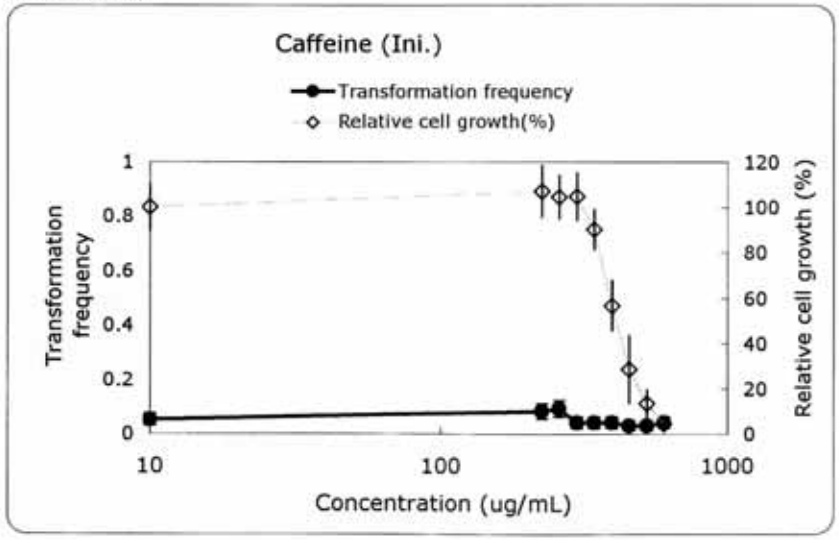
Cell Growth Assay

Crystal Violet Staining	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
OD_{540nm}		10	226	259	298	343	395	454	522	600		0.1% DMSO	MCA (1ug/mL)
1	0.065	0.342	0.390	0.331	0.363	0.295	0.214	0.102	0.082	0.069		0.334	0.232
2	0.062	0.386	0.358	0.368	0.362	0.344	0.241	0.127	0.097	0.077		0.325	0.209
3	0.060	0.401	0.413	0.358	0.423	0.316	0..218	0.157	0.115	0.075		0.336	0.219
4	0.059	0.377	0.328	0.370	0.414	0.351	0.269	0.134	0.100	0.088		0.315	0.216
5	0.060	0.393	0.414	0.424	0.408	0.314	0.263	0.133	0.136	0.077		0.355	0.202
6	0.060	0.347	0.368	0.411	0.350	0.321	0.229	0.191	0.117	0.072		0.334	0.201
7	0.060	0.356	0.434	0.382	0.345	0.365	0.246	0.235	0.086	0.073		0.311	0.198
8	0.065	0.309	0.380	0.377	0.362	0.369	0.170	0.105	0.083	0.072		0.299	0.197
Average	0.061	0.364	0.386	0.378	0.378	0.334	0.233	0.148	0.102	0.075	#DIV/0!	0.326	0.209
SD	0.002	0.031	0.035	0.029	0.031	0.027	0.034	0.045	0.019	0.006	#DIV/0!	0.018	0.012
Average - Blank	0.000	0.303	0.324	0.316	0.317	0.273	0.172	0.087	0.041	0.014	#DIV/0!	0.265	0.148
Relative Cell Growth (%)		100.0	107.2	104.5	104.8	90.2	56.8	28.6	13.4	4.6	#DIV/0!	100.0	55.9
SD of Relative Cell Growth		10.2	11.4	9.7	10.3	8.8	11.1	15.0	6.4	1.9	#DIV/0!	5.8	4.1

Transformation Assay

Giemsa Staining	10	226	259	298	343	395	454	522	600	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	8	9	4	4	4	3	3	4		4	55
Transformation frequency*	0.052083333	0.083333333	0.09375	0.041666667	0.041666667	0.041666667	0.03125	0.03125	0.041666667	0	0.041666667	0.572916667
SE	0.02267769	0.02820847	0.02974911	0.02039469	0.02039469	0.02039469	0.01775805	0.01775805	0.02039469	0.00000000	0.02039469	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Caffeine
Institution:	Lab 3
Test Number	

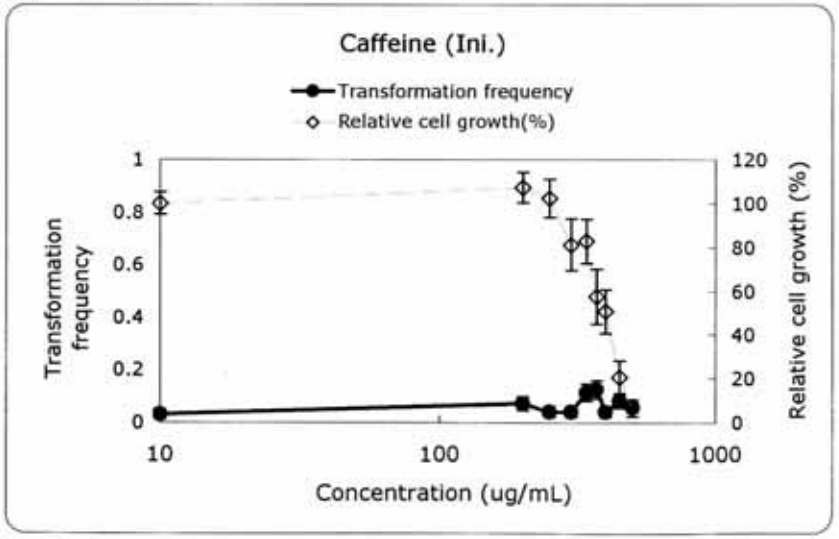
Cell Growth Assay

Crystal Violet Staining	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
OD_{540nm}		10	200	250	300	340	370	400	450	500		0.1% DMSO	MCA (1ug/mL)
1	0.085	1.226	1.252	1.158	0.916	1.061	0.629	0.718	0.208	0.100		1.107	0.572
2	0.091	1.177	1.214	1.277	1.186	1.023	0.578	0.595	0.231	0.186		1.221	0.600
3	0.097	1.193	1.386	1.108	1.100	1.050	0.803	0.748	0.290	0.143		1.284	0.613
4	0.098	1.252	1.264	1.332	0.815	1.055	0.774	0.760	0.402	0.176		1.145	0.573
5	0.094	1.153	1.408	1.166	0.989	1.110	0.692	0.769	0.408	0.245		1.250	0.672
6	0.091	1.130	1.284	1.147	0.994	0.888	1.014	0.479	0.268	0.148		1.154	0.599
7	0.098	1.300	1.251	1.378	1.089	0.791	0.711	0.537	0.437	0.180		1.199	0.533
8	0.092	1.170	1.192	1.227	0.848	1.097	0.639	0.636	0.316	0.157		1.219	0.471
Average	0.093	1.200	1.281	1.224	0.992	1.009	0.730	0.655	0.320	0.167	#DIV/0!	1.197	0.579
SD	0.004	0.056	0.077	0.097	0.129	0.111	0.137	0.111	0.086	0.042	#DIV/0!	0.059	0.059
Average - Blank	0.000	1.107	1.188	1.131	0.899	0.916	0.637	0.562	0.227	0.074	#DIV/0!	1.104	0.486
Relative Cell Growth (%)		100.0	107.3	102.2	81.2	82.8	57.5	50.8	20.5	6.7	#DIV/0!	100.0	44.0
SD of Relative Cell Growth		5.1	7.0	8.7	11.7	10.1	12.4	10.0	7.8	3.8	#DIV/0!	5.3	5.3

Transformation Assay

Giemsa Staining	10	200	250	300	340	370	400	450	500	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	3	7	4	4	11	12	4	8	6		2	41
Transformation frequency*	0.03125	0.072916667	0.041666667	0.041666667	0.114583333	0.125	0.041666667	0.083333333	0.0625	0	0.020833333	0.427083333
SE	0.01775805	0.02653610	0.02039469	0.02039469	0.03250865	0.03375386	0.02039469	0.02820847	0.02470529	0.00000000	0.01457713	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Caffeine
Institution:	Lab 2
Test Number	

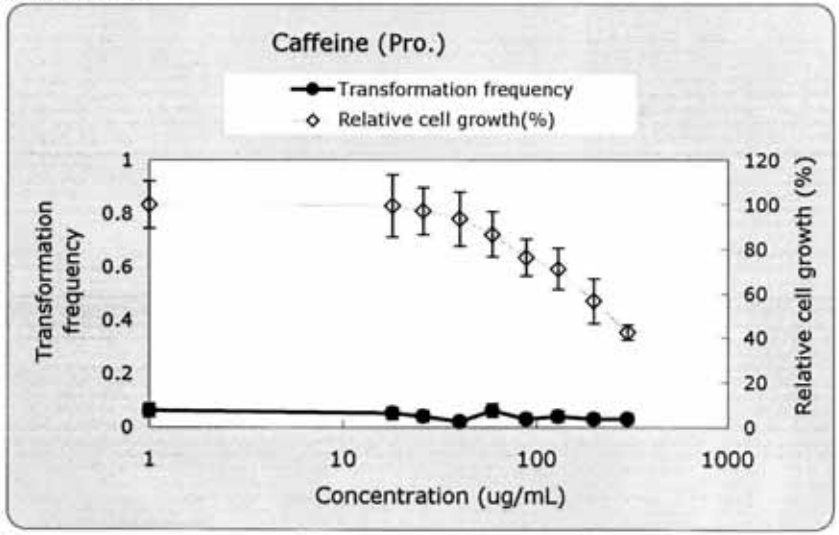
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		1	18	26	40	59	89	130	200	300		0.1% DMSO	TPA (50ng/mL)
1	0.067	0.285	0.284	0.297	0.260	0.243	0.222	0.212	0.183	0.161		0.257	0.367
2	0.067	0.318	0.308	0.314	0.288	0.285	0.269	0.273	0.221	0.187		0.287	0.388
3	0.065	0.325	0.331	0.346	0.333	0.313	0.280	0.249	0.227	0.182		0.304	0.391
4	0.065	0.362	0.370	0.332	0.338	0.294	0.258	0.251	0.197	0.175		0.294	0.418
5	0.066	0.339	0.340	0.312	0.326	0.314	0.261	0.254	0.258	0.177		0.303	0.403
6	0.065	0.326	0.341	0.334	0.290	0.279	0.281	0.273	0.191	0.170		0.284	0.404
7	0.063	0.308	0.297	0.281	0.316	0.292	0.257	0.234	0.199	0.166		0.279	0.376
8	0.063	0.281	0.261	0.270	0.263	0.252	0.233	0.214	0.191	0.166		0.243	0.358
Average	0.065	0.318	0.317	0.311	0.302	0.284	0.258	0.245	0.208	0.173	#DIV/0!	0.281	0.388
SD	0.002	0.027	0.035	0.027	0.031	0.026	0.021	0.024	0.025	0.009	#DIV/0!	0.022	0.020
Average - Blank	0.000	0.253	0.251	0.246	0.237	0.219	0.193	0.180	0.143	0.108	#DIV/0!	0.216	0.323
Relative Cell Growth (%)		100.0	99.4	97.1	93.6	86.6	76.1	71.1	56.6	42.7	#DIV/0!	100.0	149.4
SD of Relative Cell Growth		10.6	14.0	10.5	12.2	10.2	8.3	9.3	10.0	3.5	#DIV/0!	8.5	8.0

Transformation Assay

Giemsa Staining	1	18	26	40	59	89	130	200	300	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	5	4	2	6	3	4	3	3		7	68
Transformation frequency*	0.0625	0.052083333	0.041666667	0.020833333	0.0625	0.03125	0.041666667	0.03125	0.03125	0	0.072916667	0.708333333
SE	0.02470529	0.02267769	0.02039469	0.01457713	0.02470529	0.01775805	0.02039469	0.01775805	0.01775805	0.00000000	0.02653610	0.04639024

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Caffeine
Institution:	Lab 3
Test Number	

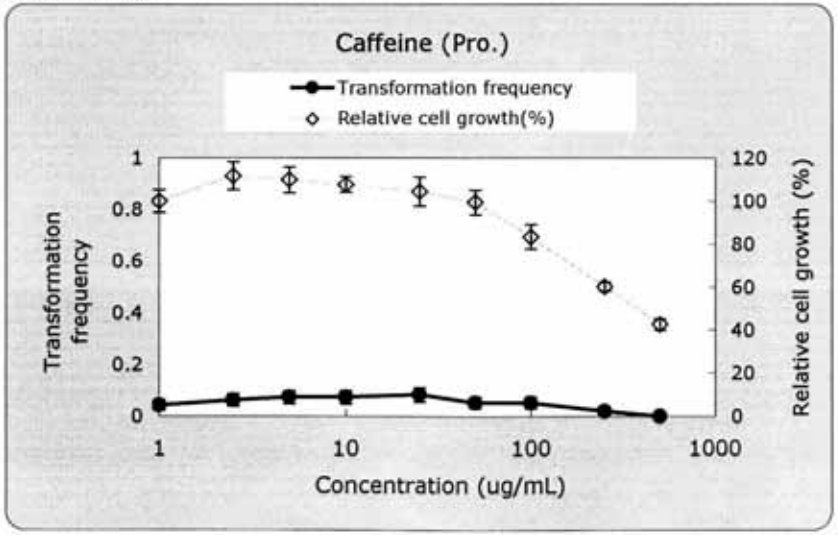
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.5	5	10	25	50	100	250	500		0.1% DMSO	TPA (50ng/mL)
1	0.084	0.995	1.194	1.271	1.136	1.000	0.959	0.986	0.696	0.477		1.170	1.614
2	0.087	1.041	1.165	1.183	1.194	1.234	1.085	0.943	0.661	0.522		1.105	1.498
3	0.087	1.127	1.181	1.141	1.148	1.133	1.154	0.904	0.696	0.507		1.095	1.594
4	0.089	1.158	1.221	1.157	1.196	1.134	1.101	0.915	0.683	0.544		1.190	1.522
5	0.088	1.105	1.176	1.207	1.193	1.119	1.085	0.977	0.724	0.556		1.154	1.541
6	0.084	1.081	1.368	1.137	1.224	1.193	1.137	0.903	0.700	0.507		1.152	1.490
7	0.091	1.117	1.187	1.298	1.167	1.138	1.080	0.970	0.716	0.528		1.110	1.600
8	0.087	1.130	1.197	1.158	1.116	1.153	1.091	0.813	0.674	0.482		1.144	1.479
Average	0.087	1.094	1.211	1.194	1.172	1.138	1.087	0.926	0.694	0.515	#DIV/0!	1.140	1.542
SD	0.002	0.053	0.066	0.061	0.036	0.068	0.058	0.056	0.021	0.028	#DIV/0!	0.034	0.054
Average - Blank	0.000	1.007	1.124	1.107	1.085	1.051	0.999	0.839	0.607	0.428	#DIV/0!	1.053	1.455
Relative Cell Growth (%)		100.0	111.6	109.9	107.7	104.3	99.2	83.3	60.2	42.5	#DIV/0!	100.0	138.2
SD of Relative Cell Growth		5.3	6.5	6.0	3.6	6.7	5.8	5.6	2.1	2.8	#DIV/0!	3.3	5.3

Transformation Assay

Giemsa Staining	1	2.5	5	10	25	50	100	250	500	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	4	6	7	7	8	5	5	2	0		11	54
Transformation frequency*	0.041666667	0.0625	0.072916667	0.072916667	0.083333333	0.052083333	0.052083333	0.020833333	0	0	0.114583333	0.5625
SE	0.02039469	0.02470529	0.02653610	0.02653610	0.02820847	0.02267769	0.02267769	0.01457713	0.00000000	0.00000000	0.03250865	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Caprolactam
Institution:	Lab 1
Test Number	

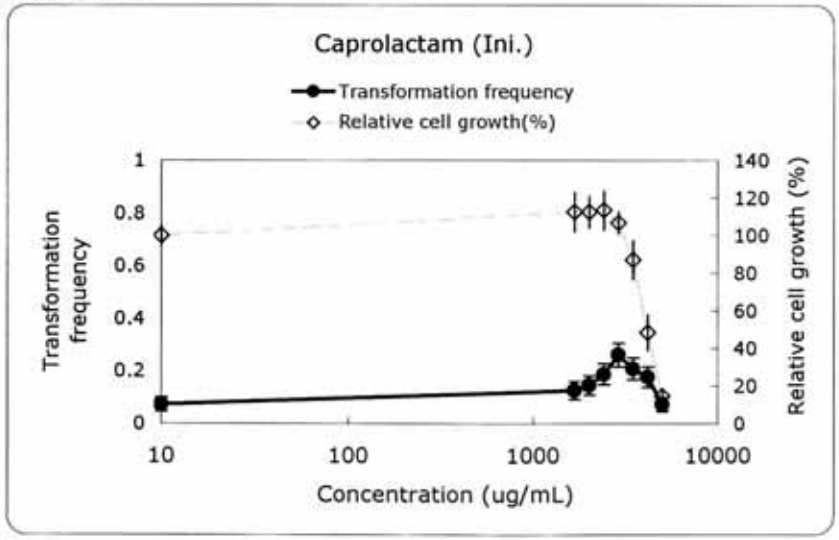
Cell Growth Assay

Crystal Violet Staining	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
OD_{540nm}		10	1670	2010	2410	2890	3470	4170	5000			0.1% DMSO	MCA (1ug/mL)
1	0.150	0.748	0.913	0.895	0.890	0.864	0.666	0.458	0.230			0.765	0.340
2	0.150	0.788	0.794	0.855	0.870	0.794	0.735	0.485	0.256			0.704	0.374
3	0.158	0.745	0.874	0.876	0.784	0.828	0.707	0.473	0.288			0.697	0.337
4	0.158	0.782	0.796	0.826	0.945	0.755	0.807	0.515	0.259			0.689	0.370
5	0.167	0.843	0.817	0.776	0.783	0.846	0.607	0.498	0.293			0.680	0.378
6	0.173	0.819	0.806	0.871	0.845	0.832	0.754	0.509	0.265			0.671	0.371
7	0.172	0.776	0.940	0.830	0.853	0.849	0.692	0.457	0.237			0.702	0.380
8	0.204	0.760	0.941	0.948	0.951	0.827	0.657	0.331	0.227			0.749	0.331
Average	0.167	0.783	0.860	0.860	0.865	0.824	0.703	0.466	0.257	#DIV/0!	#DIV/0!	0.707	0.360
SD	0.018	0.034	0.065	0.051	0.064	0.035	0.062	0.059	0.025	#DIV/0!	#DIV/0!	0.033	0.020
Average - Blank	0.000	0.616	0.694	0.693	0.699	0.658	0.537	0.299	0.090	#DIV/0!	#DIV/0!	0.541	0.194
Relative Cell Growth (%)		100.0	112.6	112.5	113.4	106.8	87.1	48.6	14.7	#DIV/0!	#DIV/0!	100.0	35.8
SD of Relative Cell Growth		5.5	10.5	8.3	10.3	5.6	10.1	9.5	4.0	#DIV/0!	#DIV/0!	5.4	3.3

Transformation Assay

Giemsa Staining	10	1670	2010	2410	2890	3470	4170	5000	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	7	12	14	18	25	20	17	7			9	43
Transformation frequency*	0.072916667	0.125	0.145833333	0.1875	0.260416667	0.208333333	0.177083333	0.072916667	0	0	0.09375	0.447916667
SE	0.02653610	0.03375386	0.03602169	0.03983609	0.04479116	0.04144908	0.03896110	0.02653610	0.00000000	0.00000000	0.02974911	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Caprolactam
Institution:	Lab 3
Test Number	

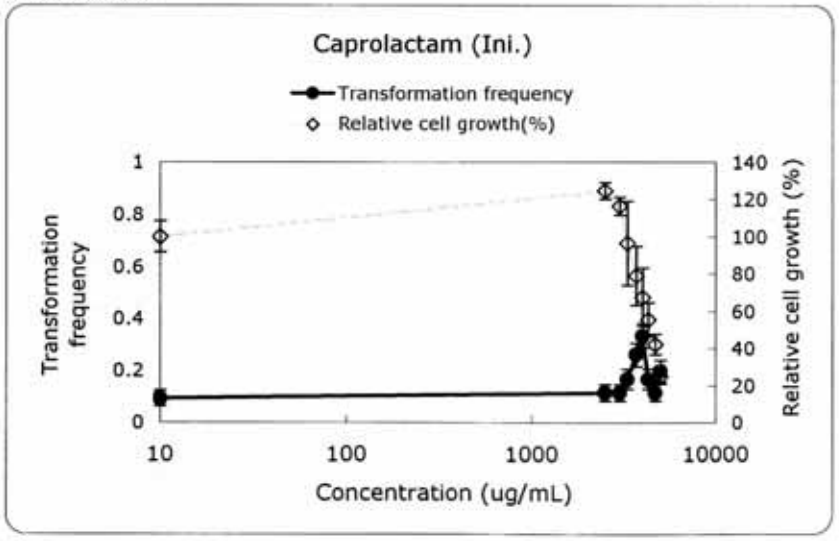
Cell Growth Assay

Crystal Violet Staining	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
OD_{540nm}		10	2500	3000	3300	3700	4000	4300	4700	5000		0.1% DMSO	MCA (1ug/mL)
1	0.086	1.188	1.542	1.434	0.584	0.822	0.708	0.551	0.593	0.398		1.292	0.545
2	0.097	1.365	1.515	1.345	1.386	0.634	0.537	0.762	0.677	0.321		1.318	0.559
3	0.089	1.359	1.488	1.420	1.297	1.025	0.871	0.823	0.595	0.387		1.243	0.564
4	0.085	1.211	1.471	1.337	1.306	1.170	1.102	0.628	0.496	0.363		1.144	0.539
5	0.090	1.214	1.442	1.394	1.228	1.078	0.989	0.721	0.538	0.387		1.142	0.517
6	0.090	1.152	1.434	1.507	1.316	1.050	0.809	0.788	0.507	0.292		1.201	0.520
7	0.095	1.169	1.529	1.390	1.235	1.133	0.954	0.634	0.603	0.443		1.180	0.551
8	0.088	1.104	1.577	1.429	1.088	0.966	0.861	0.839	0.514	0.396		1.180	0.500
Average	0.090	1.220	1.500	1.407	1.180	0.985	0.854	0.718	0.565	0.373	#DIV/0!	1.213	0.537
SD	0.004	0.094	0.050	0.054	0.256	0.178	0.175	0.104	0.062	0.048	#DIV/0!	0.066	0.022
Average - Blank	0.000	1.130	1.410	1.317	1.090	0.895	0.764	0.628	0.475	0.283	#DIV/0!	1.123	0.447
Relative Cell Growth (%)		100.0	124.7	116.5	96.4	79.2	67.6	55.6	42.1	25.1	#DIV/0!	100.0	39.8
SD of Relative Cell Growth		8.3	4.4	4.8	22.7	15.7	15.5	9.2	5.5	4.2	#DIV/0!	5.8	2.0

Transformation Assay

Giemsa Staining	10	2500	3000	3300	3700	4000	4300	4700	5000	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	11	11	16	25	32	16	11	19		8	65
Transformation frequency*	0.09375	0.114583333	0.114583333	0.166666667	0.260416667	0.333333333	0.166666667	0.114583333	0.197916667	0	0.083333333	0.677083333
SE	0.02974911	0.03250865	0.03250865	0.03803629	0.04479116	0.04811252	0.03803629	0.03250865	0.04066449	0.00000000	0.02820847	0.04772332

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Caprolactam
Institution:	Lab 1
Test Number	

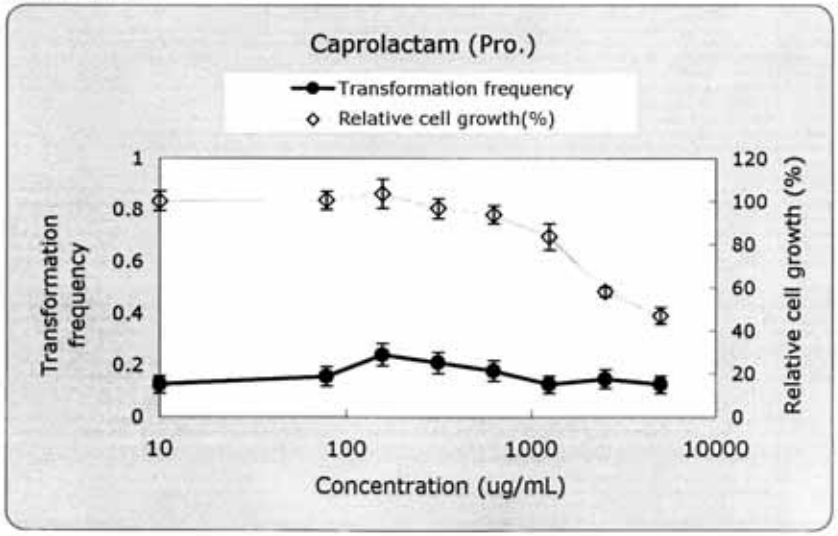
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	78.1	156	313	625	1250	2500	5000			0.1% DMSO	TPA (50ng/mL)
1	0.121	0.706	0.708	0.682	0.648	0.631	0.540	0.457	0.412			0.622	0.799
2	0.121	0.661	0.681	0.665	0.685	0.612	0.567	0.441	0.395			0.654	0.867
3	0.119	0.682	0.653	0.750	0.633	0.657	0.599	0.427	0.358			0.661	0.895
4	0.122	0.688	0.696	0.637	0.673	0.643	0.568	0.442	0.368			0.676	0.922
5	0.120	0.636	0.702	0.704	0.655	0.671	0.607	0.454	0.370			0.643	0.806
6	0.125	0.677	0.648	0.742	0.670	0.653	0.565	0.435	0.369			0.682	0.870
7	0.125	0.660	0.657	0.706	0.617	0.611	0.597	0.446	0.386			0.694	0.831
8	0.130	0.716	0.697	0.688	0.692	0.665	0.650	0.462	0.413			0.674	0.874
Average	0.123	0.678	0.680	0.697	0.659	0.643	0.587	0.446	0.384	#DIV/0!	#DIV/0!	0.663	0.858
SD	0.004	0.026	0.024	0.038	0.026	0.023	0.034	0.012	0.021	#DIV/0!	#DIV/0!	0.023	0.043
Average - Blank	0.000	0.555	0.557	0.574	0.536	0.520	0.464	0.323	0.261	#DIV/0!	#DIV/0!	0.540	0.735
Relative Cell Growth (%)		100.0	100.4	103.3	96.6	93.6	83.5	58.1	47.0	#DIV/0!	#DIV/0!	100.0	136.0
SD of Relative Cell Growth		4.7	4.4	6.8	4.6	4.1	6.1	2.1	3.8	#DIV/0!	#DIV/0!	4.2	7.7

Transformation Assay

Giemsa Staining	10	78.1	156	313	625	1250	2500	5000	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	12	15	23	20	17	12	14	12			14	63
Transformation frequency*	0.125	0.15625	0.239583333	0.208333333	0.177083333	0.125	0.145833333	0.125	0	0	0.145833333	0.65625
SE	0.03375386	0.03705794	0.04356307	0.04144908	0.03896110	0.03375386	0.03602169	0.03375386	0.00000000	0.00000000	0.03602169	0.04847529

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Caprolactam
Institution:	Lab 3
Test Number	

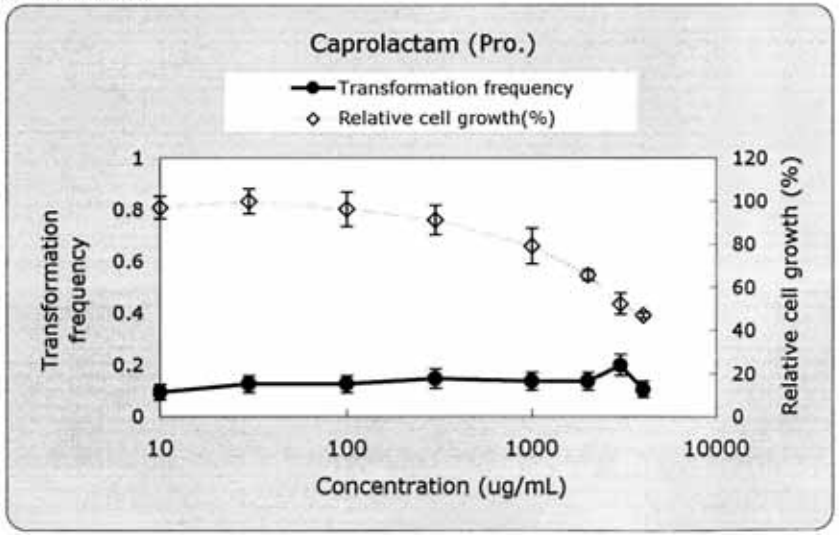
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		1	10	30	100	300	1000	2000	3000	4000		0.1% DMSO	TPA (50ng/mL)
1	0.069	1.092	1.090	1.092	1.138	1.053	0.902	0.785	0.633	0.574		1.109	1.452
2	0.076	1.226	1.237	1.232	1.125	1.111	0.940	0.797	0.623	0.570		1.131	1.589
3	0.080	1.154	1.147	1.150	1.156	1.115	0.955	0.780	0.681	0.586		1.347	1.591
4	0.084	1.224	1.142	1.085	1.200	1.043	0.970	0.844	0.653	0.602		1.178	1.607
5	0.087	1.199	1.052	1.205	1.145	1.170	1.016	0.792	0.710	0.598		1.201	1.493
6	0.087	1.119	1.115	1.187	1.146	1.082	0.959	0.804	0.672	0.594		1.136	1.627
7	0.086	1.157	1.129	1.146	1.197	1.105	1.051	0.799	0.686	0.611		1.117	1.515
8	0.081	1.186	1.180	1.255	0.924	0.920	0.752	0.756	0.538	0.602		1.102	1.624
Average	0.081	1.170	1.137	1.169	1.129	1.075	0.943	0.795	0.650	0.592	#DIV/0!	1.165	1.562
SD	0.006	0.048	0.056	0.062	0.087	0.074	0.090	0.025	0.053	0.014	#DIV/0!	0.081	0.066
Average - Blank	0.000	1.088	1.055	1.088	1.048	0.994	0.862	0.713	0.568	0.511	#DIV/0!	1.084	1.481
Relative Cell Growth (%)		100.0	97.0	99.9	96.3	91.3	79.2	65.5	52.2	46.9	#DIV/0!	100.0	136.6
SD of Relative Cell Growth		4.4	5.1	5.7	8.0	6.8	8.2	2.3	4.9	1.3	#DIV/0!	7.4	6.1

Transformation Assay

Giemsa Staining	1	10	30	100	300	1000	2000	3000	4000	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	9	12	12	14	13	13	19	10		11	54
Transformation frequency*	0.104166667	0.09375	0.125	0.125	0.145833333	0.135416667	0.135416667	0.197916667	0.104166667	0	0.114583333	0.5625
SE	0.03117758	0.02974911	0.03375386	0.03375386	0.03602169	0.03492238	0.03492238	0.04066449	0.03117758	0.00000000	0.03250865	0.05063079

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Eugenol
Institution:	Lab 2
Test Number	

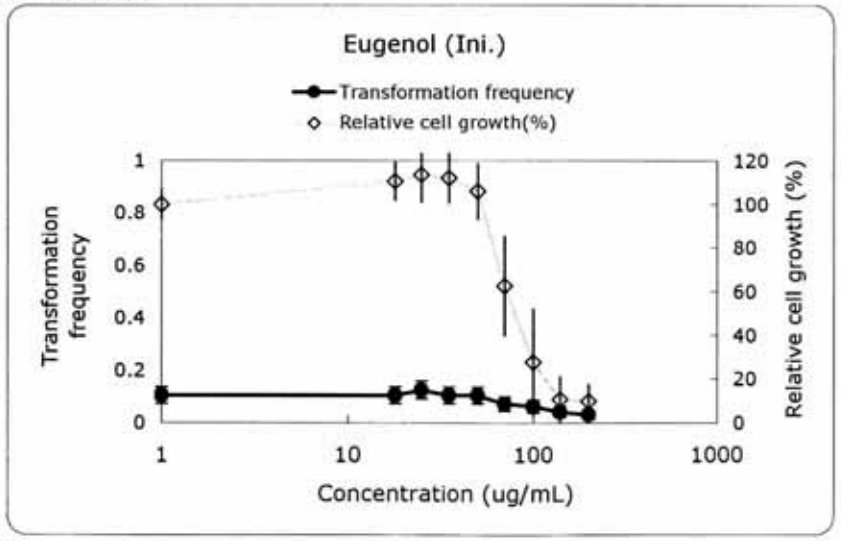
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	18	25	35	50	70	100	140	200		0.1% DMSO	MCA (1ug/mL)
1	0.065	0.383	0.450	0.414	0.429	0.478	0.462	0.365	0.182	0.160		0.383	0.197
2	0.062	0.424	0.486	0.461	0.425	0.473	0.306	0.163	0.103	0.094		0.424	0.156
3	0.060	0.432	0.414	0.408	0.465	0.381	0.231	0.118	0.097	0.085		0.432	0.105
4	0.059	0.436	0.407	0.537	0.533	0.390	0.274	0.112	0.094	0.087		0.436	0.120
5	0.060	0.423	0.486	0.487	0.445	0.446	0.255	0.113	0.079	0.081		0.423	0.125
6	0.060	0.401	0.469	0.420	0.432	0.384	0.233	0.125	0.081	0.086		0.401	0.109
7	0.060	0.422	0.448	0.471	0.493	0.421	0.208	0.124	0.088	0.082		0.422	0.133
8	0.065	0.371	0.429	0.471	0.414	0.487	0.278	0.150	0.068	0.094		0.371	0.146
Average	0.061	0.412	0.449	0.459	0.455	0.433	0.281	0.159	0.099	0.096	#DIV/0!	0.412	0.136
SD	0.002	0.024	0.031	0.044	0.041	0.044	0.080	0.085	0.035	0.026	#DIV/0!	0.024	0.030
Average - Blank	0.000	0.350	0.387	0.397	0.393	0.371	0.220	0.097	0.038	0.035	#DIV/0!	0.350	0.075
Relative Cell Growth (%)		100.0	110.6	113.5	112.3	106.0	62.7	27.8	10.7	9.9	#DIV/0!	100.0	21.4
SD of Relative Cell Growth		6.8	8.7	12.5	11.6	12.7	22.7	24.4	10.1	7.5	#DIV/0!	6.8	8.6

Transformation Assay

Giemsa Staining	1	18	25	35	50	70	100	140	200	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	10	12	10	10	7	6	4	3		10	64
Transformation frequency*	0.104166667	0.104166667	0.125	0.104166667	0.104166667	0.072916667	0.0625	0.041666667	0.03125	0	0.104166667	0.666666667
SE	0.03117758	0.03117758	0.03375386	0.03117758	0.03117758	0.02653610	0.02470529	0.02039469	0.01775805	0.00000000	0.03117758	0.04811252

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Eugenol
Institution:	Lab 3
Test Number	

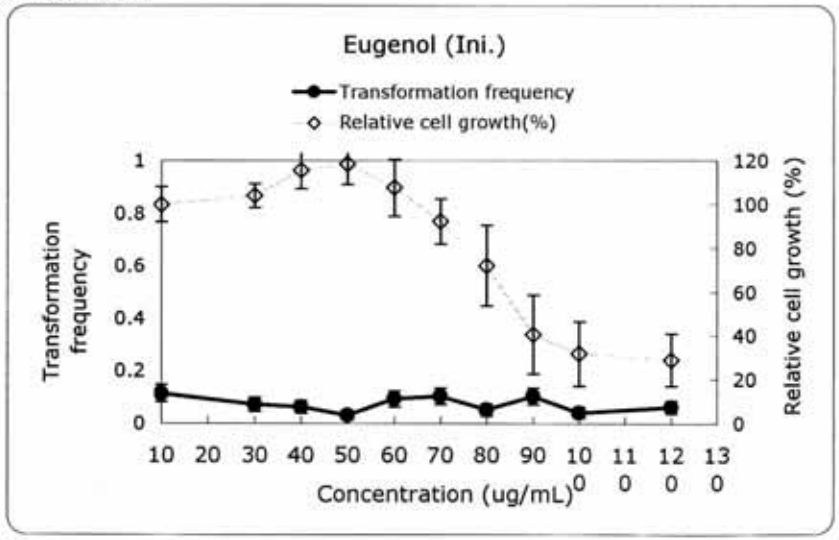
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	30	40	50	60	70	80	90	100	120	0.1% DMSO	MCA (1ug/mL)
1	0.087	0.781	0.757	0.817	0.828	0.899	0.777	0.777	0.503	0.472	0.366	0.781	0.239
2	0.088	0.747	0.791	0.874	0.757	0.747	0.722	0.551	0.383	0.312	0.377	0.747	0.251
3	0.089	0.717	0.686	0.810	0.890	0.798	0.659	0.475	0.295	0.283	0.311	0.717	0.228
4	0.090	0.734	0.706	0.785	0.847	0.756	0.640	0.509	0.257	0.273	0.228	0.734	0.167
5	0.086	0.677	0.741	0.807	0.790	0.769	0.625	0.415	0.220	0.190	0.221	0.677	0.178
6	0.081	0.677	0.717	0.853	0.813	0.640	0.631	0.483	0.213	0.182	0.204	0.677	0.169
7	0.084	0.648	0.710	0.742	0.883	0.665	0.586	0.447	0.345	0.252	0.217	0.648	0.177
8	0.082	0.643	0.710	0.714	0.728	0.729	0.603	0.590	0.477	0.295	0.201	0.643	0.184
Average	0.086	0.703	0.727	0.800	0.817	0.750	0.655	0.531	0.337	0.282	0.266	0.703	0.199
SD	0.003	0.050	0.034	0.053	0.057	0.080	0.064	0.114	0.111	0.090	0.074	0.050	0.034
Average - Blank	0.000	0.617	0.641	0.714	0.731	0.665	0.570	0.445	0.251	0.197	0.180	0.617	0.113
Relative Cell Growth (%)		100.0	103.9	115.8	118.5	107.7	92.3	72.1	40.6	31.8	29.1	100.0	18.4
SD of Relative Cell Growth		8.0	5.5	8.6	9.3	12.9	10.3	18.5	18.0	14.6	12.0	8.0	5.6

Transformation Assay

Giemsa Staining	10	30	40	50	60	70	80	90	100	120	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	7	6	3	9	10	5	10	4	6	11	59
Transformation frequency*	0.114583333	0.072916667	0.0625	0.03125	0.09375	0.104166667	0.052083333	0.104166667	0.041666667	0.0625	0.114583333	0.614583333
SE	0.03250865	0.02653610	0.02470529	0.01775805	0.02974911	0.03117758	0.02267769	0.03117758	0.02039469	0.02470529	0.03250865	0.04967296

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Eugenol
Institution:	Lab 2
Test Number	

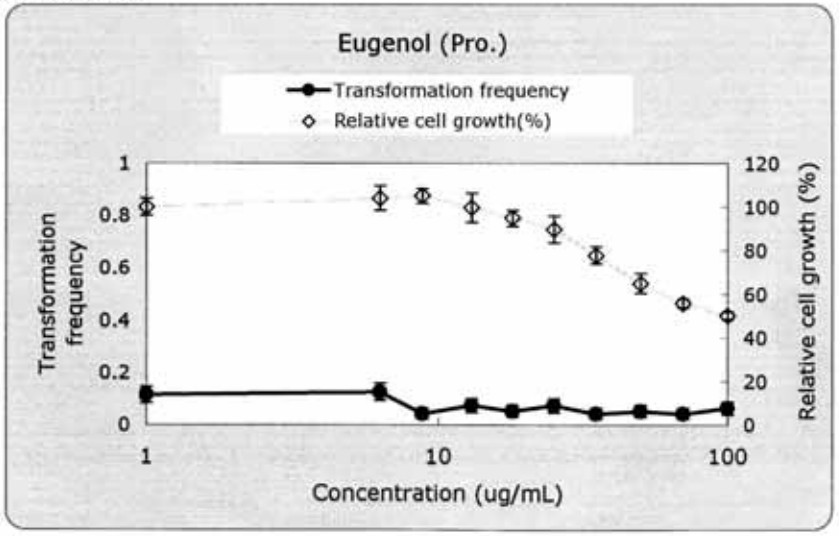
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	6.3	8.8	13	18	25	35	50	70	100	0.1% DMSO	TPA (50ng/mL)
1	0.060	0.304	0.301	0.317	0.280	0.292	0.276	0.239	0.211	0.194	0.190	0.304	0.280
2	0.063	0.303	0.330	0.316	0.331	0.297	0.312	0.264	0.227	0.207	0.187	0.303	0.283
3	0.063	0.301	0.336	0.316	0.301	0.309	0.286	0.254	0.243	0.204	0.183	0.301	0.274
4	0.065	0.331	0.322	0.338	0.304	0.301	0.294	0.254	0.218	0.197	0.184	0.331	0.264
5	0.065	0.307	0.330	0.312	0.292	0.294	0.276	0.256	0.218	0.206	0.186	0.307	0.281
6	0.066	0.310	0.305	0.322	0.318	0.295	0.282	0.262	0.225	0.195	0.182	0.310	0.258
7	0.065	0.301	0.313	0.322	0.306	0.294	0.266	0.259	0.227	0.196	0.194	0.301	0.278
8	0.064	0.304	0.302	0.315	0.319	0.277	0.267	0.240	0.208	0.199	0.182	0.304	0.273
Average	0.064	0.308	0.317	0.320	0.306	0.295	0.282	0.254	0.222	0.200	0.186	0.308	0.274
SD	0.002	0.010	0.014	0.008	0.016	0.009	0.015	0.009	0.011	0.005	0.004	0.010	0.009
Average - Blank	0.000	0.244	0.254	0.256	0.243	0.231	0.219	0.190	0.158	0.136	0.122	0.244	0.210
Relative Cell Growth (%)		100.0	104.0	105.0	99.5	94.8	89.6	77.8	64.9	55.7	50.1	100.0	86.2
SD of Relative Cell Growth		4.1	5.7	3.3	6.6	3.7	6.2	3.8	4.5	2.1	1.7	4.1	3.6

Transformation Assay

Giemsa Staining	1	6.3	8.8	13	18	25	35	50	70	100	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	11	12	4	7	5	7	4	5	4	6	11	48
Transformation frequency*	0.114583333	0.125	0.041666667	0.072916667	0.052083333	0.072916667	0.041666667	0.052083333	0.041666667	0.0625	0.114583333	0.5
SE	0.03250865	0.03375386	0.02039469	0.02653610	0.02267769	0.02653610	0.02039469	0.02267769	0.02039469	0.02470529	0.03250865	0.05103104

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Eugenol
Institution:	Lab 3
Test Number	

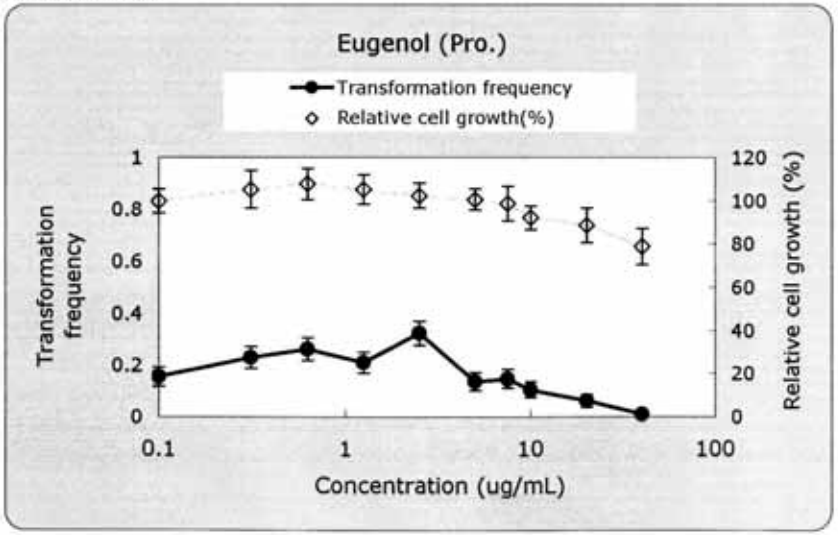
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.3125	0.625	1.25	2.5	5	7.5	10	20	40	0.1% DMSO	TPA (50ng/mL)
1	0.114	1.373	1.373	1.571	1.351	1.238	1.319	1.218	1.174	1.131	0.982	1.373	1.409
2	0.127	1.397	1.310	1.396	1.374	1.433	1.400	1.311	1.262	1.127	1.045	1.397	1.423
3	0.134	1.399	1.332	1.420	1.416	1.415	1.414	1.317	1.239	1.223	1.001	1.399	1.624
4	0.126	1.319	1.497	1.517	1.396	1.467	1.378	1.343	1.311	1.218	1.087	1.319	1.605
5	0.124	1.448	1.568	1.375	1.545	1.452	1.318	1.340	1.405	1.305	1.212	1.448	1.507
6	0.119	1.518	1.623	1.499	1.595	1.435	1.468	1.477	1.290	1.303	1.200	1.518	1.733
7	0.115	1.297	1.481	1.640	1.479	1.445	1.375	1.418	1.347	1.428	1.240	1.297	1.640
8	0.109	1.345	1.453	1.463	1.463	1.448	1.487	1.532	1.276	1.213	1.194	1.345	1.726
Average	0.121	1.387	1.455	1.485	1.452	1.417	1.395	1.370	1.288	1.244	1.120	1.387	1.583
SD	0.008	0.072	0.111	0.091	0.085	0.074	0.062	0.101	0.070	0.100	0.103	0.072	0.125
Average - Blank	0.000	1.266	1.334	1.364	1.331	1.296	1.274	1.249	1.167	1.123	0.999	1.266	1.462
Relative Cell Growth (%)		100.0	105.3	107.8	105.2	102.3	100.6	98.6	92.2	88.7	78.9	100.0	115.5
SD of Relative Cell Growth		5.6	8.8	7.2	6.7	5.8	4.9	8.0	5.5	7.9	8.2	5.6	9.9

Transformation Assay

Giemsa Staining	0.1	0.3125	0.625	1.25	2.5	5	7.5	10	20	40	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	15	22	25	20	31	13	14	10	6	1	15	85
Transformation frequency*	0.15625	0.229166667	0.260416667	0.208333333	0.322916667	0.135416667	0.145833333	0.104166667	0.0625	0.010416667	0.15625	0.885416667
SE	0.03705794	0.04289635	0.04479116	0.04144908	0.04772332	0.03492238	0.03602169	0.03117758	0.02470529	0.01036227	0.03705794	0.03250865

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Progressing Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Eugenol
Institution:	Lab 3
Test Number	

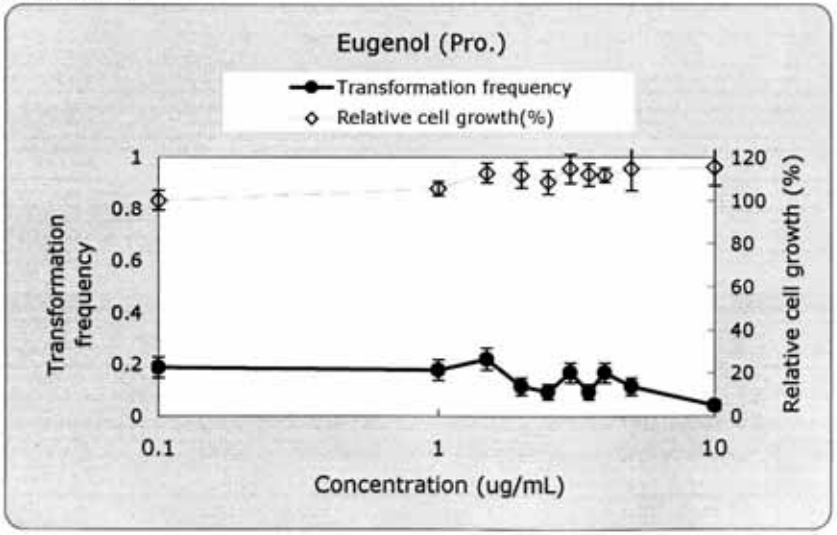
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.1% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	1	1.5	2	2.5	3	3.5	4	5	10	0.1% DMSO	TPA (50ng/mL)
1	0.064	0.612	0.578	0.617	0.610	0.594	0.601	0.612	0.640	0.624	0.653	0.612	0.798
2	0.064	0.542	0.628	0.665	0.581	0.590	0.677	0.625	0.625	0.674	0.620	0.542	0.765
3	0.064	0.568	0.606	0.654	0.633	0.615	0.688	0.640	0.653	0.621	0.670	0.568	0.767
4	0.068	0.591	0.605	0.632	0.651	0.645	0.676	0.671	0.637	0.617	0.660	0.591	0.764
5	0.067	0.584	0.607	0.676	0.665	0.601	0.660	0.606	0.666	0.649	0.671	0.584	0.753
6	0.068	0.572	0.618	0.655	0.656	0.649	0.620	0.639	0.634	0.771	0.750	0.572	0.837
7	0.069	0.601	0.588	0.608	0.662	0.665	0.681	0.639	0.611	0.638	0.623	0.601	0.821
8	0.069	0.556	0.619	0.641	0.643	0.613	0.621	0.682	0.642	0.630	0.613	0.556	0.769
Average	0.067	0.578	0.606	0.644	0.638	0.622	0.653	0.639	0.639	0.653	0.658	0.578	0.784
SD	0.002	0.023	0.017	0.023	0.029	0.028	0.034	0.026	0.017	0.051	0.044	0.023	0.031
Average - Blank	0.000	0.512	0.540	0.577	0.571	0.555	0.586	0.573	0.572	0.586	0.591	0.512	0.718
Relative Cell Growth (%)		100.0	105.4	112.8	111.6	108.5	114.6	111.9	111.8	114.6	115.5	100.0	140.3
SD of Relative Cell Growth		4.6	3.2	4.6	5.7	5.5	6.6	5.2	3.3	10.0	8.6	4.6	6.0

Transformation Assay

Giemsa Staining	0.1	1	1.5	2	2.5	3	3.5	4	5	10	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0	0	0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	18	17	21	11	9	16	9	16	11	4	18	83
Transformation frequency*	0.1875	0.177083333	0.21875	0.114583333	0.09375	0.166666667	0.09375	0.166666667	0.114583333	0.041666667	0.1875	0.864583333
SE	0.03983609	0.03896110	0.04219232	0.03250865	0.02974911	0.03803629	0.02974911	0.03803629	0.03250865	0.02039469	0.03983609	0.03492238

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	D-Mannitol
Institution:	Lab 2
Test Number	

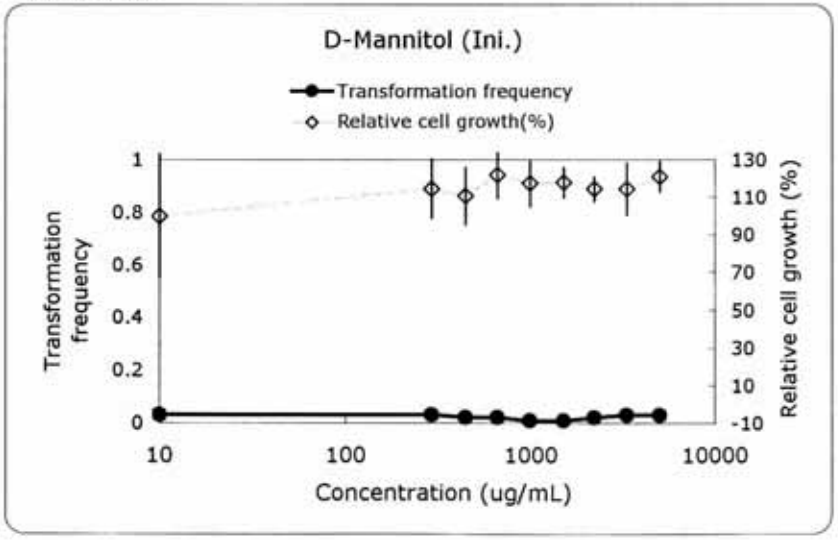
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	290	440	660	990	1500	2200	3300	5000		0.1% DMSO	MCA (1ug/mL)
1	0.104	0.299	0.308	0.275	0.307	0.276	0.297	0.309	0.305	0.301		0.257	0.174
2	0.107	0.299	0.339	0.311	0.288	0.313	0.333	0.304	0.308	0.335		0.322	0.211
3	0.104	0.340	0.301	0.311	0.318	0.292	0.306	0.325	0.368	0.318		0.329	0.212
4	0.107	0.290	0.316	0.304	0.335	0.343	0.312	0.318	0.295	0.333		0.318	0.188
5	0.097	0.321	0.345	0.297	0.359	0.335	0.326	0.304	0.290	0.314		0.318	0.215
6	0.100	0.328	0.321	0.351	0.343	0.333	0.338	0.316	0.320	0.345		0.290	0.199
7	0.095	0.234	0.299	0.317	0.308	0.318	0.304	0.316	0.293	0.318		0.240	0.216
8	0.098	0.158	0.252	0.259	0.330	0.312	0.311	0.286	0.300	0.311		0.148	0.183
Average	0.102	0.284	0.310	0.303	0.324	0.315	0.316	0.310	0.310	0.322	#DIV/0!	0.278	0.200
SD	0.005	0.060	0.029	0.028	0.023	0.023	0.015	0.012	0.025	0.015	#DIV/0!	0.062	0.016
Average - Blank	0.000	0.182	0.209	0.202	0.222	0.214	0.214	0.208	0.208	0.220	#DIV/0!	0.176	0.098
Relative Cell Growth (%)		100.0	114.6	110.7	121.9	117.4	117.7	114.3	114.4	121.0	#DIV/0!	100.0	55.7
SD of Relative Cell Growth		33.0	15.8	15.2	12.5	12.4	8.1	6.6	13.9	8.0	#DIV/0!	33.9	8.9

Transformation Assay

Giemsa Staining	10	290	440	660	990	1500	2200	3300	5000	0	Negative Control	Positive Control
No. of wells excluded	0	0	2	0	0	0	1	0	0	0	0	0
No. of wells counted	96	96	94	96	96	96	95	96	96	96	96	96
No. of wells having foci	3	3	2	2	1	1	2	3	3		4	57
Transformation frequency*	0.03125	0.03125	0.021276596	0.020833333	0.010416667	0.010416667	0.021052632	0.03125	0.03125	0	0.041666667	0.59375
SE	0.01775805	0.01775805	0.01488391	0.01457713	0.01036227	0.01036227	0.01472893	0.01775805	0.01775805	0.00000000	0.02039469	0.05012598

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	D-Mannitol
Institution:	Lab 3
Test Number	

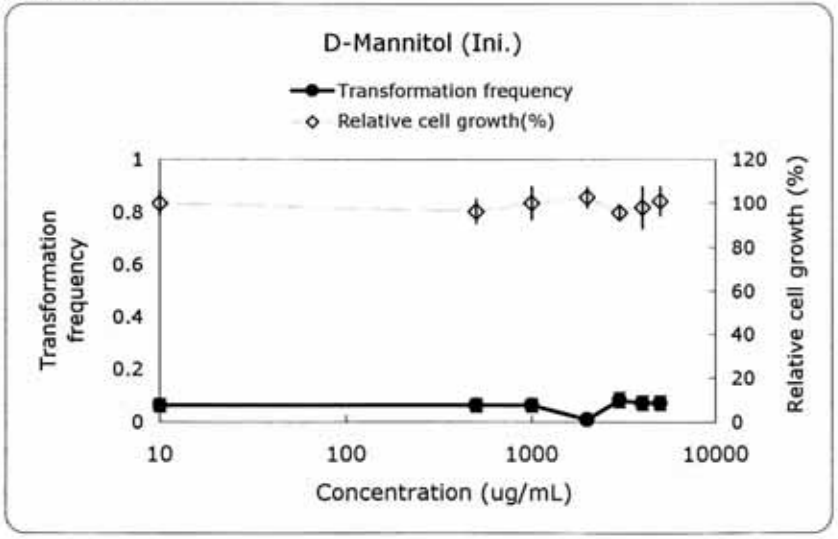
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	500	1000	2000	3000	4000	5000				0.1% DMSO	MCA (1ug/mL)
1	0.118	1.304	1.168	1.191	1.428	1.295	1.451	1.373				1.231	0.537
2	0.120	1.270	1.282	1.178	1.274	1.263	1.306	1.235				1.336	0.643
3	0.119	1.290	1.217	1.373	1.307	1.207	1.196	1.271				1.270	0.571
4	0.122	1.245	1.312	1.317	1.295	1.231	1.177	1.478				1.337	0.534
5	0.121	1.370	1.351	1.292	1.332	1.234	1.133	1.296				1.231	0.528
6	0.123	1.285	1.208	1.359	1.335	1.313	1.335	1.263				1.278	0.666
7	0.125	1.446	1.288	1.405	1.379	1.280	1.314	1.362				1.331	0.571
8	0.123	1.303	1.328	1.404	1.410	1.274	1.407	1.327				1.369	0.526
Average	0.121	1.314	1.269	1.315	1.345	1.262	1.290	1.326	#DIV/0!	#DIV/0!	#DIV/0!	1.298	0.572
SD	0.002	0.064	0.065	0.089	0.055	0.036	0.113	0.078	#DIV/0!	#DIV/0!	#DIV/0!	0.052	0.054
Average - Blank	0.000	1.193	1.148	1.194	1.224	1.141	1.169	1.204	#DIV/0!	#DIV/0!	#DIV/0!	1.177	0.451
Relative Cell Growth (%)		100.0	96.2	100.1	102.6	95.6	98.0	101.0	#DIV/0!	#DIV/0!	#DIV/0!	100.0	38.3
SD of Relative Cell Growth		5.4	5.4	7.5	4.6	3.0	9.4	6.6	#DIV/0!	#DIV/0!	#DIV/0!	4.4	4.5

Transformation Assay

Giemsa Staining	10	500	1000	2000	3000	4000	5000	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	6	6	1	8	7	7				10	46
Transformation frequency*	0.0625	0.0625	0.0625	0.010416667	0.083333333	0.072916667	0.072916667	0	0	0	0.104166667	0.479166667
SE	0.02470529	0.02470529	0.02470529	0.01036227	0.02820847	0.02653610	0.02653610	0.00000000	0.00000000	0.00000000	0.03117758	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	D-Mannitol
Institution:	Lab 2
Test Number	

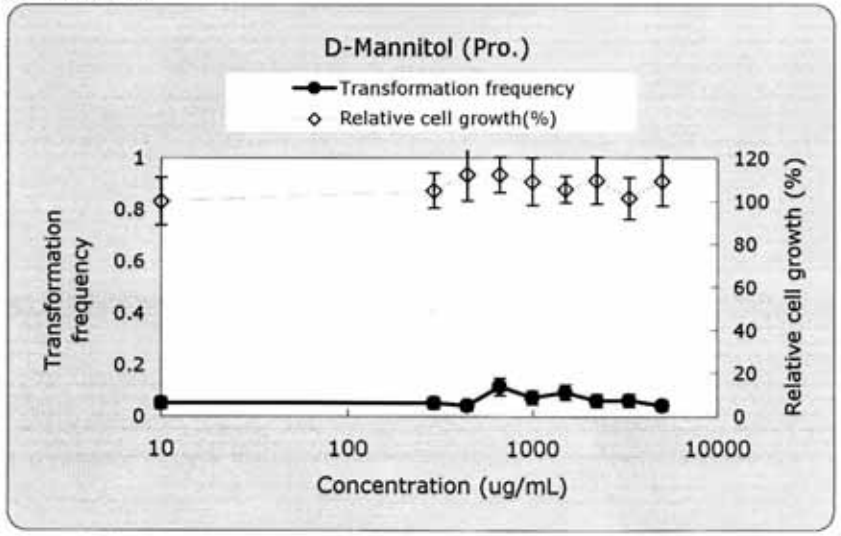
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	290	440	660	990	1500	2200	3300	5000		0.1% DMSO	TPA (50ng/mL)
1	0.104	0.330	0.350	0.355	0.366	0.388	0.348	0.399	0.359	0.355		0.335	0.500
2	0.107	0.339	0.397	0.395	0.412	0.385	0.380	0.404	0.381	0.393		0.355	0.482
3	0.104	0.357	0.363	0.421	0.376	0.399	0.381	0.399	0.335	0.380		0.336	0.458
4	0.107	0.412	0.361	0.428	0.401	0.381	0.385	0.359	0.396	0.375		0.345	0.467
5	0.097	0.347	0.371	0.392	0.398	0.407	0.363	0.401	0.330	0.430		0.339	0.466
6	0.100	0.373	0.399	0.356	0.385	0.341	0.372	0.335	0.376	0.397		0.330	0.485
7	0.095	0.355	0.352	0.391	0.395	0.389	0.377	0.384	0.352	0.342		0.335	0.440
8	0.098	0.323	0.345	0.346	0.350	0.328	0.342	0.349	0.335	0.352		0.315	0.448
Average	0.102	0.355	0.367	0.386	0.385	0.377	0.369	0.379	0.358	0.378	#DIV/0!	0.336	0.468
SD	0.005	0.028	0.021	0.031	0.020	0.028	0.016	0.027	0.024	0.029	#DIV/0!	0.012	0.020
Average - Blank	0.000	0.253	0.266	0.284	0.284	0.276	0.267	0.277	0.257	0.277	#DIV/0!	0.235	0.367
Relative Cell Growth (%)		100.0	105.0	112.3	112.2	109.0	105.5	109.6	101.4	109.3	#DIV/0!	100.0	156.2
SD of Relative Cell Growth		11.1	8.2	12.1	8.1	11.0	6.3	10.7	9.7	11.4	#DIV/0!	4.6	7.9

Transformation Assay

Giemsa Staining	10	290	440	660	990	1500	2200	3300	5000	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	5	4	11	7	9	6	6	4		8	43
Transformation frequency*	0.052083333	0.052083333	0.041666667	0.114583333	0.072916667	0.09375	0.0625	0.0625	0.041666667	0	0.083333333	0.447916667
SE	0.02267769	0.02267769	0.02039469	0.03250865	0.02653610	0.02974911	0.02470529	0.02470529	0.02039469	0.00000000	0.02820847	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	D-Mannitol
Institution:	Lab 3
Test Number	

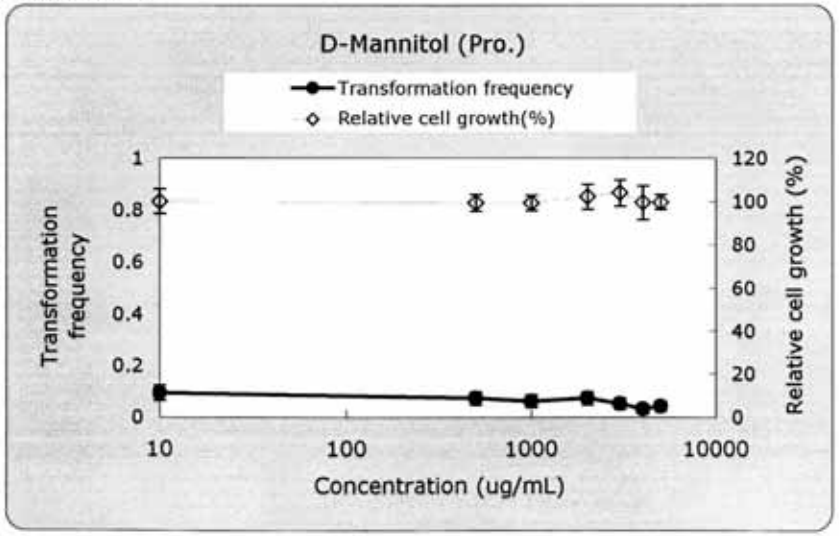
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		10	500	1000	2000	3000	4000	5000				0.1% DMSO	TPA (50ng/mL)
1	0.104	1.086	1.126	1.100	1.104	1.199	1.149	1.166				1.146	1.729
2	0.109	1.113	1.163	1.116	1.186	1.085	1.215	1.166				1.088	1.648
3	0.114	1.129	1.174	1.200	1.241	1.169	1.120	1.162				1.211	1.786
4	0.114	1.159	1.175	1.179	1.220	1.276	1.227	1.109				1.181	1.682
5	0.112	1.169	1.221	1.167	1.142	1.276	1.231	1.194				1.197	1.783
6	0.111	1.248	1.126	1.198	1.184	1.216	1.217	1.157				1.163	1.840
7	0.115	1.215	1.113	1.162	1.299	1.228	0.987	1.149				1.141	1.774
8	0.117	1.248	1.210	1.183	1.171	1.251	1.179	1.236				1.311	1.750
Average	0.112	1.171	1.164	1.163	1.193	1.213	1.166	1.167	#DIV/0!	#DIV/0!	#DIV/0!	1.180	1.749
SD	0.004	0.061	0.040	0.037	0.060	0.063	0.082	0.036	#DIV/0!	#DIV/0!	#DIV/0!	0.065	0.062
Average - Blank	0.000	1.059	1.052	1.051	1.081	1.101	1.054	1.055	#DIV/0!	#DIV/0!	#DIV/0!	1.068	1.637
Relative Cell Growth (%)		100.0	99.3	99.3	102.1	103.9	99.5	99.7	#DIV/0!	#DIV/0!	#DIV/0!	100.0	153.3
SD of Relative Cell Growth		5.8	3.8	3.5	5.7	6.0	7.8	3.4	#DIV/0!	#DIV/0!	#DIV/0!	6.2	5.8

Transformation Assay

Giemsa Staining	10	500	1000	2000	3000	4000	5000	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	7	6	7	5	3	4				8	77
Transformation frequency*	0.09375	0.072916667	0.0625	0.072916667	0.052083333	0.03125	0.041666667	0	0	0	0.083333333	0.802083333
SE	0.02974911	0.02653610	0.02470529	0.02653610	0.02267769	0.01775805	0.02039469	0.00000000	0.00000000	0.00000000	0.02820847	0.04066449

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phorbol
Institution:	Lab 1
Test Number	

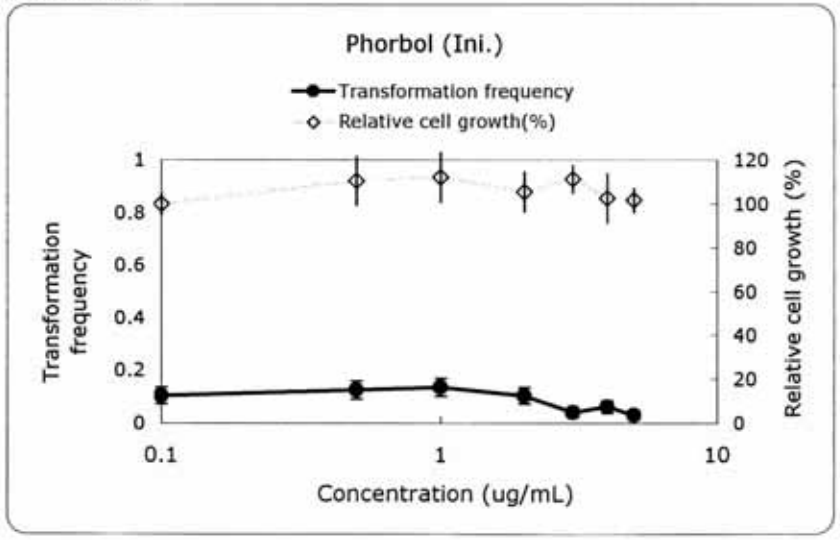
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	2	3	4	5				0.1% DMSO	MCA (1ug/mL)
1	0.107	0.829	0.776	0.898	0.808	0.888	0.816	0.815				0.866	0.369
2	0.125	0.869	0.900	0.868	0.990	0.920	0.785	0.773				0.837	0.484
3	0.114	0.867	0.911	0.810	0.920	0.890	0.757	0.864				0.761	0.512
4	0.133	0.786	0.826	0.956	0.869	0.900	0.945	0.878				0.897	0.419
5	0.121	0.802	0.940	1.075	0.854	0.860	0.821	0.845				0.839	0.462
6	0.150	0.818	1.014	0.966	0.851	1.003	0.860	0.863				0.832	0.454
7	0.142	0.859	0.967	0.947	0.922	0.931	0.980	0.885				1.009	0.471
8	0.164	0.880	0.965	0.878	0.803	0.949	0.897	0.876				0.854	0.395
Average	0.132	0.839	0.912	0.925	0.877	0.918	0.858	0.850	#DIV/0!	#DIV/0!	#DIV/0!	0.862	0.446
SD	0.019	0.035	0.078	0.080	0.063	0.044	0.078	0.038	#DIV/0!	#DIV/0!	#DIV/0!	0.071	0.048
Average - Blank	0.000	0.707	0.780	0.793	0.745	0.786	0.726	0.718	#DIV/0!	#DIV/0!	#DIV/0!	0.730	0.314
Relative Cell Growth (%)		100.0	110.4	112.2	105.4	111.2	102.7	101.6	#DIV/0!	#DIV/0!	#DIV/0!	100.0	43.0
SD of Relative Cell Growth		4.9	11.1	11.3	9.0	6.3	11.0	5.4	#DIV/0!	#DIV/0!	#DIV/0!	10.0	6.8

Transformation Assay

Giemsa Staining	0.1	0.5	1	2	3	4	5	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	12	13	10	4	6	3				5	46
Transformation frequency*	0.104166667	0.125	0.135416667	0.104166667	0.041666667	0.0625	0.03125	0	0	0	0.052083333	0.479166667
SE	0.03117758	0.03375386	0.03492238	0.03117758	0.02039469	0.02470529	0.01775805	0.00000000	0.00000000	0.00000000	0.02267769	0.05098672

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Phorbol
Institution:	Lab 3
Test Number	

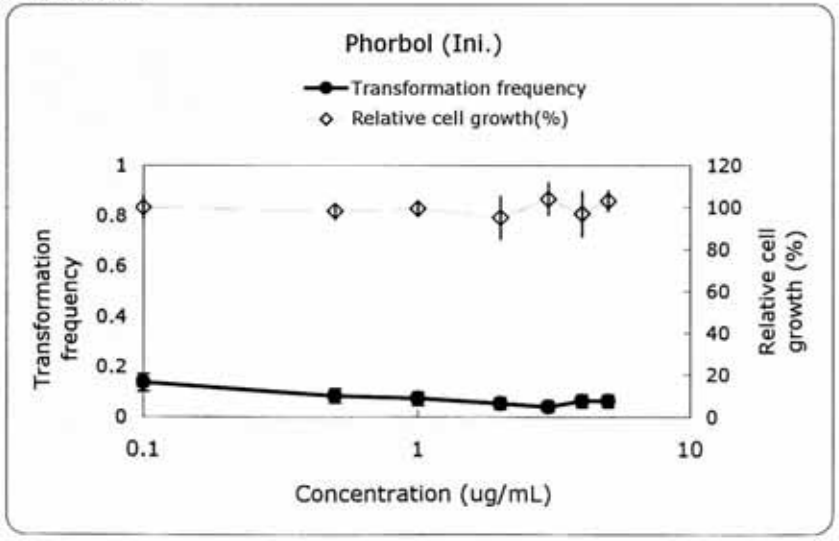
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	2	3	4	5				0.1% DMSO	MCA (1ug/mL)
1	0.125	1.211	1.190	1.273	0.976	1.243	0.959	1.301				1.261	0.687
2	0.127	1.381	1.237	1.293	1.217	1.281	1.333	1.308				1.209	0.603
3	0.126	1.197	1.299	1.284	1.243	1.327	1.206	1.345				1.279	0.606
4	0.124	1.325	1.223	1.302	1.276	1.193	1.295	1.427				1.180	0.677
5	0.119	1.338	1.274	1.201	1.314	1.401	1.239	1.280				1.186	0.722
6	0.119	1.272	1.329	1.289	1.267	1.314	1.305	1.358				1.190	0.735
7	0.116	1.248	1.284	1.321	1.362	1.429	1.348	1.257				1.308	0.671
8	0.108	1.291	1.251	1.255	1.145	1.444	1.288	1.264				1.256	0.678
Average	0.121	1.283	1.261	1.277	1.225	1.329	1.247	1.318	#DIV/0!	#DIV/0!	#DIV/0!	1.234	0.672
SD	0.006	0.064	0.045	0.036	0.119	0.090	0.125	0.057	#DIV/0!	#DIV/0!	#DIV/0!	0.049	0.048
Average - Blank	0.000	1.162	1.140	1.157	1.105	1.209	1.126	1.197	#DIV/0!	#DIV/0!	#DIV/0!	1.113	0.552
Relative Cell Growth (%)		100.0	98.1	99.5	95.0	104.0	96.9	103.0	#DIV/0!	#DIV/0!	#DIV/0!	100.0	49.6
SD of Relative Cell Growth		5.5	3.8	3.1	10.3	7.8	10.8	4.9	#DIV/0!	#DIV/0!	#DIV/0!	4.2	4.1

Transformation Assay

Giemsa Staining	0.1	0.5	1	2	3	4	5	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	8	7	5	4	6	6				9	62
Transformation frequency*	0.135416667	0.083333333	0.072916667	0.052083333	0.041666667	0.0625	0.0625	0	0	0	0.09375	0.645833333
SE	0.03492238	0.02820847	0.02653610	0.02267769	0.02039469	0.02470529	0.02470529	0.00000000	0.00000000	0.00000000	0.02974911	0.04881221

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<15 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phorbol
Institution:	Lab 1
Test Number	

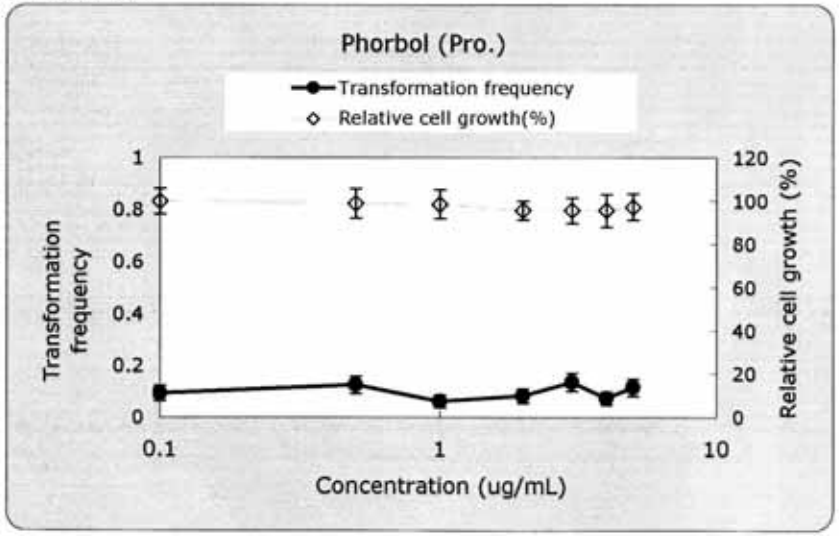
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	2	3	4	5				0.1% DMSO	TPA (50ng/mL)
1	0.107	0.809	0.774	0.780	0.801	0.765	0.729	0.777				0.759	0.982
2	0.134	0.826	0.793	0.818	0.774	0.782	0.790	0.776				0.825	1.056
3	0.127	0.794	0.865	0.772	0.839	0.836	0.876	0.887				0.785	1.090
4	0.139	0.888	0.869	0.868	0.816	0.770	0.835	0.866				0.925	1.027
5	0.131	0.897	0.764	0.830	0.855	0.844	0.804	0.803				0.802	1.058
6	0.132	0.872	0.894	0.902	0.813	0.858	0.832	0.816				0.799	1.151
7	0.126	0.819	0.852	0.862	0.798	0.841	0.856	0.844				0.761	1.066
8	0.140	0.797	0.838	0.785	0.763	0.761	0.736	0.779				0.773	1.065
Average	0.130	0.838	0.831	0.827	0.807	0.807	0.807	0.819	#DIV/0!	#DIV/0!	#DIV/0!	0.804	1.062
SD	0.010	0.042	0.048	0.047	0.031	0.041	0.053	0.043	#DIV/0!	#DIV/0!	#DIV/0!	0.054	0.048
Average - Blank	0.000	0.708	0.702	0.698	0.678	0.678	0.678	0.689	#DIV/0!	#DIV/0!	#DIV/0!	0.674	0.932
Relative Cell Growth (%)		100.0	99.1	98.5	95.7	95.7	95.7	97.3	#DIV/0!	#DIV/0!	#DIV/0!	100.0	138.3
SD of Relative Cell Growth		5.9	6.8	6.7	4.3	5.8	7.5	6.1	#DIV/0!	#DIV/0!	#DIV/0!	7.6	6.8

Transformation Assay

Giemsa Staining	0.1	0.5	1	2	3	4	5	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	12	6	8	13	7	11				10	55
Transformation frequency*	0.09375	0.125	0.0625	0.083333333	0.135416667	0.072916667	0.114583333	0	0	0	0.104166667	0.572916667
SE	0.02974911	0.03375386	0.02470529	0.02820847	0.03492238	0.02653610	0.03250865	0.00000000	0.00000000	0.00000000	0.03117758	0.05048547

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Phorbol
Institution:	Lab 3
Test Number	

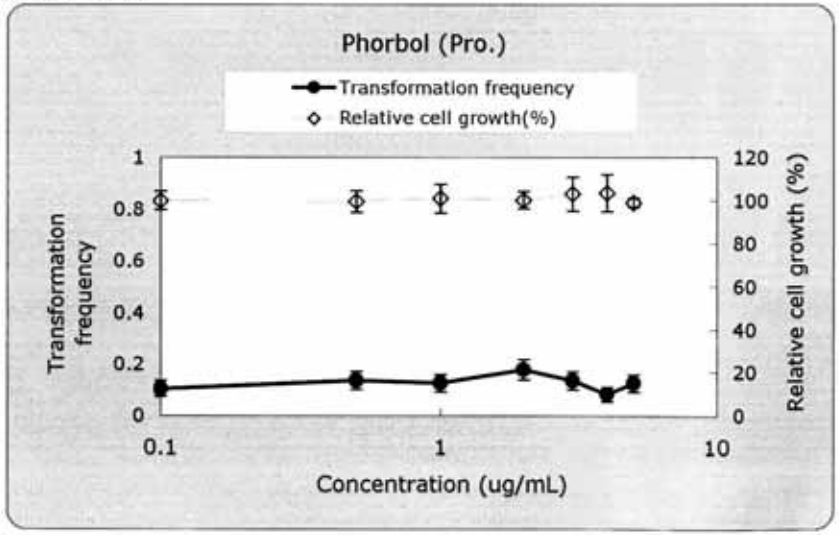
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	5% Water	Concentrations (ug/ml)									Negative Control	Positive Control
		0.1	0.5	1	2	3	4	5				0.1% DMSO	TPA (50ng/mL)
1	0.133	1.312	1.333	1.341	1.320	1.393	1.343	1.348				1.280	1.637
2	0.139	1.429	1.466	1.491	1.337	1.433	1.323	1.399				1.499	1.749
3	0.134	1.425	1.415	1.450	1.419	1.362	1.504	1.347				1.344	1.727
4	0.134	1.438	1.375	1.318	1.361	1.357	1.605	1.364				1.333	1.804
5	0.131	1.344	1.271	1.334	1.455	1.635	1.348	1.384				1.310	1.683
6	0.134	1.358	1.418	1.458	1.397	1.369	1.425	1.376				1.280	1.751
7	0.133	1.378	1.318	1.440	1.404	1.428	1.477	1.319				1.283	1.514
8	0.126	1.307	1.348	1.262	1.320	1.319	1.307	1.349				1.225	1.642
Average	0.133	1.374	1.368	1.387	1.377	1.412	1.417	1.361	#DIV/0!	#DIV/0!	#DIV/0!	1.319	1.688
SD	0.004	0.052	0.063	0.083	0.050	0.098	0.105	0.025	#DIV/0!	#DIV/0!	#DIV/0!	0.081	0.091
Average - Blank	0.000	1.241	1.235	1.254	1.244	1.279	1.284	1.228	#DIV/0!	#DIV/0!	#DIV/0!	1.186	1.555
Relative Cell Growth (%)		100.0	99.5	101.0	100.2	103.1	103.4	98.9	#DIV/0!	#DIV/0!	#DIV/0!	100.0	131.1
SD of Relative Cell Growth		4.2	5.1	6.7	4.0	7.9	8.5	2.0	#DIV/0!	#DIV/0!	#DIV/0!	6.6	7.3

Transformation Assay

Giemsa Staining	0.1	0.5	1	2	3	4	5	0	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	10	13	12	17	13	8	12				11	81
Transformation frequency*	0.104166667	0.135416667	0.125	0.177083333	0.135416667	0.083333333	0.125	0	0	0	0.114583333	0.84375
SE	0.03117758	0.03492238	0.03375386	0.03896110	0.03492238	0.02820847	0.03375386	0.00000000	0.00000000	0.00000000	0.03250865	0.03705794

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control<20 and 40<Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Pyrene
Institution:	Lab 2
Test Number	

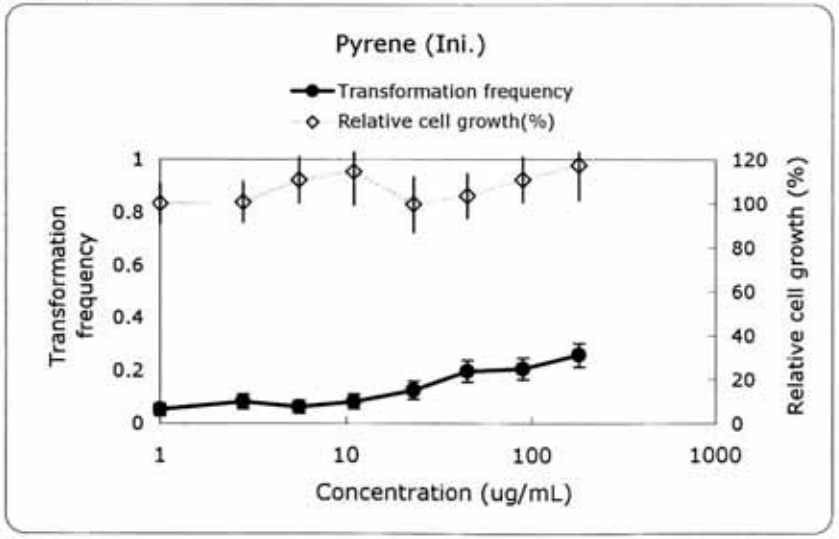
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.8	5.6	11	23	45	90	180			0.1% DMSO	MCA (1ug/mL)
1	0.065	0.321	0.325	0.347	0.387	0.350	0.362	0.375	0.385			0.320	0.208
2	0.062	0.372	0.392	0.405	0.406	0.333	0.320	0.364	0.482			0.319	0.196
3	0.060	0.364	0.341	0.356	0.377	0.377	0.344	0.354	0.357			0.356	0.226
4	0.059	0.361	0.356	0.408	0.364	0.326	0.368	0.377	0.426			0.310	0.232
5	0.060	0.413	0.357	0.399	0.425	0.349	0.383	0.438	0.372			0.376	0.221
6	0.060	0.370	0.404	0.420	0.398	0.441	0.372	0.394	0.429			0.347	0.257
7	0.060	0.347	0.380	0.435	0.503	0.370	0.415	0.429	0.475			0.338	0.212
8	0.065	0.336	0.343	0.373	0.368	0.328	0.401	0.411	0.375			0.285	0.218
Average	0.061	0.361	0.362	0.393	0.404	0.359	0.371	0.393	0.413	#DIV/0!	#DIV/0!	0.331	0.221
SD	0.002	0.028	0.027	0.031	0.045	0.038	0.030	0.031	0.048	#DIV/0!	#DIV/0!	0.029	0.018
Average - Blank	0.000	0.299	0.301	0.332	0.342	0.298	0.309	0.331	0.351	#DIV/0!	#DIV/0!	0.270	0.160
Relative Cell Growth (%)		100.0	100.6	110.8	114.4	99.6	103.4	110.8	117.4	#DIV/0!	#DIV/0!	100.0	59.2
SD of Relative Cell Growth		9.2	9.1	10.4	15.1	12.7	10.1	10.3	16.0	#DIV/0!	#DIV/0!	9.6	6.1

Transformation Assay

Giemsa Staining	1	2.8	5.6	11	23	45	90	180	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	5	8	6	8	12	19	20	25			7	53
Transformation frequency*	0.052083333	0.083333333	0.0625	0.083333333	0.125	0.197916667	0.208333333	0.260416667	0	0	0.072916667	0.552083333
SE	0.02267769	0.02820847	0.02470529	0.02820847	0.03375386	0.04066449	0.04144908	0.04479116	0.00000000	0.00000000	0.02653610	0.05075342

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Initiation Assay

Chemical Code:	Pyrene
Institution:	Lab 3
Test Number	

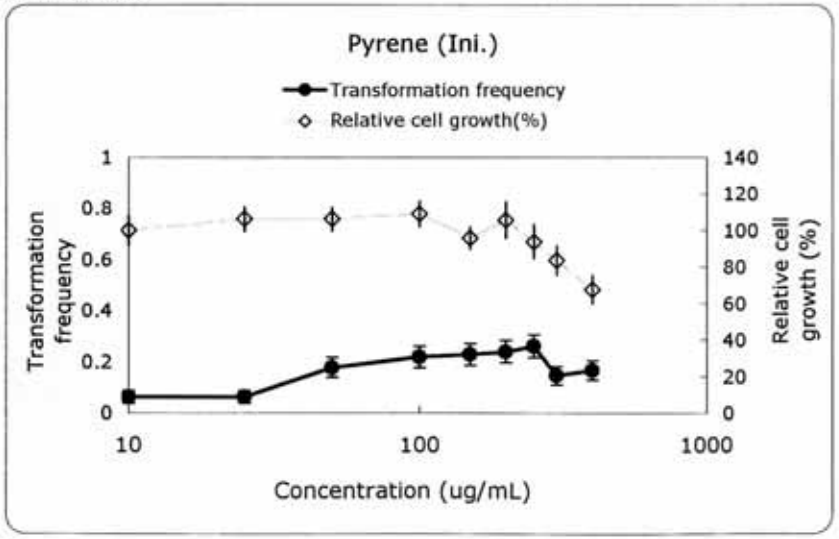
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		10	25	50	100	150	200	250	300	400		0.1% DMSO	MCA (1ug/mL)
1	0.077	0.442	0.445	0.485	0.503	0.464	0.507	0.442	0.414	0.370		0.554	0.377
2	0.098	0.484	0.494	0.502	0.504	0.420	0.503	0.480	0.411	0.368		0.518	0.301
3	0.079	0.489	0.500	0.447	0.478	0.444	0.476	0.435	0.370	0.343		0.529	0.324
4	0.090	0.442	0.480	0.482	0.520	0.426	0.424	0.383	0.384	0.308		0.458	0.314
5	0.085	0.470	0.444	0.471	0.455	0.407	0.438	0.411	0.364	0.296		0.525	0.275
6	0.087	0.401	0.485	0.437	0.498	0.449	0.466	0.387	0.368	0.307		0.504	0.306
7	0.085	0.458	0.447	0.468	0.463	0.464	0.442	0.439	0.446	0.348		0.497	0.330
8	0.099	0.429	0.497	0.500	0.454	0.418	0.524	0.456	0.380	0.334		0.432	0.261
Average	0.088	0.452	0.474	0.474	0.484	0.437	0.473	0.429	0.392	0.334	#DIV/0!	0.502	0.311
SD	0.008	0.029	0.025	0.023	0.025	0.022	0.036	0.033	0.029	0.028	#DIV/0!	0.040	0.035
Average - Blank	0.000	0.364	0.387	0.387	0.397	0.349	0.385	0.342	0.305	0.247	#DIV/0!	0.415	0.224
Relative Cell Growth (%)		100.0	106.1	106.1	108.9	95.8	105.7	93.8	83.6	67.7	#DIV/0!	100.0	53.9
SD of Relative Cell Growth		8.1	6.7	6.4	6.9	6.0	10.0	9.2	7.9	7.7	#DIV/0!	10.9	9.7

Transformation Assay

Giemsa Staining	10	25	50	100	150	200	250	300	400	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	6	6	17	21	22	23	25	14	16		13	65
Transformation frequency*	0.0625	0.0625	0.177083333	0.21875	0.229166667	0.239583333	0.260416667	0.145833333	0.166666667	0	0.135416667	0.677083333
SE	0.02470529	0.02470529	0.03896110	0.04219232	0.04289635	0.04356307	0.04479116	0.03602169	0.03803629	0.00000000	0.03492238	0.04772332

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 15 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Pyrene
Institution:	Lab 2
Test Number	

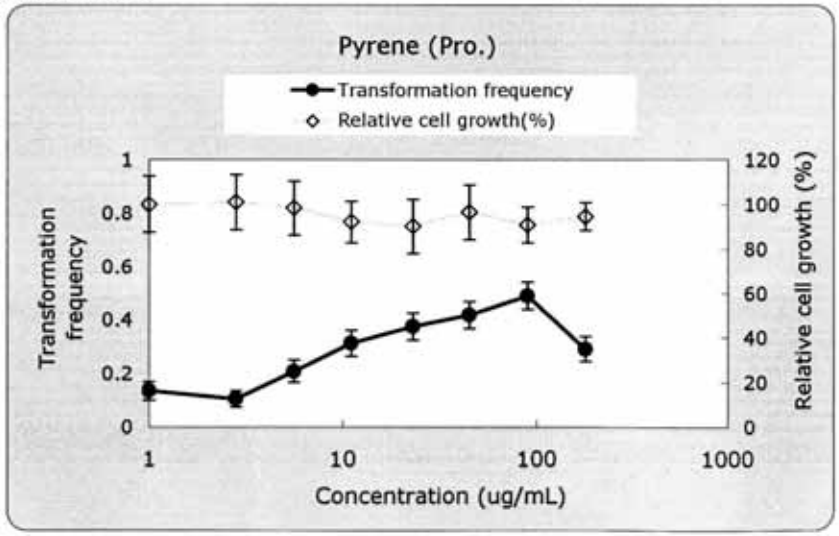
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	2.8	5.6	11	23	45	90	180			0.1% DMSO	TPA (50ng/mL)
1	0.065	0.247	0.248	0.240	0.227	0.228	0.246	0.263	0.288			0.259	0.398
2	0.062	0.291	0.288	0.273	0.282	0.239	0.259	0.261	0.266			0.306	0.433
3	0.060	0.290	0.304	0.298	0.275	0.272	0.312	0.274	0.303			0.299	0.436
4	0.059	0.304	0.315	0.327	0.294	0.301	0.290	0.282	0.274			0.297	0.447
5	0.060	0.328	0.311	0.314	0.282	0.293	0.298	0.290	0.300			0.294	0.453
6	0.060	0.299	0.294	0.294	0.279	0.296	0.325	0.303	0.279			0.332	0.438
7	0.060	0.330	0.340	0.306	0.296	0.286	0.298	0.257	0.267			0.294	0.444
8	0.065	0.258	0.266	0.267	0.266	0.249	0.256	0.246	0.270			0.266	0.389
Average	0.061	0.293	0.296	0.290	0.275	0.271	0.286	0.272	0.281	#DIV/0!	#DIV/0!	0.293	0.430
SD	0.002	0.030	0.029	0.028	0.022	0.028	0.029	0.019	0.015	#DIV/0!	#DIV/0!	0.023	0.023
Average - Blank	0.000	0.232	0.234	0.229	0.214	0.209	0.224	0.211	0.220	#DIV/0!	#DIV/0!	0.232	0.368
Relative Cell Growth (%)		100.0	101.0	98.5	92.1	90.1	96.6	90.8	94.6	#DIV/0!	#DIV/0!	100.0	158.8
SD of Relative Cell Growth		12.7	12.5	12.2	9.4	12.2	12.3	8.1	6.3	#DIV/0!	#DIV/0!	9.8	10.1

Transformation Assay

Giemsa Staining	1	2.8	5.6	11	23	45	90	180	0	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0			0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	13	10	20	30	36	40	47	28			7	52
Transformation frequency*	0.135416667	0.104166667	0.208333333	0.3125	0.375	0.416666667	0.489583333	0.291666667	0	0	0.072916667	0.541666667
SE	0.03492238	0.03117758	0.04144908	0.04730704	0.04941059	0.05031728	0.05101996	0.04639024	0.00000000	0.00000000	0.02653610	0.05085354

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Pyrene
Institution:	Lab 3
Test Number	

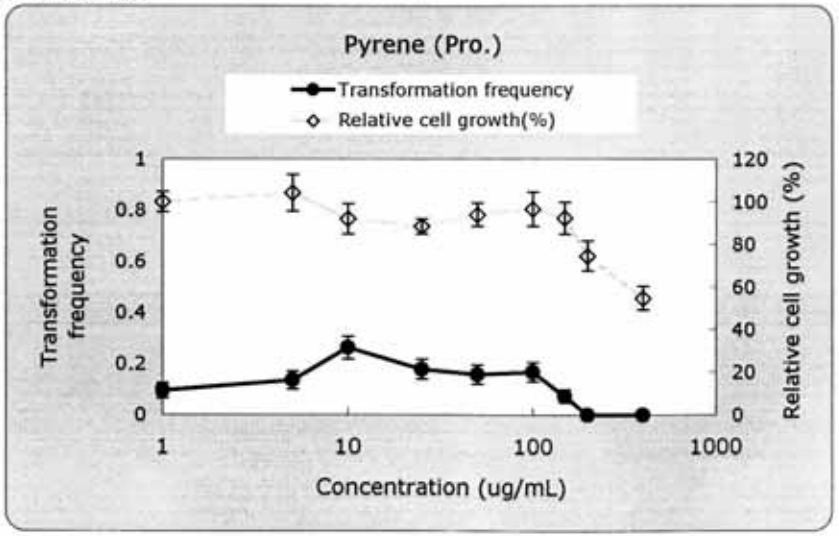
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	5	10	25	50	100	150	200	400		0.1% DMSO	TPA (50ng/mL)
1	0.118	1.040	1.084	0.928	0.942	0.954	0.945	0.914	0.741	0.628		1.111	1.510
2	0.125	1.138	1.222	1.052	1.009	1.070	1.037	0.979	0.780	0.622		1.255	1.646
3	0.124	1.131	1.176	1.154	1.023	1.016	1.166	1.029	0.956	0.579		1.196	1.672
4	0.125	1.109	1.216	1.034	1.004	1.097	1.072	1.042	0.886	0.688		1.357	1.516
5	0.123	1.175	1.189	1.060	1.000	1.072	1.094	1.127	0.887	0.751		1.241	1.747
6	0.122	1.145	1.277	1.066	1.033	1.118	1.171	1.112	0.854	0.679		1.340	1.880
7	0.116	1.152	1.087	1.056	1.042	1.101	1.160	1.109	0.885	0.714		1.349	1.691
8	0.110	1.054	1.023	0.953	0.961	1.025	1.016	1.013	0.908	0.663		1.246	1.581
Average	0.120	1.118	1.159	1.038	1.002	1.057	1.083	1.041	0.862	0.666	#DIV/0!	1.262	1.655
SD	0.005	0.048	0.086	0.070	0.035	0.055	0.081	0.074	0.070	0.055	#DIV/0!	0.085	0.123
Average - Blank	0.000	0.998	1.039	0.918	0.881	0.936	0.962	0.920	0.742	0.545	#DIV/0!	1.142	1.535
Relative Cell Growth (%)		100.0	104.1	92.0	88.3	93.8	96.5	92.2	74.4	54.6	#DIV/0!	100.0	134.5
SD of Relative Cell Growth		4.8	8.6	7.0	3.5	5.5	8.2	7.4	7.0	5.5	#DIV/0!	8.5	12.4

Transformation Assay

Giemsa Staining	1	5	10	25	50	100	150	200	400	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0				0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	9	13	25	17	15	16	7	Tox	Tox		12	58
Transformation frequency*	0.09375	0.135416667	0.260416667	0.177083333	0.15625	0.166666667	0.072916667	#VALUE!	#VALUE!	0	0.125	0.604166667
SE	0.02974911	0.03492238	0.04479116	0.03896110	0.03705794	0.03803629	0.02653610	#VALUE!	#VALUE!	0.00000000	0.03375386	0.04991131

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Progressing Data

Data Sheet for Cell Transformation Assay on Promotion Assay

Chemical Code:	Pyrene
Institution:	Lab 3
Test Number	

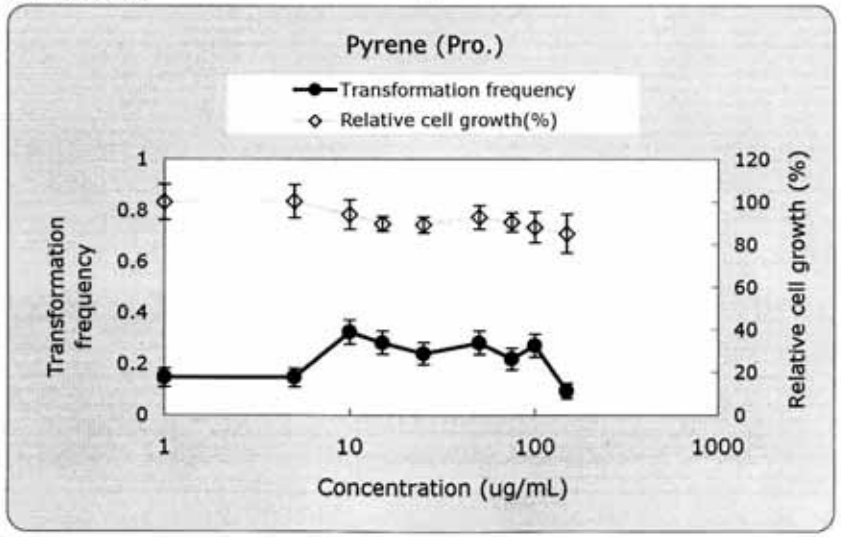
Cell Growth Assay

Crystal Violet Staining OD_{540nm}	Blank	0.5% DMSO	Concentrations (ug/ml)									Negative Control	Positive Control
		1	5	10	15	25	50	75	100	150		0.1% DMSO	TPA (50ng/mL)
1	0.093	0.908	0.959	0.900	0.920	0.884	0.939	0.974	0.768	0.861		0.804	1.455
2	0.091	0.992	0.979	0.966	0.961	0.946	1.060	0.886	0.969	0.936		0.991	1.643
3	0.091	0.942	1.106	0.933	0.939	0.939	0.975	0.954	0.931	0.692		0.945	1.550
4	0.098	1.091	1.060	1.085	0.908	0.953	0.896	0.938	0.900	0.913		0.958	1.493
5	0.094	1.093	1.052	0.985	0.881	0.949	0.933	0.911	0.947	0.973		1.023	1.531
6	0.092	1.112	1.075	0.975	0.964	0.888	0.927	0.949	0.954	0.903		1.001	1.575
7	0.096	1.017	1.026	0.963	0.901	0.885	0.942	0.954	0.917	0.864		0.913	1.531
8	0.088	0.963	0.884	0.877	0.882	0.876	0.912	0.847	0.854	0.880		0.962	1.529
Average	0.093	1.015	1.018	0.961	0.920	0.915	0.948	0.927	0.905	0.878	#DIV/0!	0.950	1.538
SD	0.003	0.077	0.073	0.063	0.033	0.034	0.051	0.042	0.066	0.084	#DIV/0!	0.068	0.056
Average - Blank	0.000	0.922	0.925	0.868	0.827	0.822	0.855	0.834	0.812	0.785	#DIV/0!	0.857	1.446
Relative Cell Growth (%)		100.0	100.3	94.1	89.7	89.2	92.8	90.4	88.1	85.1	#DIV/0!	100.0	168.7
SD of Relative Cell Growth		8.3	7.9	6.8	3.5	3.7	5.5	4.6	7.2	9.1	#DIV/0!	7.4	6.0

Transformation Assay

Giemsa Staining	1	5	10	15	25	50	75	100	150	0	Negative Control	Positive Control
No. of wells excluded	0	0	0	0	0	0	0	0	0		0	0
No. of wells counted	96	96	96	96	96	96	96	96	96	96	96	96
No. of wells having foci	14	14	31	27	23	27	21	26	9		18	71
Transformation frequency*	0.145833333	0.145833333	0.322916667	0.28125	0.239583333	0.28125	0.21875	0.270833333	0.09375	0	0.1875	0.739583333
SE	0.03602169	0.03602169	0.04772332	0.04588805	0.04356307	0.04588805	0.04219232	0.04535538	0.02974911	0.00000000	0.03983609	0.04479116

* No. of wells having foci/ No. of wells counted



Remarks

Acceptance Criteria (1)
Negative Control < 20 and 40 < Positive Control
Pass

Acceptance Criteria (2)
Concurrent Cell Growth Assay
Pass

The Final Data

Annex 12

Recommended Protocol for the Bhas 42 Cell Transformation Assay (2012.7.27)

1. Introduction

Bhas 42 cells were established from BALB/c 3T3 A31-1-1 cells (BALB/c mouse embryo cells) by transfection of v-Ha-*ras* oncogene [1]. The cells are used as a sensitive and stable cell line for the cell transformation assay (CTA), the endpoint of which is focus formation of morphologically transformed cells [2]. The Bhas 42 CTA consists of an initiation assay and a promotion assay [3]. These assays can detect genotoxic and non-genotoxic carcinogens, respectively [4-8]. In the initiation assay, the cells are treated with chemicals in the beginning of growth phase and in the promotion assay the treatment is started at subconfluence of cell growth. Cell growth assay is carried out prior to the transformation assay for the determination of appropriate test concentrations, and concurrently with the transformation assay to confirm the toxic effects of chemical treatment (Fig. 1).

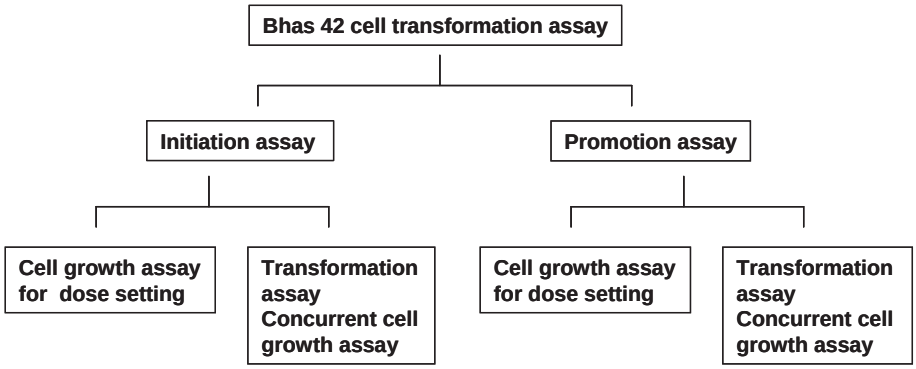


Fig. 1. Schematic of the Bhas 42 cell transformation assay.

There are two methods, a 6-well method and a 96-well method followed in the Bhas 42 CTA. The schematics of both methods are the same since their procedures are the basically the same except for some differences associated with the use of 6-well micro-plates vs. 96-well micro-plates. Furthermore, similar results are obtained with both methods.

2. Materials and preparation

The assay itself and all the preliminary steps (i.e. cell stock preparation, reagent and treatment solution preparations) should be performed under sterile conditions.

2.1 Equipment

Micro-plates with 6 wells and 96 wells are used for the 6-well method and 96-well method, respectively. Multichannel micropipettes are used for the 96-well method. In addition, general cell culture equipment is necessary.

2.2 Culture media

Eagle's minimal essential medium (with 2.2 g/L $NaHCO_3$ and 0.292 g/L L-glutamine) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin G sodium and 100 µg/mL streptomycin sulfate) (M10F) is used for the proliferation of Bhas 42 cells, cell cryo-preservation and the first culture after cell thawing. Dulbecco's modified Eagle's medium/F12 (with 1.2 g/L $NaHCO_3$) supplemented with 5% FBS and antibiotics (100 units/mL penicillin G sodium and 100 µg/mL streptomycin sulfate) (DF5F) is used for second culture after cell thawing, cell growth assays and transformation assays. (Other antibiotics could be used after examination for applicability.)

2.3 Culture

The cultures are maintained in a humidified incubator with a 5% CO_2 atmosphere at 37 ºC.

2.4 Reagents and solutions

The following reagents and solutions are used:

- 0.25% trypsin: Used for passage
- 0.02% EDTA-PBS(-): Used for passage
- Dimethylsulfoxide (DMSO): Used for cell cryo-preservation and as solvent for test chemicals
- Formalin (37% formaldehyde): Used for fixing cells.
- Methanol: Used for fixing cells.
- Crystal violet (CV) solution: Used for staining cells in cell growth assays. In 50 mL of ethanol 1 g of crystal violet is dissolved, and the total volume is adjusted to 1 L with distilled water.
- Dye extraction solution (0.02 mol/L HCl and 50% ethanol): Used for extracting CV in cell growth assays. 480 mL distilled water + 500 mL ethanol + 20 mL 1 M HCl.
- 5% Giemsa solution: Used for staining cells in transformation assays

2.5 Chemicals

2.5.1 Test chemicals and solvent/vehicle

Test chemicals are dissolved or suspended in an appropriate solvent or vehicle and diluted with the solvent/vehicle to each individual concentration before being added to cell cultures or culture media so that all chemical treatment media contain an equal concentration of the solvent/vehicle. Distilled water, DMSO, acetone, and ethanol can be used to dissolve chemicals, and the final solvent/vehicle concentrations in the medium should not exceed 5%, 0.5%, 0.5% and 0.1 %, respectively. Although the concentration of DMSO can be as high as 0.5%, it is recommended that it does not exceed 0.1%. The absence of cytotoxicity should dictate the highest allowable concentration of solvent/vehicle.

Test-chemical solutions are prepared before use as a general rule. To reduce labor, stock and/or working solutions may be preserved in aliquots at -20 °C for less than 10 days and thawed before use, but must not

be re-frozen. The suspensions of test chemicals must be prepared before use and cannot be stored for the future use.

2.5.2 Test chemical concentrations

The highest concentration of test chemical to be tested is 5 mg/mL or 10 mM, whichever is the lowest [9,10]. In the case of chemicals difficult to dissolve, the highest concentration(s) may be one or two precipitating dose levels which exceed the highest soluble concentration.

Five to nine concentrations should be tested and these are determined according to the results of cell growth assay.

2.5.3 Negative and Positive controls

The solvent/vehicle for a test chemical is used as the negative control. A known tumor-initiator, 3-methylcholanthrene (MCA, final concentration of 1 μg/mL), and a known tumor-promoter, 12-*O*-tetradecanoylphorbol-13- acetate (TPA, final concentration of 50 ng/mL), are used for the positive controls in the initiation assay and promotion assay, respectively. MCA and TPA are dissolved in DMSO. When the solvent/vehicle for the test chemical is not DMSO, DMSO is still necessary as the negative control for MCA or TPA.

The stock solutions of MCA and TPA in DMSO can be stored in aliquots at -20 ℃ for at least two years, provided they are not thawed.

2.6 Stock cells

The cells should be obtained from a reliable source. Health Science Research Resources Bank (HSRRB, Osaka, Japan) [http://www.jhsf.or.jp/index_b.html]. supplies frozen Bhas 42 cells. The cells are expanded by growing them up to 50-70% confluence in culture plates with M10F so as to avoid the increase of transformed variants. It is important to maintain a sub-confluent state in all the area of the cultures since the transformed cells, which lose the characteristic of cell-to-cell contact inhibition, preferentially multiply at confluence. The cells collected from cell populations that do not exceed 50-70 % confluence are washed once with 0.02% EDTA-PBS(-), trypsinized using 0.25% trypsin and subcultured. After two passages, the expanded cells are suspended at a cell density of 5 x 10^5 cells/mL in cold fresh M10F containing 5% DMSO and cryo-preserved in 0.5 mL aliquots to make a master stock in liquid nitrogen. Using one tube of the master stock, working cell stock for transformation assays are prepared using the same procedures and stored in liquid nitrogen until required for experiments. Generally, large numbers of tubes of the master and working stock cells are prepared so as not to be depleted prematurely.

The transformation assay must be started using the cryo-preserved stock cells. Conventionally, the cells at higher passages can be used for the cell growth assay for dose setting, but should not be used for the transformation assays themselves in order to avoid excessive spontaneous transformation.

2.7 Checking of the Bhas 42 cells

In order to determine the utility of the cryo-preserved cells, every batch of working stock cells must be confirmed for (a) adequate plating efficiency (≥50%), (b) low background of spontaneous transformation and (c) their ability to be transformed by archetypical chemical carcinogens. To be considered suitable for

the transformation assay, the fulfilment of acceptance criteria described below must be achieved in the 6-well method and/or 96-well method of the Bhas 42 cell transformation assay using the negative control DMSO and the positive controls, 1 μg/mL MCA and 50 ng/mL TPA.

2.8 Selection of FBS

Although most batches of FBS are generally usable for the transformation assay, it is recommended that at least 3 batches be screened for their ability to support plating efficiencies ≥50% as well as chemically-induced transformation. The batch of FBS which results in lowest transformation frequency in the negative control groups and highest transformation frequency in positive control groups in the 6-well method and/or 96-well method of Bhas 42 cell transformation assay should be selected. The FBS batches should fulfil all of the acceptance criteria described below (4.3 and 6.3) in the 6-well method and/or 96-well method of the Bhas 42 cell transformation assay using the negative control DMSO and the positive controls, 1 μg/mL MCA and 50 ng/mL TPA.

3. Procedures of 6-well method [3]

3.1 Initiation assay

3.1.1 Cell growth assay

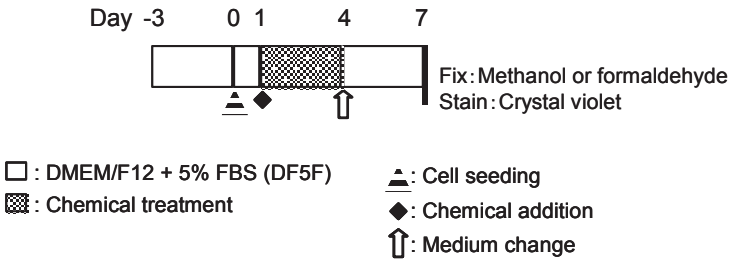


Fig. 2. Schematic protocol of cell growth assay in the initiation assay.

The cells at ≤ 70% confluence in DF5F are trypsinized, and 4000 cells are seeded into a well with 2 mL of DF5F (Day 0). Three wells are prepared for each treatment condition. Wells containing medium alone are also prepared for the blank control in photometry (The blank control can be shared among different assays performed on different chemicals provided they are tested simultaneously). At 20-24 hours after seeding, a test chemical solution at various concentrations or solvent/vehicle alone is added to each well without replacement of medium, which is the same practice used in the initiation transformation assay (Section 3.1.3). The medium is changed with fresh medium on Day 4. On Day 7, the cultures are fixed with methanol or 3.7% formaldehyde for 30 min, washed and dried. The cells are stained with 1.5 mL of crystal violet(CV) solution for 15 min, rinsed well with water and dried (Fig. 2).

CV is extracted from cells with 2 mL of dye extraction solution, and the optical density (OD) is measured at a wavelength between 540-570 nm. The relative cell growth of cultures treated with a chemical is calculated as follows:.

relative cell growth = [(At – Ab)/(Ac – Ab)] x 100

where At is the absorbance of CV extract from a well with the chemically-treated cells, Ac is the absorbance of CV extract from a well with the solvent/vehicle-treated cells and Ab is the absorbance of CV extract from a well with the medium only.

3.1.2 Dose setting for the transformation assay

Five to nine concentrations are set up based on the results of cell growth assays. These concentrations cover a range from the highest cytotoxicity (less than 20% survival compared to the negative control) to little or no toxicity. Ideally, one concentration below the no observed effect level (NOEL), two concentrations between the NOEL and the 50% inhibitory concentration (IC50) and two concentrations between the IC50 and the IC90 are selected. The ratio between neighboring concentrations should be less than square root 10 (Fig. 3).

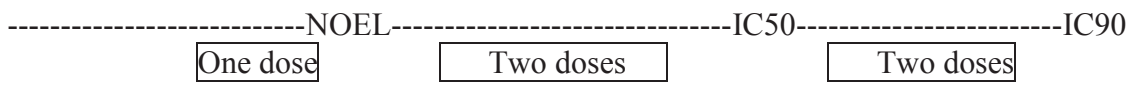


Fig. 3. Dose setting for the transformation assay in the initiation assay

3.1.3 Transformation assay

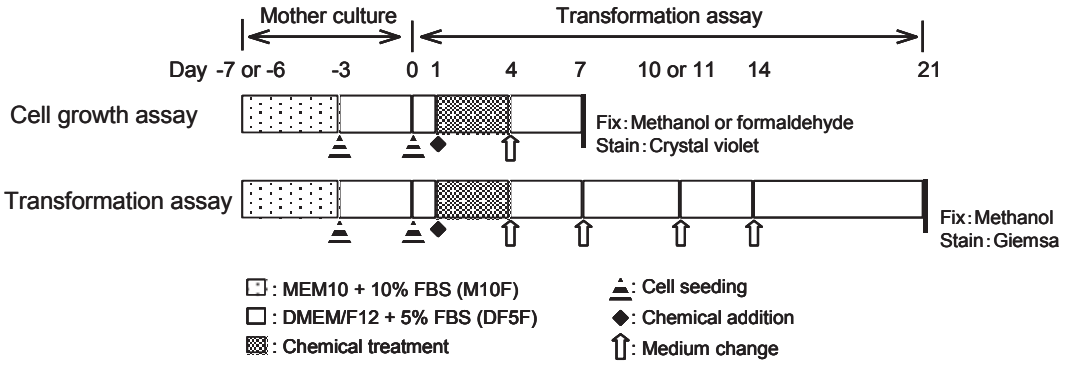


Fig. 4. Schematic protocol of transformation assay in the initiation assay

The frozen stock cells are rapidly thawed, suspended in M10F and cultured in Φ100-mm culture plates at a volume of 10 mL medium. When the cells reached about 70% confluence, they are trypsinized, suspended in DF5F at an appropriate density (7,000 to 10,000 cells /mL is suggested) and cultured in Φ 100-mm culture plates (Day -3). The cells at about 70% confluence are trypsinized, and suspended in DF5F at 2,000 cells/mL. The cell suspension is seeded into each well at a volume of 2 mL (4,000 cells/well) for the transformation assay and the concurrent cell growth assay (Day 0). Nine wells are prepared for each dose (one plate of 6 wells for the transformation assay and 3 wells for the concurrent cell growth assay). 20-24 hours after seeding, a chemical solution is added to each well without replacement of medium. The cells are exposed to the chemical for three days (Day 1-4). The medium is changed with fresh DF5F on Day 4, 7, 10 (or 11) and 14. On Day 21 the cells are fixed with methanol, and stained with 5%

Giemsa solution (Fig. 4). The positive control (1 μg/mL MCA) and the negative control are included in the transformation assay for each chemical (when the solvent/vehicle of test chemical is not DMSO, DMSO is still necessary as the negative control for MCA). The cell growth assay is carried out in parallel with the transformation assay.

3.2 Promotion assay

3.2.1 Cell growth assay

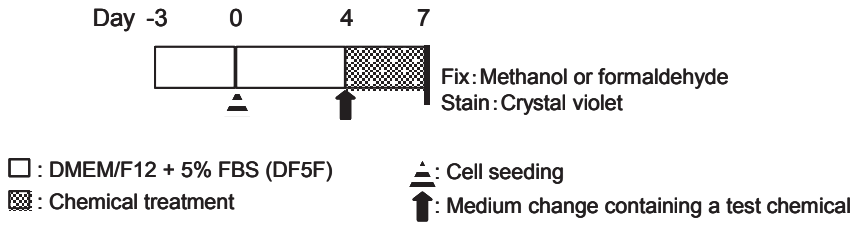


Fig. 5. Schematic protocol of cell growth assay in the promotion assay

The cells at ≤ 70% confluence in DF5F are trypsinized, and 14000 cells are seeded into a well in 2 mL of medium (Day 0). Three wells are prepared for each treatment condition. Wells containing medium alone are also prepared for the blank control in photometry (The blank control can be shared with the cell growth assay in the initiation assay which is performed simultaneously). On Day 4, chemical treatment is started by exchanging existing medium with fresh medium containing a test chemical solution or solvent/vehicle alone. On Day 7, the cultures are fixed with methanol or 3.7% formaldehyde for 30 min, washed and dried. The cells are stained with 1.5 mL of crystal violet solution for 15 min, rinsed well with water and dried (Fig. 5).

The following procedures are the same as those in the cell growth assay in the initiation assay (section 3.1.1) .

3.2.2 Dose setting for the transformation assay

For the chemicals that exhibit marked growth enhancement, concentrations are selected to cover the range from little effect to enhancement on cell growth. Ideally, one concentration below the NOEL, three concentrations in the range of growth enhancement, and one concentration in the range of weak growth inhibition are assessed (Fig. 6).

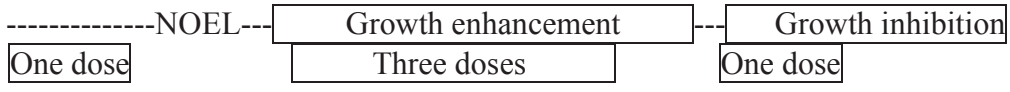


Fig. 6. Dose setting for transformation assay in the promotion assay of chemicals that exhibit marked growth enhancement.

For the chemicals that inhibit growth, concentrations are selected to cover the range from the NOEL to the level below 50% inhibition on cell growth. Ideally, two concentrations below the NOEL, three

concentrations between the NOEL and the IC50 and one concentration above the IC50 are evaluated (Fig. 7).

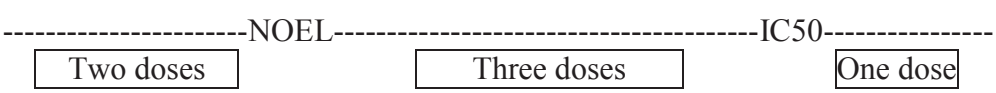


Fig. 7. Dose setting for transformation assay in the promotion assay of chemicals that inhibit growth

Whether a chemical shows growth enhancement or inhibition, other experimental conditions, such as the number of concentrations set up and the ratio between neighboring concentrations, are the same as described in the initiation assay.

3.2.3 Transformation assay

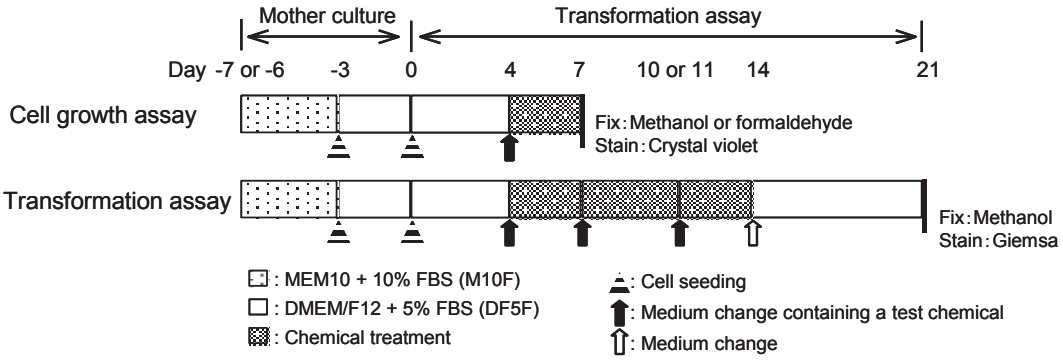


Fig. 8. Schematic protocol of transformation assay in the promotion assay.

The transformation assay is carried out in the same manner as in the initiation assay except for the following (Fig. 8):

- The cells are suspended in medium at 7,000 cells/mL, and 14,000 cells are plated into each well (Day 0)
- The cells are exposed to a test chemical for 10 days from Day 4 to Day 14.
- The chemical treatment is carried out by exchanging existing medium with fresh medium containing a chemical solution or solvent/vehicle alone on Day 4, 7 and 10 (or 11).
- On day 14 the medium is changed with the normal medium containing neither solvent/vehicle nor test chemical..
- TPA (50 ng/mL) is used for the positive control instead of MCA.

4. Evaluation of the results in the 6-well method

4.1 Record of transformation frequency

Transformed foci are scored using a stereomicroscope. Transformed foci are characterized by the following morphological properties: (a) more than 100 cells, (b) spindle-shaped cells differing in appearance from the contact-inhibited monolayer cells, (c) deep basophilic staining, (d) random orientation of cells at the edge of foci (criss-cross), (e) dense multilayering of cells (piling up) and (f) invasive growth into the surrounding monolayer of contact-inhibited cells. It should be noted that there are transformed foci that do not exhibit some of these characteristics.

The number of transformed foci in each well are recorded for every concentration.

4.2 Statistical analysis

For the increases in the number of the transformed foci produced by a test chemical, a statistical analysis is performed by multiple comparison using the one-sided Dunnett test ($p<0.05$). For the positive controls, the statistical significance is evaluated by one-sided t-test or Aspin-Welch test ($p<0.05$) depending on the result of F-test for homoscedasticity (homogeneity of variance).

4.3 Assay acceptance criteria

The following criteria must be fulfilled for a given assay to be considered valid. The initiation or promotion assay is repeated independently, as needed, to satisfy the assay acceptance criteria.

4.3.1 Concurrent cell growth assay

- When contamination or technical problems are observed, two undamaged wells are necessary at the minimum for each concentration for cell growth assessment.

4.3.2 Transformation assay

4.3.2.1 Initiation assay and promotion assay

- If a given chemical concentration results in excessive cell death and/or cells fail to reach confluence at the end of transformation assay because of chemical toxicity, the concentration is not valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment, and "toxicity" is recorded in the data sheet.
- When contamination or technical problems are observed, if, for a given concentration, the number of damaged wells is two or more, the concentration is not considered valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment. In such cases, "contamination", "accident", "technical error", etc. are recorded in the data sheet.
- In the positive control, there must be a statistically significant increase in the number of transformed foci per well compared to the corresponding negative control (one-sided t-test or Aspin-Welch test, $p<0.05$).

4.3.2.2 Initiation assay

- In the negative control, the number of transformed foci must be ten or less per well.
- If there is no statistically significant increase in the number of transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and three concentrations in the range between the NOEL and the IC_{90} in the concurrent cell growth assay.

4.3.2.3 Promotion assay

- In the negative control, the number of transformed foci must be twelve or less per well.
- If there is no statistically significant increase in the number of transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increases cell density in the cell growth assay). When the chemical does not induce growth enhancement but induces cytotoxicity, the cytotoxicity observed in the concurrent cell growth assay may not be similar to that observed in the transformation assay, since the durations of chemical exposure to the cells are different between the cell growth assay (3 days) and the transformation assay (10 days). Consequently, chemical toxicity is sometimes accumulated over the 10 day duration of the transformation promotion assay and the valid plates may be lost because of chemical toxicity. In such cases, four valid plates are also necessary in the concentration range where cells are not killed and are confluent at the end of the transformation assay. If excessive toxicity is encountered, it may be necessary to repeat the assay in a lower concentration range.

4.4 Assessment criteria

The assay results are judged as follows:

- The results in the initiation and promotion assays are judged positive if there exist two or more sequential doses that induce statistically significant increases in the number of transformed foci per well relative to the corresponding vehicle control (multiple comparison using one-sided Dunnett test, $p<0.05$).
- The results in the initiation and promotion assays are judged negative if there is no dose showing a statistically significant increase in the number of transformed foci per well.
- If the statistically significant increase is at only one or non-sequential doses, the assay result is regarded as equivocal, and then the initiation or promotion assay is repeated together with the concomitant cell growth assay, including the dose that caused the statistically significant increase of transformation frequency in the first assay. The chemical is judged to be positive if a statistically significant increase in the number of transformed foci per well is induced at one or more concentrations in the second assay; otherwise, the chemical is judged to be negative.
- A test chemical is positive in the Bhas 42 CTA, if it is positive in either the initiation or promotion assay.

5. Procedures of 96-well method

5.1 Medium change

The final volume of medium is 0.1 mL per well. The medium is removed by inverting the plates and shaking out the medium over paper towels or disposable diapers three times to drain off the medium

completely. The medium is replaced by gently dispensing it down the walls of the wells over the cells using micro-tips attached to a multichannel micropipette.

5.2 Initiation assay

5.2.1 Cell growth assay

The cell growth assay is carried out in the same manner as in the initiation assay of the 6-well method except for the following (see section 3.1.1):

- Into each well, 200 cells are seeded with 0.05 mL of DF5F (Day 0).
- For each treatment condition, 8 wells are prepared.
- The cultures are treated by 0.05 mL of medium containing a test chemical solution or solvent/vehicle alone at two times the final concentration added to each well so that the final volume of medium is 0.1 mL.
- The volumes of crystal violet solution and dye extraction solution are 0.1 mL/well.

5.2.2 Dose setting for the transformation assay

The concentrations to be tested in the transformation assay are determined in the same way that they are in the initiation assay of 6-well method (see section 3.1.2).

5.2.3 Transformation assay

The transformation assay is carried out in the same manner as in the initiation assay of the 6-well method except for the following:

- Into each well, 200 cells are seeded with 0.05 mL of DF5F (Day 0).
- For each treatment condition, one 96-well plate is prepared for the transformation assay and 8 wells are prepared for the cell growth assay, respectively.
- The cultures are treated by the addition of 0.05 mL of medium containing a chemical solution or solvent/vehicle alone at two times the final concentration added to each well so that the final volume of medium is 0.1 mL.

5.3. Promotion assay

5.3.1 Cell growth assay

A cell growth assay is carried out in the same manner as in the promotion assay of the 6-well method except for the following (see section 3.2.1):

- Into each well, 400 cells are seeded with 0.1 mL of DF5F (Day 0).
- For each treatment condition, 8 wells are prepared.
- The volumes of crystal violet solution and dye extraction solution are 0.1 mL/well.

5.3.2 Dose setting for the transformation assay

The concentrations to be tested in the transformation assay are determined in the same was that they are in the promotion assay of 6-well method (See section 3.2.2).

5.3.3 Transformation assay

The transformation assay is carried out in the same manner as in the promotion assay of the 6-well method except for the following (see section 3.2.3):

- Into each well, 400 cells are seeded with 0.1 mL of DF5F (Day 0).
- For each treatment condition, one 96-well plate is prepared for the transformation assay and 8 wells are prepared for the cell growth assay, respectively.

6. Evaluation of the results in the 96-well method

6.1 Record of transformation frequency

Transformed foci are judged using the same criteria as in the 6-well method.

The number of wells having transformed foci versus the number of wells observed is recorded for every concentration: a well having one focus is counted as one and a well having two or more foci is also counted as one.

6.2 Statistical analysis

For the increases in the proportion of wells with transformed foci in the plate treated with a test chemical, a statistical analysis is performed by chi-square test with Bonferroni adjustment (p-value<0.05, upper-sided) [11]. For multiplicity, the number of concentrations that satisfy the assay acceptance criteria is considered by this methodology. For the positive controls, the statistical significance is evaluated by one-sided chi-square test ($p<0.05$, upper-sided).

6.3 Assay acceptance criteria

The following criteria must be fulfilled for a given assay to be considered valid. The initiation or promotion assay is repeated independently, as needed, to satisfy the assay acceptance criteria.

6.3.1 Concurrent cell growth assay

- When contamination or technical problems are observed, four undamaged wells are necessary at the minimum for each concentration for cell growth assessment.

6.3.2 Transformation assay

6.3.2.1 Initiation assay and promotion assay

- If a given chemical concentration results in excessive cell death and/or cells fail to reach confluence at the end of transformation assay because of chemical toxicity, the concentration is not valid for transformation assessment and excluded from focus-counting, statistical analysis and judgment, and “toxicity” is recorded in the data sheet.

- When contamination or technical problems are observed, if, for a given concentration, the number of damaged wells is ≥7, the concentration is not considered valid for transformation assessment and is excluded from focus-counting, statistical analysis and judgment. In such cases, "contamination", "accident", "technical error", etc. are recorded in the data sheet.
- In the positive control, there must be a statistically significant increase in the proportion of wells having transformed foci compared to the corresponding negative control (one-sided chi-square test, $p<0.05$, upper-sided).

6.3.2.2 Initiation assay

- In the negative control, the number of wells having transformed foci must be 15 wells/plate or less (if there exist damaged wells, ≤15.625% of undamaged wells).
- If there is no statistically significant increase in the proportion of wells having transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and three concentrations in the range between the NOEL and the IC_{90} in the concurrent cell growth assay.

6.3.2.3 Promotion assay

- In the negative control, the number of wells having transformed foci must be 20 wells/plate or less (if there exist damaged wells, ≤20.833% of undamaged wells).
- If there is no statistically significant increase in the proportion of wells having transformed foci at any dose (in case of negative results), four valid test chemical concentrations are necessary, at a minimum, to accept the transformation assay for evaluating a chemical. Those concentrations should include at least one concentration near the NOEL and two concentrations in the range of growth enhancement when the chemical enhances cell growth (increases cell density in the cell growth assay). When the chemical does not induce growth enhancement but induces cytotoxicity, the cytotoxicity observed in the concurrent cell growth assay may not be similar to that observed in the transformation assay, since the durations of chemical exposure to the cells are different between the cell growth assay (3 days) and the transformation assay (10 days). Consequently, chemical toxicity is sometimes accumulated over the 10 day duration of the transformation promotion assay and the valid plates may be lost because of chemical toxicity. In such cases, four valid plates are also necessary in the concentration range where cells are not killed and are confluent at the end of the transformation assay. If excessive toxicity is encountered, it may be necessary to repeat the assay in a lower concentration range.

6.4 Assessment criteria

The assessment criteria are the same as those of 6-well method except that a statistical analysis is performed for the proportion of wells having transformed foci (number of wells having transformed foci/number of wells observed) using chi-square test with Bonferroni adjustment, $p<0.05$, upper-sided.

7. References

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(10) OECD Guidelines for the Testing of Chemicals. Test No. 476: *In vitro* Mammalian Cell Gene Mutation Test.
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Annex 13

Table 57.
Transformation frequency of negative and positive controls

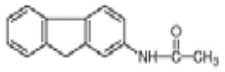
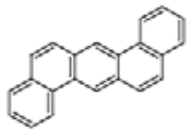
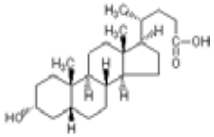
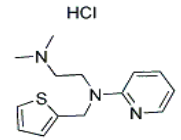
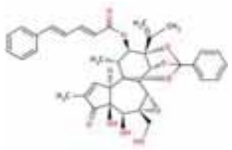
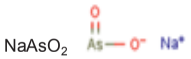
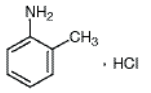
6-Well Method																			
Data source	FBS	DMSO control							H_2O control							Positive control					
		Count	Number of foci						Count	Number of foci						Count	Number of foci				
			AV	SD	MAX	MIN	MEDIAN	AV+SDx2		AV	SD	MAX	MIN	MEDIAN	AV+SDx2		AV	SD	MAX	MIN	MEDIAN
Initiation assay																					
6-Well Validation study	Moregate	35	2.8	1.7	7.8	0.8	2.3	6.2	20	2.6	1.3	6.8	1.2	2.5	5.3	38	26.8	12.9	50.0	9.2	22.9
6-Well pre-validation study	Moregate	24	2.0	1.6	8.5	0.3	1.8	5.2	3	1.4	0.4	1.7	1.0	1.5	2.1	27	24.5	10.5	50.7	6.5	24.3
In-house study on 98 chemicals	Moregate	99	2.6	1.6	8.8	0.5	2.2	5.8	49	2.2	1.4	6.8	0.5	1.8	5.0	99	25.8	8.9	49.2	11.0	25.3
In-house study in HRI	GIBCO	37	4.2	2.1	9.7	0.5	4.0	8.4	17	3.4	1.6	7.3	1.3	3.2	6.6	37	30.8	8.9	49.2	13.8	31.7
Promotion assay																					
6-Well Validation study	Moregate	39	4.4	2.9	14.8	0.7	3.3	10.2	24	3.5	3.2	16.3	0.7	2.6	9.8	42	21.8	12.6	50.2	8.3	17.5
6-Well pre-validation study	Moregate	24	3.6	3.0	15.8	0.8	3.5	9.6	3	2.2	0.6	2.7	1.5	2.3	3.4	24	14.9	5.2	32.2	6.8	15.7
In-house study on 98 chemicals	Moregate	99	4.4	2.1	9.5	1.0	3.8	8.5	49	3.7	1.8	9.2	0.8	3.5	7.4	99	22.2	8.2	41.7	4.7	21.8
In-house study in HRI	GIBCO	36	6.5	2.8	12.3	2.0	6.3	12.0	17	4.7	1.8	7.5	2.2	4.5	8.3	36	27.2	9.5	41.8	7.8	28.2
96-Well method																					
Data source	FBS	DMSO control							H_2O control							Positive control					
		Count	Number of wells having foci/96 wells						Count	Number of wells having foci/96 wells						Count	Number of wells having foci/96 wells				
			AV	SD	MAX	MIN	MEDIAN	AV+SDx2		AV	SD	MAX	MIN	MEDIAN	AV+SDx2		AV	SD	MAX	MIN	MEDIAN
Initiation assay																					
In-house study in HRI	Moregate	62	2.5	1.5	7	0	2	5.6	28	2.4	1.4	5	0	2	5.1	61	35.7	8.9	68	18	35
96-Well validation study	GIBCO	66	8.0	3.2	14	1	8	14.5	20	7.7	3.6	13	1	8	14.9	66	54.3	10.8	83	33	53
In-house study in HRI	GIBCO	11	5.8	2.9	10	2	5	11.6	5	5.8	3.4	11	2	5	12.6	11	46.8	16.1	64	23	53
Promotion assay																					
In-house study in HRI	Moregate	56	5.3	3.6	13	0	5	12.5	28	4.0	2.5	10	1	4	9.0	57	32.3	10.4	51	11	32
96-Well validation study	GIBCO	69	11.2	3.5	21	4	11		22	10.2	3.9	18	4	10		69	60.9	13.0	88	36	58
In-house study in HRI	GIBCO	11	9.2	4.4	18	3	9	18.0	6	6.8	4.0	14	3	6	14.8	11	49.5	11.9	67	26	49

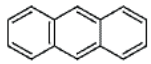
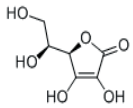
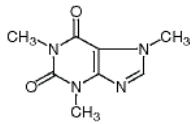
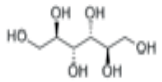
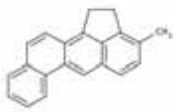
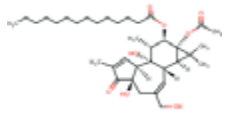
Count: Number of Assay
AV: Average
SD: Standard deviation
MAX: Maximum value
MIN: Minimum value

Annex 14

Table 58.

Chemical properties and classes of 12 coded test chemicals and positive controls for the 6-well method validation study[a]

#	Chemical Name	M.W.	Chemical structure	CAS	IARC [as of May 2010]	Physical appearance	Water solublity[b]	Solubility (Other solvents)	Vapor pressure (Volatility)	Chemical class
1	2-Acetylaminofluorene	223		53-96-3	Not listed (C[c])	Solid	144 mg/L at 25 °C (est) <very poor>	Sol in ether, acetic acid	(Possibly Non-volatile)	Aromatic amine; requires metabolic activation
2	Cadmium chloride	183		10108-64-2	1 (as Cadmium compounds) (C)	Solid	140 g/100 mL at 20°C <very good>	Soluble in acetone	10 mm Hg at 656 °C (Non-volatile)	Inorganic cadmium compound
3	Dibenz[*a,h*]anthracene	278		53-70-3	2A (C)	Solid	0.0005 mg/L at 27°C <very poor (almost insoluble)>	Slightly soluble in ethanol, soluble in acetone and benzene.	1.1X10-10 mm Hg (est) (Possibly Non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation
4	Lithocholic acid	377		434-13-9	Not listed (TP[d])	Solid	0.38 mg/L at 20 °C <very poor (almost insoluble)>	Freely soluble in hot alcohol	(Unknown, Possibly Non-volatile)	Steroid derivative (Bile acid)
5	Methapyrilene hydrochloride	298		135-23-9	Not listed (TP)	Solid	1 gm dissolves in 0.5 mL water <very good>	1 gm dissolves in 5 mL alcohol, in 3 mL chloroform	(Unknown, Possibly Non-volatile)	Heterocyclic amine; Anti-histamine drug
6	Mezerein	655		34807-41-5	Not listed (TP)	Solid	Not soluble <very poor (almost insoluble)>	Soluble in DMSO or ethanol	(Unknown, Possibly Non-volatile)	Diterpene compound Protein kinase activator (Non-phorbol type)
7	Sodium arsenite	130		7784-46-5	1 (as Arsenic compounds) (C)	Solid	Freely soluble <very good>	Slightly soluble in alcohol	0% volatiles by volume at 21 °C (Possibly Non-volatile)	Inorganic arsenite compound
8	*o* -Toluidine hydrochloride	144		636-21-5	1 (as *o* -Toluidine) (C)	Solid	Very soluble <very good>	Soluble in alcohol; insoluble in ether, benzene	as VOC (Possibly Volatile)	Aromatic amine

#	Chemical Name	M.W.	Chemical structure	CAS	IARC [as of May 2010]	Physical appearance	Water solublity[b]	Solubility (Other solvents)	Vapor pressure (Volatility)	Chemical class
9	Anthracene	178		120-12-7	3 (NC[e])	Solid	0.04-1.3 mg/L at 25°C <very poor>	1 gm dissolves in 67 mL absolute alcohol, 70 mL methanol, 62 mL benzene, 85 mL chloroform	2.67X10-6 mm Hg at 25°C (eat) (Non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation
10	L-Ascorbic acid	176		50-81-7	Not listed (NC)	Solid	0.33 g/mL <good>	0.02 mg/mL in ethanol; insoluble in ether, chloroform, benzene	7.9179 Pa @ 465.15 deg K= 0.06 mm Hg at 192 °C (Non-volatile)	Lactone structure; Antioxidant
11	Caffeine	194		58-08-2	3 (NC)	Solid	2.16X10+4 mg/L at 25°C <moderate>	1 gm dissolves in 66 mL alcohol, 50 mL acetone, 5.5 mL chloroform, 530 mL ether, 100 mL benzene	7.3X10-9 mm Hg at 25°C (est) (Non-volatile)	Purine alkaloid
12	D-Mannitol	182		69-65-8	Not listed (NC)	Solid	2.16X10+5 mg/L at 25°C <good>	Insoluble in ether	(Non-volatile)	Sugar alcohol
	3-Methylcholanthrene	268		56-49-5	Not listed (C)	Solid	2.9X10-3 mg/L at 25°C <very poor>	Soluble in xylene, toluene, and benzene	4.3X10-8 mm Hg at 25°C (Non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation
	12-*O*-tetradecanoylphorbol-13-acetate (TPA)	617		16561-29-8	Not listed (TP)	Solid	No data available	Soluble to 100 mM in DMSO	(Possibly Non-volatile)	Diterpene compound Protein kinase activator (phorbol type)

[a] Chemical property data from Hazardous Substances Data Bank (HSDB) in Toxicology Data Network (TOXNET: http://toxnet.nlm.nih.gov/index.html) except for methapyrilene HCL and mezerein.

[b] Very good >1000 g/L; good 100-1000 g/L; moderate 10-100 g/L; poor 1-10 g/L; very poor <1 g/L

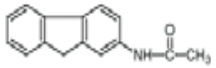
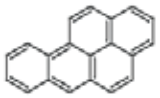
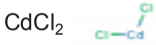
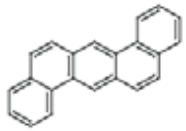
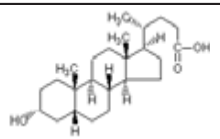
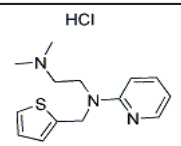
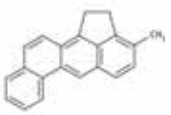
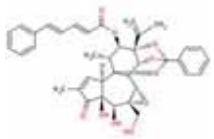
[c] Carcinogen

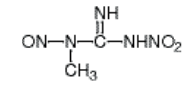
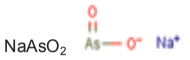
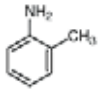
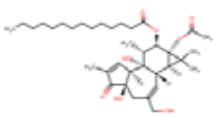
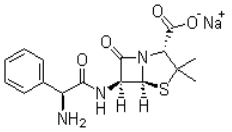
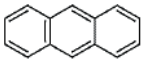
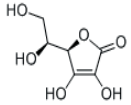
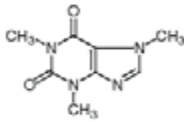
[d] Tumor-promoter

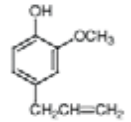
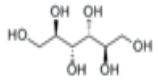
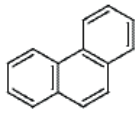
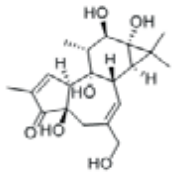
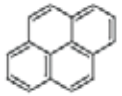
[e] Non-carcinogen

Annex 15

Chemical properties and classes of test chemicals for the pre-validation phase, phase I and phase II of 96-well method validation study[a]

#	Chemical Name	M.W.	Chemical structure	CAS	IARC [as of May 2010]	Physical appearance	Water solublity[b]	Solubility (Other solvents)	Vapor pressure (Volatility)	Chemical class
1	2-Acetylaminofluorene	223		53-96-3	Not listed (C[c])	Solid	144 mg/L at 25 ℃ (est) <very poor>	Soluble in ether, acetic acid	Possibly non-volatile	Aromatic amine; requires metabolic activation
2	Benzo[*a*]pyrene	252		50-32-8	1 (C)	Solid	1.60X10-3 mg/L at 25℃ <very poor (almost insoluble)>	Soluble in benzene, toluene, xylene, ether	5.49X10-9 mm Hg at 25 ℃ (extrapolated) (Non-volatile)	Polycyclic aromatic hydrocarbon; Metabolic activation
3	Cadmium chloride	183		10108-64-2	1 (as Cadmium compounds) (C)	Solid	140 g/100 mL at 20℃ <very good>	Soluble in acetone	10 mm Hg at 656 ℃ (Non-volatile)	Inorganic cadmium compound
4	Dibenz[*a,h*]anthracene	278		53-70-3	2A (C)	Solid	0.0005 mg/L at 27 ℃ <very poor (almost insoluble)>	Slightly soluble in ethanol, soluble in acetone and benzene.	1.1X10-10 mm Hg (est) (Possibly non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation
5	Lithocholic acid	377		434-13-9	Not listed (TP[d])	Solid	0.38 mg/L at 20 ℃ <very poor (almost insoluble)>	Freely soluble in hot alcohol	(Unknown, Possibly non-volatile)	Steroid derivative (Bile acid)
6	Methapyrilene hydrochloride	298		135-23-9	Not listed (TP)	Solid	1 gm dissolves in 0.5 mL water <very good>	1 gm dissolves in 5 mL alcohol, in 3 mL chloroform	(Unknown, Possibly non-volatile)	Heterocyclic amine; Anti-histamine drug
7	3-Methylcholanthrene	268		56-49-5	Not listed (C)	Solid	2.9X10-3 mg/L at 25℃ <very poor>	Soluble in xylene, toluene, and benzene	4.3X10-8 mm Hg at 25 ℃ (Non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation
8	Mezerein	655		34807-41-5	Not listed (TP)	Solid	Not soluble <very poor (almost insoluble)>	Soluble in DMSO or ethanol	(Unknown, Possibly non-volatile)	Diterpene compound Protein kinase activator (Non-phorbol type)

#	Chemical Name	M.W.	Chemical structure	CAS	IARC [as of May 2010]	Physical appearance	Water solublity[b]	Solubility (Other solvents)	Vapor pressure (Volatility)	Chemical class
9	*N*-Methyl-*N´*-nitro-*N*-nitrosoguanidine (MNNG)	147		70-25-7	1 (C)	Solid	Slightly soluble (< 0.5%) <poor>	Soluble in DMSO	No data available (Possibly non-volatile)	Nitrosoguanidine; Alkylating agent
10	Sodium arsenite	130		7784-46-5	1 (as Arsenic compounds) (C)	Solid	Freely soluble <very good>	Slightly soluble in alcohol	0% volatile by volume at 21 °C (Possibly non-volatile)	Inorganic arsenite compound
11	o-Toluidine	107		95-53-4	1 (C)	Liquid	Slightly soluble; 1.66X10+4 mg/L at 25°C <moderate (close to poor)>	Soluble in alcohol, ether;	2.60X10-1 mm Hg at 25 °C; 0.32 torr; Volatile with steam; Volatile organic compound	Aromatic amine
12	12-*O*-tetradecanoylphorbol-13-acetate (TPA)	617		16561-29-8	Not listed (TP)	Solid	No data available	Soluble to 100 mM in DMSO	(Possibly non-volatile)	Diterpene compound; Protein kinase activator (phorbol type)
13	Ampicillin sodium salt	371		69-52-3	3 (as Ampicillin, 69-53-4) (NC[e])	Solid	50 mg/mL <moderate>	No data available	(Unknown, Possibly non-volatile)	Beta-lactam antibiotics
14	Anthracene	178		120-12-7	3 (NC)	Solid	0.04-1.3 mg/L at 25°C <very poor>	1 gm dissolves in 67 mL absolute alcohol, 70 mL methanol, 62 mL benzene, 85 mL	2.67X10-6 mm Hg at 25 °C (eat???) (Non-volatile)	Polycyclic aromatic hydrocarbon
15	L-Ascorbic acid	176		50-81-7	Not listed (NC)	Solid	0.33 g/mL <good>	0.02 mg/mL in ethanol; insoluble in ether, chloroform, benzene	7.9179 Pa at 465.15 deg K= 0.06 mm Hg at 192 °C (Non-volatile)	Lactone structure; Antioxidant
16	Caffeine	194		58-08-2	3 (NC)	Solid	2.16X10+4 mg/L at 25°C <moderate>	1 gm dissolves in 66 mL alcohol, 50 mL acetone, 5.5 mL chloroform, 530 mL ether, 100 mL benzene	7.3X10-9 mm Hg at 25 °C (est) (Non-volatile)	Purine alkaloid

#	Chemical Name	M.W.	Chemical structure	CAS	IARC [as of May 2010]	Physical appearance	Water solublity[b]	Solubility (Other solvents)	Vapor pressure (Volatility)	Chemical class
17	Caprolactam	113		105-60-2	4 (NC)	Solid	5.25X10+6 mg/L at 25°C <very good>	Freely soluble in methanol, ethanol; Soluble in benzene, ethanol, chloroform	1.9X10-3 mm Hg at 25 °C (Non-volatile)	Amid; Lactam
18	Eugenol	164		97-53-0	3 (NC)	Liquid	2.463X10+3 mg/L at 25°C <poor>	Miscible with alcohol, chloroform, ether	2.26X10-2 mm Hg at 25 °C (Possibly volatile)	Phenylpropanoid compound
19	D-Mannitol	182		69-65-8	Not listed (NC)	Solid	2.16X10+5 mg/L at 25 °C <good>	Insoluble in ether	(Non-volatile)	Sugar alcohol
20	Phenanthrene	178		85-01-8	3 (NC)	Solid	0.977 - 1.18 mg/L at 25 °C <very poor>	Soluble in ethanol, diethyl ether, acetone, benzene, and carbon disulfide	1.21X10-4 mm Hg at 25 °C (Non-volatile)	Polycyclic aromatic hydrocarbon
21	Phorbol	364		17673-25-5	Not listed (NC)	Solid	Quite soluble <good/very good>	Highly soluble in polar solvents	(Possibly non-volatile)	Diterpene compound
22	Pyrene	202		129-00-0	3 (NC)	Solid	0.135 mg/L at 25 °C <very poor (almost insoluble)>	Soluble in alcohol, ether, benzene	8.92X10-5 mm Hg at 25 °C (Non-volatile)	Polycyclic aromatic hydrocarbon; requires metabolic activation

[a] Chemical property data from Hazardous Substances Data Bank (HSDB) in Toxicology Data Network (TOXNET: http://toxnet.nlm.nih.gov/index.html) except for methapyrilene HCL and mezer

[b] very good >1000 g/L; good 100-1000 g/L; moderate 10-100 g/L; poor 1-10 g/L; very poor <1 g/L

[c] Carcinogen

[d] Tumor-promoter

[e] Non-carcinogen

Annex 16

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A Bhas 42 cell transformation assay on 98 chemicals: The characteristics and performance for the prediction of chemical carcinogenicity

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ABSTRACT

The Bhas 42 cell transformation assay is a short-term system using a clone of the BALB/c 3T3 cells transfected with an oncogenic murine *ras* gene (v-Ha-*ras*). The assay has previously been reported to be capable of detecting the tumor-initiating and tumor-promoting activities of chemical carcinogens according to the different protocols, an initiation assay and a promotion assay, respectively. We applied this short-term assay to 98 chemicals to characterize the assay and evaluate its performance for the detection of chemical carcinogenicity. When the assay results were compared with the existing genotoxicity data, the Bhas 42 cell transformation assay could detect a considerable number of Ames-negative and Ames-discordant carcinogens: and the promotion assay detected most of those Ames-negative and -discordant carcinogens. This fact suggested that the Bhas 42 cells behaved as initiated cells in the transformation assay. The performance indices were calculated from the assay results of 52 carcinogens and 37 non-carcinogens. The concordance was 78%, sensitivity 73%, specificity 84%, positive predictivity 86%, negative predictivity 69%, false negative 27% and false positive 16%. Of these values, the concordance, specificity, negative predictivity and false positive were superior and the other performance indices were equivalent to those of conventional genotoxicity tests. From overall results, we concluded that the accuracy of prediction of chemical carcinogenicity would be improved by introducing the Bhas 42 cell transformation assay into the battery of *in vitro* assays.

1. Introduction

The carcinogenicity of chemicals has been predicted with genotoxicity assays. *In vitro* cell transformation assays can detect chemical carcinogens but are classified into a category different from genotoxicity assays [1–4]. The cell transformation assays attract attention as an additional *in vitro* method to predict the carcinogenicity of chemicals, since there are concerns about the carcinogens to which conventional genotoxicity assays are insensitive [5]. An analysis estimated non-genotoxic carcinogens to be present in 12% of carcinogens classified into Group 1, 2A and 2B by the International Agency for Research on Cancer (IARC) [6].

BALB/c 3T3 cells and C3H 10T1/2 cells have been used for the cell transformation assays [7]. The assays using these cell lines require longer period compared with the conventional genotoxicity assays, but achieve production of visible transformed foci. Also, in the assay there are initiation and promotion stages that mimic the two stages in the carcinogenicity tests in experimental animals, and most of the non-genotoxic carcinogens can be detected as promoters of cell transformation in the two-stage cell transformation assay, where the target cells are initiated with a known tumor initiator prior to treatment with a test chemical [8–10].

Recently a short-term cell transformation assay has been developed [11], using the Bhas 42 cells which were established from the BALB/c 3T3 cells through the transfection with a plasmid pBR322 containing Ha-MuSV-DNA, clone H1 (v-Ha-*ras*) [12–14]. The Bhas 42 cells are transformed by known tumor promoters, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA), okadaic acid and lithocholic acid, without initiating treatment with a known tumor initiator such as 3-methylcholanthrene (MCA) [11], and are presumed to be initiated toward transformation by the introduced viral *ras* sequence [15]. Thus the Bhas 42 cell transformation assay does not require pretreatment to detect tumor-promoting activity, and the assay period is 3 weeks instead of 6 weeks in the BALB/c 3T3 cell transformation assay. Because of the increased sensitivity of cells to transformation, the assay scales such as the dish size, the dish number, the medium volume, the serum concentration in the cultures and the number of medium exchange have been diminished, so that the cost performance is improved. The Bhas 42 cell transformation assay has been proved by Asada et al. to

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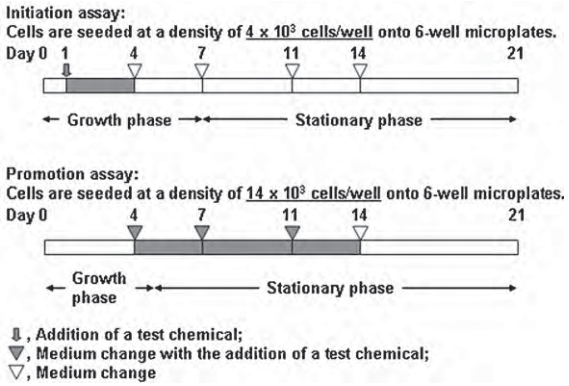

Fig. 1. Illustration of the Bhas 42 cell transformation assay.

be capable of detecting tumor-initiating activity as well as tumor-promoting activity of chemicals depending on a change between the two protocols, the initiation assay and the promotion assay [16]. The protocols are illustrated in Fig. 1. In the initiation assay, the cells are inoculated at a low density and treated with a test chemical in the beginning of the assay period so that the target cells can undergo cell division several times before contact inhibition of growth at confluence, and thus DNA damage is fixed as mutations in the genes. In the promotion assay, the cells are seeded more densely than in the initiation assay, and the treatment with a test chemical is started just before confluence (at sub-confluence) and continued longer than in the initiation assay.

Employing arsenic compounds, Muramatsu et al. verified that the sensitivity of Bhas 42 cell transformation assay for the detection of initiating activity is similar to that of the conventional BALB/c 3T3 cell transformation assay, and the sensitivity for the detection of promoting activity is equivalent to that of the two-stage BALB/c 3T3 cell transformation assay where the target cells are initiated with a sub-threshold dose of MCA [17]. Tanaka et al. conducted an interlaboratory collaborative study on the Bhas 42 cell transformation assay by 6 laboratories, and demonstrated that the assay on 9 chemicals is reproducible and applicable to the detection of both initiating activity and promoting activity of carcinogens [18].

We applied the Bhas 42 cell transformation assay to 98 chemicals including known carcinogens and non-carcinogens to evaluate its performance for predicting the carcinogenicity of chemical substances under a project financed by New Energy and Industrial Technology Development Organization (NEDO) in Japan. In this paper, we present the assay results of individual chemicals and the performance indices calculated from the results. We also compared the assay results and performances with existing genotoxicity data to characterize this assay.

2. Materials and methods

2.1. Cell culture

Bhas 42 cells were expanded and cryopreserved in a large batch prior to transformation assays. The cells were proliferated in Eagle's minimum essential medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Moregate Biotech, Bulimba, Australia, and GIBCO™, Invitrogen, Carlsbad, CA, USA) (M10F) in a humidified 5% CO_2 incubator at 37 °C, and subcultured using 0.25% trypsin (Invitrogen) when the cultures reached about 70% confluence. The multiplied cells were suspended at 5×10^5 cells/mL in fresh M10F containing 5% dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Osaka, Japan), frozen in 0.5 mL volumes at −80 °C and stored in liquid nitrogen. The transformation assays were always started from this frozen stock. Dulbecco's modified Eagle's medium/Ham's F12 (Invitrogen) supplemented with 5% FBS (DF5F) was used for the transformation assays. The two batches of FBS, which were prescreened to produce a minimal number of transformed foci in the Bhas 42 cells treated with DMSO alone and an appropriate number of foci in the cells treated with 1 μg/mL of MCA or 0.05 μg/mL of TPA in the transformation assay, were used [2,7].

2.2. Chemicals

Benz[*a*]anthracene [56-55-3], 5-azacytidine [320-67-2], barium chromate [10294-40-3], benzo[*a*]pyrene [50-32-8], cyclophosphamide [50-18-0], cyclosporin A [59865-13-3], 2,4-diaminotoluene [95-80-7], melphalan [148-82-3], MCA [56-49-5], mitomycin C [50-07-7], sterigmatocystin [10048-13-2], thio-TEPA [52-24-4], chenodeoxycholic acid [474-25-9], 4-chloro-*o*-toluidine hydrochloride [3165-93-3], cholic acid [81-25-4], deoxycholic acid [83-44-3], dichlorvos [62-73-7], epichlorohydrin [106-89-8], D-limonene [5989-27-5], methapyrilene hydrochloride [135-23-9], mezerein [34807-41-5], phorbol 12,13-didecanoate [24928-17-4], sodium arsenate, [10048-95-0], styrene oxide [96-09-3], TPA [16561-29-8], *o*-toluidine [95-53-4], zinc chloride [7646-85-7], *o*-anisidine [90-04-0], benzene [71-43-2], cobalt sulfate heptahydrate [10026-24-1], diethylstilbestrol [56-53-1], 1,4-dioxane [123-91-1], urethane (ethyl carbamate) [51-79-6], methyl carbamate [598-55-0], nickel monooxide [1313-99-1], 4-acetylaminofluorene [28322-02-3], chromotrope FB (acid red 14) [3567-69-9], ampicillin sodium salt [69-52-3], anthracene [120-12-7], L-ascorbic acid [50-81-7], benzoin [119-53-9], caffeine [58-08-2], caprolactam [105-60-2], 2-chloroethanol [107-07-3], chromium(III) chloride, anhydrous [10025-73-7], diazepam [439-14-5], *N*,*N*-dimethylformamide [68-12-2], eugenol [97-53-0], HC blue no. 2 [33229-34-4], hydrocortisone [50-23-7], phenanthrene [85-01-8], phenol [108-95-2], *p*-phenylenediamine dihydrochloride [624-18-0], rotenone [83-79-4], sodium chloride [7647-14-5], sodium nitrite [7632-00-0], sunset yellow FCF [2783-94-0], thiabendazole [148-79-8], triphenyltin hydroxide [76-87-9], barium chloride dihydrate [10326-27-9], *tert*-butylhydroquinone [1948-33-0], 8-hydroxyquinoline [148-24-3], propyl gallate [121-79-9], tetracycline hydrochloride [64-75-5], sodium valproate [1069-66-5] and sodium orthovanadate [13721-39-6] were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2-Acetylaminofluorene [53-96-3], 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) [76180-96-6], dibenz[a,h]anthracene [53-70-3], cadmium chloride [10108-64-2], lithocholic acid [434-13-9], methylarsonic acid [124-58-3], dimethylarsinic acid [75-60-5], formaldehyde [50-00-0], nickel (II) chloride [7718-54-9], *p*-toluidine [106-49-0], aspartame [22839-47-0], 2,6-diaminotoluene [823-40-5], D-mannitol [69-65-8], methotrexate [59-05-2], phthalic anhydride [85-44-9], *m*-toluidine [108-44-1], sodium fluoride [7681-49-4], valproic acid [99-66-1], 2,3-diaminotoluene [2687-25-4], 2,5-diaminotoluene dihydrochloride [615-45-2], 3,4-diaminotoluene [496-72-0], capsaicin [404-86-4] and salicylic acid [69-72-7] were obtained from Wako Pure Chemical Industries. Quercetin [117-39-5], sodium saccharin [128-44-9], phenobarbital sodium salt [57-30-7] and 1-naphthylamine [134-32-7] were from Tokyo Chemical Industry (Tokyo, Japan). *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) [70-25-7] and ethidium bromide [1239-45-8] were from Kanto Chemical (Tokyo, Japan). Sodium arsenite [7784-46-5] solution (0.05 M) was from Merck KGaA (Darmstadt, Germany). 2-Naphthylamine [91-59-8] solution (1001 μg/mL) was from Ultra Scientific (North Kingstown, RI, USA). Furylfuramide [3688-53-7] was from Ueno Fine Chemicals Industry (Tokyo, Japan).

Test chemicals were dissolved in sterile distilled water, DMSO, methanol (Wako) or acetone (Wako) as indicated in the parenthesis following "0" of the concentration columns in Table 1. MCA and TPA were in DMSO. The final concentration of distilled water was 5% in the culture medium, that of methanol was 0.5%, that of acetone was 0.5% and that of DMSO was 0.1 or 0.5% depending on the solubility of test chemicals in DMSO.

2.3. Transformation assays

The transformation assays were conducted according to the procedures reported by Asada et al. with a slight modification [16].

2.3.1. Initiation assay to examine initiating activity of chemicals

One tube of the frozen Bhas 42 cells was rapidly thawed and proliferated once in M10F up to about 70% confluence and then subcultured in DF5F to reach about 70% confluence again. Thereafter cells were cultured in DF5F. The cells were trypsinized and suspended at a density of 2000 cells/mL and seeded onto each well of 6-well microplates in 2 mL volumes (4000 cells/well, day 0). Six wells were prepared for each treatment group. At 24 h after seeding, the cells were treated by the addition of a test chemical solution or the vehicle alone to the cultures, and the treatment in the initiation phase was continued for 72 h. Following the exposure period, all treatment media were removed and the cells were refed with medium without the test chemical (day 4) and subsequently cultured in the normal medium until day 21, receiving medium exchanges on day 7, day 11 and day 14 (Fig. 1). The cells were fixed with methanol and stained with Giemsa's solution. Each assay was performed including MCA (1 μg/mL) as the positive control and 0.1% DMSO as its negative control.

2.3.2. Promotion assay to examine promoting activity of chemicals

The promotion assay was carried out in the same manner as the initiation assay except for the following procedures. The cells were seeded at a density of 7000 cells/mL (14,000 cells/well, day 0) and cultured for 4 days without a medium exchange. On day 4, day 7, and day 11, the culture medium was replaced with a fresh medium containing a test chemical or vehicle alone and the treatment in the promotion phase was continued until day 14 (for a total of 10 days). The cells were then subsequently cultured in the normal medium without the test chemical for 1

Table 1
Results of Bhas 42 cell transformation assay of 92 chemicals.

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
Carcinogens									
2-Acetylaminofluorene (53-96-3)				+					+
	0[e] (0.1% DMSO)	100	3.3 ± 1.5			0 (0.1% DMSO)	100	7.0 ± 2.4	
	10	99	6.2 ± 3.3			1	103	8.7 ± 2.3	
	20	62	9.0 ± 3.8*			5	100	13.0 ± 1.9*	
	25	19	9.5 ± 2.6*			10	82	21.7 ± 6.3*	
	30	13	7.7 ± 1.9			15	71	16.0 ± 4.6*	
	40	9	6.7 ± 2.6			20	62	8.8 ± 2.5	
	50	9	7.5 ± 3.3			25	61	4.0 ± 2.2	
MCA[f]	1	58	37.2 ± 5.8#		TPA[g]	0.05	146	20.8 ± 3.5#	
Benz[*a*]anthracene (56-55-3)				+					+
	0 (0.1% DMSO)	100	1.0 ± 1.1			0 (0.1% DMSO)	100	3.0 ± 1.3	
	0.25	84	2.7 ± 1.6			0.25	79	4.7 ± 2.1	
	0.5	73	3.7 ± 2.0			0.5	70	6.3 ± 2.7	
	1	71	8.5 ± 2.3*			1	68	9.3 ± 2.7*	
	2.5	67	8.0 ± 3.2*			2.5	64	12.2 ± 2.7*	
	5	63	11.2 ± 2.6*			5	65	8.5 ± 3.2*	
	10	60	13.2 ± 2.8*			10	69	9.7 ± 3.7*	
MCA	1	30	15.0 ± 3.8#		TPA	0.05	151	16.5 ± 4.8#	
2-Amino-3-methylimidazo [4,5-*f*]quinoline (IQ) (76180-96-6)				+					–
	0 (0.5% DMSO)	100	4.2 ± 0.8			0 (0.5% DMSO)	100	2.2 ± 1.9	
	20	73	7.2 ± 2.3			3.2	95	2.7 ± 0.5	
	40	63	11.3 ± 3.2*			5.6	72	3.2 ± 1.8	
	60	56	13.2 ± 4.4*			10	92	1.0 ± 0.9	
	70	55	15.8 ± 2.3*			18	66	0.8 ± 1.2	
	80	48	10.8 ± 1.2*			32	65	1.0 ± 0.9	
	90	42	7.5 ± 2.8			56	51	0.5 ± 0.5	
	100	37	8.0 ± 3.0		TPA	0.05	131	17.5 ± 5.9#	
MCA	1	56	14.2 ± 1.6#						
5-Azacytidine (320-67-2)				+					–
	0 (5% Water)	100	2.0 ± 0.9			0 (5% Water)	100	5.7 ± 1.2	
	0.1	40	2.7 ± 1.8			0.01	104	6.0 ± 2.6	
	0.13	35	4.2 ± 1.0			0.018	103	8.2 ± 4.0	
	0.18	26	8.3 ± 1.5*			0.032	101	6.5 ± 1.9	
	0.25	18	7.0 ± 4.5*			0.056	104	6.2 ± 1.2	
	0.3	12	7.8 ± 3.5*			0.1	105	6.8 ± 1.3	
	0.5	2	Toxic			0.18	100	6.3 ± 2.1	
	0[h] (0.1% DMSO)	100	1.5 ± 1.0			0.32	99	4.2 ± 0.8	
MCA	1	47	18.7 ± 3.2#			0[j] (0.1% DMSO)	100	7.7 ± 2.1	
					TPA	0.05	149	33.7 ± 5.0#	
Barium chromate (10294-40-3)				+					–
	0 (5% Water)	100	1.0 ± 0.6			0 (5% Water)	100	2.7 ± 1.4	
	1	101	0.7 ± 0.8			0.5	95	2.7 ± 1.6	
	2	95	2.7 ± 0.8			1	105	1.7 ± 1.2	
	3	77	2.0 ± 0.0			2	100	2.3 ± 1.4	
	4	58	3.7 ± 1.5*			3.5	106	2.3 ± 1.9	
	5	66	4.3 ± 1.2*			5	105	2.0 ± 0.9	
	7	24	4.5 ± 2.2*			7	109	1.8 ± 1.2	
	10	15	4.5 ± 2.4*			10	108	1.8 ± 1.5	
	15	10	3.5 ± 1.8*			15	99	2.3 ± 2.6	
	0 (0.1% DMSO)	100	2.2 ± 1.5			0 (0.1% DMSO)	100	2.5 ± 1.6	
MCA	1	47	27.5 ± 3.4#		TPA	0.05	176	20.5 ± 1.6#	
Benzo[*a*]pyrene (50-32-8)				+					-
	0 (0.1% DMSO)	100	1.8 ± 1.2			0 (0.1% DMSO)	100	2.5 ± 0.8	
	0.01	75	4.3 ± 2.0*			0.01	85	1.0 ± 0.9	
	0.025	35	7.5 ± 2.7*			0.025	79	1.8 ± 1.3	
	0.05	26	4.3 ± 1.5*			0.05	70	1.3 ± 1.5	
	0.1	12	4.5 ± 2.1*			0.1	57	1.3 ± 1.5	
	0.25	4	Toxic			0.25	43	0.5 ± 0.8	
MCA	1	27	14.0 ± 3.2#		TPA	0.05	152	14.5 ± 3.9#	
Cyclophosphamide (50-18-0)				+					–
	0 (5% Water)	100	3.3 ± 2.2			0 (5% Water)	100	4.5 ± 1.6	
	100	103	5.2 ± 1.2			160	100	3.7 ± 1.9	
	500	101	10.8 ± 1.0*			250	105	2.8 ± 1.6	
	1100	55	14.8 ± 3.6*			390	108	4.2 ± 2.5	
	1300	37	9.7 ± 4.3*			620	97	3.5 ± 1.0	
	1500	26	9.8 ± 3.3*			1000	95	1.8 ± 1.7	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	2000	16	1.2 ± 0.8			1600	87	0.0 ± 0.0	
	2200	13	Toxic			2500	81	0.0 ± 0.0	
	2400	10	Toxic			0 (0.1% DMSO)	100	3.7 ± 1.9	
	0 (0.1% DMSO)	100	6.5 ± 3.1		TPA	0.05	154	18.5 ± 4.1[#]	
MCA	1	63	44.5 ± 2.1[#]						
Cyclosporin A (59865-13-3)				+					–
First experiment				±					–
	0 (0.1% DMSO)	100	1.7 ± 0.5			0 (0.1% DMSO)	100	4.5 ± 1.8	
	2	95	4.0 ± 1.7			0.0625	100	7.5 ± 3.3	
	2.5	89	4.7 ± 2.6[*]			0.125	98	6.0 ± 4.1	
	3	89	2.5 ± 2.2			0.25	99	6.8 ± 2.1	
	3.5	86	2.7 ± 1.2			0.5	93	6.3 ± 3.4	
	4	89	1.0 ± 1.1			1	90	3.5 ± 3.2	
	4.5	79	1.0 ± 1.1			2	83	1.8 ± 1.8	
	5	78	1.3 ± 1.0			4	79	2.3 ± 1.8	
MCA	1	45	21.7 ± 2.3[#]			6	74	1.2 ± 1.0	
					TPA	0.05	140	37.8 ± 6.6[#]	
Second experiment				+					
	0 (0.1% DMSO)	100	2.8 ± 0.8						
	0.5	104	4.3 ± 1.5						
	1	107	4.7 ± 1.0						
	1.6	106	5.5 ± 2.4						
	2.5	108	7.0 ± 3.2[*]						
	4	99	4.8 ± 2.4						
	6	86	5.8 ± 1.0						
	10	40	6.2 ± 1.9[*]						
	15	31	4.0 ± 1.3						
MCA	1	35	33.0 ± 3.0[#]						
2,4-Diaminotoluene (95-80-7)				+					–
	0 (0.1% DMSO)	100	5.2 ± 1.0			0 (0.1% DMSO)	100	6.7 ± 1.4	
	10	90	8.0 ± 2.2			1	92	7.3 ± 1.8	
	15	98	7.5 ± 2.3			3	92	8.5 ± 2.2	
	20	92	10.2 ± 4.2[*]			5	89	7.2 ± 1.5	
	25	90	10.5 ± 3.8[*]			7.5	78	7.5 ± 3.9	
	30	80	9.3 ± 2.9			10	75	5.2 ± 1.9	
	35	67	12.3 ± 4.4[*]			15	75	3.0 ± 1.1	
	40	52	12.2 ± 3.1[*]			20	67	0.7 ± 1.2	
	45	45	12.7 ± 2.1[*]		TPA	0.05	138	38.0 ± 5.9[#]	
	50	33	8.0 ± 2.4						
MCA	1	42	45.8 ± 3.8[#]						
Dibenz[*a*,*h*]anthracene (53-70-3)				+					–
	0 (0.1% DMSO)	100	1.0 ± 1.3			0 (0.1% DMSO)	100	3.0 ± 1.1	
	0.003	109	2.8 ± 1.2			0.0003	85	1.0 ± 1.1	
	0.01	86	2.2 ± 1.2			0.001	78	3.0 ± 1.8	
	0.03	65	6.5 ± 1.9[*]			0.003	79	2.8 ± 0.8	
	0.1	36	13.5 ± 3.1[*]			0.01	79	4.2 ± 2.6	
	0.3	22	13.8 ± 2.5[*]			0.03	70	2.0 ± 2.2	
	1	24	12.0 ± 2.2[*]			0.1	62	2.3 ± 1.2	
	3	20	10.5 ± 3.6[*]			0.3	56	2.7 ± 1.9	
	10	26	Toxic			1	63	1.0 ± 1.1	
MCA	1	43	11.7 ± 5.3[#]		TPA	0.05	227	10.8 ± 2.6[#]	
Melphalan (148-82-3)				+					–
	0 (5% Water)	100	3.2 ± 1.0			0 (5% Water)	100	8.2 ± 4.0	
	0.1	99	2.3 ± 1.8			0.05	93	5.0 ± 2.8	
	0.25	107	5.5 ± 3.3			0.1	98	8.2 ± 4.3	
	0.5	105	6.2 ± 1.6			0.25	93	4.0 ± 1.5	
	0.75	109	8.7 ± 2.3[*]			0.5	94	0.7 ± 0.8	
	1	80	9.5 ± 2.9[*]			0.75	87	Toxic	
	1.5	48	15.3 ± 4.1[*]			1	90	Toxic	
	2	20	8.5 ± 3.4[*]			0 (0.1% DMSO)	100	4.8 ± 1.5	
	0 (0.1% DMSO)	100	2.2 ± 1.0		TPA	0.05	131	33.3 ± 3.1[#]	
MCA	1	61	29.7 ± 3.4[#]						
3-Methylcholanthrene (MCA) (56-49-5)				+					–
	0 (0.1% DMSO)	100	2.2 ± 1.5			0 (0.1% DMSO)	100	2.3 ± 1.0	
	0.001	96	1.0 ± 1.5			0.001	95	0.8 ± 1.0	
	0.008	97	1.0 ± 0.9			0.008	93	0.5 ± 0.8	
	0.016	92	2.5 ± 1.0			0.016	90	0.5 ± 0.5	
	0.031	93	4.0 ± 1.8			0.031	87	1.0 ± 0.9	
	0.063	81	4.8 ± 1.6			0.063	83	1.5 ± 1.5	
	0.125	77	7.7 ± 2.2[*]			0.125	81	2.7 ± 1.6	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	0.25	66	12.7 ± 2.7*			0.25	73	1.3 ± 1.2	
	0.5	53	18.8 ± 3.9*			0.5	70	0.3 ± 0.8	
	1	42	24.8 ± 3.4*			1	68	0.5 ± 0.5	
	2	35	28.7 ± 5.1*			2	69	0.5 ± 0.5	
MCA	1	45	21.2 ± 3.6#		TPA	0.05	148	10.2 ± 3.4#	
Mitomycin C (50-07-7)				+					–
	0 (0.1% DMSO)	100	2.0 ± 1.8			0 (0.1% DMSO)	100	3.2 ± 2.1	
	0.006	84	1.2 ± 1.2			0.004	94	2.8 ± 1.8	
	0.01	64	3.7 ± 1.6			0.006	98	3.0 ± 1.7	
	0.016	37	5.3 ± 1.0*			0.01	94	1.0 ± 0.6	
	0.025	8	5.2 ± 2.8*			0.016	95	0.5 ± 0.5	
	0.039	3	Toxic			0.025	90	0.2 ± 0.4	
	0.062	2	Toxic			0.039	86	0.0 ± 0.0	
	0.1	2	Toxic			0.062	88	0.0 ± 0.0	
MCA	1	36	14.7 ± 3.0#			0.1	84	0.0 ± 0.0	
					TPA	0.05	123	19.5 ± 3.6#	
N-Methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (70-25-7)				+					–
	0 (0.1% DMSO)	100	1.7 ± 1.2			0 (0.1% DMSO)	100	2.2 ± 1.3	
	0.01	92	0.5 ± 0.5			0.01	92	1.0 ± 1.3	
	0.05	94	0.5 ± 0.5			0.05	97	2.2 ± 1.6	
	0.1	99	1.7 ± 0.8			0.1	101	2.3 ± 1.2	
	0.15	94	2.2 ± 1.5			0.15	108	1.8 ± 1.0	
	0.22	93	3.0 ± 1.3			0.22	103	1.0 ± 0.6	
	0.32	80	5.0 ± 1.8			0.32	117	1.7 ± 1.2	
	0.47	47	6.0 ± 1.8*			0.47	103	2.5 ± 1.5	
	0.7	15	9.7 ± 4.8*			0.7	116	2.0 ± 1.5	
	1	6	3.8 ± 3.3			1	112	0.7 ± 0.8	
	2	2	Toxic			2	109	0.0 ± 0.0	
MCA	1	36	27.7 ± 3.8#		TPA	0.05	143	13.8 ± 5.9#	
Sterigmatocystin (10048-13-2)				+					–
	0 (0.1% DMSO)	100	1.2 ± 1.5			0 (0.1% DMSO)	100	7.8 ± 3.0	
	0.005	118	5.8 ± 3.3			0.00003	100	7.3 ± 2.4	
	0.01	112	9.5 ± 2.5*			0.0001	100	7.2 ± 3.5	
	0.02	82	13.8 ± 5.5*			0.0003	95	7.2 ± 1.9	
	0.03	56	18.0 ± 4.1*			0.001	95	8.5 ± 3.4	
	0.04	37	23.5 ± 7.3*			0.003	102	5.2 ± 1.6	
	0.05	36	19.8 ± 4.4*			0.01	99	1.7 ± 0.5	
	0.1	11	Toxic			0.03	89	0.0 ± 0.0	
MCA	1	62	30.0 ± 5.8#		TPA	0.05	142	35.8 ± 6.0#	
Thio-TEPA (52-24-4)				+					–
	0 (5% Water)	100	1.0 ± 1.1			0 (5% Water)	100	1.2 ± 0.8	
	0.06	98	0.7 ± 0.8			0.02	96	1.2 ± 1.0	
	0.1	82	1.7 ± 1.9			0.04	95	0.7 ± 0.8	
	0.16	79	1.7 ± 1.0			0.06	93	1.5 ± 1.0	
	0.25	67	5.2 ± 2.3*			0.08	88	1.5 ± 1.5	
	0.4	43	8.8 ± 2.6*			0.1	84	0.3 ± 0.5	
	0.6	16	3.5 ± 2.8			0 (0.1% DMSO)	100	2.7 ± 1.4	
	1	3	Toxic		TPA	0.05	114	12.0 ± 5.0#	
	0 (0.1% DMSO)	100	1.0 ± 1.5						
MCA	1	42	15.2 ± 5.3#						
Cadmium chloride (10108-64-2)				–					+
	0 (5% Water)	100	2.3 ± 1.8			0 (5% Water)	100	2.8 ± 1.8	
	0.5	86	3.2 ± 2.3			0.05	106	1.7 ± 1.0	
	0.63	87	2.8 ± 2.1			0.07	103	2.0 ± 1.9	
	0.8	76	1.8 ± 2.6			0.1	106	3.7 ± 2.1	
	1	76	2.8 ± 1.7			0.2	106	7.0 ± 2.0*	
	1.3	40	1.2 ± 1.9			0.3	109	7.3 ± 2.2*	
	1.6	14	0.7 ± 0.8			0.4	113	12.5 ± 3.1*	
	2	0	Toxic			0.5	119	19.2 ± 4.0*	
	0 (0.1% DMSO)	100	3.3 ± 2.3			0 (0.1% DMSO)	100	3.8 ± 1.2	
MCA	1	36	16.5 ± 6.4#		TPA	0.05	119	21.5 ± 4.8#	
Chenodeoxycholic acid (474-25-9)				–					+
	0 (0.1% DMSO)	100	0.5 ± 0.5			0 (0.1% DMSO)	100	2.7 ± 0.8	
	10	94	1.2 ± 1.2			10	74	3.0 ± 0.9	
	12	90	1.2 ± 1.0			12	87	6.8 ± 3.8*	
	14	100	2.0 ± 0.6			14	99	5.2 ± 2.0	
	16	105	1.2 ± 0.4			16	100	5.3 ± 1.4	
	19	103	1.0 ± 1.3			19	101	6.7 ± 2.6	
	23	103	1.5 ± 1.5			23	101	10.7 ± 4.0*	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	27	109	1.0 ± 1.5			27	112	14.3 ± 2.7*	
	32	122	1.0 ± 0.9			32	115	18.3 ± 3.9*	
	37	72	2.2 ± 1.2			37	139	0.2 ± 0.4	
MCA	1	48	27.8 ± 6.7#		TPA	0.05	126	22.2 ± 4.2#	
4-Chloro-*o*-toluidine hydrochloride (3165-93-3)				–					+
	0 (5% Water)	100	1.0 ± 1.1			0 (5% Water)	100	1.7 ± 0.8	
	3	72	0.7 ± 0.8			1.8	87	5.2 ± 1.0	
	6	76	0.0 ± 0.0			3	80	4.8 ± 2.1	
	10	90	1.7 ± 1.6			6	78	4.8 ± 2.9	
	18	84	1.0 ± 1.3			10	72	6.7 ± 3.1*	
	30	71	1.5 ± 1.8			18	61	6.7 ± 1.9*	
	60	57	1.7 ± 1.2			30	62	6.0 ± 1.5*	
	100	42	0.3 ± 0.5			60	43	8.3 ± 3.4*	
	180	50	0.2 ± 0.4			100	39	9.8 ± 1.9*	
	0 (0.1% DMSO)	100	1.0 ± 1.5			0 (0.1% DMSO)	100	2.2 ± 1.6	
MCA	1	42	15.2 ± 5.3#		TPA	0.05	227	11.8 ± 2.8#	
Cholic acid (81-25-4)				–					+
	0 (0.1% DMSO)	100	2.0 ± 0.9			0 (0.1% DMSO)	100	4.3 ± 1.2	
	10	64	0.3 ± 0.5			18	74	5.5 ± 2.4	
	18	80	0.7 ± 0.8			24	87	5.0 ± 1.7	
	32	90	0.7 ± 0.8			32	99	7.7 ± 2.4	
	56	96	1.2 ± 0.8			42	100	10.2 ± 2.8*	
	100	100	1.2 ± 1.2			56	101	10.2 ± 3.9*	
	180	109	2.0 ± 1.1			75	101	10.2 ± 2.8*	
	320	23	2.0 ± 1.4			100	112	12.7 ± 3.6*	
MCA	1	48	33.3 ± 3.8#			130	115	14.5 ± 4.8*	
						180	139	20.5 ± 2.1*	
					TPA	0.05	152	24.5 ± 3.4#	
Deoxycholic acid (83-44-3)				–					+
	0 (0.1% DMSO)	100	2.3 ± 1.4			0 (0.1% DMSO)	100	2.7 ± 0.8	
	6	100	0.7 ± 0.8			19	94	3.8 ± 2.0	
	10	97	1.2 ± 1.5			23	94	6.8 ± 2.8	
	15	109	1.3 ± 1.4			27	112	14.0 ± 6.2*	
	22	82	2.0 ± 1.3			32	125	18.0 ± 4.2*	
	32	46	2.0 ± 1.7			37	141	14.0 ± 4.6*	
	47	24	2.2 ± 1.5			44	100	0.0 ± 0.0	
MCA	1	48	25.2 ± 8.0#			52	41	0.0 ± 0.0	
						61	9	Toxic	
					TPA	0.05	152	27.2 ± 6.7#	
Dichlorvos (62-73-7)				–					+
First experiment				–					±
	0 (0.1% DMSO)	100	1.7 ± 1.0			0 (0.1% DMSO)	100	4.5 ± 1.4	
	5.5	92	1.2 ± 1.2			5.5	100	3.7 ± 1.0	
	7	88	2.0 ± 1.3			7	100	5.5 ± 2.7	
	10	32	1.8 ± 1.2			10	104	4.8 ± 2.6	
	13	16	0.8 ± 0.8			13	105	6.3 ± 2.7	
	16	5	0.3 ± 0.8			16	105	6.3 ± 1.8	
	20	2	0.2 ± 0.4			20	116	8.2 ± 3.3	
	24	1	0.5 ± 1.2			24	123	8.5 ± 2.0*	
	30	0	Toxic			30	123	4.2 ± 2.7	
MCA	1	36	14.5 ± 3.7#		TPA	0.05	116	22.8 ± 4.5#	
Second experiment									±
						0 (0.1% DMSO)	100	4.7 ± 1.0	
						4	93	3.3 ± 3.3	
						7	95	4.2 ± 2.2	
						10	102	4.3 ± 2.5	
						13	106	6.8 ± 1.0	
						16	101	5.2 ± 2.9	
						20	109	4.5 ± 2.3	
						24	124	5.5 ± 2.3	
						30	126	8.7 ± 2.4*	
					TPA	0.05	147	21.8 ± 3.2#	
Epichlorohydrin (106-89-8)				–					+
	0 (0.1% DMSO)	100	1.8 ± 1.3			0 (0.1% DMSO)	100	4.7 ± 1.4	
	20	121	2.7 ± 0.5			10	108	7.0 ± 4.0	
	40	103	2.0 ± 1.3			20	99	7.7 ± 1.5	
	60	116	1.5 ± 1.5			30	116	15.0 ± 5.1*	
	80	42	2.8 ± 1.8			40	113	19.2 ± 7.5*	
	100	3	Toxic			50	115	Toxic	
MCA	1	56	21.5 ± 1.5#			60	145	Toxic	
						70	117	Toxic	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]	Concentration (μg/mL)		CG	Foci/well	Judgment
					TPA	0.05	163	35.0 ± 5.0[#]	
D-Limonene (5989-27-5)				−					+
	0 (0.5% DMSO)	100	6.2 ± 2.0			0 (0.5% DMSO)	100	4.5 ± 2.0	
	10	104	9.2 ± 3.1			10	98	3.8 ± 2.1	
	20.5	48	6.2 ± 2.8			25	92	6.3 ± 3.5	
	21.5	35	6.3 ± 2.3			26	99	3.5 ± 2.2	
	22.5	12	2.2 ± 1.2			27	95	5.0 ± 3.9	
	23.5	18	2.2 ± 1.3			28	102	10.7 ± 2.3[*]	
	24.5	6	1.3 ± 1.0			29	81	14.2 ± 8.3[*]	
	25	11	1.2 ± 1.9			30	83	9.8 ± 3.7	
	26	3	0.7 ± 1.0			32	57	2.2 ± 2.4	
MCA	1	66	35.3 ± 7.3[#]			34	22	Toxic	
					TPA	0.05	132	17.8 ± 4.2[#]	
Lithocholic acid (434-13-9)				−					+
	0 (0.1% DMSO)	100	1.0 ± 0.6			0 (0.1% DMSO)	100	1.8 ± 1.5	
	12	109	1.0 ± 0.9			12	94	15.8 ± 3.3[*]	
	14	108	0.7 ± 0.8			14	88	17.7 ± 3.6[*]	
	16	119	0.8 ± 0.8			16	89	18.5 ± 3.0[*]	
	19	120	0.2 ± 0.4			19	92	24.5 ± 4.5[*]	
	23	129	0.8 ± 0.8			23	139	9.0 ± 1.7[*]	
	27	63	1.5 ± 1.4			27	34	0.0 ± 0.0	
MCA	1	48	23.7 ± 3.6[#]		TPA	0.05	152	22.8 ± 3.7[#]	
Methapyrilene hydrochloride (135-23-9)				−					+
	0 (5% Water)	100	2.3 ± 2.5			0 (5% Water)	100	2.5 ± 2.0	
	100	105	2.3 ± 1.8			10	114	7.0 ± 3.0	
	150	88	2.2 ± 1.5			25	127	8.5 ± 1.9[*]	
	200	67	2.7 ± 1.0			50	155	18.5 ± 6.3[*]	
	250	30	0.8 ± 0.8			75	165	9.5 ± 3.9[*]	
	300	5	0.5 ± 0.8			100	162	7.8 ± 1.8[*]	
	350	1	1.0 ± 1.3			150	143	2.0 ± 0.9	
	400	1	Toxic			200	125	0.5 ± 0.5	
	0 (0.1% DMSO)	100	2.5 ± 2.0			250	109	1.0 ± 0.6	
MCA	1	29	17.2 ± 3.2[#]			0 (0.1% DMSO)	100	2.7 ± 1.5	
					TPA	0.05	150	17.3 ± 3.4[#]	
Methylarsonic acid (124-58-3)				−					+
	0 (5% Water)	100	2.5 ± 2.3			0 (5% Water)	100	5.8 ± 2.6	
	700	103	1.7 ± 1.2			1	101	5.3 ± 3.2	
	900	100	2.7 ± 2.3			3	94	8.3 ± 2.2	
	1000	96	1.8 ± 0.8			7	100	14.3 ± 4.8[*]	
	1100	80	2.8 ± 1.9			10	97	19.2 ± 1.7[*]	
	1200	60	2.3 ± 2.2			30	107	43.3 ± 3.6[*]	
	1300	41	0.5 ± 0.8			70	107	55.0 ± 3.3[*]	
	1400 (10.0 mM)	23	1.7 ± 1.4			100	115	60.8 ± 3.3[*]	
	0 (0.1% DMSO)	100	3.2 ± 1.6			140	119	56.3 ± 3.5[*]	
MCA	1	54	24.5 ± 2.4[#]			280	124	36.3 ± 3.0[*]	
						400	123	Toxic	
						500	111	Toxic	
						0 (0.1% DMSO)	100	8.2 ± 1.0	
					TPA	0.05	140	30.0 ± 3.7[#]	
Mezerein (34807-41-5)				−					+
	0 (0.1% DMSO)	100	2.2 ± 1.3			0 (0.1% DMSO)	100	4.3 ± 2.0	
	0.00001	86	3.0 ± 1.4			0.00001	98	4.2 ± 2.1	
	0.00003	89	0.3 ± 0.5			0.00003	96	5.2 ± 3.2	
	0.00005	87	2.3 ± 0.5			0.00005	99	7.0 ± 2.2	
	0.0001	86	0.7 ± 1.2			0.0001	99	9.3 ± 2.9[*]	
	0.0003	84	1.7 ± 1.6			0.0003	123	19.5 ± 4.2[*]	
	0.0005	81	1.5 ± 0.5			0.0005	152	33.7 ± 3.3[*]	
	0.001	83	1.5 ± 1.4			0.001	191	40.2 ± 3.1[*]	
	0.003	114	3.0 ± 1.9			0.003	163	Toxic	
MCA	1	31	17.2 ± 3.5[#]		TPA	0.05	150	20.0 ± 3.3[#]	
2-Naphthylamine (91-59-8)				±					+
	0 (0.5% Methanol)	100	2.2 ± 1.5			0 (0.5% Methanol)	100	0.7 ± 1.2	
	0.01	111	1.8 ± 1.8			0.01	71	1.2 ± 0.8	
	0.03	113	1.8 ± 1.8			0.03	74	0.8 ± 0.4	
	0.1	116	4.2 ± 2.4			0.1	72	0.5 ± 0.8	
	0.3	106	2.2 ± 1.0			0.3	81	0.8 ± 0.8	
	1	99	2.2 ± 0.8			1	84	2.0 ± 1.3	
	3	76	4.2 ± 1.0			3	81	6.0 ± 2.6[*]	
	5	72	5.0 ± 2.4[*]			5	72	10.2 ± 2.5[*]	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	0 (0.1% DMSO)	100	2.3 ± 2.1			0 (0.1% DMSO)	100	2.0 ± 1.9	
MCA	1	36	12.7 ± 4.1[#]		TPA	0.05	104	4.7 ± 1.8[#]	
Phorbol 12,13-didecanoate (24928-17-4)				+					+
	0 (0.1% DMSO)	100	3.2 ± 1.8			0 (0.1% DMSO)	100	5.5 ± 2.3	
	0.01	124	1.8 ± 1.2			0.01	156	63.2 ± 10.7[*]	
	0.03	99	3.0 ± 1.4			0.03	178	65.0 ± 10.3[*]	
	0.1	97	7.5 ± 1.9			0.1	180	53.2 ± 5.4[*]	
	0.18	104	8.5 ± 2.3[*]			0.18	189	51.3 ± 2.9[*]	
	0.3	110	12.7 ± 3.5[*]			0.3	176	52.3 ± 1.6[*]	
	1	133	29.8 ± 7.2[*]			1	184	45.5 ± 4.3[*]	
MCA	1	59	32.7 ± 4.8[#]		TPA	0.05	125	22.7 ± 3.1[#]	
Quercetin (117-39-5)				−					+
	0 (0.1% DMSO)	100	3.5 ± 2.2			0 (0.1% DMSO)	100	7.3 ± 1.0	
	2	98	4.5 ± 2.0			0.5	98	8.5 ± 2.1	
	3	98	3.3 ± 2.3			1	93	9.5 ± 3.7	
	4	77	1.8 ± 1.2			2	84	13.5 ± 3.1[*]	
	5	27	3.3 ± 2.7			3	70	16.8 ± 4.2[*]	
	6	11	2.0 ± 1.3			5	32	40.8 ± 6.9[*]	
	7	3	1.8 ± 0.8			7	25	Toxic	
	8	1	2.0 ± 1.7			10	26	Toxic	
MCA	1	42	27.8 ± 2.6[#]		TPA	0.05	120	35.2 ± 5.6[#]	
Sodium arsenate (10048-95-0)				−					+
	0 (5% Water)	100	1.5 ± 1.4			0 (5% Water)	100	4.5 ± 2.0	
	0.5	102	1.5 ± 1.0			0.5	103	6.0 ± 2.2	
	0.7	97	1.0 ± 1.3			0.7	110	5.8 ± 1.9	
	1	86	1.5 ± 0.5			1	106	9.7 ± 3.7[*]	
	1.5	52	2.3 ± 2.0			1.5	103	17.0 ± 4.4[*]	
	2.2	31	1.7 ± 0.8			1.7	111	13.2 ± 3.1[*]	
	3.2	12	1.0 ± 0.9			2.2	102	11.0 ± 1.8[*]	
	5	6	0.8 ± 1.6			3.2	108	8.3 ± 2.5	
	0 (0.1% DMSO)	100	1.2 ± 1.5			5	102	1.7 ± 1.2	
MCA	1	49	27.7 ± 1.9[#]			0 (0.1% DMSO)	100	3.8 ± 1.7	
					TPA	0.05	117	26.7 ± 2.9[#]	
Sodium arsenite (7784-46-5)				±					+
	0 (5% Water)	100	0.5 ± 0.5			0 (5% Water)	100	4.7 ± 1.9	
	0.056	117	0.5 ± 0.5			0.01	92	3.3 ± 1.4	
	0.1	109	1.2 ± 0.8			0.056	100	6.3 ± 1.0	
	0.18	76	2.2 ± 1.2			0.1	94	10.2 ± 4.5[*]	
	0.32	33	2.8 ± 2.0[*]			0.16	100	17.0 ± 5.0[*]	
	0.56	10	1.3 ± 1.2			0.18	93	23.3 ± 5.7[*]	
	1	0	Toxic			0.25	93	12.0 ± 3.3[*]	
	0 (0.1% DMSO)	100	1.3 ± 1.4			0.32	97	5.3 ± 2.3	
MCA	1	48	21.0 ± 4.0[#]			0.39	98	2.2 ± 2.3	
						0.56	94	0.2 ± 0.4	
						0.62	98	0.2 ± 0.4	
						1	85	Toxic	
						0 (0.1% DMSO)	100	3.7 ± 1.5	
					TPA	0.05	121	23.5 ± 3.3[#]	
Sodium saccharin (128-44-9)				−					+
	0 (5% Water)	100	3.7 ± 1.9			0 (5% Water)	100	4.7 ± 1.4	
	410	100	3.2 ± 1.2			410	95	4.5 ± 0.8	
	821	100	3.3 ± 1.2			821	85	4.0 ± 1.4	
	1231	107	3.2 ± 1.8			1231	75	6.0 ± 2.8	
	1641	108	2.8 ± 1.3			1641	71	11.3 ± 2.7[*]	
	1847	109	2.3 ± 1.2			1847	70	15.2 ± 4.6[*]	
	2052 (10 mM)	108	3.8 ± 2.0			2052 (10 mM)	70	18.3 ± 6.6[*]	
	0 (0.1% DMSO)	100	3.2 ± 2.5			0 (0.1% DMSO)	100	6.0 ± 2.8	
MCA	1	57	49.2 ± 5.5[#]		TPA	0.05	146	41.3 ± 3.9[#]	
Styrene oxide (96-09-3)				−					+
	0 (0.1% DMSO)	100	1.3 ± 0.8			0 (0.1% DMSO)	100	4.5 ± 2.6	
	10	107	1.3 ± 1.5			10	94	5.8 ± 3.3	
	15	111	0.8 ± 1.2			20	97	5.8 ± 1.0	
	20	111	3.2 ± 2.3			30	95	12.7 ± 4.2[*]	
	25	104	3.2 ± 2.5			40	91	13.7 ± 5.6[*]	
	30	102	3.5 ± 1.9			50	88	11.8 ± 7.3[*]	
	40	104	4.0 ± 3.6			70	85	Toxic	
	50	96	3.7 ± 3.1		TPA	0.05	121	19.5 ± 3.9[#]	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]	Concentration (μg/mL)		CG	Foci/well	Judgment
MCA	1	49	32.5 ± 5.0[#]						
12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (16561-29-8)				±					+
	0 (0.1% DMSO)	100	0.8 ± 1.2		0 (0.1% DMSO)		100	1.3 ± 1.4	
	0.0003	97	1.8 ± 1.2		0.0003		102	1.8 ± 0.8	
	0.001	81	1.3 ± 1.5		0.001		112	2.7 ± 1.0	
	0.003	83	0.7 ± 0.5		0.003		117	3.7 ± 2.9	
	0.01	84	0.2 ± 0.4		0.01		139	8.2 ± 3.7[*]	
	0.03	86	0.5 ± 0.5		0.03		143	13.0 ± 2.5[*]	
	0.05	76	0.3 ± 0.8		0.05		164	13.8 ± 2.3[*]	
	0.1	81	0.5 ± 0.8		0.1		194	12.7 ± 4.9[*]	
	0.3	75	0.5 ± 0.5		0.3		212	20.8 ± 5.5[*]	
	0.5	85	1.3 ± 1.2		0.5		220	24.3 ± 2.3[*]	
	1	88	1.5 ± 1.0		1		231	34.8 ± 5.5[*]	
	3	88	2.8 ± 2.2[*]		3		211	47.5 ± 5.2[*]	
MCA	1	36	19.8 ± 2.5[#]		TPA	0.05	158	10.3 ± 2.7[#]	
o-Toluidine (95-53-4)				–					+
	0 (0.5% DMSO)	100	1.8 ± 1.7		0 (0.5% DMSO)		100	4.8 ± 1.5	
	100	99	1.7 ± 1.6		100		82	5.3 ± 1.6	
	200	92	3.5 ± 2.3		200		69	2.5 ± 2.9	
	300	87	1.5 ± 1.2		300		63	8.3 ± 1.9[*]	
	400	76	4.5 ± 2.0		400		54	8.0 ± 1.3[*]	
	600	50	3.3 ± 2.6		600		39	1.8 ± 1.5	
	800	36	2.7 ± 2.0		800		40	0.7 ± 0.8	
	0 (0.1% DMSO)	100	4.2 ± 1.3		0 (0.1% DMSO)		100	7.0 ± 2.6	
MCA	1	59	17.5 ± 2.3[#]		TPA	0.05	142	15.3 ± 4.4[#]	
Zinc chloride (7646-85-7)				–					+
	0 (5% Water)	100	0.8 ± 0.8		0 (5% Water)		100	1.3 ± 1.0	
	7	94	0.7 ± 0.5		1		112	1.3 ± 1.2	
	10	104	0.0 ± 0.0		3		126	2.2 ± 1.5	
	11	98	0.7 ± 0.8		7		131	4.0 ± 2.0	
	12	73	0.2 ± 0.4		10		162	14.0 ± 3.7[*]	
	13	21	0.5 ± 0.8		11		162	15.3 ± 2.5[*]	
	14	3	0.0 ± 0.0		13		175	19.8 ± 4.4[*]	
	15	1	Toxic		15		100	8.5 ± 3.2[*]	
	0 (0.1% DMSO)	100	1.3 ± 1.5		0 (0.1% DMSO)		100	2.5 ± 1.5	
MCA	1	47	25.0 ± 6.2[#]		TPA	0.05	47	11.2 ± 2.9[#]	
o-Anisidine (90-04-0)				–					–
	0 (0.1% DMSO)	100	2.0 ± 0.9		0 (0.1% DMSO)		100	3.5 ± 2.2	
	60	100	2.0 ± 1.8		10		91	2.7 ± 0.5	
	80	97	3.8 ± 2.8		30		89	1.3 ± 1.9	
	100	79	2.7 ± 0.8		70		88	1.0 ± 1.1	
	130	58	3.0 ± 2.0		130		60	1.7 ± 1.2	
	170	32	4.2 ± 1.8		170		67	4.3 ± 3.1	
	220	10	4.0 ± 1.3		220		53	4.3 ± 3.1	
	260	8	4.3 ± 2.2		TPA	0.05	169	25.7 ± 2.3[#]	
MCA	1	49	29.5 ± 4.2[#]						
Benzene (71-43-2)				–					–
	0 (0.5% DMSO)	100	2.3 ± 2.5		0 (0.5% DMSO)		100	2.7 ± 1.4	
	31.25	94	3.7 ± 3.3		31.25		95	2.3 ± 2.1	
	62.5	97	1.0 ± 1.3		62.5		104	3.8 ± 2.5	
	125	106	0.8 ± 1.6		125		111	3.8 ± 3.1	
	250	100	1.5 ± 1.4		250		107	5.0 ± 3.2	
	500	104	1.8 ± 1.5		500		89	3.3 ± 2.0	
	750	98	2.3 ± 2.0		750		104	1.7 ± 1.2	
	1000 (12.8 mM)	109	2.2 ± 0.8		1000 (12.8 mM)		100	2.3 ± 1.2	
MCA	1	34	19.7 ± 6.4[#]		TPA	0.05	106	15.8 ± 4.5[#]	
Cobalt sulfate heptahydrate (10026-24-1)				–					–
	0 (5% Water)	100	4.8 ± 1.8		0 (5% Water)		100	7.5 ± 3.1	
	1	101	4.3 ± 2.2		1		105	6.0 ± 3.0	
	3	113	2.8 ± 1.7		3		101	6.8 ± 1.5	
	10	125	2.3 ± 1.9		10		99	6.0 ± 1.1	
	15	133	3.0 ± 1.3		15		104	8.3 ± 3.6	
	20	126	3.0 ± 1.4		20		98	6.3 ± 0.8	
	30	118	3.5 ± 1.5		30		96	4.7 ± 1.9	
	40	84	3.7 ± 1.4		40		95	0.7 ± 0.8	
	0 (0.1% DMSO)	100	3.8 ± 1.3		0 (0.1% DMSO)		100	5.2 ± 1.2	
MCA	1	60	39.8 ± 4.8[#]		TPA	0.05	131	34.2 ± 6.5[#]	
Diethylstilbestrol (56-53-1)				–					–

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	0 (0.1% DMSO)	100	2.3 ± 2.6			0 (0.1% DMSO)	100	7.2 ± 3.5	
	0.7	94	2.8 ± 1.6			0.01	94	4.5 ± 1.9	
	1	94	1.8 ± 1.9			0.022	95	3.8 ± 1.3	
	1.3	67	4.8 ± 3.3			0.046	94	5.0 ± 1.7	
	1.5	39	4.2 ± 2.6			0.1	95	3.3 ± 1.4	
	1.7	16	3.3 ± 1.9			0.22	90	3.8 ± 2.9	
	2	11	3.0 ± 1.9			0.46	84	2.3 ± 2.2	
	3	20	3.2 ± 2.4			1	68	0.0 ± 0.0	
MCA	1	48	42.8 ± 5.2[#]		TPA	0.05	136	20.0 ± 2.5[#]	
Dimethylarsinic acid (75-60-5)				–					–
	0 (5% Water)	100	3.3 ± 1.6			0 (5% Water)	100	2.8 ± 1.2	
	140	119	2.3 ± 2.1			0.3	98	1.8 ± 1.2	
	200	88	2.3 ± 1.2			0.7	94	2.7 ± 2.0	
	280	60	2.0 ± 1.3			1	93	2.7 ± 2.3	
	400	35	1.8 ± 1.0			3	94	0.8 ± 1.2	
	630	21	2.0 ± 1.3			7	92	0.8 ± 0.4	
	1000	13	1.3 ± 1.6			10	94	0.8 ± 0.4	
	1380	9	0.5 ± 0.5			30	94	0.0 ± 0.0	
	0 (0.1% DMSO)	100	3.3 ± 2.0			0 (0.1% DMSO)	100	1.8 ± 1.8	
MCA	1	60	23.3 ± 4.2[#]		TPA	0.05	118	17.0 ± 3.2[#]	
1,4-Dioxane (123-91-1)				–					–
	0 (5% Water)	100	1.2 ± 0.4			0 (5% Water)	100	3.7 ± 1.5	
	150	102	1.7 ± 1.6			150	100	2.7 ± 1.5	
	300	91	1.7 ± 1.4			300	101	2.7 ± 2.0	
	625	97	1.7 ± 1.9			625	99	3.2 ± 1.0	
	1250 (14.2 mM)	95	1.8 ± 1.5			1250 (14.2 mM)	94	3.0 ± 1.4	
	0 (0.1% DMSO)	100	1.8 ± 0.8			0 (0.1% DMSO)	100	2.8 ± 1.3	
MCA	1	36	12.0 ± 3.9[#]		TPA	0.05	119	23.8 ± 3.6[#]	
Ethyl carbamate (Urethane) (51-79-6)				–					–
	0 (5% Water)	100	1.3 ± 1.9			0 (5% Water)	100	1.2 ± 1.2	
	89	104	1.0 ± 1.3			89	100	1.8 ± 0.8	
	151	102	0.7 ± 0.5			151	99	1.7 ± 1.0	
	294	97	0.7 ± 0.8			294	95	1.3 ± 0.8	
	445	96	0.7 ± 0.8			445	94	1.5 ± 1.4	
	597	90	1.5 ± 1.5			597	90	1.2 ± 1.0	
	739	95	0.7 ± 0.8			739	93	0.8 ± 0.8	
	891 (10 mM)	92	1.3 ± 0.8			891 (10 mM)	89	1.5 ± 1.6	
	0 (0.1% DMSO)	100	1.5 ± 1.5			0 (0.1% DMSO)	100	3.0 ± 1.5	
MCA	1	40	23.8 ± 7.5[#]		TPA	0.05	114	13.7 ± 3.4[#]	
Formaldehyde (50-00-0)				–					–
	0 (5% Water)	100	1.3 ± 0.5			0 (5% Water)	100	2.3 ± 1.9	
	0.5	102	1.7 ± 0.8			0.1	98	1.0 ± 0.6	
	0.7	103	0.3 ± 0.5			0.15	97	0.7 ± 0.8	
	0.8	104	1.0 ± 1.3			0.2	98	2.0 ± 1.4	
	1	90	1.7 ± 0.8			0.3	105	1.8 ± 1.2	
	1.2	70	1.3 ± 1.0			0.5	90	0.8 ± 0.8	
	1.4	25	1.3 ± 1.0			0.7	95	1.7 ± 0.8	
	1.6	11	1.5 ± 1.0			1	99	0.7 ± 0.8	
	1.8	6	1.0 ± 1.5			1.4	97	1.2 ± 0.8	
	2	6	0.8 ± 1.2			2	96	1.0 ± 1.3	
	0 (0.1% DMSO)	100	1.0 ± 0.9			3	99	1.0 ± 0.9	
MCA	1	53	19.2 ± 1.3[#]			5	96	0.0 ± 0.0	
						7	47	Toxic	
						10	13	Toxic	
						0 (0.1% DMSO)	100	2.3 ± 2.0	
					TPA	0.05	134	12.2 ± 4.1[#]	
Furylfuramide (AF-2) (3688-53-7)				–					–
	0 (0.1% DMSO)	100	2.3 ± 2.3			0 (0.1% DMSO)	100	6.5 ± 1.9	
	1	88	2.8 ± 1.7			0.6	95	7.7 ± 3.7	
	1.25	90	2.8 ± 1.5			1.25	94	3.2 ± 1.6	
	1.5	75	3.7 ± 2.9			2	92	2.7 ± 1.6	
	1.75	61	3.8 ± 1.2			3	88	1.2 ± 1.0	
	2	52	3.0 ± 0.9			5	100	0.5 ± 0.5	
	2.25	24	3.2 ± 1.9			7.5	104	0.2 ± 0.4	
	2.5	12	1.5 ± 0.5			10	94	Toxic	
MCA	1	43	28.7 ± 3.1[#]			20	21	Toxic	
					TPA	0.05	151	33.0 ± 6.2[#]	
Methyl carbamate (Methyl urethane) (598-55-0)				–					–
	0 (5% Water)	100	1.2 ± 0.8			0 (5% Water)	100	3.2 ± 2.0	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	100	79	1.3 ± 0.8			100	101	3.2 ± 2.1	
	200	74	3.0 ± 1.8			200	94	3.0 ± 2.8	
	500	81	1.2 ± 1.5			500	104	3.0 ± 2.1	
	1000 (13.3 mM)	78	2.2 ± 1.3			1000 (13.3 mM)	99	2.2 ± 1.7	
	0 (0.1% DMSO)	100	1.3 ± 0.5			0 (0.1% DMSO)	100	3.2 ± 2.2	
MCA	1	48	37.3 ± 6.2[#]		TPA	0.05	152	28.5 ± 3.1[#]	
Nickel (II) chloride (7718-54-9)				–					–
	0 (5% Water)	100	1.5 ± 1.2			0 (5% Water)	100	0.8 ± 1.2	
	0.69	114	2.3 ± 1.5			0.4	114	1.8 ± 1.3	
	1	102	0.5 ± 0.5			0.6	116	1.5 ± 1.0	
	1.5	114	1.0 ± 1.3			1	121	1.0 ± 2.3	
	2.2	109	1.2 ± 1.0			1.5	119	1.2 ± 1.2	
	3.2	104	1.2 ± 1.2			2.5	126	0.8 ± 0.8	
	4.7	87	1.8 ± 1.5			4	126	Toxic	
	6.9	53	1.5 ± 1.9			6	126	Toxic	
	10	14	0.7 ± 0.8			0 (0.1% DMSO)	100	1.2 ± 0.8	
	16	0	Toxic		TPA	0.05	138	10.2 ± 1.5[#]	
	25	0	Toxic						
	0 (0.1% DMSO)	100	1.2 ± 1.5						
MCA	1	14	17.8 ± 5.6[#]						
Nickel monooxide (1313-99-1)				–					–
	0 (5% Water)	100	3.0 ± 0.9			0 (5% Water)	100	3.5 ± 2.1	
	1.875	104	2.5 ± 1.6			1.875	104	2.0 ± 1.7	
	3.75	105	1.3 ± 1.4			3.75	108	2.5 ± 1.9	
	7.5	115	3.0 ± 1.9			7.5	100	1.2 ± 1.5	
	10	107	2.5 ± 1.9			10	98	0.8 ± 0.8	
	15	117	1.3 ± 1.4			15	87	0.8 ± 0.8	
	20	105	2.3 ± 1.9			20	93	Toxic	
	30	63	1.0 ± 1.1			30	87	Toxic	
	60	34	0.5 ± 0.8			0 (0.1% DMSO)	100	3.8 ± 1.0	
	0 (0.1% DMSO)	100	4.3 ± 2.3		TPA	0.05	112	28.5 ± 4.1[#]	
MCA	1	60	40.3 ± 4.8[#]						
Phenobarbital sodium salt (57-30-7)				–					–
	0 (5% Water)	100	3.3 ± 2.7			0 (5% Water)	100	2.8 ± 1.5	
	100	106	3.8 ± 1.2			100	94	1.0 ± 1.1	
	300	109	3.0 ± 1.3			300	85	1.3 ± 0.8	
	500	104	2.7 ± 1.0			500	81	0.7 ± 0.8	
	700	79	1.7 ± 1.9			700	77	1.0 ± 1.3	
	1000	49	1.5 ± 1.0			1000	77	0.2 ± 0.4	
	1500	15	0.8 ± 1.2			1500	60	0.8 ± 0.8	
	2000	3	0.0 ± 0.0			2000	31	Toxic	
	0 (0.1% DMSO)	100	3.2 ± 1.6			0 (0.1% DMSO)	100	2.3 ± 2.0	
MCA	1	62	29.2 ± 3.7[#]		TPA	0.05	131	14.8 ± 3.2[#]	
p-Toluidine (106-49-0)				–					–
	0 (0.1% DMSO)	100	2.7 ± 0.8			0 (0.1% DMSO)	100	2.8 ± 2.6	
	10	101	1.8 ± 2.2			5	89	3.0 ± 0.6	
	25	92	3.0 ± 0.9			10	97	2.5 ± 1.8	
	50	88	3.0 ± 0.9			25	87	2.5 ± 2.3	
	75	84	2.7 ± 2.3			50	84	1.8 ± 1.3	
	100	81	3.0 ± 2.3			75	82	1.7 ± 0.5	
	150	65	2.3 ± 1.6			150	80	1.0 ± 0.9	
	200	32	4.5 ± 2.3			300	62	3.5 ± 1.8	
	300	13	5.2 ± 3.5		TPA	0.05	117	17.8 ± 2.2[#]	
MCA	1	42	42.0 ± 8.2[#]						
Non-carcinogens									
4-Acetylaminofluorene (28322-02-3)				–					–
	0 (0.1% DMSO)	100	0.8 ± 1.2			0 (0.1% DMSO)	100	1.5 ± 0.8	
	50	115	1.3 ± 1.0			5	85	1.3 ± 1.2	
	75	106	1.7 ± 1.0			10	78	1.0 ± 0.9	
	100	92	1.7 ± 1.2			25	70	1.3 ± 1.4	
	120	87	2.0 ± 1.7			50	59	0.2 ± 0.4	
	135	73	2.2 ± 2.0			100	54	0.0 ± 0.0	
	150	76	2.5 ± 1.4			150	60	0.0 ± 0.0	
MCA	1	44	19.5 ± 4.1[#]		TPA	0.05	129	13.8 ± 2.6[#]	
Acid red 14 (Chromotrope FB) (3567-69-9)				–					–
	0 (5% Water)	100	4.2 ± 3.8			0 (5% Water)	100	4.8 ± 2.1	
	30	94	5.8 ± 3.7			30	105	3.7 ± 1.8	
	50	98	4.5 ± 3.2			70	110	2.7 ± 1.4	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	100	105	4.2 ± 1.7			100	100	2.0 ± 2.1	
	300	150	4.5 ± 2.4			300	97	4.7 ± 3.1	
	500	104	7.3 ± 2.7			700	97	4.3 ± 1.0	
	700	82	6.5 ± 1.4			1000	106	5.2 ± 3.4	
	1000	24	2.2 ± 1.6			1500	110	5.3 ± 1.9	
	0 (0.1% DMSO)	100	2.3 ± 1.5			0 (0.1% DMSO)	100	5.3 ± 2.7	
MCA	1	62	35.7 ± 2.1[#]		TPA	0.05	122	27.2 ± 4.1[#]	
Ampicillin sodium salt (69-52-3)				–					–
	0 (5% Water)	100	1.5 ± 0.8			0 (5% Water)	100	5.0 ± 1.9	
	300	102	2.2 ± 1.6			700	102	7.2 ± 1.3	
	700	99	1.5 ± 1.8			1000	95	3.5 ± 2.3	
	1000	67	1.7 ± 1.2			1500	98	4.8 ± 2.3	
	1200	29	1.0 ± 0.9			3000	90	4.2 ± 3.1	
	1250	27	1.3 ± 1.2			5000	87	Toxic	
	1300	17	2.3 ± 1.0			0 (0.1% DMSO)	100	8.8 ± 4.2	
	1500	6	3.5 ± 1.8		TPA	0.05	120	28.5 ± 5.0[#]	
	1750	2	1.8 ± 1.0						
	0 (0.1% DMSO)	100	1.7 ± 1.9						
MCA	1	42	32.7 ± 8.4[#]						
Anthracene (120-12-7)				–					–
	0 (0.5% DMSO)	100	5.3 ± 1.5			0 (0.5% DMSO)	100	6.3 ± 1.0	
	2.5	96	7.7 ± 2.7			2.5	116	6.7 ± 2.2	
	5	89	7.3 ± 1.5			5	106	6.3 ± 2.1	
	10	108	7.3 ± 3.8			10	111	6.8 ± 2.4	
	25	99	6.8 ± 1.5			25	121	6.0 ± 1.8	
	50	105	8.2 ± 1.5			50	115	7.2 ± 3.3	
	100	100	7.3 ± 1.8			100	114	6.2 ± 2.1	
	0 (0.1% DMSO)	100	5.7 ± 2.0			0 (0.1% DMSO)	100	8.8 ± 3.5	
MCA	1	37	23.8 ± 3.9[#]		TPA	0.05	141	27.2 ± 2.1[#]	
L-Ascorbic acid (50-81-7)				–					–
	0 (5% Water)	100	3.0 ± 3.2			0 (5% Water)	100	3.0 ± 1.3	
	80	94	2.5 ± 1.0			100	104	1.0 ± 0.9	
	100	88	2.0 ± 1.3			140	107	0.5 ± 0.5	
	130	79	1.5 ± 1.0			190	106	0.5 ± 0.5	
	170	64	3.8 ± 1.7			260	103	0.2 ± 0.4	
	220	33	3.0 ± 1.3			370	101	0.8 ± 1.0	
	280	20	4.0 ± 2.4			520	97	Toxic	
	370	4	3.2 ± 1.5			720	45	Toxic	
	0 (0.1% DMSO)	100	1.2 ± 1.2			0 (0.1% DMSO)	100	3.5 ± 1.6	
MCA	1	49	26.5 ± 7.3[#]		TPA	0.05	119	22.5 ± 2.4[#]	
Aspartame (22839-47-0)				–					–
	0 (5% Water)	100	1.0 ± 0.6			0 (5% Water)	100	2.3 ± 1.2	
	80	97	1.3 ± 0.8			80	107	2.3 ± 1.2	
	100	107	1.7 ± 2.0			100	120	1.8 ± 1.8	
	200	100	0.3 ± 0.5			200	106	2.8 ± 1.8	
	500	109	1.3 ± 1.2			500	108	2.3 ± 1.0	
	1000	103	1.3 ± 1.0			1000	101	1.5 ± 1.0	
	2000	111	1.8 ± 1.2			2000	94	0.0 ± 0.0	
	3000 (10.2 mM)	99	1.3 ± 0.8			3000 (10.2 mM)	79	0.2 ± 0.4	
	0 (0.1% DMSO)	100	0.8 ± 0.8			0 (0.1% DMSO)	100	1.3 ± 0.8	
MCA	1	49	27.7 ± 4.6[#]		TPA	0.05	150	21.7 ± 2.9[#]	
Benzoin (119-53-9)				–					–
	0 (0.1% DMSO)	100	8.8 ± 1.0			0 (0.1% DMSO)	100	3.8 ± 1.9	
	1	94	9.8 ± 4.4			12.5	92	4.3 ± 2.0	
	10	98	9.3 ± 2.5			25	86	4.5 ± 1.9	
	30	102	11.2 ± 4.0			50	85	4.2 ± 2.0	
	50	109	7.7 ± 2.7			100	83	1.5 ± 0.8	
	70	106	10.0 ± 1.8			200	84	0.0 ± 0.0	
	100	105	8.3 ± 4.6			400	87	0.2 ± 0.4	
	200	67	10.2 ± 2.3			800	82	0.8 ± 1.0	
MCA	1	66	42.2 ± 3.5[#]		TPA	0.05	158	14.5 ± 2.9[#]	
Caffeine (58-08-2)				–					–
	0 (5% Water)	100	0.5 ± 0.5			0 (5% Water)	100	3.5 ± 2.2	
	50	108	1.7 ± 2.1			30	105	2.7 ± 0.8	
	75	89	1.5 ± 0.8			50	95	1.5 ± 1.4	
	100	95	1.2 ± 0.8			75	94	1.5 ± 1.4	
	150	79	1.2 ± 1.5			100	83	1.2 ± 1.5	
	200	68	0.7 ± 1.2			150	77	1.0 ± 1.1	
	300	48	1.0 ± 0.6			200	70	0.0 ± 0.0	
	400	13	1.5 ± 1.4			300	60	0.0 ± 0.0	
	500	3	Toxic			400	52	0.0 ± 0.0	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	0 (0.1% DMSO)	100	0.5 ± 0.5			0 (0.1% DMSO)	100	4.3 ± 1.4	
MCA	1	36	18.5 ± 6.1[#]		TPA	0.05	119	24.3 ± 4.4[#]	
Caprolactam (105-60-2)				–					–
	0 (5% Water)	100	1.2 ± 1.2			0 (5% Water)	100	3.3 ± 2.0	
	300	120	1.8 ± 1.7			300	93	4.2 ± 1.5	
	500	115	3.5 ± 2.7			500	89	2.8 ± 1.5	
	1000	125	1.2 ± 1.5			1000	82	4.8 ± 1.3	
	2000 (17.7 mM)	128	1.8 ± 1.0			2000 (17.7 mM)	67	4.8 ± 2.3	
	0 (0.1% DMSO)	100	1.5 ± 1.8			0 (0.1% DMSO)	100	4.3 ± 1.2	
MCA	1	49	17.7 ± 3.3[#]		TPA	0.05	144	14.7 ± 1.9[#]	
2-Chloroethanol (107-07-3)				–					–
	0 (5% Water)	100	5.3 ± 1.5			0 (5% Water)	100	5.8 ± 3.4	
	10	100	6.0 ± 3.5			300	93	6.8 ± 1.8	
	30	99	5.0 ± 2.3			500	92	7.8 ± 4.7	
	100	102	5.2 ± 2.6			1000 (12.4 mM)	94	7.7 ± 2.6	
	300	98	5.7 ± 2.1			2000 (24.8 mM)	86	6.3 ± 3.6	
	1000 (12.4 mM)	100	4.3 ± 2.2			3000 (37.3 mM)	86	7.0 ± 3.3	
	0 (0.1% DMSO)	100	5.0 ± 1.4			0 (0.1% DMSO)	100	8.3 ± 3.9	
MCA	1	70	40.2 ± 3.5[#]		TPA	0.05	139	41.7 ± 3.5[#]	
Chromium(III) chloride (10025-73-7)				–					–
	0 (5% Water)	100	6.8 ± 1.2			0 (5% Water)	100	7.8 ± 2.4	
	100	106	6.8 ± 1.6			100	102	6.0 ± 1.8	
	300	100	8.8 ± 1.9			300	97	6.3 ± 1.5	
	700	118	6.2 ± 2.1			700	101	5.7 ± 1.0	
	1000	105	7.8 ± 2.1			1000	99	3.7 ± 1.5	
	2000 (12.6 mM)	111	6.2 ± 3.8			2000 (12.6 mM)	104	4.0 ± 2.6	
	0 (0.1% DMSO)	100	7.5 ± 2.1			0 (0.1% DMSO)	100	9.5 ± 1.9	
MCA	1	74	40.2 ± 5.9[#]		TPA	0.05	116	36.3 ± 2.9[#]	
2,6-Diaminotoluene (823-40-5)				–					–
	0 (5% Water)	100	1.2 ± 1.5			0 (5% Water)	100	4.8 ± 1.5	
	100	54	4.0 ± 2.0			2.5	94	3.8 ± 0.8	
	125	37	3.2 ± 2.5			5	87	6.0 ± 2.0	
	150	25	3.8 ± 2.7			10	85	6.7 ± 2.1	
	175	14	3.0 ± 1.8			25	81	5.7 ± 0.8	
	200	9	3.8 ± 1.2			50	72	4.2 ± 1.5	
	250	2	1.8 ± 1.7			100	60	1.3 ± 1.2	
	300	1	1.2 ± 0.8			200	54	0.3 ± 0.5	
	0 (0.1% DMSO)	100	1.8 ± 1.2			0 (0.1% DMSO)	100	5.5 ± 1.8	
MCA	1	51	26.3 ± 3.5[#]		TPA	0.05	120	26.5 ± 4.0[#]	
Diazepam (439-14-5)				–					–
	0 (0.1% DMSO)	100	1.8 ± 1.6			0 (0.1% DMSO)	100	7.8 ± 2.4	
	10	86	1.8 ± 0.8			1	93	10.3 ± 2.7	
	15	67	1.2 ± 0.8			2	101	11.8 ± 2.4	
	20	56	2.2 ± 1.5			3	92	10.8 ± 2.9	
	30	18	1.0 ± 0.6			4	102	9.5 ± 4.2	
	70	8	1.8 ± 1.2			5	90	9.8 ± 2.9	
	100	6	0.5 ± 0.5			7	89	7.0 ± 3.3	
	150	1	1.0 ± 1.5			10	72	5.8 ± 1.6	
MCA	1	36	20.0 ± 5.8[#]		TPA	0.05	147	27.8 ± 4.7[#]	
N,*N*-Dimethylformamide (68-12-2)				–					–
	0 (5% Water)	100	0.8 ± 0.8			0 (5% Water)	100	3.2 ± 1.9	
	100	72	1.7 ± 1.2			100	83	2.8 ± 1.5	
	200	73	2.5 ± 1.8			200	80	2.2 ± 1.7	
	500	71	0.8 ± 1.2			500	75	3.5 ± 2.1	
	1000 (13.7 mM)	70	1.7 ± 0.8			1000 (13.7 mM)	70	2.3 ± 1.9	
	0 (0.1% DMSO)	100	1.0 ± 1.1			0 (0.1% DMSO)	100	2.5 ± 0.8	
MCA	1	48	32.3 ± 4.3[#]		TPA	0.05	152	27.5 ± 4.0[#]	
Eugenol (97-53-0)				–					–
	0 (0.1% DMSO)	100	2.3 ± 0.8			0 (0.1% DMSO)	100	5.0 ± 2.5	
	20	103	2.3 ± 1.2			5	107	5.5 ± 0.8	
	25	112	1.8 ± 0.8			10	100	5.3 ± 1.5	
	32	80	3.0 ± 0.6			25	88	2.8 ± 1.8	
	40	56	3.3 ± 1.9			35	90	1.5 ± 1.0	
	50	41	3.5 ± 2.6			50	75	1.3 ± 0.5	
	60	33	3.7 ± 1.9			100	60	0.5 ± 0.8	
	80	23	3.3 ± 1.8		TPA	0.05	142	21.3 ± 6.0[#]	
MCA	1	37	23.5 ± 4.1[#]						
HC Blue no. 2 (33229-34-4)				–					–
	0 (5% Water)	100	3.2 ± 1.2			0 (5% Water)	100	9.2 ± 3.2	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]	Concentration (μg/mL)		CG	Foci/well	Judgment
	30	90	4.3 ± 1.4		75		91	7.8 ± 3.9	
	70	92	3.2 ± 1.6		150		88	7.8 ± 2.1	
	100	87	2.7 ± 1.8		300		84	4.7 ± 1.9	
	200	84	4.0 ± 2.1		500		77	2.3 ± 1.5	
	400	81	4.3 ± 1.8		750		73	0.2 ± 0.4	
	700	87	3.8 ± 1.5		1000		78	Toxic	
	1000	44	Toxic		1250		78	Toxic	
	0 (0.1% DMSO)	100	3.7 ± 2.6		1500		76	Toxic	
MCA	1	49	32.2 ± 4.4#		0 (0.1% DMSO)		100	9.3 ± 2.3	
					TPA	0.05	143	39.0 ± 5.8#	
Hydrocortisone (50-23-7)				–					–
	0 (0.5% DMSO)	100	6.5 ± 1.6		0 (0.5% DMSO)		100	0.8 ± 0.4	
	1	84	6.8 ± 2.3		3		81	2.8 ± 1.8	
	10	75	7.0 ± 2.8		10		85	2.5 ± 2.1	
	50	79	7.2 ± 1.6		25		79	2.7 ± 2.3	
	100	75	6.5 ± 2.4		50		109	1.7 ± 1.4	
	150	68	4.3 ± 2.2		100		90	1.2 ± 1.5	
	300	25	4.2 ± 1.6		250		96	1.0 ± 0.9	
	500	7	4.2 ± 1.9		500		45	Toxic	
	0 (0.1% DMSO)	100	3.5 ± 2.1		0 (0.1% DMSO)		100	1.0 ± 0.9	
MCA	1	74	30.0 ± 7.5#		TPA	0.05	152	10.8 ± 2.8#	
D-Mannitol (69-65-8)				–					–
	0 (5% Water)	100	0.5 ± 0.5		0 (5% Water)		100	2.5 ± 1.2	
	100	105	0.5 ± 0.8		100		112	2.8 ± 1.2	
	200	101	1.8 ± 1.2		200		107	1.7 ± 1.4	
	500	101	0.3 ± 0.5		500		108	3.5 ± 1.4	
	1000	102	0.7 ± 0.5		1000		102	4.0 ± 2.7	
	2000 (11.0 mM)	103	0.8 ± 0.8		2000 (11.0 mM)		116	2.7 ± 1.2	
	0 (0.1% DMSO)	100	1.5 ± 0.5		0 (0.1% DMSO)		100	2.8 ± 1.8	
MCA	1	42	13.2 ± 3.3#		TPA	0.05	227	17.3 ± 3.4#	
Methotrexate (59-05-2)				–					–
	0 (5% Water)	100	1.5 ± 2.1		0 (5% Water)		100	2.3 ± 2.3	
	0.01	88	1.5 ± 1.2		0.0003		96	1.5 ± 1.0	
	0.03	105	1.5 ± 1.4		0.001		98	0.8 ± 0.8	
	0.1	94	0.8 ± 0.8		0.003		91	2.5 ± 1.9	
	0.2	102	0.8 ± 0.8		0.01		102	1.2 ± 1.2	
	0.5	105	0.7 ± 0.8		0.03		94	1.0 ± 1.5	
	1	40	0.2 ± 0.4		0.1		84	0.0 ± 0.0	
	3	44	Toxic		0.3		77	0.0 ± 0.0	
	0 (0.1% DMSO)	100	1.0 ± 0.6		0 (0.1% DMSO)		100	2.5 ± 1.0	
MCA	1	50	17.2 ± 2.9#		TPA	0.05	122	12.2 ± 3.3#	
1-Naphthylamine (134-32-7)				–					–
	0 (0.1% DMSO)	100	5.5 ± 2.4		0 (0.1% DMSO)		100	5.0 ± 4.1	
	2.5	100	6.5 ± 2.4		2.5		90	6.0 ± 3.1	
	5	96	5.7 ± 2.4		5		94	6.3 ± 1.5	
	10	93	3.8 ± 1.5		10		83	5.2 ± 2.3	
	20	79	6.8 ± 3.3		20		80	3.5 ± 1.9	
	30	64	8.5 ± 1.5		30		77	1.0 ± 0.9	
	50	34	4.5 ± 2.1		50		60	2.2 ± 2.0	
	70	15	3.0 ± 2.1		100		43	Toxic	
	100	17	2.8 ± 2.5		TPA	0.05	118	21.8 ± 6.2#	
MCA	1	56	34.7 ± 3.5#						
Phenanthrene (85-01-8)				–					–
	0 (0.1% DMSO)	100	4.0 ± 3.0		0 (0.1% DMSO)		100	6.2 ± 1.8	
	10	97	4.5 ± 2.7		2.5		99	5.7 ± 2.8	
	20	87	4.8 ± 1.9		5		101	7.2 ± 1.9	
	40	70	4.3 ± 2.0		10		90	8.7 ± 1.9	
	60	77	4.5 ± 2.9		20		84	8.8 ± 2.9	
	100	103	5.8 ± 2.4		40		83	7.8 ± 2.7	
	150	98	4.7 ± 2.4		60		85	2.7 ± 1.5	
MCA	1	44	27.8 ± 5.0#		TPA	0.05	91	29.7 ± 4.2#	
Phenol (108-95-2)				–					–
	0 (5% Water)	100	1.8 ± 1.8		0 (5% Water)		100	3.5 ± 1.4	
	50	89	2.2 ± 1.6		5		98	2.3 ± 1.2	
	75	88	1.3 ± 2.0		10		99	1.8 ± 1.5	
	100	71	1.0 ± 1.5		25		93	1.0 ± 0.6	
	125	61	3.2 ± 1.3		50		84	0.8 ± 0.8	
	150	48	1.5 ± 1.0		100		75	1.3 ± 1.0	
	175	18	1.8 ± 1.0		200		51	0.2 ± 0.4	
	200	20	1.3 ± 1.2		300		45	Toxic	
	300	4	2.8 ± 1.2		500		38	Toxic	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	0 (0.1% DMSO)	100	1.8 ± 1.8			0 (0.1% DMSO)	100	3.2 ± 1.3	
MCA	1	42	21.8 ± 3.5[#]		TPA	0.05	120	18.2 ± 3.0[#]	
p-Phenylenediamine dihydrochloride (624-18-0)				–					–
	0 (5% Water)	100	2.7 ± 1.6			0 (5% Water)	100	3.8 ± 1.6	
	1	93	1.2 ± 0.8			0.1	98	4.2 ± 1.7	
	2.5	95	3.0 ± 1.7			0.25	101	3.7 ± 2.3	
	5	81	3.8 ± 1.5			0.5	97	3.3 ± 1.4	
	6	78	2.7 ± 1.5			1	99	5.8 ± 1.5	
	7	63	2.0 ± 1.3			2.5	89	4.2 ± 2.2	
	8	49	3.5 ± 1.4			5	77	3.3 ± 1.9	
	10	26	2.7 ± 2.0			0 (0.1% DMSO)	100	4.2 ± 2.5	
	20	12	Toxic		TPA	0.05	148	26.0 ± 2.1[#]	
	0 (0.1% DMSO)	100	5.0 ± 2.3						
MCA	1	69	30.3 ± 3.2[#]						
Phthalic anhydride (85-44-9)				–					–
	0 (0.5% Acetone)	100	1.7 ± 1.5			0 (0.5% Acetone)	100	8.8 ± 3.1	
	100	105	1.5 ± 1.4			1	108	7.8 ± 2.6	
	150	99	2.8 ± 1.7			2.5	107	6.0 ± 2.9	
	225	90	1.7 ± 1.0			5	102	9.0 ± 3.9	
	240	90	1.8 ± 1.0			10	135	7.7 ± 2.6	
	500	68	1.8 ± 1.5			30	133	6.8 ± 2.2	
	750	6	0.7 ± 0.8			100	100	7.2 ± 3.4	
	1200	6	Toxic			300	84	2.8 ± 1.8	
	0 (0.1% DMSO)	100	3.7 ± 2.4			600	70	0.5 ± 0.5	
MCA	1	77	26.0 ± 2.6[#]			0 (0.1% DMSO)	100	6.5 ± 2.2	
					TPA	0.05	44	29.3 ± 5.0[#]	
Rotenone (83-79-4)				–					–
	0 (0.1% DMSO)	100	3.2 ± 1.8			0 (0.1% DMSO)	100	5.8 ± 1.5	
	0.01	96	3.7 ± 2.3			0.0001	110	4.3 ± 3.3	
	0.03	75	2.7 ± 1.6			0.00025	113	6.7 ± 1.8	
	0.05	59	3.0 ± 2.5			0.0005	111	3.5 ± 2.1	
	0.1	22	2.7 ± 1.8			0.001	113	4.8 ± 1.7	
	0.3	6	Toxic			0.0025	123	2.3 ± 1.0	
	1	4	Toxic			0.005	130	0.0 ± 0.0	
	3	0	Toxic			0.01	133	0.0 ± 0.0	
MCA	1	51	17.2 ± 4.8[#]		TPA	0.05	147	20.8 ± 2.9[#]	
Sodium chloride (7647-14-5)				–					–
	0 (5% Water)	100	1.3 ± 0.8			0 (5% Water)	100	2.8 ± 1.3	
	156.3	98	1.3 ± 0.8			156.3	100	2.0 ± 1.1	
	312.5	100	1.2 ± 1.0			312.5	93	1.7 ± 1.6	
	625(10.7 mM)	101	2.2 ± 1.5			625 (10.7 mM)	92	2.0 ± 1.9	
	1250 (21.4 mM)	106	2.0 ± 2.4			1250 (21.4 mM)	90	5.2 ± 1.5	
	0 (0.1% DMSO)	100	1.7 ± 2.7			0 (0.1% DMSO)	100	3.8 ± 1.8	
MCA	1	43	25.2 ± 2.8[#]		TPA	0.05	140	17.5 ± 4.1[#]	
Sodium nitrite (7632-00-0)				–					–
	0 (5% Water)	100	1.5 ± 1.0			0 (5% Water)	100	2.7 ± 1.0	
	200	91	3.2 ± 1.6			25	98	4.2 ± 1.5	
	300	96	2.5 ± 1.9			50	108	3.0 ± 1.5	
	500	76	2.8 ± 1.5			100	105	2.2 ± 2.1	
	700 (10.1 mM)	46	3.5 ± 1.0			150	108	2.5 ± 1.0	
	1000 (14.5 mM)	20	2.3 ± 0.8			200	120	1.5 ± 1.2	
	0 (0.1% DMSO)	100	1.3 ± 1.0			300	93	0.5 ± 0.8	
MCA	1	49	32.5 ± 2.5[#]			500	95	0.2 ± 0.4	
						0 (0.1% DMSO)	100	2.8 ± 1.5	
					TPA	0.05	144	13.7 ± 2.4[#]	
Sunset yellow FCF (2783-94-0)				–					–
	0 (5% Water)	100	2.5 ± 0.8			0 (5% Water)	100	3.3 ± 2.8	
	100	91	2.7 ± 1.2			250	98	4.5 ± 1.9	
	160	95	2.5 ± 1.6			400	93	4.0 ± 2.2	
	250	91	1.7 ± 1.4			630	93	3.3 ± 3.3	
	400	98	1.2 ± 1.2			1000	89	3.5 ± 2.6	
	630	109	0.8 ± 0.4			2000	82	3.7 ± 1.8	
	1000	109	1.3 ± 1.4			3000	85	2.2 ± 1.3	
	2000	51	3.2 ± 1.3			5000 (11.1 mM)	75	1.5 ± 1.4	
	0 (0.1% DMSO)	100	3.0 ± 1.5			0 (0.1% DMSO)	100	3.8 ± 2.5	
MCA	1	60	24.7 ± 7.0[#]		TPA	0.05	131	19.2 ± 2.6[#]	
Thiabendazole (148-79-8)				–					–
	0 (0.1% DMSO)	100	1.2 ± 1.2			0 (0.1% DMSO)	100	5.0 ± 1.3	
	10	107	1.8 ± 1.2			1	109	2.8 ± 2.8	
	14	81	1.3 ± 0.5			3	102	3.5 ± 2.5	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]		Concentration (μg/mL)	CG	Foci/well	Judgment
	19	83	2.2 ± 1.9			5	101	5.5 ± 2.6	
	27	82	2.8 ± 2.9			10	98	2.8 ± 1.5	
	37	82	1.3 ± 2.0			50	83	0.0 ± 0.0	
	50	76	3.8 ± 2.6			100	72	Toxic	
	75	36	3.7 ± 2.3			500	73	Toxic	
MCA	1	68	18.7 ± 3.9[#]		TPA	0.05	139	19.5 ± 6.0[#]	
m-Toluidine (108-44-1)				−					−
	0 (0.1% DMSO)	100	2.0 ± 1.3			0 (0.1% DMSO)	100	7.0 ± 2.2	
	100	104	2.7 ± 1.4			100	74	5.3 ± 2.3	
	200	96	2.3 ± 2.0			200	67	3.0 ± 2.8	
	300	87	2.8 ± 1.6			300	54	5.0 ± 2.1	
	400	54	2.2 ± 1.5			400	60	5.2 ± 2.1	
	500	63	2.8 ± 1.2			500	44	2.8 ± 0.4	
	600	40	2.7 ± 1.6			600	60	5.0 ± 1.7	
	800	33	2.0 ± 1.4			700	63	2.2 ± 2.0	
MCA	1	45	25.3 ± 4.0[#]		TPA	0.05	107	22.5 ± 4.7[#]	
Triphenyltin hydroxide (76-87-9)				−					−
	0 (0.1% DMSO)	100	4.2 ± 1.9			0 (0.1% DMSO)	100	5.7 ± 2.4	
	0.01	88	2.2 ± 1.6			0.001	138	6.2 ± 1.9	
	0.015	88	2.0 ± 1.8			0.003	131	3.5 ± 1.6	
	0.017	82	3.0 ± 1.3			0.005	137	4.5 ± 1.9	
	0.02	72	1.7 ± 1.5			0.01	131	4.3 ± 0.8	
	0.023	49	1.2 ± 1.2			0.03	119	5.3 ± 2.2	
	0.025	10	1.7 ± 1.9			0.07	117	Toxic	
	0.03	5	0.8 ± 1.2			0.08	64	Toxic	
	0.04	5	Toxic		TPA	0.05	153	31.8 ± 3.3[#]	
MCA	1	43	32.8 ± 6.5[#]						
Barium chloride dihydrate (10326-27-9)				−					+
	0 (5% Water)	100	5.3 ± 1.9			0 (5% Water)	100	3.5 ± 2.2	
	500	45	5.7 ± 3.6			100	114	4.7 ± 2.1	
	600	37	6.5 ± 2.1			300	132	9.8 ± 0.8[*]	
	700	42	2.8 ± 2.7			500	144	10.0 ± 2.4[*]	
	1000	27	4.3 ± 1.0			1000	120	6.3 ± 2.7	
	1500	25	3.2 ± 1.9			2000	83	11.7 ± 3.7[*]	
	3000 (12.3 mM)	12	5.8 ± 3.7			2500 (10.2 mM)	67	11.8 ± 2.9[*]	
	0 (0.1% DMSO)	100	5.3 ± 3.2			0 (0.1% DMSO)	100	3.3 ± 1.8	
MCA	1	72	32.0 ± 2.4[#]		TPA	0.05	144	32.7 ± 4.8[#]	
tert-Butylhydroquinone (1948-33-0)				−					+
	0 (0.1% DMSO)	100	1.2 ± 1.0			0 (0.1% DMSO)	100	8.3 ± 2.4	
	0.1	96	0.8 ± 0.8			1	102	12.0 ± 2.8	
	0.3	92	1.5 ± 0.8			2	95	24.5 ± 5.0[*]	
	0.7	91	1.8 ± 1.2			3	104	38.7 ± 2.7[*]	
	1	95	0.5 ± 0.5			4	130	49.7 ± 5.2[*]	
	1.3	91	1.0 ± 1.3			5	138	33.0 ± 4.1[*]	
	1.7	66	0.5 ± 0.5			6	130	17.8 ± 3.9[*]	
	2	26	0.5 ± 0.5			8	79	Toxic	
MCA	1	46	17.8 ± 4.4[#]		TPA	0.05	140	27.3 ± 4.0[#]	
8-Hydroxyquinoline (148-24-3)				−					+
	0 (0.1% DMSO)	100	6.3 ± 3.3			0 (0.1% DMSO)	100	7.2 ± 1.8	
	0.03	100	6.5 ± 1.5			0.3	98	5.3 ± 2.7	
	0.06	101	6.8 ± 2.6			1	105	10.2 ± 2.5	
	0.09	103	7.8 ± 3.9			2	106	17.2 ± 2.3[*]	
	0.12	104	5.3 ± 3.0			3	123	20.7 ± 3.4[*]	
	0.15	102	4.5 ± 2.0			5	131	21.3 ± 3.6[*]	
	0.18	112	4.3 ± 1.2			7	67	Toxic	
	0.21	113	4.3 ± 1.6			9	85	Toxic	
	0.24	49	2.8 ± 1.2			11	55	Toxic	
	0.28	72	8.3 ± 3.6			13	35	Toxic	
MCA	1	76	41.5 ± 2.8[#]		TPA	0.05	136	23.0 ± 3.4[#]	
Propyl gallate (121-79-9)				+					+
	0 (0.1% DMSO)	100	0.8 ± 1.3			0 (0.1% DMSO)	100	6.2 ± 2.2	
	1	71	3.2 ± 1.5[*]			0.5	98	7.5 ± 1.9	
	1.25	32	3.3 ± 1.4[*]			0.8	98	9.0 ± 3.6	
	1.5	17	3.5 ± 1.4[*]			1.3	91	9.3 ± 2.7	
	1.75	7	2.3 ± 1.2			2	83	13.8 ± 6.1[*]	
	2	5	3.0 ± 1.7			3	80	13.8 ± 2.4[*]	
	2.25	3	1.5 ± 1.8			5	64	19.0 ± 7.0[*]	
	2.5	2	1.2 ± 1.0			7.5	26	Toxic	
MCA	1	50	11.0 ± 0.6[#]			10	16	Toxic	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]	Concentration (μg/mL)		CG	Foci/well	Judgment
					TPA	0.05	120	25.0 ± 3.6[#]	
Sodium fluoride (7681-49-4)				–					+
	0 (5% Water)	100	1.8 ± 2.8			0 (5% Water)	100	5.0 ± 2.2	
	4	107	1.8 ± 1.6			18	120	7.5 ± 1.2	
	6.2	105	0.7 ± 0.5			24	149	10.8 ± 2.6[*]	
	10	112	1.0 ± 0.9			32	221	13.3 ± 1.5[*]	
	16	98	1.3 ± 1.2			42	223	8.5 ± 2.3[*]	
	25	92	2.0 ± 1.4			56	128	0.8 ± 0.8	
	40	13	1.2 ± 1.6			75	76	Toxic	
	62	0	0.5 ± 0.8			100	47	Toxic	
	100	0	0.0 ± 0.0			0 (0.1% DMSO)	100	4.0 ± 1.7	
	0 (0.1% DMSO)	100	3.2 ± 1.5		TPA	0.05	143	14.7 ± 1.9[#]	
MCA	1	42	25.5 ± 2.6[#]						
Tetracycline hydrochloride (64-75-5)				–					+
	0 (5% Water)	100	1.5 ± 2.3			0 (5% Water)	100	3.7 ± 2.1	
	5	107	1.3 ± 1.4			5	101	8.2 ± 2.0[*]	
	10	104	2.5 ± 1.9			10	117	11.3 ± 4.5[*]	
	15	113	1.3 ± 1.0			15	121	5.7 ± 1.2	
	20	110	2.3 ± 1.2			20	130	3.3 ± 1.5	
	25	101	3.3 ± 2.6			25	129	8.3 ± 2.1[*]	
	40	29	2.3 ± 2.1			40	107	25.3 ± 4.0[*]	
	50	7	3.0 ± 1.5			50	61	1.0 ± 1.3	
	60	3	3.2 ± 1.2			60	36	0.0 ± 0.0	
	0 (0.1% DMSO)	100	1.5 ± 1.4			0 (0.1% DMSO)	100	5.7 ± 1.8	
MCA	1	49	26.3 ± 3.3[#]		TPA	0.05	145	32.2 ± 3.1[#]	
Chemicals of unknown carcinogenicity									
Sodium valproate (1069-66-5)				+					+
	0 (5% Water)	100	2.2 ± 0.8			0 (5% Water)	100	2.8 ± 2.5	
	50	123	2.5 ± 1.4			6	141	12.8 ± 5.5[*]	
	100	102	5.0 ± 2.5			10	161	23.0 ± 4.6[*]	
	150	62	6.2 ± 1.7[*]			25	183	29.5 ± 2.0[*]	
	200	37	5.5 ± 2.4[*]			50	228	9.7 ± 2.1[*]	
	250	22	5.3 ± 1.2[*]			100	206	0.8 ± 0.8	
	300	12	5.2 ± 2.4[*]			200	164	0.0 ± 0.0	
	400	7	3.8 ± 1.6			500	83	Toxic	
	0 (0.1% DMSO)	100	2.5 ± 2.4			0 (0.1% DMSO)	100	5.7 ± 1.6	
MCA	1	55	18.8 ± 2.6[#]		TPA	0.05	138	20.3 ± 5.5[#]	
Valproic acid (99-66-1)				+					+
	0 (0.1% DMSO)	100	2.3 ± 1.9			0 (0.1% DMSO)	100	4.8 ± 1.5	
	25	118	3.7 ± 0.5			3.125	107	10.3 ± 4.9[*]	
	50	119	5.0 ± 2.3			6.25	119	15.7 ± 2.7[*]	
	75	104	6.8 ± 1.5[*]			12.5	138	28.7 ± 1.6[*]	
	100	80	6.0 ± 1.7[*]			25	169	24.8 ± 4.1[*]	
	140	47	5.7 ± 2.9[*]			50	193	9.5 ± 2.1[*]	
	200	21	5.5 ± 2.7			100	161	0.2 ± 0.4	
	300	11	3.3 ± 2.2			200	125	0.0 ± 0.0	
MCA	1	43	20.3 ± 3.1[#]		TPA	0.05	146	22.7 ± 2.3[#]	
2,3-Diaminotoluene (2687-25-4)				–					+
	0 (0.1% DMSO)	100	1.8 ± 1.3			0 (0.1% DMSO)	100	3.8 ± 2.7	
	3	83	3.3 ± 1.8			5	90	14.0 ± 2.6[*]	
	5	78	3.5 ± 2.5			7	98	12.2 ± 1.9[*]	
	7	75	4.7 ± 1.0			15	133	11.5 ± 4.8[*]	
	15	58	4.2 ± 2.4			20	133	9.5 ± 2.9[*]	
	20	47	3.8 ± 1.8			30	134	2.5 ± 3.0	
	30	34	4.2 ± 2.1			50	118	Toxic	
	50	4	0.8 ± 0.8			100	64	Toxic	
MCA	1	47	11.2 ± 1.9[#]		TPA	0.05	153	12.7 ± 3.6[#]	
2,5-Diaminotoluene dihydrochloride (615-45-2)				–					+
	0 (5% Water)	100	2.7 ± 1.0			0 (5% Water)	100	1.3 ± 1.0	
	1	98	3.5 ± 1.6			1	92	3.7 ± 2.2	
	2.5	96	2.7 ± 2.0			5	79	4.8 ± 2.8	
	4	100	3.7 ± 0.8			10	77	10.2 ± 2.1[*]	
	5.5	90	3.8 ± 4.3			15	76	15.0 ± 0.9[*]	
	7	95	2.7 ± 2.3			20	77	16.7 ± 3.5[*]	
	8.5	86	4.2 ± 0.8			25	81	16.2 ± 4.5[*]	
	10	79	3.2 ± 2.0			30	80	17.2 ± 2.8[*]	
	11.5	84	4.0 ± 1.1			35	87	17.3 ± 4.4[*]	
	13	76	3.7 ± 1.4			40	87	15.2 ± 3.5[*]	
	0 (0.1% DMSO)	100	3.2 ± 1.8			0 (0.1% DMSO)	100	4.5 ± 1.9	

Table 1 (*Continued*)

Chemical[a]	Initiation assay				Promotion assay				
	Concentration (μg/mL)	CG[b]	Foci/well[c]	Judgment[d]	Concentration (μg/mL)		CG	Foci/well	Judgment
MCA	1	50	25.5 ± 3.8[#]		TPA	0.05	152	10.3 ± 2.4[#]	
3,4-Diaminotoluene (496-72-0)				–					+
	0 (5% Water)	100	0.7 ± 0.5			0 (5% Water)	100	4.0 ± 2.0	
	1	88	1.2 ± 1.3			0.3	91	3.5 ± 2.9	
	2.5	88	0.8 ± 0.8			1	80	5.3 ± 2.7	
	7.5	65	1.7 ± 1.2			2.5	73	11.7 ± 4.8[*]	
	10	46	1.8 ± 1.2			5	61	15.0 ± 4.4[*]	
	13.5	30	0.5 ± 0.8			10	49	19.7 ± 1.4[*]	
	15	25	1.5 ± 0.5			20	38	1.3 ± 1.4	
	17.5	17	1.7 ± 1.6			30	32	Toxic	
	20	14	0.8 ± 1.2			0 (0.1% DMSO)	100	3.7 ± 2.0	
	0 (0.1% DMSO)	100	1.5 ± 1.6		TPA	0.05	120	25.7 ± 5.0[#]	
MCA	1	47	21.7 ± 4.5[#]						
Sodium orthovanadate (13721-39-6)				–					+
	0 (5% Water)	100	1.8 ± 2.8			0 (5% Water)	100	1.5 ± 0.8	
	0.01	95	1.0 ± 0.9			0.022	95	2.0 ± 2.3	
	0.022	95	1.3 ± 0.5			0.046	96	2.5 ± 1.6	
	0.046	92	0.8 ± 1.2			0.1	106	2.0 ± 1.3	
	0.1	92	0.5 ± 0.5			0.22	138	3.3 ± 0.8	
	0.22	110	0.8 ± 0.8			0.46	226	12.0 ± 4.7[*]	
	0.46	107	0.5 ± 0.5			1	403	15.5 ± 4.3[*]	
	1	40	0.2 ± 0.4			2.2	473	7.8 ± 2.9[*]	
	2.2	0	0.2 ± 0.4			4.6	249	Toxic	
	0 (0.1% DMSO)	100	3.2 ± 1.5			0 (0.1% DMSO)	100	2.3 ± 1.8	
MCA	1	42	25.5 ± 2.6[#]		TPA	0.05	227	9.2 ± 3.3[#]	
Capsaicin (404-86-4)				–					–
	0 (0.1% DMSO)	100	0.5 ± 0.5			0 (0.1% DMSO)	100	2.0 ± 1.7	
	10	108	0.3 ± 0.8			1	82	1.8 ± 0.8	
	30	103	1.2 ± 1.2			5	95	2.7 ± 1.0	
	50	76	0.7 ± 0.8			10	109	2.2 ± 1.2	
	53	68	0.7 ± 1.0			20	100	2.0 ± 1.1	
	57	67	0.8 ± 0.8			25	95	2.0 ± 1.7	
	60	38	0.7 ± 0.8			30	90	1.2 ± 1.2	
MCA	1	45	13.8 ± 3.5[#]		TPA	0.05	131	10.0 ± 2.2[#]	
Ethidium bromide (1239-45-8)				–					–
	0 (5% Water)	100	2.8 ± 1.5			0 (5% Water)	100	3.5 ± 1.8	
	0.01	97	1.0 ± 1.1			0.0001	92	3.2 ± 2.0	
	0.03	103	1.2 ± 1.2			0.0003	101	4.0 ± 3.0	
	0.1	112	1.2 ± 1.0			0.001	101	3.7 ± 1.2	
	0.3	96	2.3 ± 1.2			0.003	106	1.8 ± 0.8	
	1	83	1.0 ± 0.6			0.01	100	0.0 ± 0.0	
	3	73	Toxic			0.03	113	0.0 ± 0.0	
	10	36	Toxic			0.1	104	Toxic	
	15	13	Toxic			0 (0.1% DMSO)	100	3.2 ± 1.8	
	30	1	Toxic		TPA	0.05	176	26.5 ± 3.9[#]	
	0 (0.1% DMSO)	100	2.2 ± 1.3						
MCA	1	60	27.5 ± 2.4[#]						
Salicylic acid (69-72-7)				–					–
	0 (0.1% DMSO)	100	3.5 ± 1.4			0 (0.1% DMSO)	100	6.7 ± 1.5	
	100	117	3.2 ± 2.6			50	96	5.3 ± 2.3	
	200	119	1.0 ± 1.3			100	94	7.7 ± 2.3	
	250	120	1.8 ± 1.3			200	86	7.5 ± 2.1	
	300	128	0.5 ± 0.5			300	85	6.0 ± 2.4	
	400	103	2.2 ± 0.8			400	75	4.5 ± 1.5	
	600	67	1.3 ± 1.4			600	83	6.8 ± 2.1	
	800	27	0.3 ± 0.5			800	80	3.3 ± 2.2	
MCA	1	63	30.5 ± 7.6[#]		TPA	0.05	211	28.7 ± 6.0[#]	

[a] CAS registry number in parentheses.
[b] % of cell growth compared to that of solvent control.
[c] Average number of transformed foci/well ± SD.
[d] Positive or negative call for the test chemical based on the assay data presented.
[e] Solvent control: final solvent concentration of the working culture media in parentheses.
[f] Positive control in the initiation assay.
[g] Positive control in the promotion assay.
[h] Solvent control for the positive control (MCA in the initiation assay or TPA in the promotion assay).
[*] $p < 0.05$; Dunnett test, vs solvent control.
[#] $p < 0.05$; *t*-test, vs DMSO (the solvent of MCA and TPA).

Table 2
The performance of Bhas 42 cell transformation assay for the prediction of chemical carcinogenicity.

		In vivo carcinogenicity		Total		
		Carcinogen	Non-carcinogen			
(a) Contingency table of the results in the Bhas 42 cell transformation assay					(b) Performance	
Bhas 42 cell transformation assay	+	38	6	44	Concordance	78%
	–	14	31	45	Sensitivity	73
Total		52	37	89	Specificity	84
					Positive predictivity	86
					Negative predictivity	69
					False negative	27
					False positive	16

week till day 21 (Fig. 1). Each assay was conducted including TPA (0.05 μg/mL) as the positive control and 0.1% DMSO as its negative control.

2.3.3. Counting of transformed foci and statistical analysis

The transformed foci were judged on the basis of the morphological characteristics: (a) more than 100 cells, (b) spindle-shaped cells different from the contact-inhibited monolayer cells, (c) deep basophilic staining, (d) random orientation of cells at the edge of foci, (e) dense multilayering of cells and (f) invasive growth into the monolayer of surrounding contact-inhibited cells. The number of transformed foci in every well was recorded and a statistical analysis for the increase of the transformed foci was performed by multiple comparison using the Dunnett method ($p < 0.05$).

2.4. Cell growth assay

The cell growth assays, using a crystal violet (CV) staining method, were performed prior to the transformation assays to determine the doses applicable to the Bhas 42 cell transformation, and also concurrently with every transformation assay (every initiation and promotion assay) to estimate the effect of each treatment on the cell growth and survival. The cells were seeded and treated with a chemical in the same manner as in the initiation assay or in the promotion assay. Three wells were prepared for each test concentration. The cultures were fixed with 10% formalin on day 7 and stained with a 0.1% CV solution. CV was extracted from the stained cells with 50% ethanol containing 0.03 M sodium citrate and 0.02 M hydrochloric acid. The optical density of extract was measured at 540 nm. The survival rate of cells treated with a chemical was expressed as a percentage of the control cells.

2.5. Dose setting for transformation assays

Five or more concentrations were set up based on the results of cell growth assays.

In the initiation assay, the concentrations were determined to cover a range from little or no toxicity to the highest toxicity (less than 20% survival compared to the control cultures). In practice, one dose below NOEL, two doses between NOEL and IC_{50} and two doses between IC_{50} and IC_{90} were arranged, at least.

In the promotion assay, the test concentrations were selected to cover a range from little effect on cell growth to growth enhancement, for the chemicals that exhibited marked growth enhancement. In practice, one dose below NOEL, three doses in the range of growth enhancement and one dose in the range of weak growth inhibition were arranged, at least. For the chemicals that did not induce marked growth enhancement, the test concentrations were selected to range from a dose two or three levels lower than the no-effect concentration to that showing a survival less than 50%. In practice, two doses below NOEL, two doses between NOEL and IC_{50} and one dose above IC_{50} were arranged, at least.

For a chemical which caused a sharp decline of cell growth within a narrow concentration range, one or two more doses outside the predicted concentration range were set up as a precaution against the fluctuation of cell response among experiments. For compounds with little cytotoxicity, the highest concentration was 5 mg/mL or 10 mM, whichever was the lowest.

3. Results

The individual results of 98 chemicals in the Bhas 42 cell transformation assay are presented in Table 1. The tested chemicals are listed being classified into carcinogens, non-carcinogens and chemicals of unknown carcinogenicity according to the existing data of rodent *in vivo* carcinogenicity and human epidemiology. Tumor promoters are assorted into carcinogens. The bases of classification are shown in the footnote of Table 3. The results in the Bhas 42 cell transformation assays were judged positive when there existed two or more doses that induced statistically significant increases of transformed foci, and negative when there was no dose showing statistically significant increase of foci. When the statistically significant increase was at only one dose, the assay result was regarded as equivocal, and then the initiation or promotion assay together with the concomitant cell growth assay was repeated including the positive dose in the first assay. The chemical was judged to be positive, when the chemical again statistically significantly increased the number of transformed foci at one or more concentrations in the second assay (Cyclosporin A and Dichlorvos). As a rule, the assays were carried out at the concentrations less than 5 mg/mL or 10 mM, whichever was the lowest, when the test chemical did not cause cell growth inhibition or enhancement. In Table 1 the molar concentrations are indicated in the parentheses for the concentrations that are equal to 10 mM or exceptionally exceed 10 mM.

Among the 52 tested carcinogens, 2-acetylaminofluorene, benz[*a*]anthracene and phorbol 12,13-didecanoate were judged positive both in the initiation assay and in the promotion assay. IQ, 5-azacytidine, barium chromate, benzo[*a*]pyrene, cyclophosphamide, cyclosporin A, 2,4-diaminotoluene, dibenz[*a*,*h*]anthracene, melphalan, MCA, mitomycin C, MNNG, sterigmatocystin and thio-TEPA were called positive in the initiation assay. Cadmium chloride, chenodeoxycholic acid, 4-chloro-*o*-toluidine hydrochloride, cholic acid, deoxycholic acid, dichlorvos, epichlorohydrin, D-limonene, lithocholic acid, methapyrilene hydrochloride, methylarsonic acid, mezerein, 2-naphthylamine, quercetin, sodium arsenate, sodium arsenite, sodium saccharin, styrene oxide, TPA, *o*-toluidine and zinc chloride were positive in the promotion assay. However, *o*-anisidine, benzene, cobalt sulfate heptahydrate, diethylstilbestrol, dimethylarsinic acid, 1,4-dioxane, ethyl carbamate, formaldehyde, furylfuramide, methyl carbamate, nickel (II) chloride, nickel monooxide, phenobarbital sodium salt and *p*-toluidine were negative in either assay.

Out of the 37 non-carcinogens, 4-acetylaminofluorene, acid red 14, ampicillin sodium salt, anthracene, L-ascorbic acid, aspartame, benzoin, caffeine, caprolactam, 2-chloroethanol, chromium (III) chloride, 2,6-diaminotoluene, diazepam, *N*,*N*-dimethylformamide, eugenol, HC blue no. 2, hydrocortisone, D-mannitol, methotrexate, 1-naphthylamine, phenanthrene, phenol, *p*-phenylenediamine dihydrochloride, phthalic anhydride, rotenone, sodium chloride, sodium nitrite, sunset yellow FCF, thiabendazole, *m*-toluidine and triphenyltin hydroxide were judged negative both in the initiation assay and in the promotion assay. However, barium chloride dihydrate, *tert*-butylhydroquinone, 8-hydroxyquinoline, sodium fluoride and tetracycline hydrochloride were positive in the promotion assay, and propyl gallate was positive both in the initiation and in the promotion assay.

The number of chemicals of unknown carcinogenicity was 9. Valproic acid and sodium valproate were positive in both assays. 2,3-Diaminotoluene, 2,5-diaminotoluene dihydrochloride,

Table 3
Comparison of results in the Bhas 42 cell transformation assay with those in genotoxicity assays.

Chemical	Bases of classification[a]	Bhas 42 CTA[b]		Ames[c,d]	ML[c,e]	Chrom. Ab.[c,f]		MN[c,g]
		Initiation	Promotion			*In vitro*	*In vivo*	
Carcinogens								
2-Acetylaminofluorene	TA#31[h]	+	+	+	+,+,+	+	+	+
Benz[*a*]anthracene	TA#31	+	+	+,+,+	+,+,+,+/−	+/−	+/−	+
IQ	TA#31	+	−	+				−(CCRIS[i])
5-Azacytidine	TA#31	+	−	+,+/−	+,+,+,+	+,+/−		+
Barium chromate	TA#31	+	−	−				
Benzo[*a*]pyrene	TA#31	+	−	+,+	+,+,+	+	+	+
Cyclophosphamide	TA#31	+	−	+,+/−,+/−	+,+,+,+	+,+	+,+/−	+,+,+
Cyclosporin A	TA#31	+	−	−				−
2,4-Diaminotoluene	TA#31	+	−	+,+	+	+		−
Dibenz[*a*,*h*]anthracene	TA#31	+	−	+,+/−,+	+,+	+		
Melphalan	TA#31	+	−	+,+,+,+/−	+	+,+	+	+
MCA	TA#31	+	−	+,+	+	−,−		
Mitomycin C	TA#31	+	−	+(CCRIS)	+,+	+,+	+,+,+	+,+,+
MNNG	TA#31	+	−	+,+	+,+	+/−		+
Sterigmatocystin	IARC[j]	+	−	+(CCRIS)				+(CCRIS)
Thio-TEPA	TA#31	+	−	+		+	+	+
Cadmium chloride	TA#31	−	+	−,−	+	+,−		−
Chenodeoxycholic acid	Orig[k], TP[l,m]	−	+	−/+(CCRIS)				
4-Chloro-*o*-toluidine HCl	TA#31	−	+	−	+	+w[n]		
Cholic acid	TA#31, TP	−	+	+/−				
Deoxycholic acid	CCRIS, TP	−	+	−/+(CCRIS)				
Dichlorvos	TA#31	−	+w	+,+	+,+,+	+	−,−	−
Epichlorohydrin	TA#31	−	+	+,+,+/−	+,+	+,+/−	+,+/−	−
D-Limonene	NTP(347)[o]	−	+	−,−	−,+	−		
Lithocholic acid	Orig., TP[p]	−	+	−,−	+/−,+/−	−		
Methapyrilene HCl	TA#31	−	+	−	−,+,+/−	+		
Methylarsonic acid	Review, TP[q]	−	+	−(Orig.[r])	+(CCRIS)	+ (Orig.[r])		+(CCRIS)
Mezerein	TA#31, TP	−	+	−,−				
2-Naphthylamine	TA#31	±	+	+,+,+/−,+,+/−	+,+,+	+,+		−,+,−,+
Phorbol 12,13-didecanoate	CCRIS, TP	+	+					
Quercetin	TA#31	−	+	+,+,+/−,+/−	+	+,+	+	+,+
Sodium arsenate	TA#31	−	+	−(CCRIS)	−(CCRIS)			−
Sodium arsenite	TA#31	±	+	−	+,+			+
Sodium saccharin	TA#31	−	+	−,−				
Styrene oxide	TA#31	−	+	+,+,+/−,+/−	+,+,+	+	+/−	−
TPA	TA#31, TP	±	+	−				
o-Toluidine	TA#31	−	+	+,−	+,−,+/−,+/−	+,+/−		+/−,−
Zinc chloride	TA#31, TP	−	+	+		−		
o-Anisidine	TA#31	−	−	+	+,+,+	+		
Benzene	TA#31	−	−	−	−,+,+/−,+/−	−,+,+	+,+	+,+
Cobalt sulfate	TA#31	−	−	+w				
Diethylstilbestrol	TA#31	−	−	−,−,−,−	+,+,+/−,+/−	+,+/−	+	+/−,+,−
Dimethylarsinic acid	CCRIS	−	−	−(CCRIS)	+(CCRIS)	+(Orig.[r])	+(Reviw[q])	+/−(CCRIS)
1,4-Dioxane	TA#31	−	−	−,−	−,−	−		−
Ethyl carbamate	TA#31	−	−	+/−,−,−	−	−,+		+,+
Formaldehyde	TA#31	−	−	+,+/−,+/−	+	+,+	+/−	+/−
Furylfuramide	TA#31	−	−	+,+,+	+,+	+		
Methyl carbamate	TA#31	−	−	−,−,−	−,−	−		−
Nickel (II) chloride	TA#31	−	−	−	+	+	+	−
Nickel monooxide	TA#31	−	−	−				−
Phenobarbital sodium	TA#31	−	−	−,+/−				
p-Toluidine	Orig.[s]	−	−	−(CCRIS)				
Non-carcinogens								
4-Acetylaminofluorene	TA#31	−	−	+	+/−,+,+/−	−	−	
Acid red 14	TA#31	−	−	−,−	−,−	−		−
Ampicillin	TA#31	−	−	−,−	−,−	−		
Anthracene	TA#31	−	−	+w, −,+/−	+,+,+,+/−	−		
L-Ascorbic acid	TA#31	−	−	+w/−,−		−	+	+
Aspartame	CCRIS	−	−	−(NTP)[t]				−(NTP)[t]
Benzoin	TA#31	−	−	+w/−,+/−	+,−,+/−	−	−	−
Caffeine	TA#31	−	−	−,−,−		+		+
Caprolactam	TA#31	−	−	−,−	−,−,−,−	−	−	−,−
2-Chloroethanol	TA#31	−	−	+,+,+/−	+,+	+	−	−
Chromium(III) Chloride	TA#31	−	−	−		+	−	
2,6-Diaminotoluene	TA#31	−	−	+				
Diazepam	TA#31	−	−	−,−		−		
N,*N*-Dimethylformamide	TA#31	−	−	−,−	+/−,−,−,+/−	−		
Eugenol	TA#31	−	−	−	+,+,+	+		−,+
HC Blue 2	TA#31	−	−	+	+,+	−	−	
Hydrocortisone	TA#31	−	−			+		
D-Mannitol	TA#31	−	−	−,−	−,−	−	−	−
Methotrexate	TA#31	−	−	−	+,+	+	+	+

tive or discordant in the Ames test. They are barium chromate, cyclosporin A, cadmium chloride, chenodeoxycholic acid, 4-chloro-*o*-toluidine hydrochloride, cholic acid, deoxycholic acid, D-limonene, lithocholic acid, methapyrilene hydrochloride, methylarsonic acid, mezerein, sodium arsenate, sodium arsenite, sodium saccharin, TPA and *o*-toluidine. The Ames-negative and Ames-discordant carcinogens except barium chromate and cyclosporin A were judged positive in the promotion assay. They included TPA and mezerein which are typical tumor promoters in mouse skin [19,20], bile acids which are regarded as endogenous tumor promoters [21–23] and sodium saccharin which has been demonstrated to be a promoter of bladder carcinogenesis in rats [24]. Phorbol 12,13-didecanoate that was positive in the promotion assay is also a tumor promoter in mouse skin [25,26]. However, we did not underline it in Table 3 because of the absence of published data in the Ames test and the other genotoxicity tests. In the previous papers, other tumor promoters such as okadaic acid [11,16,27], *o*,*p*′-dichlorodiphenyltrichloroethane (*o*,*p*′-DDT) [11], *p*,*p*′-dichlorodiphenyl trichloroethane (*p*,*p*′-DDT) [11], fumonisin B1 [28] and T-2 toxin [28] were also reported to be positive in the promotion assay of Bhas 42 cell transformation. These facts indicated that the promotion assay of Bhas 42 cell transformation detected known tumor promoters and suggested that the Bhas 42 cells behaved as initiated cells in the transformation assay. In this paper, arsenate and arsenite were judged positive in the promotion assay. Inorganic arsenics have epidemiologically been defined to be carcinogenic in the bladder, lung and skin of human and found to increase cancer incidence in various experimental animal models [29]. However, their mutagenicity is negative in bacteria and weekly positive in mammalian cells *in vitro*, though they have been reported to induce chromosomal aberrations, aneuploidy and micronuclei formation [29–31]. Thus, the Bhas 42 cell transformation assay can detect a considerable number of the carcinogens that are negative or difficult to be detected in the conventional genotoxicity assays. The carcinogens escaping scrutiny from *in vitro* screening would be diminished in number if the Bhas 42 cell transformation assay is performed in addition to genotoxicity assays to predict chemical carcinogenicity. Incidentally, we have demonstrated that the Bhas 42 cells retain the transfected v-Ha-*ras* gene and express its mRNA at the same level as c-Ha-*ras* mRNA (in preparation for publication). Our presumption is that the expression of transfected active oncogene is involved in the high sensitivity of Bhas 42 cells to the non-genotoxic carcinogens in transformation.

According to a review paper [5], the performance indices of the Ames, mouse lymphoma, *in vitro* and *in vivo* chromosomal aberration and micronucleus assays to predict rodent carcinogenicity of chemicals are as follows: concordance, 52–72%; sensitivity, 39.5–86%; specificity, 26–79%; positive predictivity, 76.5–83%; negative predictivity, 27–45%; false negative, 14–60.5%; false positive, 21–74%. As compared to those values, the concordance, specificity, negative predictivity and false positive of Bhas 42 cell transformation assay were superior and the other performances were equivalent to those of genotoxicity assays. It must be mentioned, however, that the chemical sets used for the calculation of performances are different between the Bhas 42 cell transformation assay in the present paper and the genotoxicity assays in the review paper.

The inorganic compounds tested in this study were 13 in all. This number may not be enough to evaluate the performance of Bhas 42 cell transformation assay for the prediction of carcinogenicity of inorganic compounds. When we dared to calculate the performances for inorganic chemicals, the concordance, sensitivity, specificity, positive predictivity, negative predictivity, false negative and false positive were 62%, 63%, 60%, 71%, 50%, 38% and 40%, respectively (the contingency and performance tables are not shown). The organic carcinogens and non-carcinogens tested were 76 in total. For the organic chemicals, the concordance, sensitivity, specificity, positive predictivity, negative predictivity, false negative and false positive were 80%, 75%, 88%, 89%, 72%, 25% and 13%, respectively (the contingency and performance tables are not shown). The Bhas 42 cell transformation assay may predict the carcinogenicity of inorganic chemicals with a little lower probability than that of organic chemicals.

The Bhas 42 cell transformation assay judged diethylstilbestrol negative in the present study, and also called estradiol and zearalenone as negative in the previous reports [11,27,28]. This assay may be insensitive to carcinogenicity of female sex hormones. Progesterone, a corpus luteum hormone, has been reported to be positive in the promotion assay [11].

The Bhas 42 cell transformation could detect a considerable number of Ames-negative or Ames-discordant carcinogens. Its performances to predict the carcinogenicity of chemicals are superior or equivalent to those of genotoxicity assays. As shown in Table 3, any of the genotoxicity assays is not perfect to predict the carcinogenicity of chemicals. We consider that the carcinogens escaping from *in vitro* screening would be reduced in number and the accuracy of prediction for chemical carcinogenicity would be improved by introducing the Bhas 42 cell transformation into the battery of *in vitro* assays.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Table 3 (*Continued*)

Chemical	Bases of classification[a]	Bhas 42 CTA[b]		Ames[c,d]	ML[c,e]	Chrom. Ab.[c,f]		MN[c,g]
		Initiation	Promotion			*In vitro*	*In vivo*	
1-Naphthylamine	TA#31	–	–	+,+,+/–		+,+/–		+,+
Phenanthrene	TA#31	–	–	+w,–,+/–		–		
Phenol	TA#31	–	–	–	+,+,+	+,+	+	+
p-Phenylenediamine 2HCl	TA#31	–	–	+	+,eq.[u],+	+		
Phthalic anhydride	TA#31	–	–	–,–	+	–		
Rotenone	TA#31	–	–	–	+	+		+
Sodium chloride	TA#31	–	–	–	+/–			
Sodium nitrite	Orig.[v],NTP(495)	–	–	+,+	+	+	+	–,+,+
Sunset yellow FCF	TA#31	–	–	–	+,–	–	–	–
Thiabendazole	TA#31	–	–	+				+
m-Toluidine	Orig.[s]	–	–	–(CCRIS)				
Triphenyltin hydroxide	TA#31	–	–	–,–	+	–		
Barium chloride	TA#31	–	+	–,–	+	–		
tert-Butylhydroquinone	TA#31	–	+	–,–		+	–	–
8-Hydroxyquinoline	TA#31	–	+	+,+	+,+	+w	–	–
Propyl gallate	TA#31	+	+	–,–	+,+	+	+	+/–
Sodium fluoride	TA#31	–	+	–,–	+,+,+	+/–	–	–,+
Tetracycline HCl	TA#31	–	+	–,–	–,+/–	–		
Chemicals of unknown carcinogenicity								
Sodium valproate		+	+					
Valproic acid		+	+				+/–(human)Orig.[w]	
2,3-Diaminotoluene		–	+	+(CCRIS)				
2,5-Diaminotoluene 2HCl		–	+					
3,4-Diaminotoluene		–	+	+(CCRIS)				
Sodium orthovanadate		–	+					–(CCRIS)
Capsaicin		–	–	+/–(CCRIS)	+(CCRIS)	–(CCRIS)		–(CCRIS)
Ethidium bromide		–	–	+(CCRIS)	+/–(CCRIS)			–(CCRIS)
Salicylic acid		–	–	+/–(CCRIS)				

The underline shows the carcinogens that were positive in the Bhas 42 cell transformation assay but are negative or discordant in the Ames test.

[a] The databases, or review and original papers referred to for the *in vivo* carcinogenic potency of chemicals.

[b] The assay results obtained in the present study.

[c] Assay results obtained from existing databases, or review and original papers. Most results are taken from a review paper, TA#31[h]. In TA#31, several databases are consulted for each chemical. The results listed in TA#31 are arranged in this table so that "+" means "positive in one database", "+,+" means "positive in two deferent databases", "+,+,+" means "positive in three deferent databases", "+,–,–" means "positive in a database and negative in two other databases", "+/–" means that there are diverging results inside a database and so on. The assay results which do not exist in TA#31 are based on CCRIS[i], or review and original papers, and the sources of those data are indicated in the parentheses.

[d] Ames test.

[e] Mouse lymphoma test.

[f] Chromosomal aberration test.

[g] Micronucleus test.

[h] "OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 31: Detailed Review Paper on Cell transformation Assays for Detection of Chemical Carcinogens" [5].

[i] The Chemical Carcinogenesis Research Information System.

[j] IARC Monographs.

[k] Original paper.

[l] *In vivo* tumor promoter.

[m] Ref. [22].

[n] Weakly positive.

[o] The United State National Toxicology Program. The NTP technical report number was indicated in the parenthesis.

[p] Ref. [21].

[q] Ref. [30].

[r] Ref. [32].

[s] Ref. [33].

[t] Studies in Genetically Modified Models, GMM-01.

[u] Equivocal.

[v] Ref. [34].

[w] Ref. [35].

3,4-diaminotoluene and sodium orthovanadate were positive in the promotion assay. Capsaicin and ethidium bromide were negative in either assay.

The performance indices of Bhas 42 cell transformation assay for predicting the carcinogenicity of chemicals were calculated from the assay results of known carcinogens and non-carcinogens. As shown in Table 2, we applied the Bhas 42 cell transformation assay to 52 *in vivo* carcinogens and obtained positive judgments from 38 carcinogens and negative judgments from 14. We tested 37 *in vivo* non-carcinogens and 6 were positive and 31 were negative. Consequently, concordance was 78%, sensitivity 73%, specificity 84%, positive predictivity 86%, negative predictivity 69%, false negative 27% and false positive 16%.

4. Discussion

The results of 98 chemicals tested in the present study were summarized and compared with existing data for genotoxicity in Table 3. Among genotoxicity assays, the Ames test is the most popular and has been applied to the largest number of chemicals including carcinogens and non-carcinogens. Of the 52 carcinogens tested in this assay, about half are negative or discordant in the Ames test from our consulting of databases or review of original papers. In the Bhas 42 cell transformation assay, 17 of the Ames-negative or Ames-discordant carcinogens were judged positive. In Table 3, we underline the carcinogens that were positive in the Bhas 42 cell transformation assay but are nega-

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