Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

20-Nov-2015

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 03 November 2015

INTEGRATED SUMMARY REPORT: Validation of Two Binding Assays Using Human Recombinant Estrogen Receptor Alpha (hrERa)

Series on Testing & Assessment
No. 226

This document is available in pdf format only.

JT03386693

ENV/JM/MONO(2015)35/ANN3
Unclassified

English - Or. English

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

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Integrated Summary Report: Validation of Two Binding Assays Using Human Recombinant Estrogen Receptor Alpha (hrERa)

Environment Directorate

ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT

Paris 2015

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FOREWORD

This document is Annex 3 of the Integrated Summary Report (ISR) of the Validation of two binding assays using human recombinant estrogen receptor alpha (hrERα). It includes appendices M and N of the report.

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

Peer review of hrER binding assay validation studies

Peer Review Process -

The mechanism that was used to peer review the joint validation study for the CERI and FW hrER binding assays was the same as used to peer review other Tier 1 assays, i.e. an external letter review coordinated by the Office of Science Coordination and Policy, U.S. EPA, Washington, DC, USA. The procedures used for peer review of the Tier 1 assays were in accordance with EPA's Peer Review Handbook (PDF) A balanced peer review panel consisting of three was selected from a pool of qualified peer review candidates identified from academia, government, and the private sector, based on their subject matter expertise, availability, and lack of conflict of interest or past involvement in the project.

The peer reviewers were provided documents to include (1) an Integrated Summary Report (ISR) that summarized and synthesized the information compiled during the validation process, (2) Appendixes A – L, that provided detailed information on data analyses and assay protocols, and (3) a set of Charge Questions, provided below, to ensure that all aspects of the validation process were thoroughly evaluated by each reviewer.

1. Clarity of the stated purpose of the assay.
2. Clarity, comprehensiveness, and consistency of the data interpretation with the stated purpose of the assay.
3. Biological and toxicological relevance of the assay as related to its stated purpose.
4. Clarity and conciseness of the protocol in describing the methodology of the assay such that the laboratory can:

- Comprehend the objective;
- Conduct the assay;
- Observe and measure prescribed endpoints;
- Compile and prepare data for statistical analyses; and
- Report the results.

5. Strengths and/or limitations of the assay.
6. Impacts of the choice of (a) test substances, (b) analytical methods, and (c) statistical methods in terms of demonstrating the performance of the assay.
7. Repeatability and reproducibility of the results obtained with the assay, considering the variability inherent in biological and chemical test methods.

Documents were forwarded to the peer reviewers on September 30, 2013 and each reviewed the materials independently. Written responses to the Charge Questions along with any additional comments were returned to the U.S. EPA by each reviewer prior to November 1, 2013. The table below shows a compilation of the comments received from the peer reviewers. This Table summarizes the major points from the peer reviewers and includes responses to the comments, prepared by EPA. The full responses to the letter peer review charge questions received from the three letter peer reviewers are attached in annex to this document.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
Charge Question 1: Is the stated purpose of the assays clear?			
1	A	The stated purpose of the assays – to identify chemicals that have the potential to disrupt the estrogen hormone pathway in humans and wildlife species – is clear (ISR p9). Such assays could contribute to weight-of-evidence based Tier 1 screening efforts (ISR p10) to prioritize consideration of chemicals for Tier 2 testing.	Agreed. No response needed.
2	B	Somewhat. There appear to be three purposes mentioned on page 9 of the ISR: 1) Determine if the assay[s] can identify chemicals that compete with the endogenous [ligand for] hER receptor ; 2) Provide an updated alternative to the current rat uterine cytosol assay (ER-RUC, OSCPP 890.1250) and 3) Permit a higher through-put capability The first purpose has been addressed extensively in the ISR and appendices; the last two are not explicitly evaluated.	Agreed. Stated purpose number 1 was the stated main objective of the validation study. Purpose 2 and 3: Neither of these was an intended purpose of the validation study. However, they were stated as possible improvements of the existing assay, post-validation (ISR p.9).
3	C	The stated purpose of the assay is clear. (…) The ISR clearly states that the hER assay as described, is intended to replace OCSPP 890. 1250 Rat Uterine cytosol ER binding assay for "E" and "anti E" assessments.	Agreed. No response needed. Note: The validated assay protocols are currently envisioned as potential alternatives to the Rat Uterine cytosol ER binding assay mentioned (OCSPP 890.1250).
Charge Question 2: Are the assays biologically and toxicologically relevant to the stated purpose?			
4	A	The assays reflect the ligand - estrogen receptor (ER) interaction, which is the initial step in estrogen signaling. As inappropriate ER signaling can lead to toxicological and undesirable outcomes including cancer, impaired fertility, and abnormal fetal development (ISR p9; Appendix B p xxi), the assays are relevant.	Agreed. No response needed.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		[…] The ISR's concluding observation was that reproducibility and accuracy of classification of ER binders and ER non---binders using hrER was comparable to RUC ER (ISR p. viii, p92) and to test guidelines (Table 40; ISR p93).	
5	**B**	These assays are biologically relevant; as they measure a discrete event that is known to influence numerous physiological responses. (…) [I]n the context of the EDSP Tier 1 screening battery, which is aimed at identifying potential hazard, these assays do serve their intended purpose and are therefore "toxicologically" relevant in that context.	No response needed.
6	**C**	Assays described in the ISR, i.e. FW and CERI human recombinant estrogen receptor binding assays, both meet the criteria for biological and toxicological relevance. This statement is based upon the fact that the stated aims of the described assays are to identify chemical agents that "interact" with the estrogen receptor alpha protein, and that human estrogen receptor is the stated biological target for both the FW and CERI assays. Concordantly, in terms of toxicological relevance, the estrogen receptor alpha is documented to exhibit broad ligand fidelity (i.e. is promiscuous) and is a well-established target for receptor-chemical interactions associated with estrogenic and anti-estrogenic biological responses across taxa.	No response needed.
Charge Question 3: Do the protocols describe the methodology of the assays in a clear and concise manner so that a laboratory can: **a. comprehend the objective;** **b. conduct the assay;** **c. observe and measure the prescribed endpoints;** **d. compile and prepare data for statistical analyses, and**			

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
e. report the results? **What additional advice, if any, can be given regarding the conduct of the protocols?**			
7	**A**	Several of the laboratories participating in the validation study had no prior experience with these assays (ISR p15; Appendix D p2); this empirically attests to the clarity of the protocols in Appendices J (CERI) and K (FW). As recommended by ICCVAM (Appendix B, p xxiii), the protocols are standardized; furthermore they contain sections explicitly and clearly addressing sub-points (a) through (e) listed within this charge question's prompt.	No response needed.
8	**A**	The following paragraphs [comments 8 and 9] address additional points (i.e. additional advice regarding charge question 3) as follow-up: 1. Incomplete curves. When assigning a "positive" hit in the competitive hrER-alpha binding assay, "There must be at least one data point at which 50% or more of the radioligand has been displaced" (Appendix K, p50; likewise in Appendix J, p53; ISR Figure 2 and Table 10). For reliable assignment of an IC50 or other SAR work, a curve with its final point just below 50% radioligand bound (as in ISR Figure 2) would be insufficient. However, given the note within the protocol, as in Appendix J, p51, that "The EDSP is less concerned with proving that the interaction is, specifically, one-site competitive binding, or with accurately characterizing the strength of the binding," than assigning a test chemical to be a "positive" binder in such a circumstance would be acceptable. This is also consistent with potential use of these assays in a Tier 1 screen, wherein high assay sensitivity would be desirable, even if it resulted in slightly more false positives. It may be worthwhile to include a	Plans are to update the protocols and prepare a test guideline for the assays that will include the rationale for using a qualitative description of the interaction of a test chemical with the hrER.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		brief description of this rationale within the ISR or similar summary document going forward.	
9	**A**	2. For the CERI assay, active receptor concentration must be titrated during the saturation binding experiment such that 40 +/- 10% of the radioligand is specific-bound in the presence of 0.5 nM concentration of total radioligand (ISR p40). It appeared however, that the labs, except CERI, initially had trouble achieving this requirement (ISR p40, also Table 14). In practice, how hard will it be to consistently achieve these specific binding ratios when the assays are run on a larger scale?	The recommended range for the active receptor concentration was set during the optimization of each protocol and was based upon the performance of the lead laboratory for each respective protocol. Plans are to revise Sections 9.25 (FW Assay) and 9.3.4 (CERI) of the protocols to emphasize the importance of completing a preliminary receptor titration experiment to determine the nominal concentration that will yield the recommended percent specific binding of total radioligand added for each protocol. Upon further evaluation, the range for the CERI protocol may need modification with rationale provided.
10	**B**	The answer to all of the above is "yes, to a large extend" but some general improvements could be made, see additional comments below. [comments 11-13]	No response needed.
11	**B**	Both of the protocols are dated 2008 and should be updated to reflect learning's gained from the validation study.	Plans are to revise the protocols for the FW and CERI assays to include new options for controls and a list of proficiency chemicals, as well as to provide clarification of the procedure for data analysis and interpretation. Additional guidance to help ensure acceptable runs will also be provided in the revised protocols.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
12	**B**	If the Lovelace lab were to perform the assays today, what qualification guidance/criteria are in place to ensure that the lab is capable of running the assays…?	For a successful validation study, one would expect at least 3 labs, to perform the exercise adequately. Binding assays require a certain level of expertise, which is not available in all labs. Proficiency chemicals are there to demonstrate an adequate level of expertise, and performance criteria assist in this process. It is not uncommon to remove poorly performing labs from validation programs. In this particular case, however, the Lovelace lab was allowed to continue. Overall Lovelace had difficulty performing both the CERI and FW assays; indicating a lab specific issue rather than a protocol specific issue. Due to the breadth of labs included in the validation study, 4 labs were able to demonstrate that they could correctly run both of the assays. Notably, labs should be expected to successfully run a set of well-characterized proficiency chemicals as well as the control chemicals before running test chemicals. As indicated above in response to comments 8-9, protocol revisions and the development of additional guidance in each protocol will help ensure acceptable performance.
13	**B**	A standard reporting format for both assays should be identified and published. It is not clear whether or not Assay Performance Criteria set forth in OSCPP 890.1250 would be	Worksheets for entry of saturation and competitive binding data were provided with each protocol. Once the final

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		applied to these assays. Also, clear guidance needs to be provided regarding the interpretation of the results. Although the ISR did a very good job of describing the challenges face with data interpretation (i.e., problems with 4-parameter fit and 10% rule), the method of data analysis intended to be used in updated test guideline is not explicitly stated.	protocols are completed, and the test guidelines have been approved by EPA for use in the EDSP, the Standard Evaluation Procedure (SEP) and Data Entry Spreadsheet Template (DEST) will be provided for each of the protocols. Although similar in scope and stringency to that developed for OCSPP 890.1250, tolerance bounds and other assay performance criteria set for the human recombinant ER assays will be based upon those data produced during the current validation study. The final protocols for the FW and CERI assays will include guidance for a step-wise data analysis as well as a well-documented section to clarify data interpretation that includes figures and examples of data/competitive binding curves. A description of appropriate methods for data analysis and guidance for a step-wise technical review of the data to ensure that the best curve fit is being applied will be included in the revised protocols.
14	**C**	Based upon data derived and reported within the ISR, the inner- and intra-laboratory trials for both the CERI and FW assays appear to meet the above objectives. Review of individual protocols for CERI and FW assays also appear to provide adequate descriptions for: comprehension of the	No response needed

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		objectives of the assay, how to conduct the assay, observe and measure prescribed endpoints, and compile and review data.	
15	**C**	It is not apparent, however, how information is provided for calculating assay outcomes other than compiling data in a provided data work sheet (Appendix J references Appendix D, but this does not appear to be a worksheet in the test report (section 10.5)). Parameters for data analysis are provided, including use of nonlinear curve fitting software; use of a one site competitive binding model; unconstrained top, bottom, and slope when fitting the curve; and robust regression when determining the best fit.	Once the final protocols are completed, and the test guidelines have been approved by EPA for use in the EDSP, the Standard Evaluation Procedure (SEP) and Data Entry Spreadsheet Template (DEST) will be provided for each of the protocols.
16	**C**	Also mentioned is the application of the "10% rule" and determination of RBA. While this information is essential to for data calculation and interpretation, it may be prudent to provide a blueprint on data analysis to ensure a consistent means of assessing assay performance, assay precision and overall OA/QC for the data analysis portion of the assay. This will be an especially important parameter as multiple laboratories become involved in conducting the assay, and as slight modifications in data analysis may lead to erroneous and inconsistent ER binding results.	Revisions to each protocol will include a step-wise data analysis strategy that incorporates a technical review of how well the data fit the curve generated. Specific examples will be provided as to the appropriate use of the 10% rule and constrained tops/bottom of curve versus unconstrained fits.
17	**C**	Additionally, it appears from the ISR that final data analysis from each of the validation studies was compiled by an outside independent contractor (Battelle, Appendix E) and summarized in a "Final Report on the Saturation Binding Analysis for Human Recombinant ER alpha (hrERa)". Thus, there is some question as to whether the data analysis component for both the CERI and FW assays has been "validated" and whether sufficient guidance is in place for independent laboratories	The analyses of data from the validation study were conducted by an independent contractor by design and facilitated a non-biased assessment of the data. With the inclusion of additional guidance for the step-wise approach for data analysis, technical review and interpretation of results, it is anticipated

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		to thoroughly conduct the data analysis component of the assay.	that participating laboratories will be proficient in classifying test chemicals as ER binders or non-binders. A strong indicator of the ability to complete the data analysis and interpretation of the results will be demonstrated when conducting the proficiency chemicals that will be provided with the revised protocols.
18	**C**	Given that subsequent progress of the assay may hinge upon multiple performance criteria (See pg 27 ISR), it may be prudent to establish a decision tree for each component of the assay. For example, a decision tree may facilitate guidance to ensure compliance with each of four criteria within the saturation binding component of the assay. Only after demonstrating this compliance should the competitive binding assay be conducted. A similar decision tree may be conducted for completion of the competitive binding component of the assay (five criteria) and then for data analysis.	Guidance on the use of the measures of assay performance is provided in the protocols. These measures of assay performance provided for the FW and CERI assays are intended as a guide to evaluate whether or not the saturation and competitive binding components of the assays are functioning correctly and should be sufficient. Among those measures of assay performance provided, the ratio of specific binding to total radioligand concentration used in each respective assay, is the only endpoint where a quantitative range is recommended (i.e., 20±5% (FW) and 40±10% (CERI). This endpoint reflects the expected nominal receptor concentration that should be used to obtain optimal results from each protocol by minimizing ligand depletion and supports the assumptions of the law of mass action upon which the analyses of the competitive binding data are based.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
19	**C**	On what basis/criteria will independent laboratories know when to apply these data manipulation techniques? How will differential use of these applications be assessed to establish overall concordance of assay performance across laboratories?	A list of chemicals will be provided in the revised hrER protocols that will be used in each laboratory to demonstrate their proficiency when conducting the assay, analyzing the data, and correctly interpreting the results. These chemicals will be structurally diverse and cover a range of potencies for binding to the ER. The ability to correctly classify these proficiency chemicals will be a strong indicator that each laboratory is capable of conducting all aspects of the protocol, and demonstrate concordance among laboratories.
Charge Question 4: Have the strengths and limitations of the assays been adequately addressed?			
20	**A**	Key strengths and weaknesses of the assays have been addressed. At a high level, the strengths are that both assays are run on human recombinant ER, and are amenable to higher-throughput screening than current RUC ER assays. Additionally, assay hit-calling was reproducible across labs and consistent across FW and CERI methods. Limitations are: (1) the assays do not distinguish the functional direction of the ligand-ER interaction (agonist/antagonist); (2) the assays are cell-free so they cannot be expected to account for chemical metabolites; and (3) solubility. These limitations were acceptable, and generally outside the scope of the validation study.	No response needed
21	**A**	The ISR presents several approaches to address equivocal dose-	Agreed. See response to comments 11, 13

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		response curve occurrences, including constraining the bottom of such curves to zero (ISR p77; also see curve top/bottom issues in Appendix I, p4; vs. current unconstrained approach, e.g., Appendix K, p 48) and data point exclusion for "U-shaped" curves (ISR p29) following a "10% rule" (ISR p30, p34; Figure1). These approaches were reasonable, and analyses were conducted with and without (e.g., Appendix F) the 10% rule to test it. More generally, these questions brought up the need for an "interim technical review" step (ISR p70, p77). Based on these observations and analysis, the best general solution would indeed be to formalize and incorporate an interim technical review step (which may be initiated, for instance, when one encounters an equivocal curve with certain predefined characteristics) into the analysis process.	and 19
22	**B**	The limitations of the assay were provided in Table 41. These 4 bulleted points do not provide a comprehensive assessment of the technical and biological limitations of the assay, and should continue to be developed as the assay becomes more widely utilized and more data are available. For example, no assessment is made regarding the difference in performance of the full vs. partial receptor, which could prove to be extremely important when assessing the binding of certain classes of chemicals (a very clear difference can be noted in the Tox21 full vs. partial ER TA assay data). Likewise, no attempt was made to define an applicability domain of chemical space for which these assays should <u>not</u> be used (i.e. detergents/surfactants could produce false positive results).	Agree. Validation studies are conducted to demonstrate the ability of the assay to obtain reliable and reproducible results when testing single chemicals whose response have been well characterized and documented. However, the performance of the hrER assays when testing the binding affinity of a diverse group of chemicals will continue to be evaluated. Any observed limitations of the assays will be included in appropriate assay-related documents.
23	**C**	Page 97 of the ISR provides a list of overall strengths and weaknesses of the CERI and FW assays. Strengths and weaknesses are accurate and appropriate.	No response needed.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
24	**C**	A few additional strengths and weaknesses are noted. Additional strengths that may be added: 1. Toxicological relevance of ER as a molecular target i.e. demonstrated molecular initiating event in numerous EDC studies. 2. Substitution of human ER for animal sources of cytosolic ER. Thus, no further need for cross species extrapolation and elimination of complications due to different ligand specificities between species. Additional weaknesses that may be added: 1. Assessment of assay positive and negative control performance (i.e. acceptable or unacceptable) for individual runs appears to be based upon a subjective evaluation. Reductions in subjective determinations may further improve assay reproducibility, reliability and transferability (see pg 30, lines 23-34). 2. Necessity for further guidance on data interpretation.	Agree. These suggested strengths of the assays will be considered for inclusion in appropriate assay-related documents (e.g. test guideline). (RE: Additional weakness # 1) The acceptance of control data from individual runs will be based upon tolerance bounds for the controls (reference estrogen and 2 weak binders) and additional assay performance criteria that will be provided in the revised protocols. (RE: Additional weakness # 2) See responses to comments 11, 15 and 17. This will be corrected in the revised protocols which will include a step-wise data analysis strategy that in turn will include a technical review of how well the data fit the curve generated. Specific examples will be provided as to the appropriate use of the 10% rule and constraint of the top and/or bottom of the curves. (RE: Additional weakness # 3) The use of

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
			decision trees is not considered a weakness. (RE: Additional weakness # 4) This will not be a limitation in the revised protocols that will contain tolerance bounds and other assay performance criteria for the reference estrogens and controls.
		3. Potential use of decision trees for modular completion of assay components.	**(**RE: Additional weakness # 5) A follow-up statistical analysis for each assay was used to provide additional insight into intra- and inter-laboratory reproducibility (ISR p34). But the reviewer's comment merits some further consideration for e.g., possible improvements to the way data are analyzed for inter-laboratory variability.
		4. Defined QA/QC parameters for assay concordance across laboratories.	(RE: Criteria #6) Laboratories submitted documentation describing their level of adherence to GLP conditions. All laboratories operated under the spirit of GLP with deviations reported. In addition, all work was conducted under the scientific guidance and management of the laboratory study director. Data from each laboratory were reviewed for Quality

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		5. Compliance with validation criteria may be skewed. See criteria # 4 pg 101 and pg 33 "Within test, intra-laboratory and inter-laboratory reproducibility and how these parameters vary with time should be evaluated"… Reproducibility of the assay is evaluated on the "average classification of a compound (i.e. binder, non-binder, equivocal). This language may be misleading as individual runs within laboratories and overall scores within and between laboratories are not in full agreement. Here the statement "Overall average classification" may be misleading 6. Criteria # 6 pg 101, "data should be obtained in accordance with Good Laboratory Practices. There is no mention in the ISR that any of the participating laboratories engaged in the validation studies have been GLP certified. As such language within the ISR states, "laboratory work was conducted *in the spirit of* conformance with GLP".	Assurance prior to the submission to the US EPA. Given the results from the validation studies, the US EPA has confidence that the quality of the data from the validation effort was properly assessed.
Charge Question 5: Were the (a) test substances, (b) assay methodologies, and (c) statistical methods chosen appropriately to demonstrate the performance of the assay?			

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
25	**A**	a) The current test substance collection (Table 5) appears sufficient for this validation study, subject to a reasonable response to Clarification Question 5.a.1, below.	No response needed.
26	**A**	However, going forward, as there are a "wide array of existing potential test chemicals" and variation in their outcomes (ISR p90), I would recommend prioritizing testing of additional chemicals with known ER-alpha activity, or those that might reasonably be expected to have it. As sources for such chemicals, the ICCVAM executive summary refers to 638 ER binders confirmed by more than one assay (Appendix B, p xxii), and publicly available databases might also facilitate this effort (see a.1-2, below). Clarification Question 5.a.1: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Executive Summary report (Appendix B) states, "Proposed in vitro ER binding testing methods should be evaluated in validation studies using, at a minimum, the 53 substances listed…" followed by data generation on the remainder of a list of 78 substances (emphasis added, Appendix B, p xxiv). Was this recommendation not pragmatically feasible for the current validation study, which was run on approximately 30 chemicals? It is not clear from the CAB Report alone (Appendix D) why fewer than the ICCVAM recommended 53 substances	The requirements for the validation study as described in OECD Guidance document 34 have been met. Extended chemical lists for evaluation were outside the scope of the validation effort. We appreciate the reviewer bringing this information to our attention, however, for future reference. (RE: 5.a.1)The selection and number of chemicals used in this validation study were in compliance with OECD TG 34 such that each chemical had been well documented as either an ER binder or non-binder, and the group of chemicals covered a range of ER-binding potencies across chemical classes known to interact with the ER. (RE: comment a.1) ER competitive binding is being examined in this assay, not ER

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		were chosen for the validation subtasks, as the charge to the CAB was solely "to review and suggest modifications to the chemicals [already] nominated" (Appendix D, p 2). The following section discusses public sources of known and possible ER binders, along with approaches to structural diversity analysis: (a.1) Known ER-alpha binders. The current ChEMBL-17 public database (https://www.ebi.ac.uk/chembl/) annotates approx. 5,000 chemical-receptor interactions for human ER-alpha. These interactions are curated from the literature, and whereas they will thus not be of the same quality or certainty as those control and test chemicals selected by the Chemical Advisory Board (ISR p16) or the 638 ER binders from ICCVAM (Appendix B, p xxii), they nonetheless would allow for ongoing assay validation efforts across a more diverse selection of chemistry. Of these 5,000 interactions, some 205 unique chemicals have a reported Ki < 1 uM. A simple search on these 205 chemical structures for possible vendor sources via http://emolecules.com yields 134 purchasable known ER inhibitors that are readily available for testing.	inhibition. The reference chemicals were carefully selected due to their well characterized known activity with respect to ER binding and also considered were practical factors such as cost and international availability. The number of chemicals used in the validation study was in compliance with the OECD Guidance document 34.
27	**A**	(a.2) Weak or possible ER-alpha binders. The CAB suggested greater performance testing on compounds with weak or no ER activity (ISR p17; Appendix D p3). This remains the greatest weakness of the current chemical collection used in this validation effort, because the likely prevalence of ER-alpha binders across the space of medicinal and natural product chemistry is likely substantially lower than the prevalence of ER-	The requirements for the validation study as described in OECD Guidance document 34 have been met, and extended chemical lists for evaluation were outside the scope of the validation effort. We appreciate the reviewer bringing this information to our attention, however, for future reference.

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		alpha positive binders in the focused chemical validation collection (Table 5). Due to pragmatic considerations, I do not feel that this lack of weak and non-binders (compared to their "in the wild" prevalence) in the current validation collection should delay possible adoption of these assays for estrogen hormone disruption screening. However, if the assays are adopted, I recommend that known (see response a.1, above) and weak binders be prioritized for testing as follows: Using standard and well-accepted measures of chemical similarity (e.g., Pipeline Pilot (http://accelrys.com/products/pipeline-pilot/) ECFP4, ChemAxon (http://www.chemaxon.com/) ECFP4, or RDKit (http://www.rdkit.org/) ECFP4 fingerprints compared via Tanimoto coefficients), one could identify 500 or more readily purchasable chemicals with high similarity to the 134 purchasable known ER-alpha binders described in a.1. These could reasonably be expected to contain more binders or weak binders than would a random selection of purchasable or naturally occurring chemicals. Conversely, the chemical similarity metrics above could be used to exclude all chemicals with any appreciable similarity to the 200+ known ER-alpha binders mentioned in a.1 or listed in ISR Table 5. Using the more than 5 million purchasable chemicals readily available from vendors and freely coordinated by databases such as emolecules.com or zinc.docking.org, it would be feasible to select purchasable biologically-relevant chemicals that would be expected to not to bind to ER-alpha	Also refer to response to comment 26.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		(i.e., "expected negatives"). Finally, these standard chemical similarity techniques could be used to assess the chemical diversity of chemicals already tested, or to prioritize for new chemicals that would most rapidly increase this diversity. Other diversity methods to consider could include general physiochemical properties and predicted solubility. As noted in the CAB Report, "Chemicals with known affinity for ERa were given preference over chemicals that were structurally diverse but with no ER binding history" (Appendix D p2), and while this is sensible, it implies a possible lack of structural diversity that could be addressed through ongoing analysis. In summary for part (a), the current ~30 chemicals tested in subtasks 1-4 are pragmatically sufficient for this validation effort, but ongoing analysis should be continued if the assays are adopted.	
28	**A**	(b) Assay methods were appropriate. Key concerns were chemical solubility (see charge question 4) and ER stock solution stability (ISR p48), degradation (ISR p47), and evaluation (ISR p21). These concerns were addressed within the report, and the saturation-binding step in particular is meant to decrease uncertainty from differences in ER stock solution. The [unconfirmed] possibility of an error in stock solution in the Lovelace lab (ISR p65) suggests that even with acceptable saturation-binding test protocol (ISR p48), ongoing diligence as to quality of stock solution is merited.	Agreed. Revisions to the protocol will emphasize the importance of ongoing diligence as to the quality of stock solutions.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
29	**A**	(c) The statistical methods (including independent analysis) were chosen appropriately, and questions arising such as the fairness of excluding runs based on subjectively determined acceptability of their control curves (ISR p70, p77) were explored. Pooled statistics quantified by r^2 were appropriate, although the statistical report did note difficulty in determining reliable IC50's from several dose-response curves lacking expected top and bottom values (Appendix I, p4) within the allotted time frame.	No response needed.
30	**B**	The use of DBP as a non-binding standard was perhaps not the best choice, in hindsight. The non-binding control should change to Octyltriethoxysilane or Atrazine in the revised protocols. The classification of several test substances was changed during the validation study, and the justification for these changes was well described and referenced. Statistical methods were also modified (i.e. 10% rule), and likewise well justified.	Agreed. The goal of subtask 4 was to provide additional options of weak binders and non-binders for the future use of the hrER binding assays. Revisions will be made to the protocols to reflect this information.
31	**C**	(…) Overall selection of compounds proved to be effective at discriminating function of the both the CERI and FW assays. Selected positives controls (based on historical data) demonstrated the assays were specific, sensitive and capable of discriminating between receptor binders and non-binders. Equally, putative non-binders demonstrated reasonable selectivity of the assay.	No response needed.
32	**C**	Assay performance appeared to have the most trouble with moderate or weak binders. Inclusion of several compounds with moderate to weak ER binding (based again on historic data) proved to test assay performance and function, and ultimately the ability of both assays to discriminate weak ER ligands from	Anticipated revision of the protocols will include information for the use of additional non-binder controls based on the results of subtask 4. In addition, further guidance on evaluating chemical

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		non-binders. Solubility limits of individual compounds also impacted test results (see below) suggesting that future trials should be conducted with as broad a concentration range possible in addition to evaluation of other carrying agents on assay performance to circumvent solubility issues. As such, use of DBP-TC at 10-3 M resulted in an ambiguous curve fit and an equivocal classification for a compound that was initially utilized as a negative control. Assessments of an additional select set of compounds (Subtask 4) was conducted to identify alternate "weak" binders and non binders that could be used as positive/negative controls and evaluate both reproducibility and accuracy of the CERI and FW assays These trials were useful in that they further solidified impacts of chemical solubility on chemical classification, identified Norethindrone as a weak binder and plausible control, and provided further examples of the necessity to apply the 10% rule. Combined, it appears that further evaluation of chemical solubility limits for all compounds tested will needed to be conducted in order to maintain assay reliability.	solubility limits will be provided.
Charge Question 6: Considering the variability inherent in biological and chemical test methods, were the results obtained sufficiently repeatable and reproducible?			
33	**A**	The results were sufficiently repeatable and reproducible.	No response needed
34	**A**	Given that the goal of the assays is to identify possible estrogen hormone disruptors in a 'first pass' prior to more extensive testing, a slightly higher than desired false positive rate (as observed) is preferable over, for instance, a predilection toward false negatives (which generally did not occur).	No response needed
35	**B**	One of the best techniques to assess the performance of any assay is to compare the new assay with an accepted	A comparison of the overall classifications (i.e., binder or non-binders) for 23 test

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		(preferably validated) existing assay. This could easily have been done in the ISR by comparing the results (repeatability and reproducibility) of the hER assays with the current ER-RUC assay. Such a comparison would reduce the subjectivity of this assessment and provide a quantitative assessment regarding the relative performance of the assays (specifically, are the new assays less variable than the existing assay). Regardless of lack of this type of assessment, the assays do appear to have sufficient performance for their intended use.	chemicals reported during the respective validation studies for the hrER binding assays and OCSPP TG 890.1250 (as shown in Table 40, page 94, Integrated Summary Report) demonstrated that the performance of all three assays were comparable. In fact the comparison (in Table 40) indicates that the recombinant protocols are performing as good or better that the existing Guideline.
36	**C**	Overall, laboratories demonstrated proficiency in conducting both the CERI and FW assays. Intra-laboratory test runs for both saturation binding and competition components of the assays appeared reproducible and within a reliable standard of biological test variation. Comparisons across laboratories i.e. inter-laboratory trials also exhibited reasonable variability, although this data was inherently more variable than intra-laboratory runs. In both CERI and FW saturation assays Kd values varied significantly for one or more laboratories, and Bmax values were not consistent, suggesting laboratories were not working with similar receptor concentrations. While not inherently fatal in evaluating Kd determinations receptor concentration differences may ultimately impact data outcomes especially if ligand dilution models are applied. However considering SD values for estimates of Kd and Bmax results across most laboratories produced similar outcomes with exception of Bayer (CERI assay) and U Konstanz (FW assay). With competition studies, inter-laboratory comparisons identified numerous difficulties	No response required. See responses to comments 11, 13, 15, and 17.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		with assay performance, including deviations in top values, steep/shallow slope values and curve fitting artifacts. Application of data manipulation tools were necessary to generate consistency across both individual runs and between laboratories for both uncoded and coded compounds (tasks 1-3), suggesting that inter-laboratory trials were not uniformly consistent. Individual laboratories additionally exhibited varying degrees of difficulty in conducting both CERI and FW assays—specifically the Loveless laboratory, which reported difficulties with 17β-estradiol, indicating either problems with assay transferability or technical proficiency with this particular lab. Additionally, several laboratories appeared to have difficulties with assay performance due to issues with chemical concentration, and solubility. In particular, these issues complicated classification of compounds as non-binders such as Di-n-butyl phthalate and heptylphenol. These compounds exemplify difficulties in classifying weak binders. In cases where a complete binding curve is not produced or available, data manipulation tools may be applied to facilitate interpretation. Use of such manipulations should only be conducted with specific guidance established in the assay protocol. This is alluded to on page 77 of the ISR with the need to establish additional step-wise technical review of the data. However, this point is not mentioned again throughout the ISR, indicating the process (i.e. step-wise technical review) has not been established. This will likely be essential for subsequent chemical evaluations as most compounds tested are likely to fall into the weak or non-binder category. If sufficient rigor is not included into either the assay itself or data interpretation/manipulation process, then erroneous results are likely to ensue.	
Charge Question 7: With respect to performance measures, are the measures of assay performance for the saturation and			

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competitive binding experiments appropriate? Should the tolerance bounds developed for the reference estrogen and/or two weak binders be used to ensure proper performance of the assays?			
37	**A**	The measures of saturation and competitive binding experiment performance for the assays are appropriate, including analysis of non-concurrent run consistency intra-lab, and consistency across labs and FW and CERI assay approaches. Manual review of curve acceptability (e.g., ISR p30, p45) introduces possible subjectivity, but decision criteria are clear and subsequent analysis excluding this step remained consistent.	No response needed.
38	**A**	The tolerance bounds developed for the control chemicals could be used to flag assay runs for interim technical review of the entire run and/or repeat testing where confirmed by manual supervision. The particular criteria of their derivation, set to account for 80% of the population at 95% confidence (ISR p90), seem reasonable, but could be modified to enhance their use in a decision-support capability if warranted empirically. The ultimate goal may be to remove human supervision or review from all but the most equivocal cases, but this extra effort (i.e., human review of out-of-tolerance-bound 'flagged' results) would seem to be justified during the initial adoption of the assays.	No response needed.
39	**B**	(…) Yes, tolerance bounds should be provided as guidance (not strict cut-offs) to ensure the proper performance of the assays.	No response needed.
40	**C**	As stated previously, there remains a fair degree of subjective interpretation for assessments of assay performance. For example, Table 9 (ISR pg 27) provides multiple measures of required assay performance for both the saturation and	See responses to comments 9 and 17.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
		competitive binding components of the assay. Review of saturation binding measures includes four specific parameters to assess performance. While most of these criteria appear to be met, there is significant variability between intra- and inter-laboratory trials. For instance, laboratories report varying ranges for measure 2, with some laboratories not achieving the measured mark of 40% binding at 0.5 nM radioligand in the CERI assay (table 15; ISR) and 20% binding at 1 nM radioligand in the FW assay (table 18; ISR). These discrepancies appear to be associated with reported differences in Kd and Bmax values.	
41	**C**	Adherence to performance criteria for competitive binding also suggests difficulties in conducting the assay, including the use of DBP as a negative control, solubility and variance of IC50 values. Additionally, it appears that throughout the validation process i.e. intra- and inter-laboratory trials, that performance evaluations for competitive binding experiments with coded or uncoded compounds were based entirely upon a subjective determination of the behavior of positive and negative controls. Assessments of PRISM fit parameters Top, Bottom, slope values and curve fit for control substances were subjectively evaluated to discern overall assay performance. Concern regarding this decision process is mentioned on page 77 of the ISR in reference to the need to identify acceptable or unacceptable data limits and establish performance criteria and tolerance bounds. The development of tolerance bounds for CERI and FW assays is essential to ensure assay consistency and provide a measure of QA/QC for assay performance. The approach taken (ISR pg 90-95) to assess tolerance bounds provides an adequate measure of assay performance based upon	See responses to comments 13, 15, 16, and 17.

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		validation trials and defined a set of tolerance limits for subsequent applications. Defined criteria including Hillslope, logIC50, Syx and log10RBA will serve as an essential guide to ensure assay performance across laboratories and within replicate runs (see table 38).	Tolerance bounds for the reference estrogen and two weak binders have been developed for the CERI and FW hrER assays and will be included along with other assay performance criteria in the revised protocols.
Charge Question 8: Are the data interpretation criteria clear, comprehensive, and consistent with the stated purpose of identifying compounds as binders and non-binders for the estrogen receptor?			
42	**A**	Data interpretation criteria for ER-binder identification of compounds are clear. These criteria include analysis of dose-response curve fit (ISR Figure 2) and data point artifact cleanup (ISR Figure 1; also see charge question 4), along with a scoring system for hit-calling and multiple-run scoring consensus (ISR Tables 10-11). There is some discussion of manual (and therefore potentially subjective) techniques used to determine "acceptable" curves for known controls (ISR p30, Table 9), but this did not negatively affect accuracy or repeatability when compared to subsequent statistical post-analysis (see charge question 6). Combined with or replaced by an explicit "interim technical review" for equivocal or weak curves (see charge question 4), some degree of human supervision of curve acceptability would remain reasonable.	No response needed.
43	**B**	The rational and use of data interpretation criteria were clearly outlined in the ISR for evaluating study data.	No response needed.
44	**C**	Criteria for assessing classification based upon test chemical binding curves can be found on pg 31 of the ISR section 10.4.4	No response needed.

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		of Appendix J for the CERI assay and section 10.6.4 Appendix K for the FW assay. In each description criteria for classification of a chemical as either a binder, non-binder or equivocal is clear, comprehensive and consistent. (…) Strict adherence to defined guidelines will help facilitate consistent applications of data manipulation and thus consistency of chemical classification	
Charge Question 9: Please comment on the overall utility of the assay as a screening tool as described in the introduction of the ISR to be used by the U.S. EPA to identify chemicals that have the potential to interact with the endocrine system.			
45	**A**	Overall, the FW and CERI assays using hrERa are sufficiently accurate, repeatable, clear, and consistent with existing RUC ER and the existing ER binding guideline OPPTS 890.1250 (ISR p100, Table 40) to be used as a screening tool as described in the introduction to the ISR. They can be performed at higher throughput than assays relying on ER derived from animal tissues, allow for greater standardization than cytosolic preparations, and would be expected to be as or more relevant to human toxicology than are ligand-receptor interaction assays using animal-tissue derived ER.	No response needed.
46	**A**	As with any new assay system, the binding curve and result data should continue to be compiled if these assays are adopted for broader screening use, and subjected to periodic or ongoing statistical analysis as in ISR section 4 (ISR p77-82); also see charge question 5.a. This would allow continued evaluation of the techniques such as the 10% rule and/or other corrections (e.g., to address solubility) incorporated within a technical review step. Likewise, it would increase the chances of identifying any artifacts specific to new chemicals or chemical categories not yet represented in the collection that comprises the current validation subtasks.	Agree that continuing to evaluate the performance of laboratories as the assays are used for broader screening is a worthwhile recommendation for further consideration. Tolerance bounds for the reference estrogen and two weak binders have been developed for the CERI and FW hrER assays and will be included along with other assay performance criteria in the revised protocols. Addition of proficiency chemicals in the final protocols will provide another uniform method for assessing the

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			ability of laboratories to conduct the data analysis and as well as consistency in data interpretation.
47	**B**	I believe the most relevant question is whether or not these tests meet the performance criteria to allow their use as part of the EPA Tie 1 Screening battery. This question would most easily be address by directly comparing the performance of the new methods with that of the existing ER-RUC assay. Doing so would allow for an objective assessment as to whether the performance of these test was equal to, or better than, the existing ER-RUC method. In either of these cases, the new methods would be judged as meeting the needs of the EPA Tier 1 Screening battery. If the performance of these methods is worse than the existing method, then other factors must be taken into consideration. Consequently, the performance of the three methods (CERI, FW, ER-RUC) should be directly compared using existing data.	See response to comment 35**.**
48	**C**	As per ICCVAM validation criteria, both the CERI and FW hER alpha binding assays provide a scientific and regulatory rationale for the test method. The ISR and both assay protocols provide a clear statement of the intended purpose of the assay and clearly define biological and toxicological relevance with regards to EDC toxicity. Additionally, as per ICCVAM validation criteria, the ISR illustrates test method performance through evaluation of a series of reference chemicals, both uncoded and coded to exclude test bias. Chemicals were selected as described in the ISR and in response to Q5 above. Reliability is defined by ICCVAM "as the reproducibility of results from an assay within and between laboratories."	No response needed.

Comment Number	Reviewer (A, B, or C)	Expert Comments	Response
49		Overall, both CERI and FW assay performance proved to be predictive with accurate classification of most coded and uncoded test substrates. Individual assay trials within laboratories were reproducible and thus produced reliable outcome in chemical classifications. More variability was observed between laboratories, suggesting that there may be moderate issues with transferability. Conversely, most laboratories were capable of conducting the assays with predicted outcomes of chemical classification. Laboratories appeared to have the greatest difficulties with weak estrogen receptor binders. Thus, several data manipulation tools were applied with the stated aims of facilitating data interpretation. Once applied, overall outcomes across laboratories were significantly predictive, suggesting that the assays are robust as designed. That is to say that the assays maintain the ability to effectively perform under varied conditions. The caveat to this statement, however, is the need to have in place strict guidance criteria for assay performance and data manipulation.	No response needed. See comments 11, 13, 17, and 19.
50	**C**	Additionally, there remain unresolved questions following review of the ISR. Specifically which of the assays will be implemented for screening hER interaction? On what basis will one assay be chosen over the other or will both assays be utilized?	The results from the validation study for both the FW and CERI assays were consistent and in agreement with expected classifications even though there were differences in the form of the ER (full length ER for FW vs. ligand binding domain

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			for CERI) and subtle differences in the protocols. Further, reproducibility of both assays was comparable to the existing ER binding Guideline OPPTS 890.1250. THE US EPA is reviewing the results from this validation study as a basis for considering the two assays as potential alternates to Guideline OCSPP 890.1250. If approved as such, the Agency's established process for the development of new test guidelines will be followed. The full length human recombinant ER alpha used in the validation study is commercially available from Life Technologies, Carlsbad, CA, catalog # P1287). Other sources of full-length hrER alpha were acknowledged, but resources were not available to examine differences between products in the validation study. Theoretically, any full length hrER alpha should perform comparably. The glutathione-S-transferase fusion protein incorporating the ligand binding domain of the hrERa was developed by the

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		Secondly the source of hER is not mentioned for subsequent testing. Will the EPA continue to provide recombinant hER to contract facilities, or will individual facilities produce and purify their own? If the latter, how will QA/QC be established for this component of the assay?	Chemicals Evaluation and Research Institute, Japan, who have committed to making it commercially available upon validation of the assay. The analyses of data from the validation study were conducted by an independent contractor by design and facilitated a non-biased assessment of the data. With the inclusion of additional guidance for the step-wise approach for data analysis, technical review and interpretation of results, it is anticipated that participating laboratories will be proficient in classifying test chemicals as ER binders or non-binders. A strong indicator of the ability to complete the data analysis and interpretation of the results will be demonstrated when conducting the proficiency chemicals that will be provided in the revised protocols for the FW and CERI hrER Binding assays.

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		Also, since an outside independent laboratory conducted most of the data analysis, how will this component of the assay be validated? It may be prudent to have independent laboratories conduct a ring test on various data sets to ensure that proper guidance is provided for data analysis, interpretation, and ultimately chemical classification.	

Annex

CHARGE TO PEER REVIEWERS
for
INDEPENDENT PEER REVIEW OF THE VALIDATION OF TWO HUMAN RECOMBINANT ESTROGEN RECEPTOR BINDING ASSAYS AS POTENTIAL SCREENING ASSAYS IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY
September 30, 2013

Charge Questions:
Please respond to each of the following questions:

1. Is the stated purpose of the assays clear?

   The stated purpose of the assays – to identify chemicals that have the potential to disrupt the estrogen hormone pathway in humans and wildlife species – is clear (ISR p9). Such assays could contribute to weight-of-evidence based Tier 1 screening efforts (ISR p10) to prioritize consideration of chemicals for Tier 2 testing.

2. Are the assays biologically and toxicologically relevant to the stated purpose?

   The assays reflect the ligand - estrogen receptor (ER) interaction, which is the initial step in estrogen signaling. As inappropriate ER signaling can lead to toxicological and undesirable outcomes including cancer, impaired fertility, and abnormal fetal development (ISR p9; Appendix B p xxi), the assays are relevant.

   Furthermore the assays incorporate full-length human recombinant ER (hrER) (for FW assay) or ligand-binding-domain truncated hrER (for CERI assay) (ISR p. vii) instead of the rat uterine cytosol (RUC) ER used in the current EPA Tier 1 Battery (ISR p9). The expectation is that assays on recombinant human receptor will be more consistent and standardized than those using cytosolic preparations, and such assay approaches were prioritized by ICCVAM (Appendix B p xxiii; Appendix D p1). The ISR's concluding observation was that reproducibility and accuracy of classification of ER binders and ER non-binders using hrER was comparable to RUC ER (ISR p. viii, p92) and to test guidelines (Table 40; ISR p93).

3. Do the protocols describe the methodology of the assays in a clear and concise manner so that a laboratory can:
   a. comprehend the objective;
   b. conduct the assay;
   c. observe and measure the prescribed endpoints;
   d. compile and prepare data for statistical analyses, and
   e. report the results?

   What additional advice, if any, can be given regarding the conduct of the protocols?

   Several of the laboratories participating in the validation study had no prior experience with these assays (ISR p15; Appendix D p2); this empirically attests to the clarity of the protocols in Appendices J (CERI) and K (FW). As recommended by ICCVAM (Appendix B, p xxiii), the

protocols are standardized; furthermore they contain sections explicitly and clearly addressing sub-points (a) through (e) listed within this charge question's prompt. The following paragraphs address additional points:

1. Incomplete curves. When assigning a "positive" hit in the competitive hrER-alpha binding assay, "There must be at least one data point at which 50% or more of the radioligand has been displaced" (Appendix K, p50; likewise in Appendix J, p53; ISR Figure 2 and Table 10). For reliable assignment of an IC50 or other SAR work, a curve with its final point just below 50% radioligand bound (as in ISR Figure 2) would be insufficient. However, given the note within the protocol, as in Appendix J, p51, that "The EDSP is less concerned with proving that the interaction is, specifically, one-site competitive binding, or with accurately characterizing the strength of the binding," then assigning a test chemical to be a "positive" binder in such a circumstance would be acceptable. This is also consistent with potential use of these assays in a Tier 1 screen, wherein high assay sensitivity would be desirable, even if it resulted in slightly more false positives. It may be worthwhile to include a brief description of this rationale within the ISR or similar summary document going forward.

2. For the CERI assay, active receptor concentration must be titrated during the saturation binding experiment such that 40 +/- 10% of the radioligand is specific-bound in the presence of 0.5 nM concentration of total radioligand (ISR p40). It appeared however, that the labs, except CERI, initially had trouble achieving this requirement (ISR p40, also Table 14). In practice, how hard will it be to consistently achieve these specific binding ratios when the assays are run on a larger scale?

4. Have the strengths and limitations of the assays been adequately addressed?

Key strengths and weaknesses of the assays have been addressed. At a high level, the strengths are that both assays are run on human recombinant ER, and are amenable to higher-throughput screening than current RUC ER assays. Additionally, assay hit-calling was reproducible across labs and consistent across FW and CERI methods. Limitations are: (1) the assays do not distinguish the functional direction of the ligand-ER interaction (agonist/antagonist); (2) the assays are cell-free so they cannot be expected to account for chemical metabolites; and (3) solubility. These limitations were acceptable, and generally outside the scope of the validation study.

Solubility concerns (limitation #3) were most relevant to the validation effort. In some cases, lack of solubility resulted in artefactual data points within dose-response curves at the highest chemical concentration(s) tested (e.g., 1e-3 M) and it could be difficult to determine chemical solubility limits (ISR p86). This particularly affected some weak or negative binders such as DBP, which yielded equivocal results when coded and tested up to 1e-3 M (ISR p76). By contrast, when used as a negative control in previous subtasks, it was only tested up to 1e-4 M, at which no solubility issue arose, and was thus correctly assigned as a non-binder.

The ISR presents several approaches to address equivocal dose-response curve occurrences, including constraining the bottom of such curves to zero (ISR p77; also see curve top/bottom issues in Appendix I, p4; vs. current unconstrained approach, e.g., Appendix K, p 48) and data point exclusion for "U-shaped" curves (ISR p29) following a "10% rule" (ISR p30, p34; Figure 1). These approaches were reasonable, and analyses were conducted with and without (e.g.,

Appendix F) the 10% rule to test it. More generally, these questions brought up the need for an "interim technical review" step (ISR p70, p77). Based on these observations and analysis, the best general solution would indeed be to formalize and incorporate an interim technical review step (which may be initiated, for instance, when one encounters an equivocal curve with certain predefined characteristics) into the analysis process.

5. Were the (a) test substances, (b) assay methodologies, and (c) statistical methods chosen appropriately to demonstrate the performance of the assay?

(a) The current test substance collection (Table 5) appears sufficient for this validation study, subject to a reasonable response to Clarification Question 5.a.1, below. However, going forward, as there are a "wide array of existing potential test chemicals" and variation in their outcomes (ISR p90), I would recommend prioritizing testing of additional chemicals with known ER-alpha activity, or those that might reasonably be expected to have it. As sources for such chemicals, the ICCVAM executive summary refers to 638 ER binders confirmed by more than one assay (Appendix B, p xxii), and publicly available databases might also facilitate this effort (see a.1-2, below).

Clarification Question 5.a.1: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Executive Summary report (Appendix B) states, "Proposed in vitro ER binding testing methods should be evaluated in validation studies using, **at a minimum, the 53 substances** listed…" followed by data generation on the remainder of a list of 78 substances (emphasis added, Appendix B, p xxiv). Was this recommendation not pragmatically feasible for the current validation study, which was run on approximately 30 chemicals? It is not clear from the CAB Report alone (Appendix D) why fewer than the ICCVAM recommended 53 substances were chosen for the validation subtasks, as the charge to the CAB was solely "to review and suggest modifications to the chemicals [already] nominated" (Appendix D, p 2).

The following section discusses public sources of known and possible ER binders, along with approaches to structural diversity analysis:

(a.1) Known ER-alpha binders. The current ChEMBL-17 public database (https://www.ebi.ac.uk/chembl/) annotates approx. 5,000 chemical-receptor interactions for human ER-alpha. These interactions are curated from the literature, and whereas they will thus not be of the same quality or certainty as those control and test chemicals selected by the Chemical Advisory Board (ISR p16) or the 638 ER binders from ICCVAM (Appendix B, p xxii), they nonetheless would allow for ongoing assay validation efforts across a more diverse selection of chemistry. Of these 5,000 interactions, some 205 unique chemicals have a reported Ki < 1 uM. A simple search on these 205 chemical structures for possible vendor sources via http://emolecules.com yields 134 purchasable known ER inhibitors that are readily available for testing.

(a.2) Weak or possible ER-alpha binders. The CAB suggested greater performance testing on compounds with weak or no ER activity (ISR p17; Appendix D p3). This remains the greatest weakness of the current chemical collection used in this validation effort, because the likely prevalence of ER-alpha binders across the space of medicinal and natural product chemistry is likely substantially lower than the prevalence of ER-alpha positive binders in the focused

chemical validation collection (Table 5).

Due to pragmatic considerations, I do not feel that this lack of weak and non-binders (compared to their "in the wild" prevalence) in the current validation collection should delay possible adoption of these assays for estrogen hormone disruption screening. However, if the assays are adopted, I recommend that known (see response a.1, above) and weak binders be prioritized for testing as follows:

- Using standard and well-accepted measures of chemical similarity (e.g., Pipeline Pilot (http://accelrys.com/products/pipeline-pilot/) ECFP4, ChemAxon (http://www.chemaxon.com/) ECFP4, or RDKit (http://www.rdkit.org/) ECFP4 fingerprints compared via Tanimoto coefficients), one could identify 500 or more readily purchasable chemicals with high similarity to the 134 purchasable known ER-alpha binders described in a.1. These could reasonably be expected to contain more binders or weak binders than would a random selection of purchasable or naturally occurring chemicals.
- Conversely, the chemical similarity metrics above could be used to **exclude** all chemicals with any appreciable similarity to the 200+ known ER-alpha binders mentioned in a.1 or listed in ISR Table 5. Using the more than 5 million purchasable chemicals readily available from vendors and freely coordinated by databases such as emolecules.com or zinc.docking.org, it would be feasible to select purchasable biologically-relevant chemicals that would be expected to not to bind to ER-alpha (i.e., "expected negatives").
- Finally, these standard chemical similarity techniques could be used to assess the chemical diversity of chemicals already tested, or to prioritize for new chemicals that would most rapidly increase this diversity. Other diversity methods to consider could include general physiochemical properties and predicted solubility. As noted in the CAB Report, "Chemicals with known affinity for ERa were given preference over chemicals that were structurally diverse but with no ER binding history" (Appendix D p2), and while this is sensible, it implies a possible lack of structural diversity that could be addressed through ongoing analysis.

In summary for part (a), the current ~30 chemicals tested in subtasks 1-4 are pragmatically sufficient for this validation effort, but ongoing analysis should be continued if the assays are adopted. One good way to perform this ongoing analysis would be to prioritize chemicals known to have, or expected **not** to have, ER-alpha binding interactions, by drawing on the extensive datasets now publicly available.

(b) Assay methods were appropriate. Key concerns were chemical solubility (see charge question 4) and ER stock solution stability (ISR p48), degradation (ISR p47), and evaluation (ISR p21). These concerns were addressed within the report, and the saturation-binding step in particular is meant to decrease uncertainty from differences in ER stock solution. The [unconfirmed] possibility of an error in stock solution in the Lovelace lab (ISR p65) suggests that even with acceptable saturation-binding test protocol (ISR p48), ongoing diligence as to quality of stock solution is merited.

The potential for promiscuous small-molecule inhibition of ER activity was not discussed, but as this activity has not been fully explored for nuclear hormone receptors in general, and as curve acceptability evaluation protocols already flag particularly "steep" Hill slopes (e.g., ISR

p50), this omission is acceptable.

(c) The statistical methods (including independent analysis) were chosen appropriately, and questions arising such as the fairness of excluding runs based on subjectively determined acceptability of their control curves (ISR p70, p77) were explored. Pooled statistics quantified by r^2 were appropriate, although the statistical report did note difficulty in determining reliable IC50's from several dose-response curves lacking expected top and bottom values (Appendix I, p4) within the allotted time frame.

6. Considering the variability inherent in biological and chemical test methods, were the results obtained sufficiently repeatable and reproducible?

The results were sufficiently repeatable and reproducible. Within labs the results were stable to repetition (e.g., Tables 23 and 27), although one of the labs, Lovelace, experienced the greatest degree of variation (e.g., ISR p50, CERI subtask 1; Tables 23-25; FW subtask 1, Tables 27 & 29) and this was borne out in subsequent r^2 analysis of pooled data (Figures 14 and 16).

Results within labs were averaged across multiple wells and runs into a single binding score (Table 11). Score-based binding classifications were also consistent across assays approaches (Table 31) and labs, except for Lovelace (e.g., Table 32; ISR p65; Table 33). This may have been due to an error in Lovelace stock solution as per discussion on ISR p65 and Figure 7-8.

The peformance results above applied to subtasks 1 and 2 (assays on control and uncoded chemicals). For subtask 3 (coded chemicals), classification errors arose slightly more along chemical rather than lab lines, as with DBP, as previously discussed (Tables 34 & 35).

When comparing results across labs, analysis was done with and without subjective-review-based exclusion of "unacceptable" (ISR p30, p34, p77) dose response curves. In a conservative retrospective analysis without subjective review of result acceptability (but respecting a lab's assignment of unacceptability, if present; ISR p78), all labs showed good or acceptable r^2, although Lovelace and U Konstanz had higher false positive rates (ISR p82, Figures 15 & 17).

Given that the goal of the assays is to identify possible estrogen hormone disruptors in a 'first pass' prior to more extensive testing, a slightly higher than desired false positive rate (as observed) is preferable over, for instance, a predilection toward false negatives (which generally did not occur). Lovelace was consistently most susceptible to false negatives (ISR p80), but as this was the lab showing the greatest variability overall and as per previous discussion, this may have been a lab-specific issue.

7. With respect to performance measures, are the measures of assay performance for the saturation and competitive binding experiments appropriate? Should the tolerance bounds developed for the reference estrogen and/or two weak binders be used to ensure proper performance of the assays?

The measures of saturation and competitive binding experiment performance for the assays are appropriate, including analysis of non-concurrent run consistency intra-lab, and consistency across labs and FW and CERI assay approaches. Manual review of curve

acceptability (e.g., ISR p30, p45) introduces possible subjectivity, but decision criteria are clear and subsequent analysis excluding this step remained consistent.

The tolerance bounds developed for the control chemicals could be used to flag assay runs for interim technical review of the entire run and/or repeat testing where confirmed by manual supervision. The particular criteria of their derivation, set to account for 80% of the population at 95% confidence (ISR p90), seem reasonable, but could be modified to enhance their use in a decision-support capability if warranted empirically. The ultimate goal may be to remove human supervision or review from all but the most equivocal cases, but this extra effort (i.e., human review of out-of-tolerance-bound 'flagged' results) would seem to be justified during the initial adoption of the assays.

8. Are the data interpretation criteria clear, comprehensive, and consistent with the stated purpose of identifying compounds as binders and non-binders for the estrogen receptor?

Data interpretation criteria for ER-binder identification of compounds are clear. These criteria include analysis of dose-response curve fit (ISR Figure 2) and data point artifact cleanup (ISR Figure 1; also see charge question 4), along with a scoring system for hit-calling and multiple-run scoring consensus (ISR Tables 10-11).

There is some discussion of manual (and therefore potentially subjective) techniques used to determine "acceptable" curves for known controls (ISR p30, Table 9), but this did not negatively affect accuracy or repeatability when compared to subsequent statistical post-analysis (see charge question 6). Combined with or replaced by an explicit "interim technical review" for equivocal or weak curves (see charge question 4), some degree of human supervision of curve acceptability would remain reasonable.

9. Please comment on the overall utility of the assay as a screening tool as described in the introduction of the ISR to be used by the U.S. EPA to identify chemicals that have the potential to interact with the endocrine system.

Overall, the FW and CERI assays using hrERa are sufficiently accurate, repeatable, clear, and consistent with existing RUC ER and the existing ER binding guideline OPPTS 890.1250 (ISR p100, Table 40) to be used as a screening tool as described in the introduction to the ISR. They can be performed at higher throughput than assays relying on ER derived from animal tissues, allow for greater standardization than cytosolic preparations, and would be expected to be as or more relevant to human toxicology than are ligand-receptor interaction assays using animal-tissue derived ER.

As with any new assay system, the binding curve and result data should continue to be compiled if these assays are adopted for broader screening use, and subjected to periodic or ongoing statistical analysis as in ISR section 4 (ISR p77-82); also see charge question 5.a. This would allow continued evaluation of the techniques such as the 10% rule and/or other corrections (e.g., to address solubility) incorporated within a technical review step. Likewise, it would increase the chances of identifying any artifacts specific to new chemicals or chemical categories not yet represented in the collection that comprises the current validation subtasks.

CHARGE TO PEER REVIEWERS
for
INDEPENDENT PEER REVIEW OF THE VALIDATION OF TWO HUMAN RECOMBINANT ESTROGEN RECEPTOR BINDING ASSAYS AS POTENTIAL SCREENING ASSAYS IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY
September 30, 2013

Charge Questions:
Please respond to each of the following questions:

1. **Is the stated purpose of the assays clear?** Somewhat. There appear to be three purposes mentioned on page 9 of the ISR:
   1) Determine if the assay can identify chemicals that compete with the endogenous hER receptor
   2) Provide an updated alternative to the current rat uterine cytosol assay (ER-RUC, OSCPP 890.1250) and
   3) Permit a higher through-put capability

   Although related, these three purposes are distinct and should be considered separately. The first purpose has been addressed extensively in the ISR and appendicies; the last two are not explicitly evaluated.

2. **Are the assays biologically and toxicologically relevant to the stated purpose?** These assays are biologically relevant; as they measure a discrete event that is known to influence numerous physiological responses. The true toxicological relevance is difficult to ascertain from these assays when used in isolation, and would require an evaluation of the relationship between binding and adverse health effects. However, in the context of the EDSP Tier 1 screening battery, which is aimed at identifying potential hazard, these assays do serve their intended purpose and are therefore "toxicologically" relevant in that context.

3. **Do the protocols describe the methodology of the assays in a clear and concise manner so that a laboratory can:**
   a. **comprehend the objective;**
   b. **conduct the assay;**
   c. **observe and measure the prescribed endpoints;**
   d. **compile and prepare data for statistical analyses,and**
   e. **report the results?**

   The answer to all of the above is "yes, to a large extend" but some general improvements could be made, see additional comments below.

   What additional advice, if any, can be given regarding the conduct of the protocols?

   Both of the protocols are dated 2008 and should be updated to reflect learning's gained from the validation study. For instance, Octyltriethoxysilane or Atrizine should be listed as the negative (non-binding) control in both protocols and updated statistical analysis techniques should be included. Also consider adding a list of proficiency chemicals that should be run by any laboratory that is naive to the test method (this is separate from the list of concurrently run controls). Ideally, these should be run in a blinded manner, although the practical challenges of acquiring and managing coded chemicals is acknowledged. If the Lovelace lab were to perform the assays today, what qualification guidance/criteria are in place to ensure that the lab is capable of running the assays – running concurrent controls is most likely not sufficient, as labs seem to become proficient at running known chemicals and then struggle with unknowns.

A standard reporting format for both assays should be identified and published. It is not clear whether or not Assay Performance Criteria set forth in OSCPP 890.1250 would be applied to these assays. Also, clear guidance needs to be provided regarding the interpretation of the results. Although the ISR did a very good job of describing the challenges face with data interpretation (i.e., problems with 4-parameter fit and 10% rule), the method of data analysis intended to be used in updated test guideline is not explicitly stated.

4. **Have the strengths and limitations of the assays been adequately addressed?**
The limitations of the assay were provided in Table 41. These 4 bulleted points do not provide a comprehensive assessment of the technical and biological limitations of the assay, and should continue to be developed as the assay becomes more widely utilized and more data are available. For example, no assessment is made regarding the difference in performance of the full vs partial receptor, which could prove to be extremely important when assessing the binding of certain classes of chemicals (a very clear difference can be noted in the Tox21 full vs partial ER TA assay data). Likewise, no attempt was made to define an applicability domain of chemical space for which these assays should not be used (i.e. detergents/surfactants could produce false positive results).

5. **Were the (a) test substances, (b) assay methodologies, and (c) statistical methods chosen appropriately to demonstrate the performance of the assay?** The use of DBT as a non-binding standard was perhaps not the best choice, in hindsight. The non-binding control should change to Octyltriethoxysilane or Atrizine in the revised protocols. The classification of several test substances was changed during the validation study, and the justification for these changes was well described and referenced. Statistical methods were also modified (i.e. 10% rule), and likewise well justified.

6. **Considering the variability inherent in biological and chemical test methods, were the results obtained sufficiently repeatable and reproducible?** One of the best techniques to assess the performance of any assay is to compare the new assay with an accepted (preferably validated) existing assay. This could easily have been done in the ISR by comparing the results (repeatability and reproducibility) of the hER assays with the current ER-RUC assay. Such a comparison would reduce the subjectivity of this assessment and provide a quantitative assessment regarding the relative performance of the assays (specifically, are the new assays less variable than the existing assay). Regardless of lack of this type of assessment, the assays do appear to have sufficient performance for their intended use.

7. **With respect to performance measures, are the measures of assay performance for the saturation and competitive binding experiments appropriate? Should the tolerance bounds developed for the reference estrogen and/or two weak binders be used to ensure proper performance of the assays?** See comments above (#6) re: comparison to existing ER-RUC method. Yes, tolerance bounds should be provided as guidance (not strict cut-offs) to ensure the proper performance of the assays.

8. **Are the data interpretation criteria clear, comprehensive, and consistent with the stated purpose of identifying compounds as binders and non-binders for the estrogen receptor?** The rational and use of data interpretation criteria were clearly outlined in the ISR for evaluating study data. However, it was not immediately clear how this guidance would be conveyed in the new (?) protocols or test guidelines.

9. **Please comment on the overall utility of the assay as a screening tool as described in the introduction of the ISR to be used by the U.S. EPA to identify chemicals that have the potential to interact with the endocrine system.** I believe the most relevant question is

whether or not these tests meet the performance criteria to allow their use as part of the EPA Tie 1 Screening battery. This question would most easily be address by directly comparing the performance of the new methods with that of the existing ER-RUC assay. Doing so would allow for an objective assessment as to whether the performance of these test was equal to, or better than, the existing ER-RUC method. In either of these cases, the new methods would be judged as meeting the needs of the EPA Tier 1 Screening battery. If the performance of these methods is worse than the existing method, then other factors must be taken into consideration. Consequently, the performance of the three methods (CERI, FW, ER-RUC) should be directly compared using existing data.

CHARGE TO PEER REVIEWERS

INDEPENDENT PEER REVIEW OF THE VALIDATION OF TWO HUMAN RECOMBINANT ESTROGEN RECEPTOR BINDING ASSAYS AS POTENTIAL SCREENING ASSAYS IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY
September 30, 2013

Charge Questions:
Please respond to each of the following questions:

Completed 11/25/2013

1. Is the stated purpose of the assays clear?

The stated purpose of the assay is clear. Section 1A of the ISR provides a comprehensive summary of the overall intent for conducting the hER binding assay in relation to the U.S. EPA Tier I Screening Battery and the practical use of in vitro assays for screening purposes as described by ICCVAM. The anticipated goal of validation process is stated with the intent to "establish a performance based test guideline for ER binding in collaboration with OECD". Part B of the ISR provides context for use of the hER assay as a component of the Tier 1 EDSP focused on identifying potential chemicals that may interact with estrogen, androgen or thyroid pathways. The ISR clearly states that the hER assay as described, is intended to replace OCSPP 890.1250 Rat Uterine cytosol ER binding assay for "E" and "anti E" assessments.

2. Are the assays biologically and toxicologically relevant to the stated purpose?

Assays described in the ISR, i.e. FW and CERI human recombinant estrogen receptor binding assays, both meet the criteria for biological and toxicological relevance. This statement is based upon the fact that the stated aims of the described assays are to identify chemical agents that "interact" with the estrogen receptor alpha protein, and that human estrogen receptor is the stated biological target for both the FW and CERI assays. Concordantly, in terms of toxicological relevance, the estrogen receptor alpha is documented to exhibit broad ligand fidelity (i.e. is promiscuous) and is a well-established target for receptor-chemical interactions associated with estrogenic and anti-estrogenic biological responses across taxa. Utilization of the FW and CERI assays as a tier one screen and as a replacement of OCSPP 890.1250 Rat Uterine cytosol ER binding assay, provide a direct means to assess for chemical interaction between exogenous xenobiotic compounds that putatively function as an E or anti E ligands for hER alpha.

3. Do the protocols describe the methodology of the assays in a clear and concise manner so that a laboratory can: a. comprehend the objective; b. conduct the assay; c. observe and measure the prescribed endpoints; d. compile and prepare data for statistical analyses, and e. report the results?

Based upon data derived and reported within the ISR, the inner- and intra-laboratory trials for both the CERI and FW assays appear to meet the above objectives. Review of individual protocols for CERI and FW assays also appear to provide adequate descriptions for: comprehension of the objectives of the assay, how to conduct the assay, observe and measure prescribed endpoints, and compile and review data. It is not apparent, however, how information is provided for calculating assay outcomes other than compiling data in a provided data work sheet (Appendix J references Appendix D, but this does not appear to be a worksheet in the test report (section 10.5)). Parameters for data analysis are provided, including use of nonlinear curve fitting software; use of a one site competitive binding model; unconstrained top, bottom, and slope when fitting the curve; and robust regression when determining the best fit. Also mentioned is the application of the "10% rule" and determination of RBA. While this information is essential to for data calculation and interpretation, it may be prudent to provide a blueprint on data analysis to ensure a consistent means of assessing assay performance, assay precision and overall OA/QC for the data analysis portion of the assay. This will be an especially important parameter as multiple laboratories become involved in conducting the assay, and as slight modifications in data analysis may lead to erroneous and inconsistent ER binding results. Additionally, it appears from the ISR that final data analysis from each of the validation studies was compiled by an outside-independent contractor (Battelle, Appendix E) and summarized in a "Final Report on the Saturation Binding Analysis for Human Recombinant ER alpha (hrERa)". Thus, there is some question as to whether the data analysis component for both the CERI and FW assays has been "validated" and whether sufficient guidance is in place for independent laboratories to thoroughly conduct the data analysis component of the assay.

What additional advice, if any, can be given regarding the conduct of the protocols?

In review of the CERI and FW assays protocols, it appears that there are multiple points in the progression of the assay protocol where subjective and/or objective judgments are required to further the test run. These include assessments of performance for both saturation binding and competitive binding in an effort to establish general guidance for laboratories when evaluating success of each test run. Given that subsequent progress of the assay may hinge upon multiple performance criteria (See pg 27 ISR), it may be prudent to establish a decision tree for each component of the assay. For example, a decision tree may facilitate guidance to ensure compliance with each of four criteria within the saturation binding component of the assay. Only after demonstrating this compliance should the competitive binding assay be conducted. A similar decision tree may be conducted for completion of the competitive binding component of the assay (five criteria) and then for data analysis. This last component of the assay may be most important and provide proper guidance when utilization/application of various data manipulation tools are necessary, such as application of the 10% rule, 4 parameter curve fit, single binding model, ligand dilution model, etc. On what basis/criteria will independent laboratories know when to apply these data manipulation techniques? How will differential use of these applications be assessed to establish overall concordance of assay performance across laboratories?

4. Have the strengths and limitations of the assays been adequately addressed?

Page 97 of the ISR provides a list of overall strengths and weaknesses of the CERI and FW assays. Strengths and weaknesses are accurate and appropriate. A few additional strengths and weaknesses are noted.

Stated strengths include:

1. Relevance of hER assay to biological functions
2. Historical value of in vitro ER assays for use to identify putative endocrine disrupting compounds
3. Replacement assay for cytosolic sources of mammalian estrogen receptor
4. Ability to cover a broad range of potencies
5. Update alternative to OPPTS 890.1250

Additional strengths that may be added:

1. Toxicological relevance of ER as a molecular target i.e. demonstrated molecular initiating event in numerous EDC studies (see question 2)
2. Substitution of human ER for animal sources of cytosolic ER. Thus, no further need for cross species extrapolation and elimination of complications due to different ligand specificities between species.

Stated weaknesses:

1. Assays limited to assessment of chemicals that interact with ER and do not distinguish between ER agonists and ER antagonists
2. NO metabolic activation of test compounds
3. Concentration range of test compound limited by solubility in EtOH or DMSO
4. Use of radioactive materials

Additional weaknesses that may be added:

1. Assessment of assay positive and negative control performance (i.e. acceptable or unacceptable) for individual runs appears to be based upon a subjective evaluation. Reductions in subjective determinations may further improve assay reproducibility, reliability and transferability (see pg 30 lines 23-34)
2. Necessity for further guidance on data interpretation.
3. Potential use of decision trees for modular completion of assay components.
4. Defined QA/QC parameters for assay concordance across laboratories.
5. Compliance with validation criteria may be skewed. See criteria # 4 pg 101 and pg 33 "Within test, intra-laboratory and inter-laboratory reproducibility and how these parameters vary with time should be evaluated"… Reproducibility of the assay is evaluated on the "average classification of a compound (i.e. binder, non-binder, equivocal). This language may be misleading as individual runs within laboratories and overall scores within and between laboratories are not in full agreement. Here the statement "Overall average classification" may be misleading
6. Criteria # 6 pg 101, "data should be obtained in accordance with Good Laboratory Practices. There is no mention in the ISR that any of the participating laboratories engaged in the validation studies have been GLP certified. As such language within the ISR states, "laboratory work was conducted ***in the spirit of*** conformance with GLP".

5. Were the (a) test substances, (b) assay methodologies, and (c) statistical methods chosen appropriately to demonstrate the performance of the assay?

All test substances were selected by the SMT and VMG-NA and approved by the Chemical Advisory Board (CAB). Appendix D provides a summary of the Chemical Approval Board (CAB) Report. The committee was comprised of Dr. Taisen Iguchi, Okazaki Institute for Integrative Bioscience, NIBB; Dr. William Kelce, Pozen Pharmaceuticals; Dr. Weida Tong, NCTR, US FDA. Test substances were selected to represent multiple chemical classes, a range of potencies and positive and negative controls based on historical knowledge as ER binders and non-ER binders (see ICCVAM reports referenced on page 16 of the ISR). Chemicals were assigned to defined tasks to facilitate the validation process (see pg 18 of the ISR). Within the validation study several compounds proved to be equivocal i.e. neither a binder or a non-binder, specifically weak and non-binders demonstrated ER interactions at higher concentrations. Overall selection of compounds proved to be effective at discriminating function of the both the CERI and FW assays. Selected positives controls (based on historical data) demonstrated the assays were specific, sensitive and capable of discriminating between receptor binders and non-binders. Equally, putative non-binders demonstrated reasonable selectivity of the assay. Assay performance appeared to have the most trouble with moderate or weak binders. Inclusion of several compounds with moderate to weak ER binding (based again on historic data) proved to test assay performance and function, and ultimately the ability of both assays to discriminate weak ER ligands from non-binders. Solubility limits of individual compounds also impacted test results (see below) suggesting that future trails should be conducted with as broad a concentration range possible in addition to evaluation of other carrying agents on assay performance to circumvent solubility issues. As such, use of DBP-TC at 10-3 M resulted in an ambiguous curve fit and an equivocal classification for a compound that was initially utilized as a negative control. Assessments of an additional select set of compounds (Subtask 4) was conducted to identify alternate “weak” binders and non binders that could be used as positive/negative controls and evaluate both reproducibility and accuracy of the CERI and FW assays. These trials were useful in that they further solidified impacts of chemical solubility on chemical classification, identified Norethindrone as a weak binder and plausible control, and provided further examples of the necessity to apply the 10% rule. Combined, it appears that further evaluation of chemical solubility limits for all compounds tested will needed to be conducted in order to maintain assay reliability.

6. Considering the variability inherent in biological and chemical test methods, were the results obtained sufficiently repeatable and reproducible?

Overall, laboratories demonstrated proficiency in conducting both the CERI and FW assays. Intra-laboratory test runs for both saturation binding and competition components of the assays appeared reproducible and within a reliable standard of biological test variation. Comparisons across laboratories i.e. inter-laboratory trials also exhibited reasonable variability, although this data was inherently more variable than intra-laboratory runs. In both CERI and FW saturation assays Kd values varied significantly for one or more laboratories, and Bmax values were not consistent, suggesting laboratories were not working with similar receptor concentrations. While not inherently fatal in evaluating Kd determinations receptor concentration differences may ultimately impact data outcomes especially if ligand dilution models are applied. However considering SD values for estimates of Kd and Bmax results across most laboratories produced similar outcomes with exception of Bayer (CERI assay) and U Konstanz (FW assay). With competition studies, inter-laboratory comparisons identified numerous difficulties with assay performance, including deviations in top values, steep/shallow slope values and curve fitting artifacts. Application of data manipulation tools were necessary to generate consistency across both individual runs and between laboratories for both uncoded and coded compounds (tasks 1-3), suggesting that inter-laboratory trials were not uniformly consistent. Individual laboratories additionally exhibited varying degrees of difficulty in conducting both CERI and FW assays—specifically the Loveless laboratory, which reported difficulties with 17β-estradiol, indicating either problems with assay transferability or technical proficiency with this particular lab. Additionally, several laboratories appeared to have difficulties with assay performance due to issues with chemical concentration, and solubility. In particular, these issues complicated classification of compounds as non-binders such as Di-n-butyl phthalate and heptylphenol. These compounds exemplify difficulties in classifying weak binders. In cases where a complete binding curve is not produced/available, data manipulation tools may be applied to facilitate interpretation. Use of such manipulations should only be conducted with specific guidance established in the assay protocol. This is alluded to on page 77 of the ISR with the need to establish additional step-wise technical review of the data. However, this point is not mentioned again throughout the ISR, indicating the process (i.e. step-wise technical review) has not been established. This will likely be essential for subsequent chemical evaluations as most compounds tested are likely to fall into the weak or non-binder category. If sufficient rigor is not included into either the assay itself or data interpretation/manipulation process, then erroneous results are likely to ensue.

7. With respect to performance measures, are the measures of assay performance for the saturation and competitive binding experiments appropriate? Should the tolerance bounds developed for the reference estrogen and/or two weak binders be used to ensure proper performance of the assays?

As stated previously, there remains a fair degree of subjective interpretation for assessments of assay performance. For example, Table 9 (ISR pg 27) provides multiple measures of required assay performance for both the saturation and competitive binding components of the assay. Review of saturation binding measures includes four specific parameters to assess performance. While most of these criteria appear to be met, there is significant variability between intra- and inter-laboratory trials. For instance, laboratories report varying ranges for measure 2, with some laboratories not achieving the measured mark of 40% binding at 0.5 nM radioligand in the CERI assay (table 15; ISR) and 20% binding at 1 nM radioligand in the FW assay (table 18; ISR). These discrepancies appear to be associated with reported differences in Kd and Bmax values.

Adherence to performance criteria for competitive binding also suggests difficulties in conducting the assay, including the use of DBP as a negative control, solubility and variance of IC50 values. Additionally, it appears that throughout the validation process i.e. intra- and inter-laboratory trials, that performance evaluations for competitive binding experiments with coded or uncoded compounds were based entirely upon a subjective determination of the behavior of positive and negative controls. Assessments of PRISM fit parameters Top, Bottom, slope values and curve fit for control substances were subjectively evaluated to discern overall assay performance. Concern regarding this decision process is mentioned on page 77 of the ISR in reference to the need to identify acceptable or unacceptable data limits and establish performance criteria and tolerance bounds. The development of tolerance bounds for CERI and FW assays is essential to ensure assay consistency and provide a measure of QA/QC for assay performance. The approach taken (ISR pg 90-95) to assess tolerance bounds provides an adequate measure of assay performance based upon validation trials and defined a set of tolerance limits for subsequent applications. Defined criteria including Hillslope, logIC50, Syx and log10RBA will serve as an essential guide to ensure assay performance across laboratories and within replicate runs (see table 38). Again, however, specific guidance needs to be provided when data manipulation applications are required. For instance, even when establishing tolerance limits, the 10% rule was applied to assessments of norethindrone. Subsequent consolidation of mixed effects assessment of variance, within run variance, between run variance, and between laboratory variance appears sufficient to estimate tolerance bounds. Again, without defined guidance on when and how to apply data manipulation tools (i.e curve fit parameters/adjustments), erroneous results may ensue.

8. Are the data interpretation criteria clear, comprehensive, and consistent with the stated purpose of identifying compounds as binders and non-binders for the estrogen receptor?

Criteria for assessing classification based upon test chemical binding curves can be found on pg 31 of the ISR section 10.4.4 of Appendix J for the CERI assay and section 10.6.4 Apendix K for the FW assay. In each description criteria for classification of a chemical as either a binder, non-binder or equivocal is clear, comprehensive and consistent. As stated previously, however, interpretation of raw data may be more ambiguous, specifically with regard as to when to apply data manipulation tools. As demonstrated throughout the ISR, slight modifications of raw data with data manipulation approaches i.e. curve fit parameters, may significantly alter assay outcome and thus overall chemical classification. Strict adherence to defined guidelines will help facilitate consistent applications of data manipulation and thus consistency of chemical classification.

9. Please comment on the overall utility of the assay as a screening tool as described in the introduction of the ISR to be used by the U.S. EPA to identify chemicals that have the potential to interact with the endocrine system.

As per ICCVAM validation criteria, both the CERI and FW hER alpha binding assays provide a scientific and regulatory rationale for the test method. The ISR and both assay protocols provide a clear statement of the intended purpose of the assay and clearly define biological and toxicological relevance with regards to EDC toxicity. Additionally, as per ICCVAM validation criteria, the ISR illustrates test method performance through evaluation of a series of reference chemicals, both uncoded and coded to exclude test bias. Chemicals were selected as described in the ISR and in response to Q5 above. Reliability is defined by ICCVAM "as the reproducibility of results from an assay within and between laboratories." Overall, both CERI and FW assay performance proved to be predictive with accurate classification of most coded and uncoded test substrates. Individual assay trials within laboratories were reproducible and thus produced reliable outcome in chemical classifications. More variability was observed between laboratories, suggesting that there may be moderate issues with transferability. Conversely, most laboratories were capable of conducting the assays with predicted outcomes of chemical classification. Laboratories appeared to have the greatest difficulties with weak estrogen receptor binders. Thus, several data manipulation tools were applied with the stated aims of facilitating data interpretation. Once applied, overall outcomes across laboratories were significantly predictive, suggesting that the assays are robust as designed. That is to say that the assays maintain the ability to effectively perform under varied conditions. The caveat to this statement, however, is the need to have in place strict guidance criteria for assay performance and data manipulation.

Additionally, there remain unresolved questions following review of the ISR. Specifically which of the assays will be implemented for screening hER interaction? On what basis will one assay be chosen over the other or will both assays be utilized? Secondly the source of hER is not mentioned for subsequent testing. Will the EPA continue to provide recombinant hER to contract facilities, or will individual facilities produce and purify their own? If the latter, how will QA/QC be established for this component of the assay? Also, since an outside independent laboratory conducted most of the data analysis, how will this component of the assay be validated? It may be prudent to have independent laboratories conduct a ring test on various data sets to ensure that proper guidance is provided for data analysis, interpretation, and ultimately chemical classification.

Appendix N

Data supporting the assessment of reliability and accuracy of the FW and CERI in vitro hrER binding assays in the validation studies

(as determined in Annex 2 of the Performance Standards)

Table of content

PART 1: Introduction

1. Performance Standards (PS) (1) have been developed in parallel to the development of the Performance Based Test Guideline (PBTG) for hrER in vitro assays to detect chemicals with ER binding affinity (2). The purpose of the PS is to communicate the basis by which new proprietary (i.e. copyrighted, trademarked, registered) or non-proprietary test methods can be determined to have sufficient accuracy (i.e., agreement between a test method result and an accepted reference value) and reliability (i.e., extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol) for a specific testing purpose.

2. These PS include a summary of the reliability and accuracy values obtained during the validation studies for the FW and CERI In vitro hrER Binding Assays (Annex 2 of the PS). Data/information from validation study reports and the ISR have been collated and reviewed to calculate the validated assays reliability and accuracy values. The objective of this annex is to explain the basis of which data these values have been calculated on.

Selection of laboratories

3. In line with the peer review comments (appendix M), the data from Lovelace laboratory were not included in the analysis, as well as those from the University of Konstanz laboratory, which conducted the assay with the FW protocol only. The 4 selected laboratories for the assessment were thus CeeTox, CERI, Bayer and University of Missouri. These laboratories completed at least 3 runs for the test substances using both the FW and CERI assays.

Selection of runs

4. The number of test chemicals used to assess within (intra-) laboratory replication over time was limited during the initial analysis since some labs did not have 3 *qualifying* runs for some of the test chemicals. As described in the ISR (Section III, Data Analysis and Statistical Analysis, and Appendixes F, H), each laboratory submitted a minimum of 3 independent runs for each test chemical with 3 technical replicates at each concentrations within a run. Each independent run was tested on a different date, and included a full concentration curve for each of the assay controls (E2, norethynodrel (NE), and di-n-butyl-phthalate (DBP)). During the original standardized data analysis conducted by an independent contractor, each run was subjected to qualifying criteria that included (i) the designation as a good run by the submitting laboratory, (ii) a review of the results from the PRISM

model fit curves for the positive controls (E2 and NE) and the subjective judgment as an acceptable curve based upon the top, bottom, slope, residual variability, and logIC50 for the curve, and (iii) for any given run, both E2 and NE control curves were required to be acceptable. Runs that did not meet all qualifying criteria were excluded from the initial analysis included in the ISR. With several runs being excluded, the assessment of intra- laboratory reproducibility was made impossible (because of a too limited number of test chemicals with 3 runs) for one laboratory and a reduced number of test chemicals with 3 runs for several other laboratories that could be used to develop the PS. Excluded runs (see Appendix C, PRISM Fit data for all accepted and excluded runs) had been typically excluded because one or in a few cases, both positive control(s) (E2 and/or NE control) for a particular run were considered unacceptable based on the top or bottom estimates for the curve.

5. However, a preliminary reanalyse of the excluded data showed that the criteria for accepting the control curves were quite stringent, and that several sets of data for the test chemicals might need to be re-included in the analysis for the PS.

6. The excluded runs were thus re-analysed further, in a consistent manner, and intra and inter-laboratory reproducibility for the CERI and FW protocols were re-assessed based on these potentially new data. Tables PS-5 and PS-6 in Part 3 of this document contain all runs for the controls and test chemicals that were submitted from the 4 laboratories (Ceetox, Bayer, CERI, U.Missouri) which were (i) acceptable by the submitting laboratory, and (ii) contained data for at least 6 concentrations of the test chemical. The data that have been included during the re-analysis have been highlighted in yellow. In addition, Part 4 provides the control (E2, NE) curves for all the runs (including those excluded in the original data analysis) in subtasks 1, 2 and 3, for the 4 laboratories, for the 2 protocols.

7. These curves and data were reviewed by the VMG NA at their meeting on 2-4 December 2014 (Paris, France). The group supported the approach used for the analysis and development of the Performance Standards. As a consequence, it was agreed to move away from the use of the set of tolerance bounds that had been originally proposed in the ISR for use as acceptability criteria for the two assays included in the PBTG (see ISR, Section VI, B-5, and Appendix F) . Rather, the expert group recommended a recalculation of the mean ± STD and 95% confidence intervals for the curve fit estimates using all of the runs for the reference estrogen and control weak binder from the four laboratories that participated during each phase of the validation study and provided data for at least 3 runs/test substance. The revised assay acceptability criteria has been based upon the newly developed 95% confidence intervals using the larger, more inclusive, set of competitive binding curves for the control substances (see Tables PS-1 and PS-2).

References

(1) OECD (2015), *Performance Standards For Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity*, Series on Testing and Assessment No. 222, OECD, Paris.

(2) OECD (2015), *Test No. 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris.

PART 2 - TABLE PS-1. (CERI Assay), Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable (E2/NE)	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	Ceetox	st1	20090207	A/A	E2	2.84	107.00	-8.78	-1.18	2.36	2.86	0.04	0.13	20	0.984	5.78	24
2	Ceetox	st1	20090208	A/A	E2	2.85	101.40	-8.77	-1.16	2.58	3.03	0.04	0.15	20	0.980	6.22	24
3	Ceetox	st1	20090211	A/A	E2	3.41	109.50	-8.74	-1.24	2.52	2.94	0.04	0.15	20	0.983	6.22	24
4	Ceetox	st2	20090215	A/A	E2	2.45	110.90	-8.73	-1.18	2.35	2.77	0.03	0.13	20	0.986	5.71	24
5	Ceetox	st2	20090218	U/U	E2	-1.48	127.40	-8.62	-0.69	6.41	9.44	0.10	0.15	17	0.953	11.70	21
6	Ceetox	st2	20090221	A/A	E2	1.28	115.10	-8.69	-1.09	3.20	3.82	0.05	0.15	20	0.977	7.53	24
7	Ceetox	st3	20090228B	A/U	E2	2.01	119.90	-8.72	-1.01	3.44	4.43	0.05	0.14	20	0.975	8.00	24
8	Ceetox	st3	20090305A	A/U	E2	0.07	125.50	-8.57	-1.25	3.02	3.27	0.04	0.15	20	0.984	7.28	24
9	Ceetox	st3	20090310A	A/A	E2	1.87	111.20	-8.64	-1.31	2.54	2.73	0.03	0.15	20	0.984	6.22	24
10	Ceetox	st3	20090401A	A/U	E2	3.77	122.40	-8.61	-1.13	3.48	4.04	0.05	0.16	20	0.975	8.25	24
11	Ceetox	st3	20090415	A/A	E2	0.93	105.60	-9.55	-1.60	1.43	2.84	0.02	0.16	20	0.988	4.70	24
12	Ceetox	st4	20090903A	A/A	E2	1.73	113.90	-8.35	-1.58	3.21	2.74	0.04	0.23	20	0.979	7.67	24
13	Ceetox	st4	20090905A	A/A	E2	2.84	116.10	-8.65	-1.30	2.69	2.99	0.04	0.16	20	0.983	6.64	24
14	Ceetox	st4	20090907A	A/A	E2	5.99	176.80	-8.41	-1.37	5.80	6.27	0.07	0.24	20	0.967	14.49	24
15	Ceetox	st4	20090930A	A/A	E2	9.64	106.40	-8.39	-1.76	4.07	3.51	0.05	0.41	20	0.954	10.00	24
16	Freyberger	st2	B	A/A	E2	-0.49	101.00	-9.00	-1.09	0.54	0.74	0.01	0.03	20	0.999	1.33	24
17	Freyberger	st2	C	A/A	E2	-0.53	99.55	-9.02	-1.10	0.53	0.74	0.01	0.03	20	0.999	1.32	24
18	Freyberger	st2	D	A/A	E2	-0.26	99.27	-9.02	-1.07	0.53	0.74	0.01	0.03	20	0.999	1.31	24
19	Freyberger	st2	E	A/A	E2	-1.39	102.70	-9.03	-1.13	0.66	0.92	0.01	0.04	20	0.999	1.66	24
20	Freyberger	st2	F	A/A	E2	-0.61	102.80	-9.10	-1.16	0.67	0.99	0.01	0.04	20	0.998	1.76	24
21	Freyberger	st2	G	A/A	E2	0.60	88.15	-8.95	-1.59	1.33	1.52	0.02	0.14	20	0.993	3.55	24
22	Freyberger	st3	CODE A1	A/U	E2	0.27	95.85	-9.09	-1.12	1.13	1.61	0.02	0.07	20	0.995	2.87	24
23	Freyberger	st3	CODE A2	A/A	E2	-0.56	100.80	-9.09	-1.04	0.98	1.46	0.02	0.05	20	0.997	2.44	24
24	Freyberger	st3	CODE A3	A/A	E2	-1.27	99.95	-9.09	-1.11	0.88	1.28	0.01	0.05	20	0.997	2.24	24
25	Freyberger	st3	CODE B1	A/A	E2	-0.37	98.76	-9.10	-1.14	0.74	1.07	0.01	0.05	20	0.998	1.91	24
26	Freyberger	st3	Code B2	A/A	E2	-0.21	99.68	-9.05	-1.12	0.87	1.22	0.01	0.05	20	0.997	2.19	24

PART 2 - TABLE PS-1. (CERI Assay), Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable	Chemical	Bottom	Top	LogIC$_{50}$	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$	Hillslope	DF	R2	Syx	N
27	Freyberger	st3	CODE B3	A/A	E2	0.17	99.20	-9.10	-1.16	0.84	1.21	0.01	0.05	20	0.997	2.16	24
28	Freyberger	st3	CODE C1	A/A	E2	-0.96	103.00	-9.01	-1.20	1.06	1.45	0.02	0.06	20	0.996	2.73	24
29	Freyberger	st3	CODE C2	A/A	E2	-0.74	103.60	-9.02	-1.14	0.65	0.90	0.01	0.04	20	0.999	1.64	24
30	Freyberger	st3	CODE C3	A/A	E2	-0.77	96.78	-9.04	-1.14	0.76	1.04	0.01	0.05	20	0.998	1.91	24
31	JapanCERI	st1	91224	A/A	E2	-0.93	92.45	-8.96	-1.37	1.32	1.59	0.02	0.10	20	0.994	3.42	24
32	JapanCERI	st1	100107	A/A	E2	-0.86	99.42	-8.99	-1.42	1.15	1.46	0.02	0.09	20	0.996	3.07	24
33	JapanCERI	st1	100108	A/A	E2	-3.53	101.80	-8.96	-1.28	2.03	2.58	0.03	0.13	20	0.988	5.21	24
34	JapanCERI	st2	100119	A/A	E2	4.42	108.00	-8.67	-1.42	3.93	4.20	0.06	0.28	20	0.959	9.89	24
35	JapanCERI	st2	100126	A/A	E2	4.01	109.70	-8.80	-1.38	1.82	2.17	0.03	0.13	20	0.991	4.70	24
36	JapanCERI	st2	100129	A/A	E2	1.34	101.00	-8.84	-1.14	2.80	3.42	0.04	0.16	20	0.976	6.77	24
37	JapanCERI	st3	100203	A/A	E2	-0.41	137.80	-8.70	-1.22	1.80	2.21	0.02	0.08	20	0.995	4.49	24
38	JapanCERI	st3	100208	A/A	E2	-2.68	126.60	-8.70	-1.21	1.81	2.15	0.02	0.09	20	0.994	4.46	24
39	JapanCERI	st3	100215	A/A	E2	4.30	102.10	-8.79	-1.41	1.64	1.87	0.02	0.12	20	0.991	4.20	24
40	JapanCERI	st3	100223	A/A	E2	4.08	97.33	-8.89	-1.46	1.81	2.15	0.03	0.16	20	0.988	4.76	24
41	JapanCERI	st3	100224	A/A	E2	6.04	106.70	-8.80	-1.53	1.76	2.02	0.03	0.15	20	0.990	4.64	24
42	JapanCERI	st3	100225	A/A	E2	3.02	93.76	-8.86	-1.51	1.27	1.42	0.02	0.11	20	0.994	3.31	24
43	JapanCERI	st3	100226	A/A	E2	2.95	100.20	-8.82	-1.41	1.09	1.25	0.02	0.08	20	0.996	2.81	24
44	JapanCERI	st3	100301	A/A	E2	1.15	90.77	-8.83	-1.22	0.67	0.75	0.01	0.04	20	0.998	1.61	24
45	JapanCERI	st3	100303	A/A	E2	-0.69	115.10	-8.73	-1.33	1.91	2.18	0.03	0.11	20	0.992	4.81	24
46	JapanCERI	st3	100305	A/A	E2	3.41	98.48	-8.76	-1.11	1.36	1.58	0.02	0.08	20	0.994	3.19	24
47	Missouri	st1	4001	A/A	E2	0.38	88.43	-9.09	-1.07	2.62	3.57	0.05	0.16	20	0.972	6.39	24
48	Missouri	st1	4002	A/A	E2	0.86	105.70	-9.11	-0.85	1.93	3.23	0.03	0.08	20	0.989	4.43	24
49	Missouri	st1	4003	A/A	E2	0.74	102.70	-8.85	-1.21	5.00	6.10	0.08	0.30	20	0.930	12.39	24
50	Missouri	st1	4005	A/A	E2	-0.20	95.96	-9.13	-1.06	2.28	3.37	0.04	0.13	20	0.981	5.70	24
51	Missouri	st1	4006	A/A	E2	-0.69	102.50	-9.08	-1.03	2.16	3.21	0.04	0.11	20	0.985	5.34	24
52	Missouri	st1	4007	A/A	E2	0.45	95.29	-9.15	-0.96	2.02	3.09	0.04	0.10	20	0.985	4.86	24
53	Missouri	st1	5003	A/A	E2	-1.04	94.90	-8.97	-1.37	2.41	2.97	0.04	0.19	20	0.980	6.28	24

PART 2 - TABLE PS-1. (CERI Assay), Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$	Hillslope	DF	R2	Syx	N
54	Missouri	st1	5005	A/U	E2	0.74	107.20	-8.91	-1.39	1.61	2.01	0.02	0.12	19	0.992	4.23	23
55	Missouri	st2	5006	A/A	E2	-1.10	103.90	-9.20	-1.10	1.28	2.07	0.02	0.07	20	0.994	3.37	24
56	Missouri	st1	5007	A	E2	1.33	100.20	-9.14	-1.17	1.39	2.10	0.02	0.09	20	0.993	3.68	24
57	Missouri	st2	5008	A/A	E2	1.56	96.94	-8.96	-1.56	1.81	2.21	0.03	0.18	20	0.988	4.91	24
58	Missouri	st2	5009	A/A	E2	-1.14	111.80	-9.02	-1.17	1.69	2.43	0.03	0.09	20	0.992	4.39	24
59	Missouri	st2	5010	A/A	E2	-1.60	100.40	-9.27	-1.16	1.58	2.59	0.03	0.10	20	0.990	4.28	24
60	Missouri	st3	6001	A/U	E2	3.80	98.78	-8.89	-1.54	1.85	2.17	0.03	0.17	20	0.988	4.94	24
61	Missouri	st3	6002	A/U	E2	-2.22	94.92	-9.04	-1.07	1.16	1.58	0.02	0.06	20	0.995	2.83	24
62	Missouri	st3	6005	A/A	E2	1.66	98.80	-9.06	-1.44	1.51	2.06	0.02	0.13	20	0.991	4.16	24
63	Missouri	st3	6006	A/A	E2	2.62	95.60	-9.11	-1.22	2.55	3.41	0.04	0.17	17	0.978	6.23	21
64	Missouri	st3	6007	A/A	E2	-1.25	109.30	-8.87	-1.39	2.48	3.02	0.03	0.17	20	0.984	6.49	24
65	Missouri	st3	6009	A/A	E2	-1.87	107.30	-9.26	-0.87	2.10	3.89	0.04	0.09	20	0.986	5.04	24
66	Missouri	st3	6010	A/A	E2	-2.83	85.70	-9.45	-0.84	1.76	3.12	0.04	0.08	20	0.986	4.06	24
67	Missouri	st3	6011	A/A	E2	4.72	110.70	-8.98	-1.21	2.52	3.33	0.04	0.15	19	0.983	6.10	23
68	Missouri	st3	6502	A/A	E2	-1.91	102.10	-9.12	-0.98	1.86	2.86	0.03	0.09	20	0.989	4.51	24
69	Missouri	st3	6503	A/A	E2	-1.05	91.40	-9.05	-1.20	1.21	1.59	0.02	0.08	20	0.994	3.08	24
70	Missouri	st3	6504	A/A	E2	-0.90	89.96	-8.98	-1.07	4.18	5.27	0.07	0.24	20	0.939	9.94	24

*Refers to the control runs that were classified as unacceptable during the 2011 SMT (US EPA) review and the initial independent, standardized data analysis by Battelle, Columbus, OH, USA.(See ISR).

[b]shaded rows indicate runs that were excluded from the original data analysis because either the reference estrogen or NE was considered unacceptable (See ISR, Appendix F, H and N).

PART 2 - TABLE PS-1. (CERI Assay), Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

Statistic	Estimates for PRISM Curve Fit Parameters, CERI Assay, Reference Estrogen											
	Bottom	Top	$LogIC_{50}$	Hill Slope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
Mean	0.854	104.74	-8.925	-1.221	3.268	2.554	0.031	0.121	19.886	0.986	4.947	23.886
Standard Deviation	2.411	13.124	0.225	0.200	1.192	1.459	0.017	0.069	0.526	0.014	2.700	0.526
n	70	70	70	70	70	70	70	70	70	70	70	70
Coefficient of Variation (%)	282.34	12.53	2.52	16.36	36.47	57.11	55.66	56.52	2.64	1.37	54.57	2.20

95% Confidence Intervals				
	Bottom	**Top**	LogIC50	Hill Slope
Lower	0.279	101.600	-8.979	**-1.269**
Upper	1.429	107.900	-8.871	**-1.173**

Table PS-1. (CERI Assay), Prism curve fit parameter estimates for control, weak binder, Norethynodrel.

No.	Laboratory	Subtask	Run ID	Acceptable (E2/NE) *	Chemical	PRISM Fit Parameters											
						Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	CeeTox	st1	20090207	A/A	NE	1.2	96.28	-5.94	-0.86	2.04	0.91	0.02	0.05	20	0.996	2.135	24
2	CeeTox	st1	20090208	A/A	NE	2.71	94.59	-5.86	-0.97	2.43	1.04	0.02	0.07	20	0.994	2.731	24
3	CeeTox	st1	20090211	A/A	NE	0.71	96.99	-5.73	-0.96	3.19	1.17	0.03	0.09	20	0.992	3.157	24
4	Ceetox	st2	20090215	A/A	NE	6.15	95.17	-5.90	-1.13	1.75	0.85	0.02	0.07	20	0.995	2.41	24
5	Ceetox	st2	20090218*	U/U	NE	0.00	111.80	-5.46	-0.25	0.0	22.03	0.15	0.33	17	0.924	10.35	21
6	Ceetox	st2	20090221	A/A	NE	4.21	98.11	-6.19	-0.95	1.78	1.10	0.02	0.06	20	0.995	2.52	24
7	Ceetox	st3	20090228B	A/U	NE	.[a]	101.20	.	.	.	0.95	.	.	20	0.988	3.67	24
8	Ceetox	st3	20090305A	A/U	NE	.	106.50	.	.	.	1.63	.	.	20	0.971	6.30	24
9	Ceetox	st3	20090310A	A/A	NE	2.21	94.71	-5.80	-1.02	1.82	0.75	0.02	0.06	20	0.996	2.06	24
10	Ceetox	st3	20090401A	A/U	NE	3.91	104.70	-4.76	-0.95	35.94	1.00	0.08	0.27	20	0.985	2.93	24
11	Ceetox	st3	20090415	U/A	NE	1.51	93.38	-5.73	-1.11	2.96	1.15	0.03	0.11	20	0.990	3.39	24
12	Ceetox	st3	20090903A, B	A/A	NE	3.85	96.40	-5.53	-1.36	2.72	0.99	0.02	0.14	20	0.990	3.29	24
13	Ceetox	st3	20090905A,B	A/A	NE	3.176	101	-5.794	-1.054	1.618	0.717	0.0149	0.05252	20	0.997	1.995	24
14	Ceetox	st3	20090907A	A/A	NE	12.5	135.5	-5.545	-1.309	8.782	4.099	0.0866	0.3127	20	0.9334	12.83	24
15	Ceetox	st3	20090930A	A/A	NE	14.38	90.61	-5.539	-1.235	5.406	1.983	0.062	0.287	20	0.9497	6.312	24
16	Freyberger	st2	B	A/A	NE	-1.26	97.70	-6.32	-0.95	1.31	0.86	0.01	0.04	20	0.998	1.93	24
17	Freyberger	st2	C	A/A	NE	-1.05	96.84	-6.39	-0.96	2.18	1.52	0.02	0.07	20	0.993	3.34	24
18	Freyberger	st2	D	A/A	NE	-0.80	95.48	-6.49	-1.02	1.37	1.04	0.02	0.05	20	0.997	2.29	24
19	Freyberger	st2	E	A/A	NE	-1.89	100.00	-6.55	-1.01	1.45	1.19	0.02	0.05	20	0.997	2.51	24
20	Freyberger	st2	F	A/A	NE	-1.11	101.00	-6.58	-1.02	1.10	0.93	0.01	0.04	20	0.998	1.95	24
21	Freyberger	st2	G	A/A	NE	-1.94	103.10	-6.56	-1.02	0.89	0.75	0.01	0.03	20	0.999	1.57	24
22	Freyberger	st3	CODE A1	A/U	NE	1.75	69.01	-6.59	-1.73	11.05	7.76	0.22	1.31	20	0.645	23.09	24
23	Freyberger	st3	CODE A2	A/A	NE	-2.17	99.82	-6.63	-1.01	0.79	0.69	0.01	0.03	20	0.999	1.41	24
24	Freyberger	st3	CODE A3	A/A	NE	-2.07	99.85	-6.67	-0.98	1.52	1.39	0.19	0.06	20	0.996	2.73	24
25	Freyberger	st3	CODE B1	A/A	NE	-2.94	91.46	-6.65	-0.91	1.75	1.44	0.02	0.06	20	0.995	2.81	24
26	Freyberger	st3	CODE B2	A/A	NE	-1.33	97.39	-6.62	-0.98	1.19	1.03	0.01	0.04	20	0.998	2.06	24

Table PS-1. (CERI Assay), Prism curve fit parameter estimates for control, weak binder, Norethynodrel.

No.	Laboratory	Subtask	Run ID	Acceptable (E2/NE) *	Chemical	PRISM Fit Parameters											
						Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
27	Freyberger	st3	CODE B3	A/A	NE	-1.52	101.60	-6.64	-0.89	1.05	0.95	0.01	0.03	20	0.998	1.74	24
28	Freyberger	st3	CODE C1	A/A	NE	-1.53	107.40	-6.57	-0.98	1.42	1.25	0.02	0.05	20	0.997	2.49	24
29	Freyberger	st3	CODE C2	A/A	NE	-2.88	101.00	-6.61	-0.99	1.51	1.31	0.02	0.05	20	0.996	2.67	24
30	Freyberger	st3	CODE C3	A/A	NE	-2.70	101.90	-6.67	-0.96	1.32	1.22	0.02	0.03	20	0.997	2.34	24
31	JapanCERI	st1	91224	A/A	NE	-7.02	97.85	-6.28	-0.72	3.12	1.77	0.03	0.06	20	0.993	3.205	24
32	JapanCERI	st1	100107	A/A	NE	-5.56	97.09	-6.16	-0.88	3.79	2.03	0.03	0.09	20	0.986	4.619	24
33	JapanCERI	st1	100108	A/A	NE	-4.65	100.4	-6.1	-0.93	3.88	2.08	0.04	0.1	20	0.985	4.961	24
34	JapanCERI	st2	100119	A/A	NE	10.77	118.40	-6.00	-0.96	5.34	3.38	0.06	0.15	20	0.968	7.76	24
35	JapanCERI	st2	100126	A/A	NE	9.63	104.00	-6.20	-1.29	4.54	3.03	0.05	0.23	20	0.962	8.12	24
36	JapanCERI	st2	100129	A/A	NE	9.64	100.50	-6.29	-1.19	1.64	1.16	0.02	0.08	20	0.995	2.91	24
37	JapanCERI	st3	100203	A/A	NE	3.76	139.60	-6.10	-1.02	3.27	2.34	0.03	0.09	20	0.991	5.29	24
38	JapanCERI	st3	100208	A/A	NE	-2.03	134.20	-6.17	-0.95	3.03	2.19	0.03	0.07	20	0.993	4.70	24
39	JapanCERI	st3	100215	A/A	NE	11.40	103.40	-6.33	-1.14	1.42	1.08	0.02	0.07	20	0.996	2.55	24
40	JapanCERI	st3	100223	A/A	NE	11.01	95.98	-6.40	-1.11	1.66	1.28	0.02	0.08	20	0.993	2.95	24
41	JapanCERI	st3	100224	A/A	NE	9.15	111.10	-6.28	-1.05	1.52	1.15	0.02	0.06	20	0.996	2.58	24
42	JapanCERI	st3	100225	A/A	NE	8.05	94.95	-6.41	-1.17	1.07	0.80	0.01	0.06	20	0.997	1.94	24
43	JapanCERI	st3	100226	A/A	NE	8.09	98.47	-6.33	-1.37	1.72	1.23	0.02	0.11	20	0.993	3.29	24
44	JapanCERI	st3	100301	A/A	NE	3.59	91.93	-6.08	-0.83	2.20	1.11	0.02	0.06	20	0.994	2.43	24
45	JapanCERI	st3	100303	A/A	NE	2.99	109.30	-5.90	-1.06	3.29	1.69	0.03	0.10	20	0.988	4.51	24
46	JapanCERI	st3	100305	A/A	NE	10.33	96.27	-5.97	-0.95	1.98	1.04	0.02	0.07	20	0.994	2.55	24
47	Missouri	st1	4001	A/A	NE	2.03	107.4	-6	-0.9	4.02	2.18	0.04	0.1	20	0.985	5.025	24
48	Missouri	st1	4002	A/A	NE	1.26	101.1	-6.03	-1.01	3.12	1.68	0.03	0.1	20	0.988	4.281	24
49	Missouri	st1	4003	A/A	NE	10.18	113.6	-5.92	-1.09	9.87	5.61	0.11	0.34	20	0.89	14.66	24
50	Missouri	st1	4005	A/A	NE	-3.78	104.7	-5.89	-0.76	7.64	3.17	0.06	0.13	20	0.97	6.65	24
51	Missouri	st1	4006	A/A	NE	2.72	100.9	-5.9	-1.1	4.76	2.34	0.05	0.17	20	0.972	6.503	24
52	Missouri	st1	4007	A/A	NE	0.44	95.21	-6.02	-0.92	6.59	2.75	0.05	0.16	19	0.962	6.659	23

Table PS-1. (CERI Assay), Prism curve fit parameter estimates for control, weak binder, Norethynodrel.

No.	Laboratory	Subtask	Run ID	Acceptable (E2/NE) *	Chemical	PRISM Fit Parameters											
						Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
53	Missouri	st1	5003	A/A	NE	9.2	99.19	-6.44	-0.85	7.01	5.74	0.09	0.23	20	0.918	10.62	24
54	Missouri	st1	5005	A/U	NE	-3.31	108.80	-6.03	-0.66	8.18	4.13	0.06	0.12	20	0.968	6.88	24
55	Missouri	st1	5007	A/A	NE	6.31	104	-6.16	-0.94	3.97	2.54	0.04	0.12	20	0.979	5.675	24
56	Missouri	st1	5010	A/A	NE	-1.5	99.46	-6.69	-1.06	1.14	1.07	0.01	0.05	20	0.997	2.182	24
57	Missouri	st2	5006	A/A	NE	0.82	97.13	-6.10	-1.10	4.33	2.42	0.04	0.16	20	0.973	6.42	24
58	Missouri	st2	5008	A/A	NE	5.33	98.77	-5.93	-1.33	4.30	2.22	0.04	0.21	20	0.970	6.72	24
59	Missouri	st2	5009	A/A	NE	-1.82	110.10	-5.72	-0.96	4.73	1.91	0.04	0.11	20	0.985	5.06	24
60	Missouri	st3	6005	A/A	NE	-2.50	107.20	-6.63	-0.99	4.61	2.34	0.03	0.11	17	0.990	4.35	21
61	Missouri	st3	6006	A/A	NE	8.55	94.29	-6.75	-1.53	3.61	2.94	0.05	0.27	17	0.968	7.07	21
62	Missouri	st3	6007	A/A	NE	0.50	107.50	-6.60	-1.15	4.76	2.59	0.04	0.16	17	0.985	5.43	21
63	Missouri	st3	6009	A/A	NE	0.13	88.51	-6.68	-1.40	3.20	2.80	0.05	0.23	20	0.971	6.90	24
64	Missouri	st3	6010	A/A	NE	-0.08	83.85	-6.87	-0.97	3.08	2.94	0.05	0.14	20	0.975	5.60	24
65	Missouri	st3	6011	A/A	NE	8.43	107.20	-6.43	-1.14	8.44	3.79	0.06	0.26	17	0.958	8.21	21
66	Missouri	st3	6502	A/A	NE	-0.76	102.40	-6.01	-0.99	8.73	4.54	0.08	0.26	20	0.923	11.51	24
67	Missouri	st3	6503	A/A	NE	-0.11	96.59	-6.59	-1.16	4.32	3.63	0.06	0.21	20	0.963	8.25	24
68	Missouri	st3	6504	A/A	NE	3.78	87.49	-6.08	-1.40	5.40	2.92	0.06	0.33	20	0.939	8.89	24

[a]Nonsensical parameter values for bottom of PRISM curve. Reset to 0 for PS analysis.

[b]Shaded rows indicate runs that were excluded from the original data analysis because either the reference estrogen or NE was considered unacceptable (See ISR, Appendix F, H and N).

#Missouri runs 6001, 6002 deleted as were not acceptable by laboratory's QA assessment.

Missing data for the PRISM curve fit parameters typically indicate am ambiguous fit for that particular run.

[a]Nonsensical fit parameter estimates for bottom of PRISM curve. Reset to 0 for PS analysis.

Table PS-1. (CERI Assay), Prism curve fit parameter estimates for control, weak binder, Norethynodrel.

Statistic	Estimates for PRISM Curve Fit Parameters, CERI Assay, Control Weak Binder, Norethynodrel											
	Bottom	Top	$LogIC_{50}$	Hill Slope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
Mean	2.394	101.31	-6.193	-1.040	3.720	2.313	0.042	0.141	19.765	0.976	4.984	23.765
Standard Deviation	5.013	10.546	0.395	0.207	4.652	2.758	0.039	0.169	0.788	0.046	3.620	0.788
n	68	68	68	68	68	68	68	68	68	68	68	68
Coefficient of Variation (%)	209.38	10.41	6.38	19.95	125.08	119.24	91.68	119.82	3.99	4.74	72.63	3.32
	95% Confidence Intervals											
	Bottom	Top	LogIC50	Hill Slope								
Lower	1.178	98.760	-6.289	-1.090								
Upper	3.604	103.900	-6.097	-0.990								

Table PS-1. (CERI Assay). Prism curve fit parameter estimates for the control, non-binder, Di-n-butyl phthalate (DBP).

					PRISM Fit Parameters											
No.	Laboratory	Subtask	Run ID	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	CeeTox	st1	20090207	DBP	.	.	.	.	.	.	.	.	.	.		21
2	CeeTox	st1	20090208	DBP	.	.	.	.	.	.	.	.	.	.		21
3	CeeTox	st1	20090211	DBP	.	.	.	.	.	.	.	.	.	.		21
4	Ceetox	st2	20090215	DBP	.	.	.	.	.	.	.	.	.	.		21
5	Ceetox	st2	20090218*	DBP	.	.	.	.	.	.	.	.	.	.	.	21
6	Ceetox	st2	20090221	DBP	.	.	.	.	.	.	.	.	.	.	.	21
7	Ceetox	st3	20090228B	DBP	.	.	.	.	.	.	.	.	.	.	.	21
8	Ceetox	st3	20090305A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
9	Ceetox	st3	20090310A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
10	Ceetox	st3	20090401A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
11	Ceetox	st3	20090415	DBP	.	.	.	.	.	.	.	.	.	.	.	21
12	Ceetox	st3	20090903A, B	DBP	.	.	.	.	.	.	.	.	.	.	.	21
13	Ceetox	st3	20090905A,B	DBP	.	.	.	.	.	.	.	.	.	.	.	21
14	Ceetox	st3	20090907A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
15	Ceetox	st3	20090930A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
16	Freyberger	st2	B	DBP	.	.	.	.	.	.	.	.	.	.	.	21
17	Freyberger	st2	C	DBP	.	.	.	.	.	.	.	.	.	.	.	21
18	Freyberger	st2	D	DBP	.	.	.	.	.	.	.	.	.	.	.	21
19	Freyberger	st2	E	DBP	.	.	.	.	.	.	.	.	.	.	.	21
20	Freyberger	st2	F	DBP	.	.	.	.	.	.	.	.	.	.	.	21
21	Freyberger	st2	G	DBP	.	.	.	.	.	.	.	.	.	.	.	21
22	Freyberger	st3	CODE A1	DBP												21
23	Freyberger	st3	CODE A2	DBP	.	.	.	.	.	.	.	.	.	.	.	21
24	Freyberger	st3	CODE A3	DBP	.	.	.	.	.	.	.	.	.	.	.	21
25	Freyberger	st3	CODE B1	DBP	.	.	.	.	.	.	.	.	.	.	.	21

Table PS-1. (CERI Assay). Prism curve fit parameter estimates for the control, non-binder, Di-n-butyl phthalate (DBP).

					PRISM Fit Parameters											
No.	Laboratory	Subtask	Run ID	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
26	Freyberger	st3	CODE B2	DBP	.	.	.	.	.	.	.	.	.	.	.	21
27	Freyberger	st3	CODE B3	DBP	.	.	.	.	.	.	.	.	.	.	.	21
28	Freyberger	st3	CODE C1	DBP	.	.	.	.	.	.	.	.	.	.	.	21
29	Freyberger	st3	CODE C2	DBP	.	.	.	.	.	.	.	.	.	.	.	21
30	Freyberger	st3	CODEC3	DBP	.	.	.	.	.	.	.	.	.	.	.	21
31	JapanCERI	st1	91224	DBP	.	.	.	.	.	.	.	.	.	.	.	21
32	JapanCERI	st1	100107	DBP	.	.	.	.	.	.	.	.	.	.	.	21
33	JapanCERI	st1	100108	DBP	.	.	.	.	.	.	.	.	.	.	.	21
34	JapanCERI	st2	100119	DBP	.	.	.	.	.	.	.	.	.	.	.	21
35	JapanCERI	st2	100126	DBP	.	.	.	.	.	.	.	.	.	.	.	21
36	JapanCERI	st2	100129	DBP	.	.	.	.	.	.	.	.	.	.	.	21
37	JapanCERI	st3	100203	DBP	.	.	.	.	.	.	.	.	.	.	.	21
38	JapanCERI	st3	100208	DBP	.	.	.	.	.	.	.	.	.	.	.	21
39	JapanCERI	st3	100215	DBP	.	.	.	.	.	.	.	.	.	.	.	21
40	JapanCERI	st3	100223	DBP	.	.	.	.	.	.	.	.	.	.	.	21
41	JapanCERI	st3	100224	DBP	.	.	.	.	.	.	.	.	.	.	.	21
42	JapanCERI	st3	100225	DBP	.	.	.	.	.	.	.	.	.	.	.	21
43	JapanCERI	st3	100226	DBP	.	.	.	.	.	.	.	.	.	.	.	21
44	JapanCERI	st3	100301	DBP	.	.	.	.	.	.	.	.	.	.	.	21
45	JapanCERI	st3	100303	DBP	.	.	.	.	.	.	.	.	.	.	.	21
46	JapanCERI	st3	100305	DBP	.	.	.	.	.	.	.	.	.	.	.	21
47	Missouri	st1	4001	DBP	.	.	.	.	.	.	.	.	.	.	.	21
48	Missouri	st1	4002	DBP	.	.	.	.	.	.	.	.	.	.	.	21
49	Missouri	st1	4003	DBP	.	.	.	.	.	.	.	.	.	.	.	21
50	Missouri	st1	4005	DBP	.	.	.	.	.	.	.	.	.	.	.	21

Table PS-1. (CERI Assay). Prism curve fit parameter estimates for the control, non-binder, Di-n-butyl phthalate (DBP).

					PRISM Fit Parameters											
No.	Laboratory	Subtask	Run ID	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
51	Missouri	st1	4006	DBP	.	.	.	.	.	.	.	.	.	.	.	21
52	Missouri	st1	4007	DBP	.	.	.	.	.	.	.	.	.	.	.	21
53	Missouri	st1	5003	DBP	.	.	.	.	.	.	.	.	.	.	.	20
54	Missouri	st1	5005	DBP	.	.	.	.	.	.	.	.	.	.	.	21
55	Missouri	st1	5007	DBP	.	.	.	.	.	.	.	.	.	.	.	21
56	Missouri	st1	5010	DBP	.	.	.	.	.	.	.	.	.	.	.	21
57	Missouri	st2	5006	DBP	.	.	.	.	.	.	.	.	.	.	.	21
58	Missouri	st2	5008	DBP	.	.	.	.	.	.	.	.	.	.	.	21
59	Missouri	st2	5009	DBP	.	.	.	.	.	.	.	.	.	.	.	21
60	Missouri	st3	6001	DBP	.	.	.	.	.	.	.	.	.	.	.	20
61	Missouri	st3	6002	DBP	.	.	.	.	.	.	.	.	.	.	.	21
62	Missouri	st3	6005	DBP	.	.	.	.	.	.	.	.	.	.	.	20
63	Missouri	st3	6006	DBP	.	.	.	.	.	.	.	.	.	.	.	18
64	Missouri	st3	6007	DBP	.	.	.	.	.	.	.	.	.	.	.	21
65	Missouri	st3	6009	DBP	.	.	.	.	.	.	.	.	.	.	.	21
66	Missouri	st3	6010	DBP	.	.	.	.	.	.	.	.	.	.	.	21
67	Missouri	st3	6011	DBP	.	.	.	.	.	.	.	.	.	.	.	21
68	Missouri	st3	6502	DBP	.	.	.	.	.	.	.	.	.	.	.	21
69	Missouri	st3	6503	DBP	.	.	.	.	.	.	.	.	.	.	.	21
70	Missouri	st3	6504	DBP	.	.	.	.	.	.	.	.	.	.	.	20

[a]Nonsensical parameter values for bottom of PRISM curve. Reset to 0 for PS analysis.

[b]Shaded rows indicate runs that were excluded from the original data analysis because either the reference estrogen or NE was considered unacceptable (See ISR, Appendix F, H and N)..

Missing data for the PRISM curve fit parameters typically indicate am ambiguous fit for that particular run.

Table PS-1. (CERI Assay). PRISM curve fit parameter estimates for the alternate weak control, Norethindrone.

						PRISM Fit Parameters											
NO.	Laboratory	Subtask	Run ID	Acceptable (E2/NE)	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	Ceetox	st4	20090905A	A/A	Nethdrone-1	12.94	103.90	-5.77	-1.24	3.84	2.91	0.10	0.40	20	0.959	9.16	24
2	Ceetox	st4	20090930A	A/A	Nethdrone-1	13.75	91.49	-5.25	-1.20	3.22	2.09	0.08	0.25	20	0.965	6.95	24
3	Ceetox	st4	20090903A	A/A	Nethdrone-2	4.80	88.74	-5.61	-0.88	4.99	3.16	0.12	0.22	20	0.940	9.67	24
4	Ceetox	st4	20090905A	A/A	Nethdrone-2	5.72	97.08	-5.75	-1.10	2.08	1.48	0.05	0.14	20	0.989	4.67	24
5	Ceetox	st4	20090903A	A/A	Nethdrone-3	6.19	90.46	-5.48	-0.86	1.73	1.04	0.04	0.07	20	0.993	3.21	24
6	Ceetox	st4	20090903B	A/A	Nethdrone-4	5.95	88.31	-5.39	-0.99	2.39	1.46	0.06	0.13	20	0.985	4.71	24
7	Ceetox	st4	20090905A	A/A	Nethdrone-3	6.21	102.90	-5.65	-0.92	2.01	1.39	0.05	0.09	20	0.992	4.18	24
8	Ceetox	st4	20090905B	A/A	Nethdrone-4	7.34	101.00	-5.57	-1.03	2.18	1.47	0.05	0.11	20	0.989	4.67	24
9	Ceetox	st4	20090930A	A/A	Nethdrone-2	8.70	89.86	-5.50	-0.93	1.52	0.96	0.04	0.07	20	0.994	3.01	24
10	Ceetox	st4	20090930A	A/A	Nethdrone-3	11.71	89.40	-5.35	-1.06	2.39	1.52	0.06	0.15	20	0.982	4.94	24
11	Ceetox	st4	20090930B	A/A	Nethdrone-4	5.98	89.13	-5.35	-0.79	2.69	1.44	0.06	0.10	20	0.986	4.36	24
12	Missouri	st4	2206	A/A	Nethdrone-1	26.88	90.50	-6.12	-1.28	3.37	2.62	0.10	0.36	17	0.944	7.55	21
13	Missouri	st4	2206	A/A	Nethdrone-2	29.04	98.50	-6.04	-1.37	4.85	3.82	0.14	0.54	17	0.905	11.10	21
14	Missouri	st4	2210	A/A	Nethdrone-1	17.47	81.77	-6.84	-1.75	3.05	3.13	0.13	1.21	20	0.928	9.09	24
15	Missouri	st4	2210	A/A	Nethdrone-2	17.48	82.74	-6.72	-1.50	4.08	4.14	0.17	0.77	20	0.884	11.96	24
16	Missouri	st4	2211	A/A	Nethdrone-1	24.21	96.97	-6.69	-1.30	4.09	4.50	0.19	0.71	20	0.899	12.07	24
17	Missouri	st4	2211	A/A	Nethdrone-2	28.39	94.50	-6.40	-1.19	3.46	3.54	0.17	0.39	20	0.918	9.82	24
18	Missouri	st4	2301	A/A	Nethdrone-3	26.80	88.14	-6.79	-0.98	3.45	1.85	0.06	0.16	14	0.984	4.65	18
19	Missouri	st4	2301	A/A	Nethdrone-4	21.72	74.44	-6.79	-1.69	4.42	4.47	0.23	1.50	20	0.809	13.08	24
20	Missouri	st4	2302	A/A	Nethdrone-3	18.54	96.83	-6.52	-1.44	5.56	5.62	0.22	0.69	20	0.856	16.22	24
21	Missouri	st4	2302	A/A	Nethdrone-4	47.23	106.90	-6.11	-1.89	6.23	6.32	1.28	3.07	20	0.730	18.59	24
22	Missouri	st4	2305	A/A	Nethdrone-3	21.13	95.32	-6.24	-0.77	10.62	4.16	0.17	0.29	14	0.910	9.59	18

Only 2 laboratories elected to participate in subtask IV of the validation study.

Table PS-1. (CERI Assay). PRISM curve fit parameter estimates for the alternate weak control, Norethindrone.

Statistic	Estimates for PRISM Curve Fit Parameters, CERI Assay, Alternative Weak Binder, Norethindrone											
	Bottom	Top	$LogIC_{50}$	Hill Slope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
Mean	16.518	92.266	-6.014	-1.175	3.910	2.869	0.160	0.509	18.957	0.934	8.290	22.957
Standard Deviation	10.594	7.788	0.543	0.315	2.116	1.509	0.250	0.671	2.142	0.066	4.198	2.142
n	23	23	23	23	23	23	23	23	23	23	23	23
Coefficient of Variation (%)	64.13	8.44	9.03	26.79	54.13	52.58	156.57	131.95	11.30	7.10	50.63	9.33

95% Confidence Intervals				
	Bottom	**Top**	LogIC50	Hill Slope
Lower	**11.940**	**88.900**	**-6.249**	**-1.311**
Upper	**21.100**	**95.630**	**-5.779**	**-1.039**

PART 2 - Table PS-2 (FW Assay). Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable* (E2/NE)	Chemical	Bottom	Top	*LogIC$_{50}$*	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	CeeTox	st1	20081123	A	E2	2.03	93.84	*-8.74*	-1.55	2.34	2.89	0.04	0.25	23	0.973	7.218	27
2	CeeTox	st1	20081124	U[b]	E2	-0.09	81.73	*-8.93*	-1.06	2.62	3.72	0.07	0.2	23	0.958	7.469	27
3	CeeTox	st1	20081218	A	E2	0.29	103.8	*-8.78*	-0.96	2.79	4.95	0.05	0.15	22	0.969	7.773	26
4	Ceetox	st2	20090124	A	E2	0.06	92.76	*-9.36*	-0.99	1.63	4.04	0.039	0.11	22	0.9799	4.983	26
5	Ceetox	st2	20090128	A	E2	0.87	79.71	*-9.02*	-1.17	1.25	1.82	0.033	0.11	23	0.988	3.715	27
6	Ceetox	st2	20090223	A	E2	2.00	103.20	*-8.45*	-1.12	2.83	3.39	0.05	0.18	23	0.971	7.85	27
7	Ceetox	st2	20090307	A	E2	3.23	100.60	*-8.74*	-1.41	2.70	3.52	0.05	0.24	23	0.967	8.25	27
8	Ceetox	st2	20090326	A	E2	1.84	108.40	*-8.59*	-1.07	1.69	2.29	0.03	0.10	23	0.990	4.77	27
9	Ceetox	st3	20090418	A	E2	2.25	103.10	*-8.74*	-1.25	2.60	3.57	0.05	0.19	23	0.972	7.76	27
10	Ceetox	st3	20090426	A	E2	1.67	113.20	*-8.78*	-0.95	3.23	5.31	0.06	0.16	23	0.963	9.13	27
11	Ceetox	st3	20090505	A	E2	0.51	88.48	*-8.82*	-1.21	1.57	2.12	0.03	0.13	23	0.987	4.63	27
12	Ceetox	st3	20090514	A	E2	2.71	86.70	*-8.95*	-1.35	0.93	2.75	0.04	0.19	23	0.975	5.98	27
13	Ceetox	st4	20090911	A	E2	0.68	96.71	*-9.00*	-1.14	1.54	2.52	0.03	0.11	23	0.978	6.28	27
14	Ceetox	st4	20090914	A	E2	2.32	94.99	*-8.78*	-1.36	2.07	2.73	0.04	0.18	23	0.978	6.28	27
15	Ceetox	st4	20090917	A	E2	2.49	99.40	*-8.75*	-1.26	2.26	3.05	0.04	0.18	23	0.976	6.75	27
16	Freyberger	st2	A	A	E2	0.30	97.65	*-8.89*	-1.14	0.74	1.13	0.01	0.05	23	0.997	2.21	27
17	Freyberger	st2	B	A	E2	0.56	96.22	*-8.89*	-1.13	0.66	0.99	0.01	0.05	23	0.998	1.94	27
18	Freyberger	st2	D	A	E2	0.14	106.40	*-9.01*	-0.93	0.66	1.23	0.01	0.03	23	0.998	1.92	27
19	Freyberger	st1	E	DMSO(A)	E2	-0.51	114.4	*-8.99*	-0.86	0.72	1.42	0.01	0.03	23	0.998	2.038	27
20	Freyberger	st1	F	DMSO(A)	E2	0.2	106.5	*-9.08*	-0.91	0.73	1.43	0.01	0.04	23	0.998	2.125	27
21	Freyberger	st1	G	DMSO(A)	E2	-0.2	93.13	*-9.11*	-0.95	1	1.8	0.02	0.06	23	0.994	2.898	27
22	Freyberger	st3	CODE A	A	E2	0.90	105.80	*-9.00*	-0.96	0.64	1.16	0.01	0.03	23	0.998	1.87	27
23	Freyberger	st3	CODE A1	A	E2	0.95	106.70	*-9.04*	-0.94	0.59	1.10	0.01	0.03	23	0.998	1.71	27
24	Freyberger	st3	CODE A2	A	E2	0.33	103.40	*-8.87*	-0.98	2.51	4.06	0.05	0.14	23	0.974	7.17	27
25	Freyberger	st3	CODE AB	A	E2	0.38	100.80	*-9.00*	-1.01	0.72	1.24	0.01	0.04	23	0.998	2.12	27
26	Freyberger	st3	CODE B	A	E2	0.57	97.80	*-9.14*	-0.97	0.46	0.87	0.01	0.03	23	0.999	1.37	27

PART 2 - Table PS-2 (FW Assay). Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable*	Chemical	Bottom	Top	*LogIC$_{50}$*	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
27	Freyberger	st3	CODE B1	A	E2	0.15	107.10	*-8.97*	-0.92	0.89	1.61	0.02	0.04	23	0.997	2.53	27
28	Freyberger	st3	CODE B2	A	E2	0.30	98.52	*-9.02*	-1.01	0.52	0.90	0.01	0.03	23	0.999	1.53	27
29	JapanCERI	st1	20090113_01	A	E2	0.75	94.01	*-8.83*	-1.15	0.8	1.14	0.02	0.06	23	0.997	2.34	27
30	JapanCERI	st1	20090121_02	A	E2	0.01	100.8	*-8.76*	-1.11	0.95	1.36	0.02	0.06	23	0.996	2.769	27
31	JapanCERI	st1	20090128_03	A	E2	0.76	97.34	*-8.74*	-1.16	0.7	0.95	0.01	0.05	23	0.998	2.032	27
32	JapanCERI	st2	20090422_01	A	E2	-0.08	101.60	*-8.72*	-1.00	0.71	1.03	0.01	0.04	23	0.998	1.99	27
33	JapanCERI	st2	20090427_02	A	E2	0.10	106.10	*-8.77*	-1.07	0.68	1.02	0.01	0.04	23	0.998	1.98	27
34	JapanCERI	st2	20090430_03	A	E2	-1.15	100.30	*-8.83*	-0.88	1.00	1.60	0.02	0.05	23	0.996	2.69	27
35	JapanCERI	st3	091026_02	U	E2	0.64	84.29	*-8.73*	-1.51	2.31	2.73	0.05	0.26	23	0.969	6.99	27
36	JapanCERI	st3	091104_00	U	E2	1.03	82.02	*-8.62*	-1.63	6.87	7.45	0.14	0.89	23	0.779	20.56	27
37	JapanCERI	st3	091105_03	A	E2	-0.62	101.10	*-8.73*	-1.16	0.50	0.68	0.01	0.03	23	0.999	1.45	27
38	JapanCERI	st3	091109_04	A	E2	-0.34	100.20	*-8.80*	-1.08	0.93	1.36	0.02	0.06	23	0.996	2.68	27
39	JapanCERI	st3	091110_05	DMSO(A)	E2	-0.11	103.90	*-8.84*	-1.08	1.73	2.66	0.03	0.10	23	0.988	5.07	27
40	JapanCERI	st3	091111_06	A	E2	-0.28	97.87	*-8.88*	-1.12	1.39	2.08	0.03	0.09	23	0.991	4.09	27
41	JapanCERI	st3	091112_07	DMSO(A)	E2	-1.33	103.10	*-8.77*	-0.96	0.87	1.32	0.02	0.04	23	0.997	2.41	27
42	JapanCERI	st3	091116_08	A	E2	1.53	98.81	*-8.74*	-1.25	1.43	1.91	0.03	0.11	23	0.991	4.24	27
43	JapanCERI	st3	091117_09	DMSO(A)	E2	0.76	102.50	*-8.83*	-1.14	0.68	1.01	0.01	0.05	23	0.998	2.01	27
44	JapanCERI	st3	091118_10	DMSO(A)	E2	0.77	102.20	*-8.77*	-1.03	1.09	1.62	0.02	0.06	23	0.995	3.10	27
45	JapanCERI	st3	091119_11	A	E2	-0.93	105.50	*-8.80*	-0.87	1.58	2.57	0.03	0.07	23	0.991	4.26	27
46	Missouri	st1	3006	U	E2	-5.69	155.30	*-9.52*	-0.36	5.09	47.38	0.07	0.10	23	0.968	6.31	27
47	Missouri	st1	7003	A	E2	0.56	111.3	*-8.92*	-1.09	1.03	1.03	0.02	0.08	23	0.997	3.053	27
48	Missouri	st1	7004	A	E2	-0.09	112.9	*-8.87*	-0.92	1.84	1.8	0.05	0.1	23	0.991	5.144	27
49	Missouri	st1	7005	A	E2	-0.39	105.7	*-8.92*	-0.88	2.3	2.23	0.06	0.12	23	0.985	6.29	27
50	Missouri	st1	7006	A	E2	-0.57	107.4	*-8.86*	-0.99	1.83	2.98	0.03	0.1	23	0.987	5.277	27
51	Missouri	st1	7007	A	E2	0.12	109.2	*-8.92*	-0.96	1.16	2.03	0.02	0.06	23	0.995	3.368	27
52	Missouri	st2	9003	A	E2	-1.34	106.30	*-9.11*	-0.89	1.88	3.73	0.04	0.09	23	0.984	5.41	27
53	Missouri	st2	9004	A	E2	-0.81	107.50	*-9.13*	-0.87	1.48	3.06	0.03	0.07	23	0.990	4.25	27

PART 2 - Table PS-2 (FW Assay). Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

No.	Laboratory	Subtask	Run ID	Acceptable*	Chemical	Bottom	Top	*LogIC$_{50}$*	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
54	Missouri	st2	9005	A	E2	-2.12	105.60	*-9.14*	-0.91	2.06	4.10	0.04	0.10	23	0.981	5.99	27
55	Missouri	st2	9007	A	E2	-0.62	102.90	*-9.21*	-0.98	1.61	3.22	0.03	0.09	23	0.986	4.87	27
56	Missouri	st3	8001	U	E2	0.13	88.12	*-9.09*	-0.91	1.29	2.25	0.03	0.08	23	0.990	3.62	27
57	Missouri	st3	8002	U	E2	-0.34	81.14	*-9.13*	-0.92	0.68	1.13	0.02	0.04	23	0.997	1.89	27
58	Missouri	st3	8003	A	E2	0.33	103.20	*-9.03*	-0.98	0.90	1.63	0.02	0.05	23	0.996	2.66	27
59	Missouri	st3	8004	A	E2	1.23	114.50	*-8.92*	-0.93	1.88	3.42	0.04	0.09	23	0.987	5.41	27
60	Missouri	st3	8005	A	E2	-0.45	96.83	*-9.14*	-0.85	0.60	1.17	0.01	0.03	23	0.998	1.67	27
61	Missouri	st3	8101	A	E2	0.29	96.73	*-8.99*	-1.50	0.35	1.00	0.01	0.06	23	0.998	1.34	27
62	Missouri	st3	8102	A	E2	0.74	91.49	*-9.02*	-1.35	0.24	0.73	0.01	0.04	23	0.999	0.89	27
63	Missouri	st3	8107	A	E2	0.59	96.77	*-9.00*	-1.07	1.09	1.80	0.02	0.07	23	0.994	3.25	27
64	Missouri	st3	8108	A	E2	-1.15	109.90	*-8.90*	-1.00	1.65	2.62	0.03	0.08	22	0.991	4.53	26
65	Missouri	st3	8201 DMSO	DMSO(A)	E2	0.13	86.65	*-9.06*	-1.02	0.67	1.10	0.02	0.05	23	0.997	1.96	27
66	Missouri	st3	8202 DMSO	DMSO(U)	E2	0.38	83.25	*-9.14*	-1.14	0.52	0.85	0.01	0.04	23	0.998	1.59	27
67	Missouri	st3	8203 DMSO	DMSO(A)	E2	0.17	94.21	*-9.15*	-1.03	1.85	3.36	0.04	0.12	23	0.980	5.58	27

[a] No estimate for parameters indicate no displacement of radioactive ligand and data supported the classification of chemical as a non-binder.

*Refers to the control runs that were classified as unacceptable during the 2011 SMT (US EPA) review and the initial independent, standardized data analysis by Battelle, Columbus, OH, USA.(See ISR).

[b] shaded rows indicate runs that were excluded from the original data analysis because either the reference estrogen or NE was considered unacceptable (See ISR, Appendix F, H and N).

[C] Runs C (Freyberger), 3005 and 3008 (Missouri) were deleted because data were noted as unacceptable by respective laboratories.

PART 2 - Table PS-2 (FW Assay). Prism curve fit parameter estimates for reference estrogen (17β-estradiol (E2)).

Statistic	Estimates for PRISM Curve Fit Parameters, FW Assay, Reference Estrogen											
	Bottom	Top	$LogIC_{50}$	Hill Slope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
Mean	0.291	100.441	-8.921	-1.064	1.479	2.876	0.030	0.102	22.955	0.986	4.258	26.955
Standard Deviation	1.252	10.838	0.182	0.198	1.087	5.666	0.021	0.113	0.208	0.028	2.944	0.208
n	67	67	67	67	67	67	67	67	67	67	67	67
Coefficient of Variation (%)	429.52	10.79	2.04	18.57	73.51	197.00	70.78	111.11	0.91	2.82	69.12	0.77

95% Confidence Intervals				
	Bottom	**Top**	LogIC50	Hill Slope
Lower	-0.014	97.8	-8.965	-1.112
Upper	0.596	103.1	-8.877	-1.016

Table PS-2, (FW Assay), PRISM curve fit parameter estimates for control weak binder, Norethynodrel

No.	Laboratory	Subtask	Run ID	Acceptable* (E2/NE)	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	Ceetox	st1	20081123	A	NE	9.56	87.25	-6.18	-1.59	2.76	2.52	0.05	0.28	20	0.97	6.408	24
2	Ceetox	st1	20081124	A	NE	0.52	87.7	-6.34	-0.93	4.85	4	0.06	0.18	20	0.96	7.255	24
3	Ceetox	st1	20081218	A	NE	4.3	93.44	-6.21	-1.18	3.17	2.8	0.04	0.18	20	0.976	6.063	24
4	Ceetox	st2	20090124	A	NE	6.11	83.4	-6.28	-1.32	2.6	2.35	0.04	0.2	20	0.976	5.44	24
5	Ceetox	st2	20090128	A	NE	4.69	72.8	-6.38	-1.34	3.58	3.16	0.08	0.32	20	0.944	7.45	24
6	Ceetox	st2	20090223	A	NE	10.39	94.26	-6.00	-1.54	2.02	1.61	0.03	0.18	20	0.988	4.24	24
7	Ceetox	st2	20090307	A	NE	4.01	104.30	-6.17	-0.93	2.68	2.32	0.03	0.09	20	0.990	4.17	24
8	Ceetox	st2	20090326	A	NE	6.24	107.50	-6.31	-0.78	4.47	8.44	0.07	0.18	20	0.948	8.50	24
9	Ceetox	st3	20090418	A	NE	1.54	111.80	-6.12	-0.86	4.35	3.61	0.04	0.12	20	0.981	6.04	24
10	Ceetox	st3	20090426	U	NE	2.80	108.10	-5.30	-1.66	4.53	1.50	0.03	0.21	20	0.986	5.05	24
11	Ceetox	st3	20090505	A	NE	0.60	95.98	-6.31	-0.96	2.64	2.40	0.03	0.10	20	0.988	4.36	24
12	Ceetox	st3	20090514	DMSO(A)	NE	10.48	95.51	-6.05	-1.26	2.96	2.42	0.04	0.18	20	0.978	5.63	24
13	Ceetox	st4	20090911	A	NE	5.832	99.74	-5.99	-1.14	3.28	2.44	0.04	0.16	20	0.981	5.64	24
14	Ceetox	st4	20090914	A	NE	8.42	96.23	-5.96	-1.30	3.52	2.56	0.04	0.21	20	0.974	6.35	24
15	Ceetox	st4	20090917	A	NE	10.50	92.65	-6.03	-1.33	2.83	2.23	0.04	0.19	20	0.978	5.46	24
16	Freyberger	st2	A	A	NE	2.75	101.10	-6.35	-0.95	1.29	1.32	0.02	0.05	20	0.997	2.25	24
17	Freyberger	st2	B	A	NE	3.57	97.67	-6.40	-0.95	1.39	1.46	0.02	0.06	20	0.996	2.46	24
18	Freyberger	st2	D	A	NE	1.54	108.20	-6.15	-0.89	1.47	1.24	0.02	0.04	20	0.998	2.14	24
19	Freyberger	st2	E	DMSO (A)	NE	1.36	99.88	-6.37	-0.89	1.18	1.18	0.01	0.04	20	0.998	1.93	24
20	Freyberger	st2	F	DMSO (A)	NE	3.15	100.40	-6.46	-1.00	1.70	1.96	0.02	0.08	20	0.993	3.34	24
21	Freyberger	st2	G	DMSO (A)	NE	4.95	86.43	-6.45	-1.16	1.21	1.27	0.02	0.08	20	0.995	2.53	24
22	Freyberger	st3	CODE A	A	NE	4.46	105.40	-6.12	-1.03	1.26	1.08	0.02	0.05	20	0.998	2.12	24
23	Freyberger	st3	CODE A1	A	NE	-1.73	105.90	-6.27	-0.71	4.60	3.89	0.04	0.10	20	0.983	5.12	24
24	Freyberger	st3	CODE A2	U	NE	8.22	101.00	-6.12	-1.83	3.59	3.38	0.05	0.39	20	0.961	9.01	24
25	Freyberger	st3	CODE AB	A	NE	2.80	101.20	-6.35	-0.87	3.08	3.06	0.04	0.10	20	0.985	4.88	24

Table PS-2, (FW Assay), PRISM curve fit parameter estimates for control weak binder, Norethynodrel

No.	Laboratory	Subtask	Run ID	Acceptable* (F2/NE)	Chemical	Bottom	Top	LogIC$_{50}$	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
26	Freyberger	st3	CODE B	A	NE	3.32	100.60	-6.29	-0.94	0.90	0.85	0.01	0.03	20	0.999	1.49	24
27	Freyberger	st3	CODE B1	U	NE	5.84	93.03	-6.47	-3.43	2.41	2.64	0.03	1.76	20	0.973	7.32	24
28	Freyberger	st3	CODE B2	A	NE	0.90	99.25	-6.32	-0.83	4.24	3.81	0.05	0.12	20	0.977	5.95	24
29	JapanCERI	st1	20090113_01	A	NE	1.17	90.23	-6.59	-0.93	1.48	1.69	0.02	0.07	20	0.994	2.717	24
30	JapanCERI	st1	20090121_02	A	NE	2.28	99.31	-6.58	-0.92	0.86	1.09	0.01	0.04	20	0.998	1.648	24
31	JapanCERI	st1	20090128_03	A	NE	0.94	99.9	-6.51	-0.85	1.27	1.44	0.02	0.04	20	0.997	2.121	24
32	JapanCERI	st2	20090422_01	A	NE	1.32	103.30	-6.61	-0.92	0.93	1.25	0.01	0.04	20	0.998	1.84	24
33	JapanCERI	st2	20090427_02	A	NE	3.59	104.90	-6.63	-0.96	1.14	1.62	0.02	0.05	20	0.997	2.40	24
34	JapanCERI	st2	20090430_03	A	NE	0.93	98.71	-6.61	-0.93	0.81	1.03	0.01	0.03	20	0.998	1.57	24
35	JapanCERI	st3	091021_01	A	NE	-0.64	99.69	-6.75	-0.85	1.27	1.85	0.02	0.05	20	0.996	2.42	24
36	JapanCERI	st3	091026_02	A	NE	0.02	104.80	-6.83	-0.78	1.52	2.57	0.02	0.06	20	0.995	2.79	24
37	JapanCERI	st3	091104_00	U	NE	-2.70	119.40	-6.89	-0.67	3.62	7.93	0.04	0.10	20	0.980	5.75	24
38	JapanCERI	st3	091105_03	A	NE	-0.51	103.30	-6.68	-0.85	2.98	4.19	0.04	0.11	20	0.981	5.58	24
39	JapanCERI	st3	091109_04	A	NE	-0.79	102.90	-6.73	-0.86	1.00	1.47	0.01	0.04	20	0.998	1.93	24
40	JapanCERI	st3	091110_05	DMSO(A)	NE	0.09	100.90	-6.76	-0.86	1.97	2.94	0.03	0.08	20	0.990	3.84	24
41	JapanCERI	st3	091111_06	A	NE	-1.29	103.40	-6.65	-0.87	1.31	1.77	0.02	0.05	20	0.996	2.46	24
42	JapanCERI	st3	091112_07	DMSO(A)	NE	-1.66	102.40	-6.78	-0.86	1.59	2.43	0.02	0.06	20	0.994	3.12	24
43	JapanCERI	st3	091116_08	A	NE	0.83	104.10	-6.71	-0.82	1.27	1.88	0.02	0.05	20	0.996	2.34	24
44	JapanCERI	st3	091117_09	DMSO(A)	NE	1.97	100.30	-6.83	-0.92	1.61	2.65	0.03	0.08	20	0.992	3.53	24
45	JapanCERI	st3	091118_10	DMSO(A)	NE	1.66	102.60	-6.78	-0.93	1.07	1.70	0.02	0.05	20	0.997	2.33	24
46	JapanCERI	st3	091119_11	A	NE	0.33	106.70	-6.69	-0.93	1.55	2.30	0.02	0.07	20	0.994	3.25	24
47	Missouri	st1	3006	A	NE	3.66	90.53	-6.64	-1.08	1.62	2.08	0.03	0.1	20	0.99	3.595	24
48	Missouri	st1	7003	A	NE	0.82	102.2	-6.36	-0.95	1.54	1.57	0.02	0.06	20	0.996	2.705	24
49	Missouri	st1	7004	A	NE	0.74	109.3	-6.4	-0.94	2.14	2.41	0.03	0.08	20	0.992	3.954	24
50	Missouri	st1	7005	A	NE	0.1	101.2	-6.47	-0.89	1.4	1.55	0.02	0.05	20	0.996	2.421	24
51	Missouri	st1	7006	A	NE	1.23	110.6	-6.47	-0.79	3.36	4.08	0.04	0.1	20	0.984	5.359	24
52	Missouri	st1	7007	A	NE	0.92	107.8	-6.4	-0 .84	1.45	1.58	0.02	0.04	20	0.997	2.342	24

Table PS-2, (FW Assay), PRISM curve fit parameter estimates for control weak binder, Norethynodrel

No.	Laboratory	Subtask	Run ID	Acceptable* (E2/NE)	Chemical	Bottom	Top	LogIC$_{50}$	HillSlope	Bottom SEM	Top SEM	LogIC$_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
53	Missouri	st2	9003	A	NE	0.11	105.50	-6.26	-0.95	1.26	1.19	0.01	0.04	20	0.998	2.11	24
54	Missouri	st2	9004	A	NE	1.95	102.10	-6.25	-0.97	1.97	1.82	0.02	0.07	20	0.994	3.32	24
55	Missouri	st2	9005	A	NE	-0.91	99.05	-6.33	-1.00	2.14	2.04	0.03	0.09	20	0.992	3.78	24
56	Missouri	st2	9007	A	NE	-2.79	99.57	-6.45	-0.82	2.86	2.87	0.03	0.09	20	0.989	4.27	24
57	Missouri	st3	8001	U	NE	3.92	80.63	-6.24	-1.11	2.77	2.12	0.04	0.15	20	0.981	4.62	24
58	Missouri	st3	8002	U	NE	1.49	76.31	-6.28	-1.02	1.47	1.00	0.02	0.07	20	0.995	2.11	24
59	Missouri	st3	8003	A	NE	-0.30	96.53	-5.92	-0.90	3.01	1.60	0.03	0.08	20	0.993	3.20	24
60	Missouri	st3	8004	A	NE	1.95	106.30	-6.27	-0.97	3.05	2.99	0.04	0.11	20	0.985	5.31	24
61	Missouri	st3	8005	A	NE	-0.34	92.07	-6.11	-0.86	3.39	2.11	0.03	0.09	20	0.989	3.87	24
62	Missouri	st3	8101	A	NE	-1.62	107.80	-6.39	-0.76	2.58	2.65	0.03	0.07	20	0.993	3.56	24
63	Missouri	st3	8102	A	NE	-0.19	95.74	-6.44	-0.82	2.33	2.27	0.03	0.07	20	0.992	3.43	24
64	Missouri	st3	8107	A	NE	-4.25	107.60	-6.44	-0.59	5.63	5.58	0.05	0.09	20	0.981	5.19	24
65	Missouri	st3	8108	U	NE	-8.01	123.10	-6.32	-0.54	8.21	8.19	0.05	0.10	20	0.978	6.27	24
66	Missouri	st3	8201 DMSO	DMSO(A)	NE	0.08	93.66	-6.62	-0.84	0.98	1.16	0.01	0.04	20	0.998	1.66	24
67	Missouri	st3	8202 DMSO	DMSO(U)	NE	1.23	81.45	-6.73	-0.92	0.79	0.93	0.01	0.04	20	0.998	1.46	24
68	Missouri	st3	8203 DMSO	DMSO(A)	NE	2.69	94.20	-6.56	-1.04	2.01	2.44	0.03	0.11	20	0.988	4.19	24

*Refers to the control runs that were classified as unacceptable during the 2011 SMT (US EPA) review and the initial independent, standardized data analysis by Battelle, Columbus, OH, USA.(See ISR).

[b]Shaded rows indicate runs that were excluded from the original data analysis because either the reference estrogen or NE was considered unacceptable (See ISR, Appendix F, H and N).

Freyberger (Run C) and Ceetox (20090426) were deleted because these laboratories indicated the runs were unacceptable.

Table PS-2, (FW Assay), PRISM curve fit parameter estimates for control weak binder, Norethynodrel

Statistic		Estimates for PRISM Curve Fit Parameters, FW Assay, Control Weak Binder, Norethynodrel										
	Bottom	**Top**	**LogIC$_{50}$**	**Hill Slope**	**Bottom SEM**	**Top SEM**	**LogIC$_{50}$ SEM**	**Hillslope SEM**	**DF**	**R2**	**Syx**	**N**
Mean	2.017	99.424	-6.393	-1.013	2.379	2.464	0.030	0.125	20.000	0.988	3.955	24.000
Standard Deviation	3.418	8.902	0.273	0.377	1.363	1.544	0.014	0.213	0.000	0.012	1.838	0.000
n	68	68	68	68	68	68	68	68	68	68	68	68
Coefficient of Variation (%)	169.48	8.95	4.27	37.20	57.30	62.69	47.64	170.34	0.00	1.19	46.47	0.00

95% Confidence Intervals				
	Bottom	**Top**	LogIC50	Hill Slope
Lower limit	1.190	97.270	-6.459	-1.104
Upper limit	2.844	101.600	-6.327	-0.922

Table PS-2. (FW Assay), PRISM curve fit parameter estimates for control non-binder, di-n-butyl phthalate (DBP).

No.	Laboratory	Subtask	Run ID	Acceptable* (E2/NE)	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	Ceetox	st1	20081123	A/A	DBP	.[a]	.	.	.	.	.	.	.	.	.	.	21
2	Ceetox	st1	20081124	U/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
3	Ceetox	st1	20081218	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
4	Ceetox	st2	20090124		DBP	.	.	.	.	.	.	.	.	.	.	.	21
5	Ceetox	st2	20090128		DBP	.	.	.	.	.	.	.	.	.	.	.	21
6	Ceetox	st2	20090223	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
7	Ceetox	st2	20090307	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
8	Ceetox	st2	20090326	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
9	Ceetox	st3	20090418	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
10	Ceetox	st3	20090505	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
11	Ceetox	st3	20090418 DMSO	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
12	Ceetox	st3	20090426	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
13	Ceetox	st3	20090505	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
14	Ceetox	st3	20090514	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
15	Ceetox	st4	20090911	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
16	Ceetox	st4	20090914	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
17	Ceetox	st4	20090917	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
18	Freyberger	st2	A	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
19	Freyberger	st2	B	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
20	Freyberger	st2	D	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
21	Freyberger	st2	E	DMSO (A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
22	Freyberger	st2	F	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
23	Freyberger	st2	G	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21

Table PS-2. (FW Assay), PRISM curve fit parameter estimates for control non-binder, di-n-butyl phthalate (DBP).

No.	Laboratory	Subtask	Run ID	Acceptable*	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
24	Freyberger	st3	Code A	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
27	Freyberger	st3	Code A1	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
28	Freyberger	st3	Code A2	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
29	Freyberger	st3	Code AB	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
30	Freyberger	st3	Code B	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
31	Freyberger	st3	Code B1	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
32	Freyberger	st3	Code B2	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
33	JapanCERI	st1	20090113	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
34	JapanCERI	st1	20090121	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
35	JapanCERI	st1	20090128	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
36	JapanCERI	st1	20090422	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
37	JapanCERI	st1	20090427	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
38	JapanCERI	st1	20090430	A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
39	JapanCERI	st3	91021	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
40	JapanCERI	st3	91105	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
41	JapanCERI	st3	91109	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
42	JapanCERI	st3	91110	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
43	JapanCERI	st3	91111	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
44	JapanCERI	st3	91112	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
45	JapanCERI	st3	91116	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21

Table PS-2. (FW Assay), PRISM curve fit parameter estimates for control non-binder, di-n-butyl phthalate (DBP).

No.	Laboratory	Subtask	Run ID	Acceptable*	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
46	JapanCERI	st3	91117	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
47	JapanCERI	st3	91118	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
48	JapanCERI	st3	91119	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
49	Missouri	st1	3006	U/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
50	Missouri	st1	7003	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	21
51	Missouri	st1	7004	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	20
52	Missouri	st1	7005	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	20
53	Missouri	st1	7006	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	20
54	Missouri	st1	7007	A/A	DBP	.	.	.	.	.	.	.	.	.	.	.	20
55	Missouri	st3	8201	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21
56	Missouri	st3	8203	DMSO(A)	DBP	.	.	.	.	.	.	.	.	.	.	.	21

[a]No estimate for parameters indicate no displacement of radioactive ligand and data supported the classification of chemical as a non-binder.

*Refers to the control runs that were classified as unacceptable during the 2011 SMT (US EPA) review and the initial independent, standardized data analysis by Battelle, Columbus, OH, USA.(See ISR).

Table PS-2. (FW Assay), PRISM curve fits parameter estimates for alternative control weak binder, Norethindrone.

No.	Laboratory	Subtask	Run ID	Acceptable (E2/NE)	Chemical	Bottom	Top	$LogIC_{50}$	HillSlope	Bottom SEM	Top SEM	$LogIC_{50}$ SEM	Hillslope SEM	DF	R2	Syx	N
1	Ceetox	st4	20090914	A/A	Nethdrone-1	7.50	103.20	-5.94	-1.16	1.66	1.38	0.04	0.15	20	0.992	4.12	24
2	Ceetox	st4	20090914	A/A	Nethdrone-2	8.40	95.23	-6.04	-1.43	1.88	1.67	0.04	0.34	20	0.987	5.03	24
3	Ceetox	st4	20090914	A/A	Nethdrone-3	4.10	101.90	-5.86	-0.89	1.90	1.44	0.04	0.09	20	0.992	4.11	24
4	Ceetox	st4	20090911A	A/A	Nethdrone-1	4.53	103.50	-5.92	-1.28	2.45	1.98	0.05	0.28	20	0.985	6.09	24
5	Ceetox	st4	20090911A	A/A	Nethdrone-2	2.58	95.84	-6.02	-1.25	2.18	1.81	0.05	0.25	20	0.986	5.48	24
6	Ceetox	st4	20090911A	A/A	Nethdrone-3	3.98	99.21	-6.00	-0.88	2.17	1.76	0.05	0.11	20	0.988	4.85	24
7	Ceetox	st4	20090911B	A/A	Nethdrone-4	4.12	89.94	-6.07	-1.68	2.00	1.75	0.05	0.65	20	0.985	5.41	24
8	Ceetox	st4	20090914B	A/A	Nethdrone-4	6.02	102.10	-5.88	-0.91	2.40	1.87	0.06	0.12	20	0.986	5.32	24
9	Ceetox	st4	20090917A	A/A	Nethdrone-1	9.48	87.95	-6.05	-1.80	1.86	1.64	0.04	0.87	20	0.984	5.06	24
10	Ceetox	st4	20090917A	A/A	Nethdrone-2	9.46	87.95	-6.05	-1.80	1.86	1.64	0.04	0.87	20	0.984	5.07	24
11	Ceetox	st4	20090917A	A/A	Nethdrone-3	11.50	83.12	-6.11	-1.95	1.76	1.63	0.07	1.21	20	0.982	4.98	24
12	Ceetox	st4	20090917B	A/A	Nethdrone-4	8.14	89.56	-5.96	-0.95	2.01	1.55	0.05	0.13	20	0.987	4.50	24
13	Missouri	st4	1101	A/A	Nethdrone-1	-4.91	89.42	-5.55	-0.94	7.15	2.22	0.09	0.20	20	0.955	7.96	24
14	Missouri	st4	1101	A/A	Nethdrone-2	-5.59	99.82	-5.34	-1.53	6.57	2.71	0.10	0.40	20	0.946	10.34	24
15	Missouri	st4	1102	A/A	Nethdrone-3	3.89	91.12	-5.64	-1.29	2.73	1.11	0.05	0.15	20	0.988	4.15	24
16	Missouri	st4	1102	A/A	Nethdrone-4	-2.43	100.50	-5.47	-1.37	4.64	1.88	0.07	0.21	20	0.974	7.13	24
17	Missouri	st4	1103	A/A	Nethdrone-1	-1.74	81.26	-5.76	-1.37	2.48	1.03	0.04	0.16	20	0.989	3.87	24
18	Missouri	st4	1104	A/A	Nethdrone-3	-0.14	100.60	-5.38	-1.45	2.95	1.21	0.04	0.15	20	0.988	4.61	24
19	Missouri	st4	1104	A/A	Nethdrone-4	1.28	93.47	-5.59	-1.59	5.04	2.15	0.09	0.34	20	0.960	8.21	24
20	Missouri	st4	1106	A/A	Nethdrone-1	1.97	95.17	-5.51	-1.77	5.94	2.57	0.12	0.44	20	0.945	9.88	24
21	Missouri	st4	1106	A/A	Nethdrone-2	0.04	98.99	-5.33	-1.57	3.57	1.49	0.06	0.23	20	0.981	5.70	24
22	Missouri	st4	1107	A/A	Nethdrone-3	2.02	97.55	-5.37	-1.49	3.21	1.33	0.05	0.19	20	0.984	5.06	24
23	Missouri	st4	1107	A/A	Nethdrone-4	4.49	98.16	-5.52	-1.57	2.53	1.07	0.05	0.16	20	0.990	4.10	24
24	Missouri	st4	1108	A/A	Nethdrone-1	-4.53	120.70	-5.44	-1.61	4.17	1.78	0.06	0.20	20	0.985	6.81	24
25	Missouri	st4	1108	A/A	Nethdrone-2	-7.49	110.50	-5.49	-1.10	6.41	2.37	0.07	0.19	20	0.969	8.71	24
26	Missouri	st4	1109	A/A	Nethdrone-3	-0.47	86.33	-5.74	-1.46	5.02	2.12	0.09	0.36	20	0.957	8.03	24
27	Missouri	st4	1109	A/A	Nethdrone-4	-1.93	92.73	-5.73	-1.92	3.15	1.39	0.07	0.46	20	0.985	5.33	24

Table PS-2. (FW Assay), PRISM curve fits parameter estimates for alternative control weak binder, Norethindrone.

Statistic	Estimates for PRISM Curve Fit Parameters, FW Assay, Alternate Control Weak Binder, Norethindrone											
	Bottom	**Top**	**LogIC$_{50}$**	**Hill Slope**	**Bottom SEM**	**Top SEM**	**LogIC$_{50}$ SEM**	**Hillslope SEM**	**DF**	**R2**	**Syx**	**N**
Mean	2.380	96.141	-5.731	-1.407	3.321	1.723	0.060	0.330	20	0.979	5.921	24
Standard Deviation	5.017	8.440	0.265	0.321	1.677	0.436	0.021	0.273	0.00	0.014	1.821	0.00
n	27	27	27	27	27	27	27	27	27	27	27	27
Coefficient of Variation (%)	210.81	8.78	4.62	22.80	50.51	25.30	34.55	82.89	0.00	1.42	30.75	0.00

	95% Confidence Interval			
	Bottom	**Top**	LogIC50	Hill Slope
Lower limit	0.395	92.800	-5.836	-1.534
Upper limit	4.365	99.480	-5.626	-1.280

PART 3

Table PS-5 (CERI Assay) Chemical ER Binding Classification for each Run

and

Table PS-6 (FW Assay) Chemical ER Binding Classification for each Run

Introduction

These following two tables are revised versions of Tables B-5 and B-6 that were prepared and submitted to the U.S. EPA under Contract No: EP-W-11-063, Task Order No. 10, Additional *Statistical Analysis for Human Recombinant Estrogen Receptor (hrER) Validation*, by Battelle, Columbus, Ohio (see *Integrated Summary Report for the Validation of Two hrER Binding Assays*, Appendix H).

The revised tables PS-5 and PS-6 show the chemical classification for each chemical when tested using both the CERI and FW Assays in four laboratories (CeeTox, Freyberger (Bayer), JapanCERI and U. Missouri). Each line in the table reflects the outcome of a single run of the assay for a given chemical using criteria described in the *Integrated Summary Report* (Section III-A). Each chemical was tested at 8 concentrations up to the limit of solubility but did not exceed 10-3 M, each in triplicate. Labs that participated in the validation study submitted at least 3 independent runs for each chemical. These data were used for the development of the draft performance standards that include the assessments of intra- and inter-assay reproducibility as well as accuracy for correctly identifying ER binders and non-binders.

Tables PS-5 and PS-6 contain all runs for the controls and test chemicals that were submitted from 4 laboratories that were (1) acceptable by the submitting laboratory, and (2) contained data for at least 6 concentrations of the test chemical. During the development of the draft Performance Standards, only the first 3 sequential runs (by dates conducted) for a given chemical were used for the analysis of intra-laboratory reproducibility for cases where more than 3 runs were submitted.

Classification of test chemicals were coded as numeric values as shown below to assign a final classification across the 3 runs for each test chemical.

To assign value to each run:	
Classification	**Numeric Value**
Binder	2
Equivocal	1
Non-binder	0
To classify average of numeric value across runs:	
Classification	**Numeric Value**
Binder	Average $\geq$ 1.5
Equivocal	0.5 Average < 1.5
Non-binder	Average < 0.5

Table PS-5, CERI hrER Binding Assay

Table PS-5[1]. CERI Assay, Chemical ER Binding Classification by Individual Test Runs. [2]

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	DBP	st1	20090207	0	0	0
CERI	Ceetox	DBP	st1	20090208	0	0	
CERI	Ceetox	DBP	st1	20090211	0	0	
CERI	Ceetox	DBP	st2	20090215	0		
CERI	Ceetox	DBP	st2	20090221	0		
CERI	Ceetox	DBP	st3	20090310A	0		
CERI	Ceetox	DBP	st4	20090903A	0		
CERI	Ceetox	DBP	st4	20090905A	0		
CERI	Ceetox	DBP	st4	20090930A	0		
CERI	Ceetox	E2	st1	20090207	2	2	2
CERI	Ceetox	E2	st1	20090208	2	2	
CERI	Ceetox	E2	st1	20090211	2	2	
CERI	Ceetox	NE	st1	20090207	2	2	2
CERI	Ceetox	NE	st1	20090208	2		
CERI	Ceetox	NE	st1	20090211	2		
CERI	Ceetox	ButylPar	st2	20090215	2	2	2
CERI	Ceetox	ButylPar	st2	20090221	2	2	
CERI	Ceetox	ButylPar	st2	20090218	2	2	
CERI	Ceetox	Cort	st2	20090215	0	0	0

[1] This table is an updated version of Table B-5 (see ISR, Appendix H) and now includes the equivocal runs that were reanalyzed using the 10% Rule (see PBTG, Annex 2) and new classifications as described on pages 1-4 and Table C-1 (ISR, Appendix H). Additional runs that were not used in the original data analysis have been included as reported in this document and are highlighted with yellow.

[2] Table was revised (9-29-2014) to include classification following reanalyzes using the 10% Rule as shown in Table C-1). Revised (11-21-2014) to include 2 new columns for "runs used for PS' and "final classification for lab'.

[3] A simple average of numeric values across runs was used for final classification. Classified as 'binder' if average ≥1.5, 'equivocal' if 0.5 ≤ average ≤ 1.5, or 'non-binder' if average < 0.5.

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	Cort	st2	20090221	0	0	
CERI	Ceetox	Cort	st2	20090218	0	0	
CERI	Ceetox	Equol	st2	20090215	2	2	2
CERI	Ceetox	Equol	st2	20090218	2	2	
CERI	Ceetox	Equol	st2	20090221	2	2	
CERI	Ceetox	Hexestrol	st2	20090215	2	2	2
CERI	Ceetox	Hexestrol	st2	20090218	2	2	
CERI	Ceetox	Hexestrol	st2	20090221	2	2	
CERI	Ceetox	NPhenol	st2	20090215	2	2	2
CERI	Ceetox	NPhenol	st2	20090218	2	2	
CERI	Ceetox	NPhenol	st2	20090221	1	1	
CERI	Ceetox	DDT	st2	20090215	2	2	2
CERI	Ceetox	DDT	st2	20090218	2	2	
CERI	Ceetox	DDT	st2	20090221	2	2	
CERI	Ceetox	DES	st2	20090215	2	2	2
CERI	Ceetox	DES	st2	20090218	2	2	
CERI	Ceetox	DES	st2	20090221	2	2	
CERI	Ceetox	Gen	st2	20090215	2	2	2
CERI	Ceetox	Gen	st2	20090218	2	2	
CERI	Ceetox	Gen	st2	20090221	2	2	
CERI	Ceetox	E2-TC[4]	st3	20090305A	2	2	2
CERI	Ceetox	E2-TC	st3	20090310A	2	2	
CERI	Ceetox	E2-TC	st3	2009401A	2	2	
CERI	Ceetox	E2-TC	st3	20090415	2		
CERI	Ceetox	NE-TC	st3	20090305A	2	2	2
CERI	Ceetox	NE-TC	st3	20090310A	2	2	

[4] E2-TC (TC indicates that the control was included as a coded chemical and evaluated as a “test chemical”).

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	NE-TC	st3	20090405A	2	2	
CERI	Ceetox	DBP-TC	st3	20090305A	1	1	1
CERI	Ceetox	DBP-TC	st3	20090310A	2	2	
CERI	Ceetox	DBP-TC	st3	20090401A	1	1	
CERI	Ceetox	DBP-TC	st3	20090415	2		
CERI	Ceetox	Zear	st3	20090310A	2	2	2
CERI	Ceetox	Zear	st3	2009401A	2	2	
CERI	Ceetox	Zear	st3	20090415	2	2	
CERI	Ceetox	Tamox	st3	20090310A	2	2	2
CERI	Ceetox	Tamox	st3	20090401A	2	2	
CERI	Ceetox	Tamox	st3	20090405	2	2	
CERI	Ceetox	DHT	st3	20090305A	2	2	2
CERI	Ceetox	DHT	st3	20090310A	2	2	
CERI	Ceetox	DHT	st3	20090401A	2	2	
CERI	Ceetox	BPA	st3	20090305A	2	2	2
CERI	Ceetox	BPA	st3	20090310A	2	2	
CERI	Ceetox	BPA	st3	20090401A	2	2	2
CERI	Ceetox	Heptylphenol	st3	20090305A	2	2	
CERI	Ceetox	Heptylphenol	st3	20090310A	2	2	
CERI	Ceetox	Heptyphenol	st3	20090401A	2	2	
CERI	Ceetox	Kepone	st3	20090305A	2	2	2
CERI	Ceetox	Kepone	st3	20090310A	2	2	
CERI	Ceetox	Kepone	st3	20090401A	2	2	
CERI	Ceetox	BaA	st3	20090305A	0	0	0
CERI	Ceetox	BaA	st3	20090310A	0	0	
CERI	Ceetox	BaA	st3	20090401A	0	0	
CERI	Ceetox	ELactone	st3	20090305A	2	2	2
CERI	Ceetox	ELactone	st3	20090310A	2	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	ELactone	st3	20090401A	2	2	
CERI	Ceetox	Progesterone	st3	20090305A	0	0	0
CERI	Ceetox	Progesterone	st3	20090310A	0	0	
CERI	Ceetox	Progesterone	st3	20090401A	0	0	
CERI	Ceetox	OTES	st3	20090310B	0	0	0
CERI	Ceetox	OTES	st3	20090305B	0	0	
CERI	Ceetox	OTES	st3	20090401B	0	0	
CERI	Ceetox	Atr	st3	20090228B	0	0	0
CERI	Ceetox	Atr	st3	20090305A	0	0	
CERI	Ceetox	Atr	st3	20090310B	0	0	
CERI	Ceetox	Atr	st3	20090401B	1		
CERI	Ceetox	Cort-1	st4	20090903A	0		
CERI	Ceetox	Cort-1	st4	20090905A	0		
CERI	Ceetox	Cort-1	st4	20090930A	0		
CERI	Ceetox	Dex-1	st4	20090903A	0		
CERI	Ceetox	Dex-1	st4	20090905A	0		
CERI	Ceetox	Dex-1	st4	20090930A	0		
CERI	Ceetox	Nethdrone-1	st4	20090905A	2	2	
CERI	Ceetox	Nethdrone-1	st4	20090930A	2	2	
CERI	Ceetox	HPTE-1	st4	20090903A	2		
CERI	Ceetox	HPTE-1	st4	20090905A	2		
CERI	Ceetox	HPTE-1	st4	20090930A	2		
CERI	Ceetox	Gen-1	st4	20090903A	2		
CERI	Ceetox	Gen-1	st4	20090905A	2		
CERI	Ceetox	Gen-1	st4	20090930A	2		
CERI	Ceetox	BPA-1	st4	20090903A	2		
CERI	Ceetox	BPA-1	st4	20090905A	2		
CERI	Ceetox	BPA-1	st4	20090930A	2		

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	OTES-1	st4	20090903A	0		
CERI	Ceetox	OTES-1	st4	20090905A	0		
CERI	Ceetox	OTES-1	st4	20090930A	0		
CERI	Ceetox	Nethdrone-2	st4	20090903A	2	2	2
CERI	Ceetox	Nethdrone-2	st4	20090905A	2	2	
CERI	Ceetox	Nethdrone-2	st4	20090930A	2	2	
CERI	Ceetox	BPA-2	st4	20090903A	2		
CERI	Ceetox	BPA-2	st4	20090905A	2		
CERI	Ceetox	BPA-2	st4	20090930A	2		
CERI	Ceetox	Dex-2	st4	20090903A	0		
CERI	Ceetox	Dex-2	st4	20090905A	1		
CERI	Ceetox	Dex-2	st4	20090930A	1 (10% rule, no change in classification)		
CERI	Ceetox	Nethdrone-3	st4	20090903A	2	2	2
CERI	Ceetox	Nethdrone-3	st4	20090905A	2	2	
CERI	Ceetox	Nethdrone-3	st4	20090930A	2	2	
CERI	Ceetox	Cort-2	st4	20090903A	1 (10% rule, no change in classification)		
CERI	Ceetox	Cort-2	st4	20090905A	0		
CERI	Ceetox	Cort-2	st4	20090930A	1 (10% rule, no change in classification)		
CERI	Ceetox	HPTE-2	st4	20090903B	2		
CERI	Ceetox	HPTE-2	st4	20090905B	2		
CERI	Ceetox	HPTE-2	st4	20090930B	2		
CERI	Ceetox	OTES-2	st4	20090903B	0		
CERI	Ceetox	OTES-2	st4	20090905B	0		
CERI	Ceetox	OTES-2	st4	20090930B	0		

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Ceetox	Nethdrone-4	st4	20090903B	2	2	2
CERI	Ceetox	Nethdrone-4	st4	20090905B	2	2	
CERI	Ceetox	Nethdrone-4	st4	20090930B	2	2	
CERI	Ceetox	Gen-2	st4	20090903B	2		
CERI	Ceetox	Gen-2	st4	20090905B	2		
CERI	Ceetox	Gen-2	st4	20090930B	2 (10% rule, Reclassified as 2)		
CERI	Ceetox	EE2	st2	20090215	2	2	2
CERI	Ceetox	EE2	st2	20090218	2	2	
CERI	Ceetox	EE2	st2	20090221	1 (10% rule, No change classification	1	
CERI	Freyberger	DBP	st2	B	0	0	0
CERI	Freyberger	DBP	st2	C	0	0	
CERI	Freyberger	DBP	st2	D	0	0	
CERI	Freyberger	DBP	st2	E	0		
CERI	Freyberger	DBP	st2	F	0		
CERI	Freyberger	DBP	st2	G	0		
CERI	Freyberger	DBP	st3	CODE A2	1		
CERI	Freyberger	DBP	st3	CODE A3	0		
CERI	Freyberger	DBP	st3	CODE B1	0		
CERI	Freyberger	DBP	st3	CODE B2	1		
CERI	Freyberger	DBP	st3	CODE B3	0		
CERI	Freyberger	DBP	st3	CODE C1	0		
CERI	Freyberger	DBP	st3	CODE C2	0		
CERI	Freyberger	DBP	st3	CODE C3	0		
CERI	Freyberger	ButylPar	st2	B	2	2	2
CERI	Freyberger	ButylPar	st2	C	2	2	
CERI	Freyberger	ButylPar	st2	D	2	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Freyberger	Cort	st2	B	1 (10% rule, No change classification	1	0
CERI	Freyberger	Cort	st2	C	0	0	
CERI	Freyberger	Cort	st2	D	0	0	
CERI	Freyberger	Equol	st2	B	2	2	2
CERI	Freyberger	Equol	st2	C	2	2	
CERI	Freyberger	Equol	st2	D	2	2	
CERI	Freyberger	E2	st2	B	2	2	2
CERI	Freyberger	E2	st2	C	2	2	
CERI	Freyberger	E2	st2	D	2	2	
CERI	Freyberger	Hexestrol	st2	B	2	2	2
CERI	Freyberger	Hexestrol	st2	C	2	2	
CERI	Freyberger	Hexestrol	st2	D	2	2	
CERI	Freyberger	NPhenol	st2	E	2	2	2
CERI	Freyberger	NPhenol	st2	F	2	2	
CERI	Freyberger	NPhenol	st2	G	2	2	
CERI	Freyberger	DDT	st2	E	2	2	2
CERI	Freyberger	DDT	st2	F	2	2	
CERI	Freyberger	DDT	st2	G	2	2	
CERI	Freyberger	DES	st2	E	2	2	2
CERI	Freyberger	DES	st2	F	2	2	
CERI	Freyberger	DES	st2	G	2	2	
CERI	Freyberger	Gen	st2	E	2 (10% rule, Reclassified as 2)	2	2
CERI	Freyberger	Gen	st2	F	2 (10% rule, Reclassified as 2)	2	
CERI	Freyberger	Gen	st2	G	2 (10% rule, Reclassified as 2)	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Freyberger	NE	st2	B	2	2	2
CERI	Freyberger	NE	st2	C	2	2	
CERI	Freyberger	NE	st2	D	2	2	
CERI	Freyberger	E2-TC	st3	Code A1	2 (10% rule, Reclassified as 2)	2	2
CERI	Freyberger	E2-TC	st3	CODE A2	2	2	
CERI	Freyberger	E2-TC	st3	CODE A3	2 (10% rule, Reclassified as 2)	2	
CERI	Freyberger	NE-TC	st3	CODE C1	2		
CERI	Freyberger	NE-TC	st3	CODE C2	2		
CERI	Freyberger	NE-TC	st3	CODE C3	2		
CERI	Freyberger	DBP-TC	st3	CODE B1	2	2	2
CERI	Freyberger	DBP-TC	st3	CODE B2	2	2	
CERI	Freyberger	DBP-TC	st3	CODE B3	2	2	
CERI	Freyberger	Zear	st3	CODE B1	2	2	2
CERI	Freyberger	Zear	st3	CODE B2	2	2	
CERI	Freyberger	Zear	st3	CODE B3	2	2	
CERI	Freyberger	Tamox	st3	Code A1	2	2	2
CERI	Freyberger	Tamox	st3	CODE A2	2	2	
CERI	Freyberger	Tamox	st3	CODE A3	Outlier point (-10 M) deleted, Reanalyzed & classified as 2)	2	
CERI	Freyberger	DHT	st3	CODE B1	2	2	2
CERI	Freyberger	DHT	st3	CODE B2	2	2	
CERI	Freyberger	DHT	st3	CODE B3	2	2	
CERI	Freyberger	BPA	st3	CODE 1A	2	2	2
CERI	Freyberger	BPA	st3	CODE A2	2	2	
CERI	Freyberger	BPA	st3	CODE A3	2	2	
CERI	Freyberger	Heptylphenol	st3	CODE B1	2	2	2

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Freyberger	Heptylphenol	st3	CODE B2	2	2	
CERI	Freyberger	Heptylphenol	st3	CODE B3	2	2	
CERI	Freyberger	Kepone	st3	CODE A1	2	2	2
CERI	Freyberger	Kepone	st3	CODE A2	2	2	
CERI	Freyberger	Kepone	st3	CODE A3	2	2	
CERI	Freyberger	BaA	st3	CODE C1	0	0	0
CERI	Freyberger	BaA	st3	CODE C2	0	0	
CERI	Freyberger	BaA	st3	CODE C3	0	0	
CERI	Freyberger	ELactone	st3	Code 1A	2	2	2
CERI	Freyberger	ELactone	st3	CODE A2	2	2	
CERI	Freyberger	ELactone	st3	CODE A3	2	2	
CERI	Freyberger	Progesterone	st3	CODE C1	0	0	0
CERI	Freyberger	Progesterone	st3	CODE C2	0	0	
CERI	Freyberger	Progesterone	st3	CODE C3	0	0	
CERI	Freyberger	OTES	st3	CODE B1	0	0	0
CERI	Freyberger	OTES	st3	CODE B2	0	0	
CERI	Freyberger	OTES	st3	CODE B3	0	0	
CERI	Freyberger	Atr	st3	CODE C1	0	0	0
CERI	Freyberger	Atr	st3	CODE C2	0	0	
CERI	Freyberger	Atr	st3	CODE C3	0	0	
CERI	Freyberger	EE2	st2	E	2	2	2
CERI	Freyberger	EE2	st2	F	2	2	
CERI	Freyberger	EE2	st2	G	2	2	
CERI	JapanCERI	DBP	st1	91224	0	0	0
CERI	JapanCERI	DBP	st1	100107	0	0	
CERI	JapanCERI	DBP	st1	100108	0	0	
CERI	JapanCERI	DBP	st2	100119	0		
CERI	JapanCERI	DBP	st2	100126	0		

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	JapanCERI	DBP	st2	100129	0		
CERI	JapanCERI	DBP	st3	100203	0		
CERI	JapanCERI	DBP	st3	100208	0		
CERI	JapanCERI	DBP	st3	100215	0		
CERI	JapanCERI	DBP	st3	100223	0		
CERI	JapanCERI	DBP	st3	100224	0		
CERI	JapanCERI	DBP	st3	100225	0		
CERI	JapanCERI	DBP	st3	100226	0		
CERI	JapanCERI	DBP	st3	100301	0		
CERI	JapanCERI	DBP	st3	100303	0		
CERI	JapanCERI	DBP	st3	100305	0		
CERI	JapanCERI	E2	st1	91224	2	2	2
CERI	JapanCERI	E2	st1	100107	2	2	
CERI	JapanCERI	E2	st1	100108	2	2	
CERI	JapanCERI	ButylPar	st2	100119	2	2	2
CERI	JapanCERI	ButylPar	st2	100126	2	2	
CERI	JapanCERI	ButylPar	st2	100129	2	2	
CERI	JapanCERI	Cort	st2	100119	0	0	0
CERI	JapanCERI	Cort	st2	100126	0	0	
CERI	JapanCERI	Cort	st2	100129	0	0	
CERI	JapanCERI	Equol	st2	100119	2	2	2
CERI	JapanCERI	Equol	st2	100126	2	2	
CERI	JapanCERI	Equol	st2	100129	2	2	
CERI	JapanCERI	Hexestrol	st2	100119	2	2	2
CERI	JapanCERI	Hexestrol	st2	100126	2	2	
CERI	JapanCERI	Hexestrol	st2	100129	2	2	
CERI	JapanCERI	NPhenol	st2	100119	2	2	2
CERI	JapanCERI	NPhenol	st2	100126	2	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	JapanCERI	NPhenol	st2	100129	2	2	
CERI	JapanCERI	NE	st1	91224	2	2	2
CERI	JapanCERI	NE	st1	100107	2	2	
CERI	JapanCERI	NE	st1	100108	2	2	
CERI	JapanCERI	DDT	st2	100119	2	2	2
CERI	JapanCERI	DDT	st2	100126	2	2	
CERI	JapanCERI	DDT	st2	100129	2	2	
CERI	JapanCERI	DES	st2	100119	2	2	2
CERI	JapanCERI	DES	st2	100126	2	2	
CERI	JapanCERI	DES	st2	100129	2	2	
CERI	JapanCERI	Gen	st2	100119	2	2	2
CERI	JapanCERI	Gen	st2	100126	2	2	
CERI	JapanCERI	Gen	st2	100129	1	1	
CERI	JapanCERI	E2-TC	st3	100223	2	2	2
CERI	JapanCERI	E2-TC	st3	100224	2	2	
CERI	JapanCERI	E2-TC	st3	100225	2	2	
CERI	JapanCERI	NE-TC	st3	100223	2	2	2
CERI	JapanCERI	NE-TC	st3	100224	2	2	
CERI	JapanCERI	NE-TC	st3	100225	2	2	
CERI	JapanCERI	DBP-TC	st3	100203	0	0	0
CERI	JapanCERI	DBP-TC	st3	100208	0	0	
CERI	JapanCERI	DBP-TC	st3	100215	0	0	
CERI	JapanCERI	Zear	st3	100203	2	2	2
CERI	JapanCERI	Zear	st3	100208	2	2	
CERI	JapanCERI	Zear	st3	100215	2	2	
CERI	JapanCERI	Tamox	st3	100203	2	2	2
CERI	JapanCERI	Tamox	st3	100208	2	2	
CERI	JapanCERI	Tamox	st3	100215	2	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	JapanCERI	DHT	st3	100203	2	2	2
CERI	JapanCERI	DHT	st3	100208	2	2	
CERI	JapanCERI	DHT	st3	100215	2	2	
CERI	JapanCERI	BPA	st3	100203	2	2	2
CERI	JapanCERI	BPA	st3	100208	2	2	
CERI	JapanCERI	BPA	st3	100215	2	2	
CERI	JapanCERI	BPA	st3	100226	2		
CERI	JapanCERI	Heptylphenol	st3	100223	1	1	1
CERI	JapanCERI	Heptylphenol	st3	100224	1	1	
CERI	JapanCERI	Heptylphenol	st3	100225	1	1	
CERI	JapanCERI	Heptylphenol	st3	100226	1		
CERI	JapanCERI	Kepone	st3	100203	2	2	2
CERI	JapanCERI	Kepone	st3	100208	2	2	
CERI	JapanCERI	Kepone	st3	100215	2	2	
CERI	JapanCERI	BaA	st3	100203	0	0	0
CERI	JapanCERI	BaA	st3	100208	0	0	
CERI	JapanCERI	BaA	st3	100215	0	0	
CERI	JapanCERI	ELactone	st3	100223	1	1	1
CERI	JapanCERI	ELactone	st3	100224	1	1	
CERI	JapanCERI	ELactone	st3	100225	1	1	
CERI	JapanCERI	Progesterone	st3	100203	0	0	0
CERI	JapanCERI	Progesterone	st3	100208	0	0	
CERI	JapanCERI	Progesterone	st3	100215	0	0	
CERI	JapanCERI	Progesterone	st3	100226	0		
CERI	JapanCERI	OTES	st3	100301	0	0	0
CERI	JapanCERI	OTES	st3	100303	0	0	
CERI	JapanCERI	OTES	st3	100305	0	0	
CERI	JapanCERI	Atr	st3	100223	0	0	0

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	JapanCERI	Atr	st3	100224	0	0	
CERI	JapanCERI	Atr	st3	100225	0	0	
CERI	JapanCERI	EE2	st2	100119	2	2	2
CERI	JapanCERI	EE2	st2	100126	2	2	
CERI	JapanCERI	EE2	st2	100129	2	2	
CERI	Missouri	DBP	st1	4001	0	0	0
CERI	Missouri	DBP	st1	4002	0	0	
CERI	Missouri	DBP	st1	4003	0	0	
CERI	Missouri	DBP	st1	4005	0		
CERI	Missouri	DBP	st1	4006	0		
CERI	Missouri	DBP	st1	4007	0		
CERI	Missouri	DBP	st1	5003	0		
CERI	Missouri	DBP	st1	5007	0		
CERI	Missouri	DBP	st1	5010	0		
CERI	Missouri	DBP	st2	5006	1		
CERI	Missouri	DBP	st2	5008	0		
CERI	Missouri	DBP	st2	5009	0		
CERI	Missouri	DBP	st3	6005	0		
CERI	Missouri	DBP	st3	6006	0		
CERI	Missouri	DBP	st3	6007	0		
CERI	Missouri	DBP	st3	6009	0		
CERI	Missouri	DBP	st3	6010	0		
CERI	Missouri	DBP	st3	6011	0		
CERI	Missouri	DBP	st3	6502	0		
CERI	Missouri	DBP	st3	6503	0		
CERI	Missouri	DBP	st3	6504	0		
CERI	Missouri	DBP	st4	2206	1		
CERI	Missouri	DBP	st4	2210	0		

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	DBP	st4	2211	0		
CERI	Missouri	DBP	st4	2301	0		
CERI	Missouri	DBP	st4	2302	0		
CERI	Missouri	DBP	st4	2305	0		
CERI	Missouri	E2	st1	4001	2	2	2
CERI	Missouri	E2	st1	4002	2	2	
CERI	Missouri	E2	st1	4003	2	2	
CERI	Missouri	ButylPar	st2	5006	2	2	2
CERI	Missouri	ButylPar	st2	5008	2	2	
CERI	Missouri	ButylPar	st2	5009	2	2	
CERI	Missouri	Cort	st2	5006	1 (10% rule, No change classification)	1	0
CERI	Missouri	Cort	st2	5008	0	0	
CERI	Missouri	Cort	st2	5009	0	0	
CERI	Missouri	Equol	st2	5006	2	2	2
CERI	Missouri	Equol	st2	5008	2	2	
CERI	Missouri	Equol	st2	5009	2	2	
CERI	Missouri	Hexestrol	st2	5006	2	2	2
CERI	Missouri	Hexestrol	st2	5008	2	2	
CERI	Missouri	Hexestrol	st2	5009	2	2	
CERI	Missouri	NPhenol	st2	5006	2	2	2
CERI	Missouri	NPhenol	st2	5008	2	2	
CERI	Missouri	NPhenol	st2	5009	2	2	
CERI	Missouri	NE	st1	4001	2	2	2
CERI	Missouri	NE	st1	4002	2	2	
CERI	Missouri	NE	st1	4003	2	2	
CERI	Missouri	DDT	st2	5006	2	2	2

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	DDT	st2	5008	2	2	
CERI	Missouri	DDT	st2	5009	2	2	
CERI	Missouri	DES	st2	5006	2	2	2
CERI	Missouri	DES	st2	5008	2	2	
CERI	Missouri	DES	st2	5009	2	2	
CERI	Missouri	Gen	st2	5006	2 (10% rule, No change classification)	2	2
CERI	Missouri	Gen	st2	5008	2	2	
CERI	Missouri	Gen	st2	5009	1(10% rule, No change classification)		
CERI	Missouri	E2-TC	st3	6005	2	2	2
CERI	Missouri	E2-TC	st3	6006	2 (10% rule, Reclassification to 2)	2	
CERI	Missouri	E2-TC	st3	6007	2 (10% rule, Reclassification to 2)	2	
CERI	Missouri	NE-TC	st3	6005	2	2	2
CERI	Missouri	NE-TC	st3	6006	2	2	
CERI	Missouri	NE-TC	st3	6007	2	2	
CERI	Missouri	DBP-TC	st3	6005	0	0	0
CERI	Missouri	DBP-TC	st3	6006	0	0	
CERI	Missouri	DBP-TC	st3	6007	0	0	
CERI	Missouri	Zear	st3	6005	2	2	2
CERI	Missouri	Zear	st3	6006	2	2	
CERI	Missouri	Zear	st3	6007	2	2	
CERI	Missouri	Tamox	st3	6005	2	2	2
CERI	Missouri	Tamox	st3	6006	2	2	
CERI	Missouri	Tamox	st3	6007	2 (10% rule, Reclassification to 2)	2	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	DHT	st3	6005	2		
CERI	Missouri	DHT	st3	6006	1		
CERI	Missouri	DHT	st3	6007	1		
CERI	Missouri	BPA	st3	6005	2		
CERI	Missouri	BPA	st3	6006	2		
CERI	Missouri	BPA	st3	6007	2		
CERI	Missouri	Heptylphenol	st3	6009	1	1	1
CERI	Missouri	Heptylphenol	st3	6010	2	2	
CERI	Missouri	Heptylphenol	st3	6011	0	0	
CERI	Missouri	Heptylphenol	st3	6502	1 (10% Rule, No change in classification)		
CERI	Missouri	Heptylphenol	st3	6503	1		
CERI	Missouri	Heptylphenol	st3	6504	1		
CERI	Missouri	Kepone	st3	6009	2	2	2
CERI	Missouri	Kepone	st3	6010	2	2	
CERI	Missouri	Kepone	st3	6011	2	2	
CERI	Missouri	Kepone	st3	6502	2		
CERI	Missouri	Kepone	st3	6503	2		
CERI	Missouri	Kepone	st3	6504	2		
CERI	Missouri	BaA	st3	6009	0	0	0
CERI	Missouri	BaA	st3	6010	0	0	
CERI	Missouri	BaA	st3	6011	0	0	
CERI	Missouri	BaA	st3	6502	0		
CERI	Missouri	BaA	st3	6503	0		
CERI	Missouri	BaA	st3	6504	0		
CERI	Missouri	ELactone	st3	6009	1	1	1
CERI	Missouri	ELactone	st3	6010	1	1	

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	ELactone	st3	6011	0	0	
CERI	Missouri	ELactone	st3	6502	0		
CERI	Missouri	ELactone	st3	6503	1		
CERI	Missouri	ELactone	st3	6504	1		
CERI	Missouri	Progesterone	st3	6009	0	0	0
CERI	Missouri	Progesterone	st3	6010	0	0	
CERI	Missouri	Progesterone	st3	6011	0	0	
CERI	Missouri	Progesterone	st3	6502	0		
CERI	Missouri	Progesterone	st3	6503	0		
CERI	Missouri	Progesterone	st3	6504	0		
CERI	Missouri	OTES	st3	6009	0	0	0
CERI	Missouri	OTES	st3	6010	0	0	
CERI	Missouri	OTES	st3	6011	0	0	
CERI	Missouri	OTES	st3	6502	0		
CERI	Missouri	OTES	st3	6503	0		
CERI	Missouri	OTES	st3	6504	0		
CERI	Missouri	Atr	st3	6009	0	0	0
CERI	Missouri	Atr	st3	6010	0	0	
CERI	Missouri	Atr	st3	6011	0	0	
CERI	Missouri	Atr	st3	6502	0		
CERI	Missouri	Atr	st3	6503	0		
CERI	Missouri	Atr	st3	6504	0		
CERI	Missouri	Cort-1	st4	2206	0		
CERI	Missouri	Cort-1	st4	2210	0		
CERI	Missouri	Cort-1	st4	2211	0		
CERI	Missouri	Dex-1	st4	2206	0		
CERI	Missouri	Dex-1	st4	2210	1		
CERI	Missouri	Dex-1	st4	2211	0		

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	Nethdrone-1	st4	2206	2	2	2
CERI	Missouri	Nethdrone-1	st4	2210	2	2	
CERI	Missouri	Nethdrone-1	st4	2211	2	2	
CERI	Missouri	HPTE-1	st4	2206	2		
CERI	Missouri	HPTE-1	st4	2210	2		
CERI	Missouri	HPTE-1	st4	2211	2		
CERI	Missouri	Gen-1	st4	2206	2 (10% rule, Reclassified as 2)		
CERI	Missouri	Gen-1	st4	2210	2		
CERI	Missouri	Gen-1	st4	2211	2 (10% rule, Reclassified as 2)		
CERI	Missouri	BPA-1	st4	2206	2 (10% rule, Reclassified as 2)		
CERI	Missouri	BPA-1	st4	2210	2		
CERI	Missouri	BPA-1	st4	2211	2		
CERI	Missouri	OTES-1	st4	2206	0		
CERI	Missouri	OTES-1	st4	2210	0		
CERI	Missouri	OTES-1	st4	2211	0		
CERI	Missouri	Nethdrone-2	st4	2206	2	2	2
CERI	Missouri	Nethdrone-2	st4	2210	2	2	
CERI	Missouri	Nethdrone-2	st4	2211	2	2	
CERI	Missouri	BPA-2	st4	2301	2		
CERI	Missouri	BPA-2	st4	2302	2		
CERI	Missouri	BPA-2	st4	2305	2 (10% rule, Reclassified as 2)		
CERI	Missouri	Dex-2	st4	2301	1		
CERI	Missouri	Dex-2	st4	2302	0		
CERI	Missouri	Dex-2	st4	2305	0		
CERI	Missouri	Nethdrone-3	st4	2301	2 (10% rule, Reclassified as 2)	2	2

Table PS-5, CERI hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[3]
CERI	Missouri	Nethdrone-3	st4	2302	2	2	
CERI	Missouri	Nethdrone-3	st4	2305	2 (10% rule, Reclassified as 2)	2	
CERI	Missouri	Cort-2	st4	2301	0		
CERI	Missouri	Cort-2	st4	2302	0		
CERI	Missouri	Cort-2	st4	2305	0		
CERI	Missouri	HPTE-2	st4	2301	2		
CERI	Missouri	HPTE-2	st4	2302	2		
CERI	Missouri	HPTE-2	st4	2305	2 (10% rule, Reclassified as 2)		
CERI	Missouri	OTES-2	st4	2301	1 (10% rule, No change in Classification		
CERI	Missouri	OTES-2	st4	2302	0		
CERI	Missouri	OTES-2	st4	2305	1		
CERI	Missouri	Nethdrone-4	st4	2301	2	2	2
CERI	Missouri	Nethdrone-4	st4	2302	2	2	
CERI	Missouri	Nethdrone-4	st4	2305	2 (10% rule, Reclassified as 2)	2	
CERI	Missouri	Gen-2	st4	2301	2 (10% rule, Reclassified as 2)		
CERI	Missouri	Gen-2	st4	2302	2 (10% rule, Reclassified as 2)		
CERI	Missouri	Gen-2	st4	2305	2 (10% rule, Reclassified as 2)		
CERI	Missouri	EE2	st2	5006	2		
CERI	Missouri	EE2	st2	5008	2		
CERI	Missouri	EE2	st2	5009	2		

Table PS-5, CERI hrER Binding Assay

Table PS--6.[5] FW Assay, Chemical hER Binding Classifications by Individual Test Run. [6]

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	Atr	st3	20090505	0 (10% rule used, no change in classification)	0	0
FWA	Ceetox	Atr	st3	20090418B	0	0	
FWA	Ceetox	Atr	st3	20090426	0	0	
FWA	Ceetox	BPA	st3	20090418	2	2	2
FWA	Ceetox	BPA	st3	2009426	2	2	
FWA	Ceetox	BPA	st3	20090505	2	2	
FWA	Ceetox	BPA	st4	20090914	2 (10% rule, Reanalyzed and new classification of 2)		
FWA	Ceetox	BPA	st4	20090917A	2		
FWA	Ceetox	BPA	st4	20090911A	2		
FWA	Ceetox	BaA_DMSO	st3	20090514DMSO	0	0	1
FWA	Ceetox	BaA_DMSO	st3	20090418DMSO	1	1	
FWA	Ceetox	BaA_DMSO	st3	20090505DMSO	0 (2 of 3 replicates at 10-9 M were deleted as outliers. The data were reanalyzed, and chemical classified as non-binder)	0	
FWA	Ceetox	ButylPar	st2	20090223	2	2	2
FWA	Ceetox	ButylPar	st2	20090307	2	2	

[5] Table B-6 has been updated to denote particular equivocal runs that were reanalyzed using the 10% Rule and new classification as described on pages 1-4 and Table C-1 (ISR, Appendix H). Additional runs that were not used in the original data analysis have been included as reported in this document, and are highlighted in yellow.

[6] Revised (9-29-2014) to include classification following reanalyzes using the 10% Rule as shown in Table C-1 (ISR, Appendix H). Revised (11-21-2014) to include 2 new columns for "runs used for development of the PS' and "final classification for lab'.

[7] A simple average of numeric values across runs was used for final classification. Classified as 'binder' if average ≥1.5, 'equivocal' if 0.5 ≤ average ≤ 1.5, or 'non-binder' if average < 0.5.

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	ButylPar	st2	20090326	2	2	
FWA	Ceetox	Cort	st2	20090223	0	0	0
FWA	Ceetox	Cort	st2	20090326	0	0	
FWA	Ceetox	Cort	st2	20090307	0	0	
FWA	Ceetox	Cort_b	st4	20090914	0		
FWA	Ceetox	Cort_b	st4	20090914	0		
FWA	Ceetox	Cort_b	st4	20090911A	0		
FWA	Ceetox	Cort_b	st4	20090917A	0		
FWA	Ceetox	Cort_b	st4	20090917A	0		
FWA	Ceetox	Cort_b	st4	20090911A	0		
FWA	Ceetox	DBP	st1	20090207	0	0	0
FWA	Ceetox	DBP	st1	20090208	0	0	
FWA	Ceetox	DBP	st1	20090211	0	0	
FWA	Ceetox	DBP	st2	Run20090326	0		
FWA	Ceetox	DBP	st2	Run20090223	0		
FWA	Ceetox	DBP	st2	Run20090307	0		
FWA	Ceetox	DBP	st3	Run20090418	0		
FWA	Ceetox	DBP	st3	Run20090514 DMSO	0		
FWA	Ceetox	DBP	st3	Run20090505	0		
FWA	Ceetox	DBP	st3	Run20090505 DMSO	0		
FWA	Ceetox	DBP	st3	Run20090418 DMSO	0		
FWA	Ceetox	DBP	st4	Run20090917A	0		
FWA	Ceetox	DBP	st4	Run20090911A	0		
FWA	Ceetox	DBP	st4	Run20090914A	0		
FWA	Ceetox	DBP-TC8	st3	20090426	0	0	0

[8] E2-TC (TC indicates that the control was included as a coded chemical and evaluated as a “test chemical”).

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	DBP-TC	st3	20090505	1	1	
FWA	Ceetox	DBP-TC	st3	20090418	0	0	
FWA	Ceetox	DDT	st2	20090307	2	2	2
FWA	Ceetox	DDT	st2	20090223	2	2	
FWA	Ceetox	DDT	st2	20090326	2	2	
FWA	Ceetox	DES	st2	20090223	2	2	2
FWA	Ceetox	DES	st2	20090307	2	2	
FWA	Ceetox	DES	st2	20090326	2	2	
FWA	Ceetox	DHT	st3	20090426	2	2	2
FWA	Ceetox	DHT	st3	20090505	2	2	
FWA	Ceetox	DHT	st3	20090418	2	2	
FWA	Ceetox	Dex	st4	20090917A	0	0	0
FWA	Ceetox	Dex	st4	20090914	0	0	
FWA	Ceetox	Dex	st4	20090911A	0	0	
FWA	Ceetox	Dex	st4	20090914	0		
FWA	Ceetox	Dex	st4	20090917A	0		
FWA	Ceetox	Dex	st4	20090911A	0		
FWA	Ceetox	E2	st1	20081123	2	2	2
FWA	Ceetox	E2	st1	20081218	2	2	
FWA	Ceetox	E2	st1	20081124	2	2	
FWA	Ceetox	E2-TC	st3	20090418	2	2	2
FWA	Ceetox	E2-TC	st3	20090426	2	2	
FWA	Ceetox	E2-TC	st3	20090505	2	2	
FWA	Ceetox	EE2	st2	20090307	2	2	2
FWA	Ceetox	EE2	st2	20090326	2	2	
FWA	Ceetox	EE2	st2	20090223	2	2	
FWA	Ceetox	ELactone	st3	20090505	2	2	2
FWA	Ceetox	E:actone	st3	20090426	2	2	

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	ELactone	st3	20090418	2	2	
FWA	Ceetox	Equol	st2	20090326	2	2	2
FWA	Ceetox	Equol	st2	20090223	2	2	
FWA	Ceetox	Equol	st2	20090307	2	2	
FWA	Ceetox	Gen	st2	20090326	2	2	2
FWA	Ceetox	Gen	st2	20090307	2	2	
FWA	Ceetox	Gen	st2	20090223	2	2	
FWA	Ceetox	Gen_b	st4	20090917A	2		
FWA	Ceetox	Gen_b	st4	20090911A	2		
FWA	Ceetox	Gen_b	st4	20090914	2		
FWA	Ceetox	Gen_b	st4	20090911B	2		
FWA	Ceetox	Gen_b	st4	20090917B	2		
FWA	Ceetox	Gen_b	st4	20090914B	2		
FWA	Ceetox	HPTE	st4	20090914B	2		
FWA	Ceetox	HPTE	st4	20090911A	2		
FWA	Ceetox	HPTE	st4	20090917B	2		
FWA	Ceetox	HPTE	st4	20090917A	2		
FWA	Ceetox	HPTE	st4	20090914	2		
FWA	Ceetox	HPTE	st4	20090911A	2		
FWA	Ceetox	Heptylphenol	st3	20090418	1	1	1
FWA	Ceetox	Heptylphenol	st3	20090426	1	1	
FWA	Ceetox	Heptylphenol	st3	20090505	2	2	
FWA	Ceetox	Hexestrol	st2	20090307	2	2	2
FWA	Ceetox	Hexestrol	st2	20090223	2	2	
FWA	Ceetox	Hexestrol	st2	20090326	2	2	
FWA	Ceetox	Kepone	st3	20090505	2	2	2
FWA	Ceetox	Kepone	st3	20090426	2	2	
FWA	Ceetox	Kepone	st3	20090418	2	2	

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	NE-TC	st3	20090418	2	2	2
FWA	Ceetox	NE-TC	st3	20090426	2	2	
FWA	Ceetox	NE-TC	st3	20090505	2	2	
FWA	Ceetox	NPhenol	st2	20090223	2	2	2
FWA	Ceetox	NPhenol	st2	20090326	2	2	
FWA	Ceetox	NPhenol	st2	20090307	2	2	
FWA	Ceetox	NE	st1	2008123	2	2	2
FWA	Ceetox	NE	st1	20081218	2	2	
FWA	Ceetox	NE	st1	20081124	2	2	
FWA	Ceetox	Nethdrone	st4	20090914	2	2	2
FWA	Ceetox	Nethdrone	st4	20090914	2	2	
FWA	Ceetox	Nethdrone	st4	20090917B	2	2	
FWA	Ceetox	Nethdrone	st4	20090917A	2		
FWA	Ceetox	Nethdrone	st4	20090914	2		
FWA	Ceetox	Nethdrone	st4	20090911B	2		
FWA	Ceetox	Nethdrone	st4	20090917A	2		
FWA	Ceetox	Nethdrone	st4	20090911A	2		
FWA	Ceetox	Nethdrone	st4	20090911A	2		
FWA	Ceetox	Nethdrone	st4	20090914B	2		
FWA	Ceetox	Nethdrone	st4	20090911A	2		
FWA	Ceetox	OTES	st3	20090426	0	0	1
FWA	Ceetox	OTES	st3	20090418	1 (10% rule, No change in classification)	1	
FWA	Ceetox	OTES	st3	20090505	0	0	
FWA	Ceetox	OTES	st4	20090911A	0		
FWA	Ceetox	OTES	st4	20090917A	0		
FWA	Ceetox	OTES	st4	20090914B	0		
FWA	Ceetox	OTES	st4	20090917B	0		
FWA	Ceetox	OTES	st4	20090914	0		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Ceetox	OTES	st4	20090911B	0		
FWA	Ceetox	Progesterone	st3	20090418	0 (Reanalyzed after removal of outlier replicates at 10-9 M, reclassified as Non-binder)	0	0
FWA	Ceetox	Progesterone	st3	20090426	0	0	
FWA	Ceetox	Progesterone	st3	20090505	0	0	
FWA	Ceetox	Tamox	st3	20090418	2	2	2
FWA	Ceetox	Tamox	st3	20090426	2	2	
FWA	Ceetox	Tamox	st3	20090505	2	2	
FWA	Ceetox	Zear9	st3	20090426	1		
FWA	Ceetox	Zear	st3	20090505	2		
FWA	Freyberger	Atr	st3	Code A1	0	0	0
FWA	Freyberger	Atr	st3	Code AB	0	0	
FWA	Freyberger	Atr	st3	Code A	0	0	
FWA	Freyberger	BPA	st3	Code B	2	2	2
FWA	Freyberger	BPA	st3	Code B1	2	2	
FWA	Freyberger	BPA	st3	Code B2	2	2	
FWA	Freyberger	BaA_Ethol	st3	Code AB	0	0	0
FWA	Freyberger	BaA_Ethol	st3	Code A	0	0	
FWA	Freyberger	BaA_Ethol	st3	Code A1	0	0	
FWA	Freyberger	ButylPar	st2	D	2	2	2
FWA	Freyberger	ButylPar	st2	B	2	2	
FWA	Freyberger	ButylPar	st2	A	2	2	
FWA	Freyberger	Cort	st2	B	0	0	0
FWA	Freyberger	Cort	st2	F	0	0	
FWA	Freyberger	Cort	st2	D	0	0	

[9] There are only 2 runs for Zear submitted from the CeeTox laboratory. Chemical not used for assessment of intra-laboratory reproducibility in the Performance Standards.

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Freyberger	Cort	st2	E	0		
FWA	Freyberger	Cort	st2	G	0		
FWA	Freyberger	Cort	st2	A	0		
FWA	Freyberger	DBP	st2	E	0	0	0
FWA	Freyberger	DBP	st2	F	0	0	
FWA	Freyberger	DBP	st2	D	0	0	
FWA	Freyberger	DBP	st2	B	0		
FWA	Freyberger	DBP	st2	A	0		
FWA	Freyberger	DBP	st2	G	0		
FWA	Freyberger	DBP	st3	Code AB	0		
FWA	Freyberger	DBP	st3	Code A	0		
FWA	Freyberger	DBP	st3	Code B2	(10% rule, too few points for classification, Run deleted)		
FWA	Freyberger	DBP	st3	Code B	0		
FWA	Freyberger	DBP	st3	Code A1	0		
FWA	Freyberger	DBP-TC	st3	Code A1	1	1	1
FWA	Freyberger	DBP-TC	st3	Code A	0	0	
FWA	Freyberger	DBP-TC	st3	Code A1	0	0	
FWA	Freyberger	DDT	st2	G	2	2	2
FWA	Freyberger	DDT	st2	F	2	2	
FWA	Freyberger	DDT	st2	E	2	2	
FWA	Freyberger	DDT	st2	A	2		
FWA	Freyberger	DDT	st2	B	2		
FWA	Freyberger	DDT	st2	D	2		
FWA	Freyberger	DES	st2	A	2		
FWA	Freyberger	DES	st2	B	2		
FWA	Freyberger	DES	st2	D	2		
FWA	Freyberger	DHT	st3	Code A	2		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Freyberger	DHT	st3	Code A1	2		
FWA	Freyberger	DHT	st3	Code AB	2		
FWA	Freyberger	E2	st1	A	2	2	2
FWA	Freyberger	E2	st1	B	2	2	
FWA	Freyberger	E2	st1	D	2	2	
FWA	Freyberger	E2-TC	st3	Code AB	1 (10% rule, No change in classification)	1	1
FWA	Freyberger	E2-TC	st3	Code B2	1 (10% rule, No change in classification)	1	
FWA	Freyberger	E2-TC	st3	Code B	2	2	
FWA	Freyberger	EE2	st2	A	2		
FWA	Freyberger	EE2	st2	B	2		
FWA	Freyberger	EE2	st2	D	2		
FWA	Freyberger	ELactone	st3	Code B	1	1	2
FWA	Freyberger	ELactone	st3	Code B1	2	2	
FWA	Freyberger	ELactone	st3	Code B2	2	2	
FWA	Freyberger	Equol	st2	D	2	2	2
FWA	Freyberger	Equol	st2	A	2	2	
FWA	Freyberger	Equol	st2	B	2	2	
FWA	Freyberger	Gen	st2	E	2	2	2
FWA	Freyberger	Gen	st2	F	2	2	
FWA	Freyberger	Gen	st2	G	2	2	
FWA	Freyberger	Gen	st2	D	2		
FWA	Freyberger	Gen	st2	B	1		
FWA	Freyberger	Gen	st2	A	2		
FWA	Freyberger	Heptylphenol	st3	Code AB	2	2	2
FWA	Freyberger	Heptylphenol	st3	Code A1	2	2	
FWA	Freyberger	Heptylphenol	st3	Code A	2	2	
FWA	Freyberger	Hexestrol	st2	A	2	2	2

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Freyberger	Hexestrol	st2	B	2	2	
FWA	Freyberger	Hexestrol	st2	D	2	2	
FWA	Freyberger	Kepone	st3	Code B	2	2	2
FWA	Freyberger	Kepone	st3	Code B1	2	2	
FWA	Freyberger	Kepone	st3	Code B2	2	2	
FWA	Freyberger	NE	st1	A	2	2	2
FWA	Freyberger	NE	st1	B	2	2	
FWA	Freyberger	NE	st1	D	2	2	
FWA	Freyberger	NE-TC	st3	Code A1	2	2	2
FWA	Freyberger	NE-TC	st3	Code A	2	2	
FWA	Freyberger	NE-TC	st3	Code AB	2	2	
FWA	Freyberger	NPhenol	st2	D	2	2	2
FWA	Freyberger	NPhenol	st2	A	2	2	
FWA	Freyberger	NPhenol	st2	B	2	2	
FWA	Freyberger	OTES	st3	Code B	0	0	0
FWA	Freyberger	OTES	st3	Code B2	0	0	
FWA	Freyberger	OTES	st3	Code AB	0	0	
FWA	Freyberger	Progesterone	st3	Code A	0	0	0
FWA	Freyberger	Progesterone	st3	Code A1	0	0	
FWA	Freyberger	Progesterone	st3	Code A2	0 (Outlier point at 10-8 M deleted. Data reanalyzed and Run classified as non-binder.	0	
FWA	Freyberger	Tamox	st3	Code B	2	2	2
FWA	Freyberger	Tamox	st3	Code B1	2	2	
FWA	Freyberger	Tamox	st3	Code B2	2	2	
FWA	Freyberger	Zear	st3	Code A1	1 (10% rule, No change in Classification)	1	2
FWA	Freyberger	Zear	st3	Code AB	2	2	
FWA	Freyberger	Zear	st3	Code A	2	2	

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	JapanCERI	Atr	st3	91110	0	0	0
FWA	JapanCERI	Atr	st3	91112	0	0	
FWA	JapanCERI	Atr	st3	91117	0	0	
FWA	JapanCERI	Atr	st3	91118	0		
FWA	JapanCERI	BPA	st3	91116	2	2	2
FWA	JapanCERI	BPA	st3	91119	2	2	
FWA	JapanCERI	BPA	st3	91021	2	2	
FWA	JapanCERI	BaA_DMSO	st3	91110	0 (10% rule, Not appropriate use) Classification corrected to 0)	0	0
FWA	JapanCERI	BaA_DMSO	st3	91112	0	0	
FWA	JapanCERI	BaA_DMSO	st3	91117	0	0	
FWA	JapanCERI	BaA_DMSO	st3	91118	0		
FWA	JapanCERI	ButylPar	st2	20090430	2	2	2
FWA	JapanCERI	ButylPar	st2	20090427	2	2	
FWA	JapanCERI	ButylPar	st2	20090422	2	2	
FWA	JapanCERI	Cort	st2	20090422	0	0	0
FWA	JapanCERI	Cort	st2	20090427	0	0	
FWA	JapanCERI	Cort	st2	20090430	0	0	
FWA	JapanCERI	DBP	st1	20090121	0	0	0
FWA	JapanCERI	DBP	st1	20090128	0	0	
FWA	JapanCERI	DBP	st1	20090113	0	0	
FWA	JapanCERI	DBP	st2	20090422	0		
FWA	JapanCERI	DBP	st2	20090427	0		
FWA	JapanCERI	DBP	st2	20090430	0		
FWA	JapanCERI	DBP	st3	91105	0		
FWA	JapanCERI	DBP	st3	91109	0		
FWA	JapanCERI	DBP	st3	91119	0		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	JapanCERI	DBP	st3	91117	0		
FWA	JapanCERI	DBP	st3	91112	0		
FWA	JapanCERI	DBP	st3	91118	0		
FWA	JapanCERI	DBP	st3	91116	0		
FWA	JapanCERI	DBP	st3	91111	0		
FWA	JapanCERI	DBP	st3	91110	0		
FWA	JapanCERI	DBP	st3	91021	0		
FWA	JapanCERI	DBP-TC	st3	91116	1	1	1
FWA	JapanCERI	DBP-TC	st3	91021	1	1	
FWA	JapanCERI	DBP-TC	st3	91026	1	1	
FWA	JapanCERI	DDT	st2	20090430	2	2	2
FWA	JapanCERI	DDT	st2	20090427	2	2	
FWA	JapanCERI	DDT	st2	20090422	2	2	
FWA	JapanCERI	DES	st2	20090427	2		
FWA	JapanCERI	DES	st2	20090430	2		
FWA	JapanCERI	DES	st2	20090422	2		
FWA	JapanCERI	DHT	st3	91021	2		
FWA	JapanCERI	DHT	st3	91116	2		
FWA	JapanCERI	DHT	st3	91026	2		
FWA	JapanCERI	E2	st1	20090113	2	2	2
FWA	JapanCERI	E2	st1	20090121	2	2	
FWA	JapanCERI	E2	st1	20090128	2	2	
FWA	JapanCERI	E2-TC	st3	91109	2	2	2
FWA	JapanCERI	E2-TC	st3	91110	1	1	
FWA	JapanCERI	E2-TC	st3	91111	2	2	
FWA	JapanCERI	E2-TC	st3	91112	2		
FWA	JapanCERI	E2-TC	st3	91117	2		
FWA	JapanCERI	E2-TC	st3	91118	2		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	JapanCERI	EE2	st2	20090427	2	2	2
FWA	JapanCERI	EE2	st2	20090430	2	2	
FWA	JapanCERI	EE2	st2	20090422	2	2	
FWA	JapanCERI	ELactone	st3	91109	2	2	2
FWA	JapanCERI	ELactone	st3	91105	2	2	
FWA	JapanCERI	ELactone	st3	91111	2	2	
FWA	JapanCERI	Equol	st2	20090427	2	2	2
FWA	JapanCERI	Equol	st2	20090430	2	2	
FWA	JapanCERI	Equol	st2	20090422	2	2	
FWA	JapanCERI	Gen	st2	20090427	2	2	2
FWA	JapanCERI	Gen	st2	20090422	2	2	
FWA	JapanCERI	Gen	st2	20090430	2	2	
FWA	JapanCERI	Heptylphenol	st3	91021	2	2	2
FWA	JapanCERI	Heptylphenol	st3	91116	2	2	
FWA	JapanCERI	Heptylphenol	st3	91119	2	2	
FWA	JapanCERI	Hexestrol	st2	20090427	2	2	2
FWA	JapanCERI	Hexestrol	st2	20090430	2	2	
FWA	JapanCERI	Hexestrol	st2	20090422	2	2	
FWA	JapanCERI	Kepone	st3	91021	2	2	2
FWA	JapanCERI	Kepone	st3	91026	2	2	
FWA	JapanCERI	Kepone	st3	91116	2	2	
FWA	JapanCERI	NE-TC	st3	91105	2	2	2
FWA	JapanCERI	NE-TC	st3	91111	2	2	
FWA	JapanCERI	NE-TC	st3	91109	2	2	
FWA	JapanCERI	NPhenol	st2	20090422	2	2	2
FWA	JapanCERI	NPhenol	st2	20090427	2	2	
FWA	JapanCERI	NPhenol	st2	20090430	2	2	
FWA	JapanCERI	NE	st1	20090113	2	2	2

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	JapanCERI	NE	st1	20090121	2	2	
FWA	JapanCERI	NE	st1	20090128	2	2	
FWA	JapanCERI	OTES	st3	91109	0	0	0
FWA	JapanCERI	OTES	st3	91111	0	0	
FWA	JapanCERI	OTES	st3	91105	0	0	
FWA	JapanCERI	OTES	st3	91119	0		
FWA	JapanCERI	OTES	st3	91116	0		
FWA	JapanCERI	Progesterone	st3	91021	1 (10% rule, No change in classification)	1	0
FWA	JapanCERI	Progesterone	st3	91105	0	0	
FWA	JapanCERI	Progesterone	st3	91109	0	0	
FWA	JapanCERI	Progesterone	st3	91111	0		
FWA	JapanCERI	Tamox	st3	91105	2	2	2
FWA	JapanCERI	Tamox	st3	91116	2	2	
FWA	JapanCERI	Tamox	st3	91021	2	2	
FWA	JapanCERI	Zear	st3	91116	2	2	2
FWA	JapanCERI	Zear	st3	91021	2	2	
FWA	JapanCERI	Zear	st3	91109	2	2	
FWA	JapanCERI	Zear	st3	91105	2		
FWA	Missouri	Atr	st3	8004	0	0	0
FWA	Missouri	Atr	st3	8005	0	0	
FWA	Missouri	Atr	st3	8003	0	0	
FWA	Missouri	BPA	st3	8101	2	2	2
FWA	Missouri	BPA	st3	8102	2	2	
FWA	Missouri	BPA	st3	8107	2	2	
FWA	Missouri	BPA	st4	1109	2		
FWA	Missouri	BPA	st4	1108	2		
FWA	Missouri	BPA	st4	1102	2		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	BPA	st4	1107	2		
FWA	Missouri	BPA	st4	1104	2		
FWA	Missouri	BPA	st4	1106	2		
FWA	Missouri	BPA	st4	1101	2		
FWA	Missouri	BaA_DMSO	st3	8203	0		
FWA	Missouri	BaA_DMSO	st3	8201	0		
FWA	Missouri	BaA_Ethol	st3	8004	0		
FWA	Missouri	BaA_Ethol	st3	8003	0		
FWA	Missouri	ButylPar	st2	9003	2		
FWA	Missouri	ButylPar	st2	9004	2		
FWA	Missouri	ButylPar	st2	9005	2		
FWA	Missouri	ButylPar	st2	9007	2		
FWA	Missouri	Cort	st2	9007	0		
FWA	Missouri	Cort	st2	9003	0		
FWA	Missouri	Cort	st2	9005	0		
FWA	Missouri	Cort	st2	9004	0		
FWA	Missouri	Cort_b	st4	1109	0		
FWA	Missouri	Cort_b	st4	1101	0		
FWA	Missouri	Cort_b	st4	1104	0		
FWA	Missouri	Cort_b	st4	1106	0		
FWA	Missouri	Cort_b	st4	1108	0		
FWA	Missouri	Cort_b	st4	1107	0		
FWA	Missouri	Cort_b	st4	1103	1		
FWA	Missouri	Cort_b	st4	1102	0		
FWA	Missouri	DBP	st1	7004	0		
FWA	Missouri	DBP	st1	7003	0		
FWA	Missouri	DBP	st1	7006	0		
FWA	Missouri	DBP	st1	7005	0		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	DBP	st1	7007	0		
FWA	Missouri	DBP	st2	9005	0		
FWA	Missouri	DBP	st2	9004	0		
FWA	Missouri	DBP	st2	9003	0		
FWA	Missouri	DBP	st2	9007	0		
FWA	Missouri	DBP	st3	8003	0		
FWA	Missouri	DBP	st3	8101	0		
FWA	Missouri	DBP	st3	8102	0		
FWA	Missouri	DBP	st3	8107	0		
FWA	Missouri	DBP	st3	8201	0		
FWA	Missouri	DBP	st3	8005	0		
FWA	Missouri	DBP	st3	8203	0		
FWA	Missouri	DBP	st3	8004	0		
FWA	Missouri	DBP	st4	1109	0		
FWA	Missouri	DBP	st4	1104	0		
FWA	Missouri	DBP	st4	1107	0		
FWA	Missouri	DBP	st4	1102	0		
FWA	Missouri	DBP	st4	1108	0		
FWA	Missouri	DBP	st4	1106	0		
FWA	Missouri	DBP	st4	1101	0		
FWA	Missouri	DBP	st4	1103	0		
FWA	Missouri	DBP-TC	st3	8101	1	1	1
FWA	Missouri	DBP-TC	st3	8102	1	1	
FWA	Missouri	DBP-TC	st3	8107	0	0	
FWA	Missouri	DDT	st2	9007	2	2	2
FWA	Missouri	DDT	st2	9004	2	2	
FWA	Missouri	DDT	st2	9003	2	2	
FWA	Missouri	DDT	st2	9005	2		

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	DES	st2	9002	2	2	2
FWA	Missouri	DES	st2	9004	2	2	
FWA	Missouri	DES	st2	9005	2	2	
FWA	Missouri	DES	st2	9007	2		
FWA	Missouri	DHT	st3	8101	2	2	2
FWA	Missouri	DHT	st3	81027	2	2	
FWA	Missouri	DHT	st3	8107-1	2	2	
FWA	Missouri	Dex	st4	1104	0		
FWA	Missouri	Dex	st4	1106	0		
FWA	Missouri	Dex	st4	1108	0		
FWA	Missouri	Dex	st4	1109	0		
FWA	Missouri	Dex	st4	1103	1		
FWA	Missouri	Dex	st4	1107	0		
FWA	Missouri	Dex	st4	1102	0		
FWA	Missouri	Dex	st4	1101	0		
FWA	Missouri	E2	st1	7003	2	2	2
FWA	Missouri	E2	st1	7004	2	2	
FWA	Missouri	E2	st1	7005	2	2	
FWA	Missouri	E2-TC	st3	8102	2	2	2
FWA	Missouri	E2-TC	st3	8107	2	2	
FWA	Missouri	E2-TC	st3	8101	2	2	
FWA	Missouri	EE2	st2	9004	2	2	2
FWA	Missouri	EE2	st2	9005	2	2	
FWA	Missouri	EE2	st2	9007	2	2	
FWA	Missouri	EE2	st2	9003	2		
FWA	Missouri	ELactone	st3	8003	2	2	2
FWA	Missouri	ELactone	st3	8004	2	2	
FWA	Missouri	Equol	st2	9004	2	2	2

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	Equol	st2	9007	2	2	
FWA	Missouri	Equol	st2	9005	2	2	
FWA	Missouri	Equol	st2	9003	2		
FWA	Missouri	Gen	st2	9007	2	2	2
FWA	Missouri	Gen	st2	9003	2	2	
FWA	Missouri	Gen	st2	9005	2	2	
FWA	Missouri	Gen	st2	9004	2		
FWA	Missouri	GenW	st2	9004	2		
FWA	Missouri	GenW	st2	9007	2		
FWA	Missouri	GenW	st2	9003	2		
FWA	Missouri	GenW	st2	9005	2		
FWA	Missouri	Gen_b	st4	1101	2		
FWA	Missouri	Gen_b	st4	1106	2		
FWA	Missouri	Gen_b	st4	1109	1		
FWA	Missouri	Gen_b	st4	1102	2		
FWA	Missouri	Gen_b	st4	1108	2		
FWA	Missouri	Gen_b	st4	1104	2		
FWA	Missouri	Gen_b	st4	1107	2		
FWA	Missouri	HPTE	st4	1102	2		
FWA	Missouri	HPTE	st4	1101	1		
FWA	Missouri	HPTE	st4	1107	2		
FWA	Missouri	HPTE	st4	1106	2		
FWA	Missouri	HPTE	st4	1103	2		
FWA	Missouri	HPTE	st4	1108	2		
FWA	Missouri	HPTE	st4	1109	2		
FWA	Missouri	HPTE	st4	1104	2		
FWA	Missouri	Heptylphenol	st3	8001	0	0	0
FWA	Missouri	Heptylphenol	st3	8002	0	0	

Table PS-6, FW hrER Binding Assay

Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	Heptylphenol	st3	8004	0	0	
FWA	Missouri	Heptylphenol	st3	8003	0		
FWA	Missouri	Hexestrol	st2	9003	2	2	2
FWA	Missouri	Hexestrol	st2	9004	2	2	
FWA	Missouri	Hexestrol	st2	9007	2	2	
FWA	Missouri	Hexestrol	st2	9005	2		
FWA	Missouri	Kepone	st2	8001	2	2	2
FWA	Missouri	Kepone	st2	8002	2	2	
FWA	Missouri	Kepone	st3	8004	2	2	
FWA	Missouri	Kepone	st3	8003	2		
FWA	Missouri	E2	st1	7003	2	2	2
FWA	Missouri	E2	st1	7004	2	2	
FWA	Missouri	E2	st1	7005	2	2	
FWA	Missouri	NE-TC	st3	8107	2	2	2
FWA	Missouri	NE-TC	st3	8102	2	2	
FWA	Missouri	NE-TC	st3	8101	2	2	
FWA	Missouri	NPhenol	st2	9007	2	2	2
FWA	Missouri	NPhenol	st2	9004	2	2	
FWA	Missouri	NPhenol	st2	9005	2	2	
FWA	Missouri	NPhenol	st2	9003	2		
FWA	Missouri	Nethdrone	st4	1101	2	2	2
FWA	Missouri	Nethdrone	st4	1108	2	2	
FWA	Missouri	Nethdrone	st4	1102	2	2	
FWA	Missouri	Nethdrone	st4	1108	2		
FWA	Missouri	Nethdrone	st4	1106	2		
FWA	Missouri	Nethdrone	st4	1106	2		
FWA	Missouri	Nethdrone	st4	1103	2		
FWA	Missouri	Nethdrone	st4	1102	2		

Table PS-6, FW hrER Binding Assay

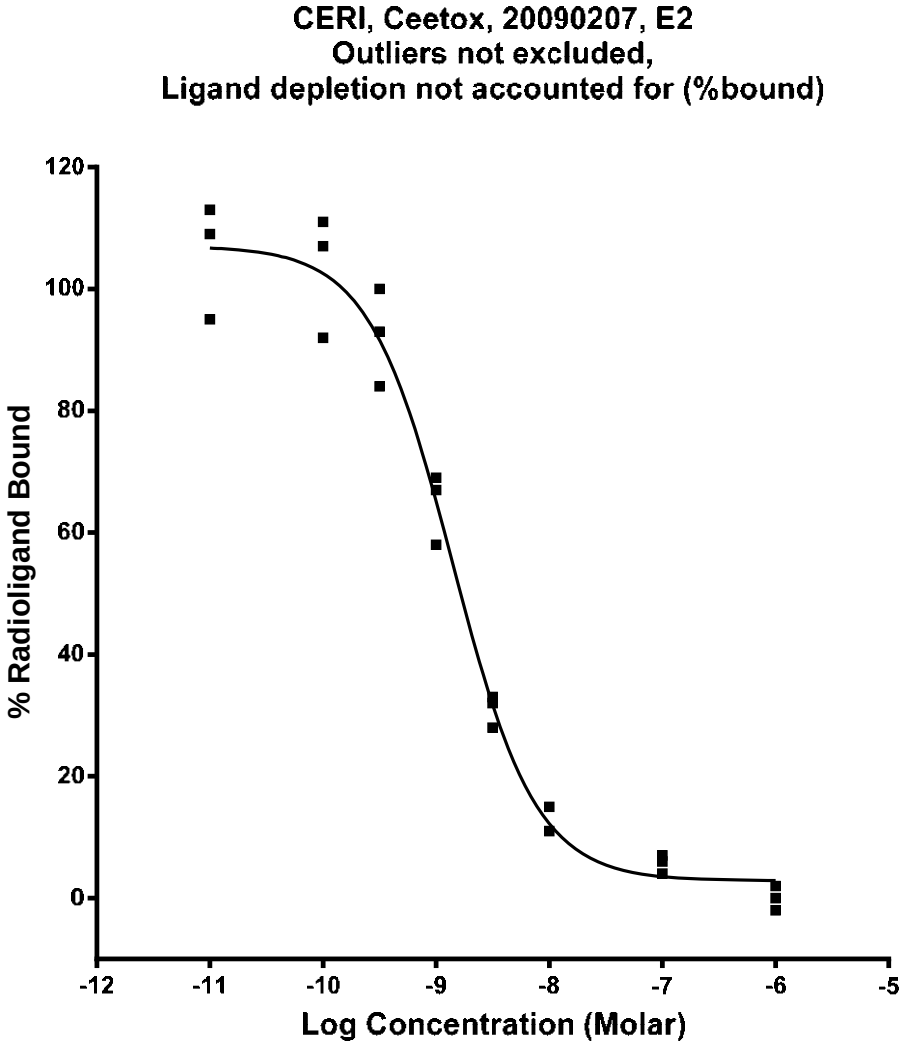
Protocol	Laboratory	Chemical	SubTask	Test	Individual Run Classification Vote	Runs (3) Used for Performance Standards	Final Classification for Lab[7]
FWA	Missouri	Nethdrone	st4	1109	2		
FWA	Missouri	Nethdrone	st4	1104	2		
FWA	Missouri	Nethdrone	st4	1109	2		
FWA	Missouri	Nethdrone	st4	1104	2		
FWA	Missouri	Nethdrone	st4	1107	2		
FWA	Missouri	Nethdrone	st4	1107	2		
FWA	Missouri	Nethdrone	st4	1101	2		
FWA	Missouri	OTES	st3	8004	0	0	0
FWA	Missouri	OTES	st3	8003	0	0	
FWA	Missouri	OTES	st3	8005	0	0	
FWA	Missouri	OTES	st4	1107	0		
FWA	Missouri	OTES	st4	1102	0		
FWA	Missouri	OTES	st4	1109	0		
FWA	Missouri	OTES	st4	1108	0		
FWA	Missouri	OTES	st4	1101	0		
FWA	Missouri	OTES	st4	1104	0		
FWA	Missouri	OTES	st4	1106	0		
FWA	Missouri	Progesterone	st3	8003	0	0	0
FWA	Missouri	Progesterone	st3	8004	0	0	
FWA	Missouri	Progesterone	st3	8005	0	0	
FWA	Missouri	Tamox	st3	8107	2	2	2
FWA	Missouri	Tamox	st3	8102	2	2	
FWA	Missouri	Tamox	st3	8101	2	2	
FWA	Missouri	Zear	st3	8101	2	2	2
FWA	Missouri	Zear	st3	8107	2	2	
FWA	Missouri	Zear	st3	8102	2	2	

Table PS-6, FW hrER Binding Assay

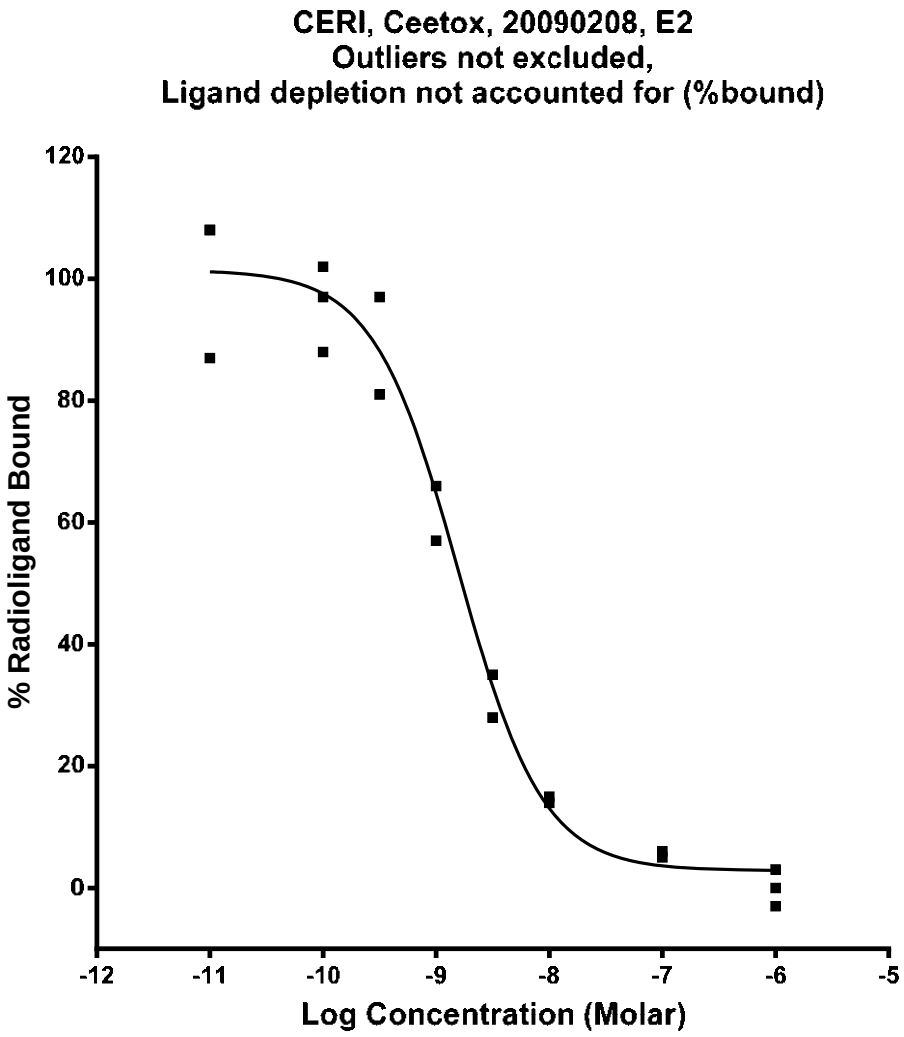
PART 4 :

Graphs of binding curves for control E2 and NE (4 laboratories, 2 protocols)

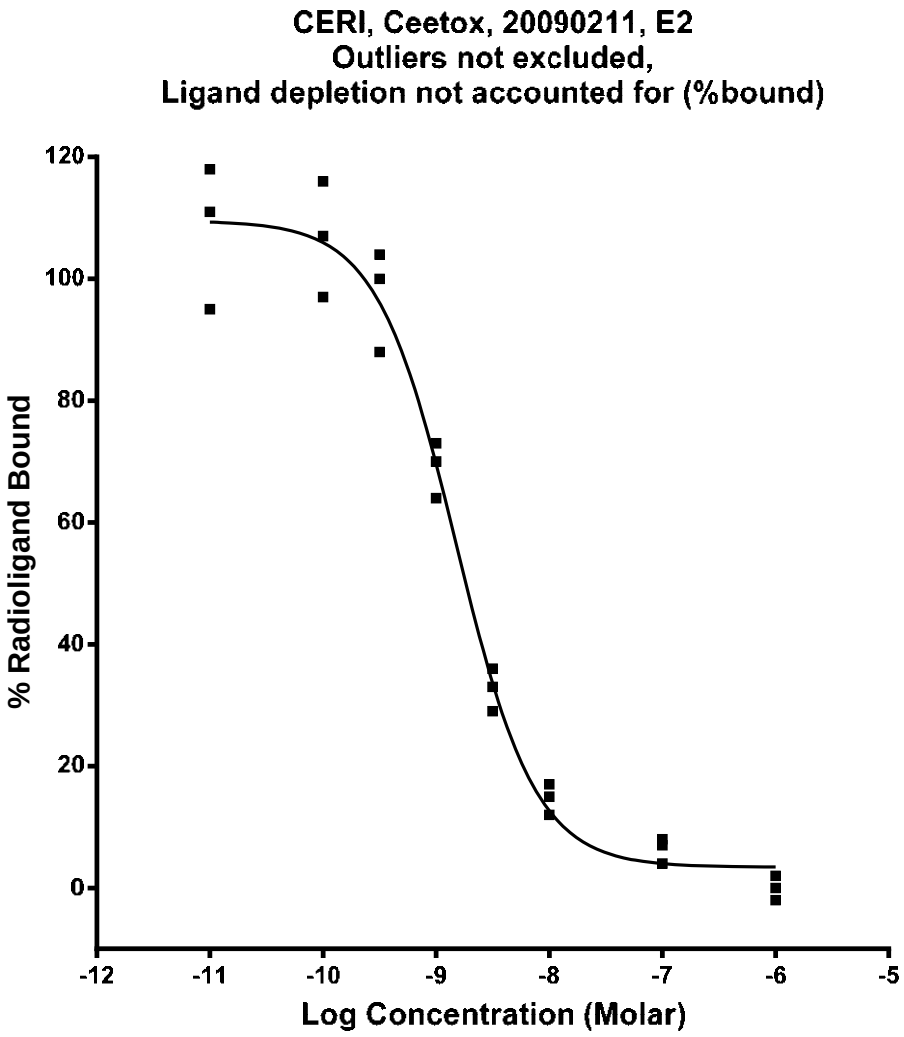
Subtask 1


CERI, Ceetox, 20090207, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

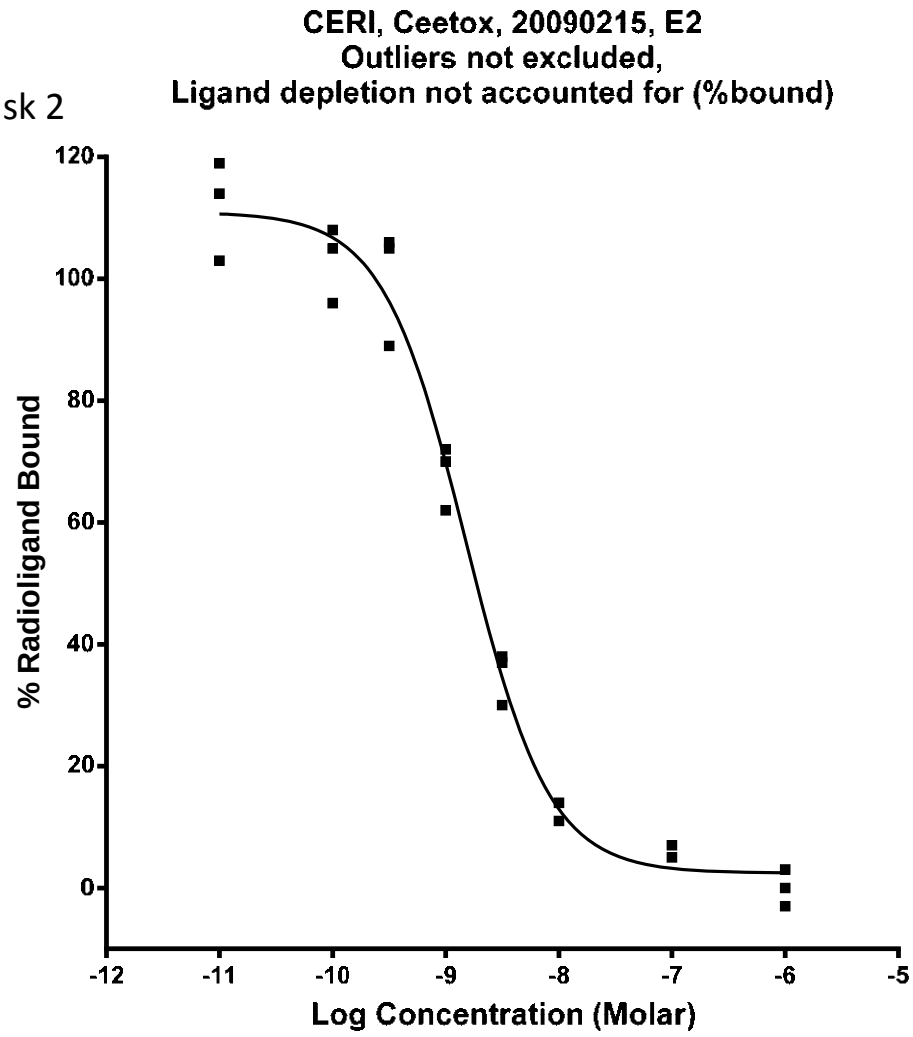
Subtask 1

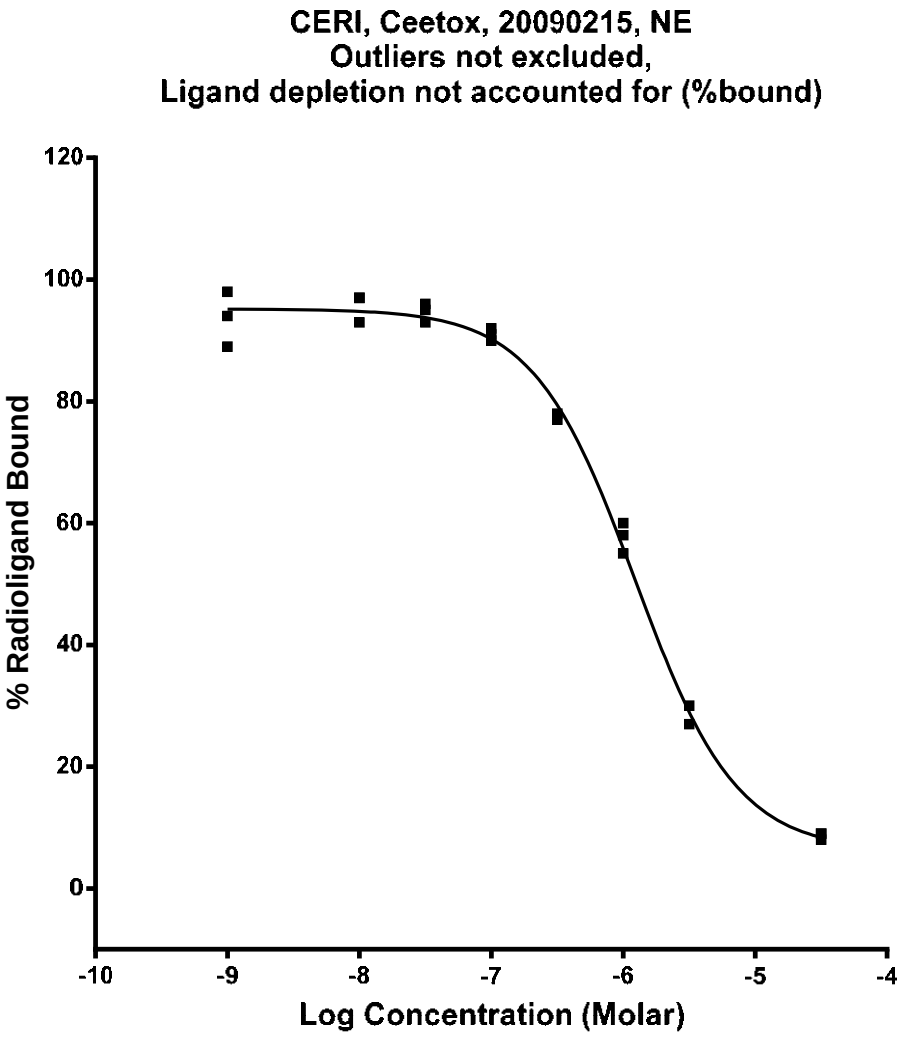

CERI, Ceetox, 20090208, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 1

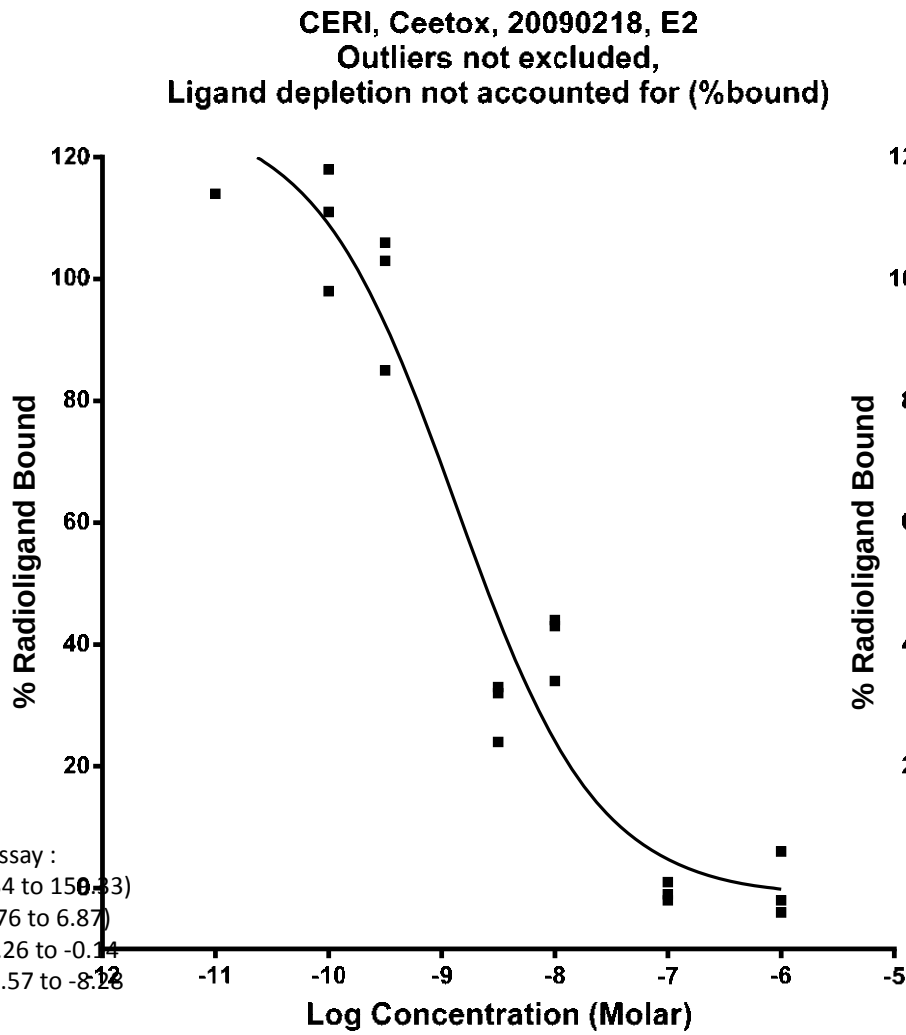

CERI, Ceetox, 20090211, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

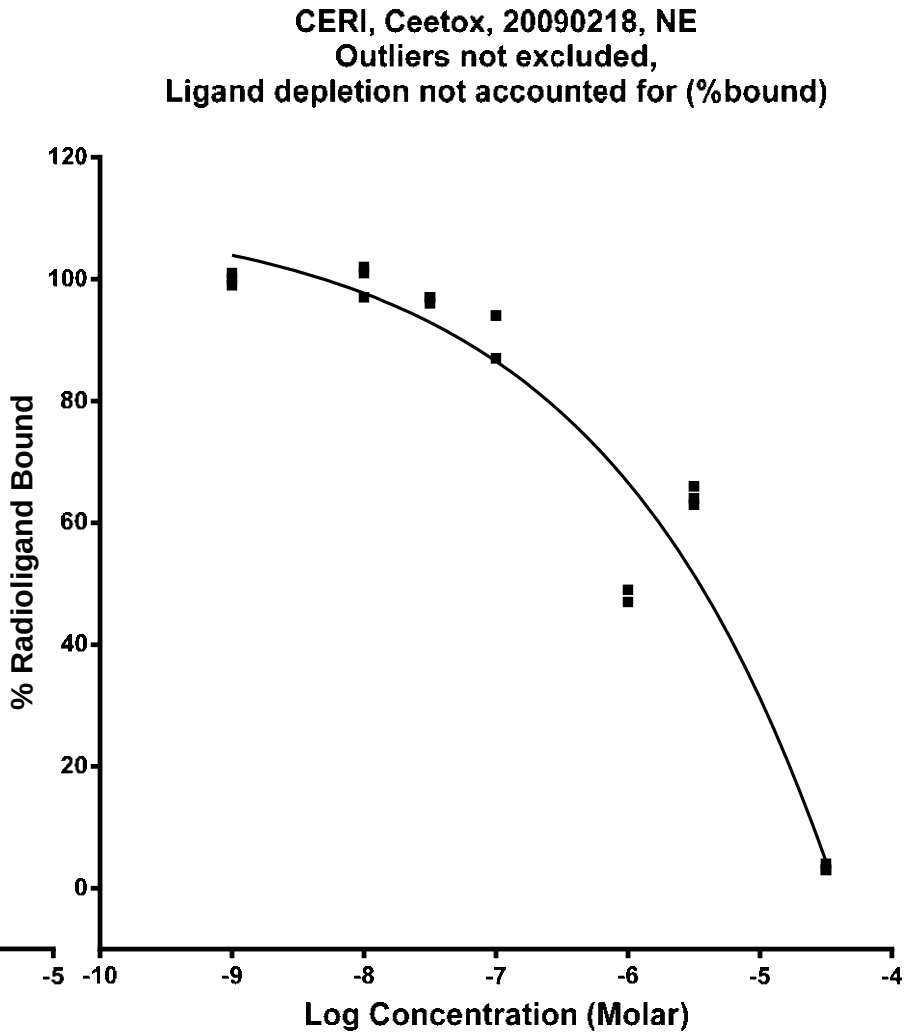
Subtask 2


CERI, Ceetox, 20090215, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)


CERI, Ceetox, 20090215, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log Concentration (Molar)

Subtask 2





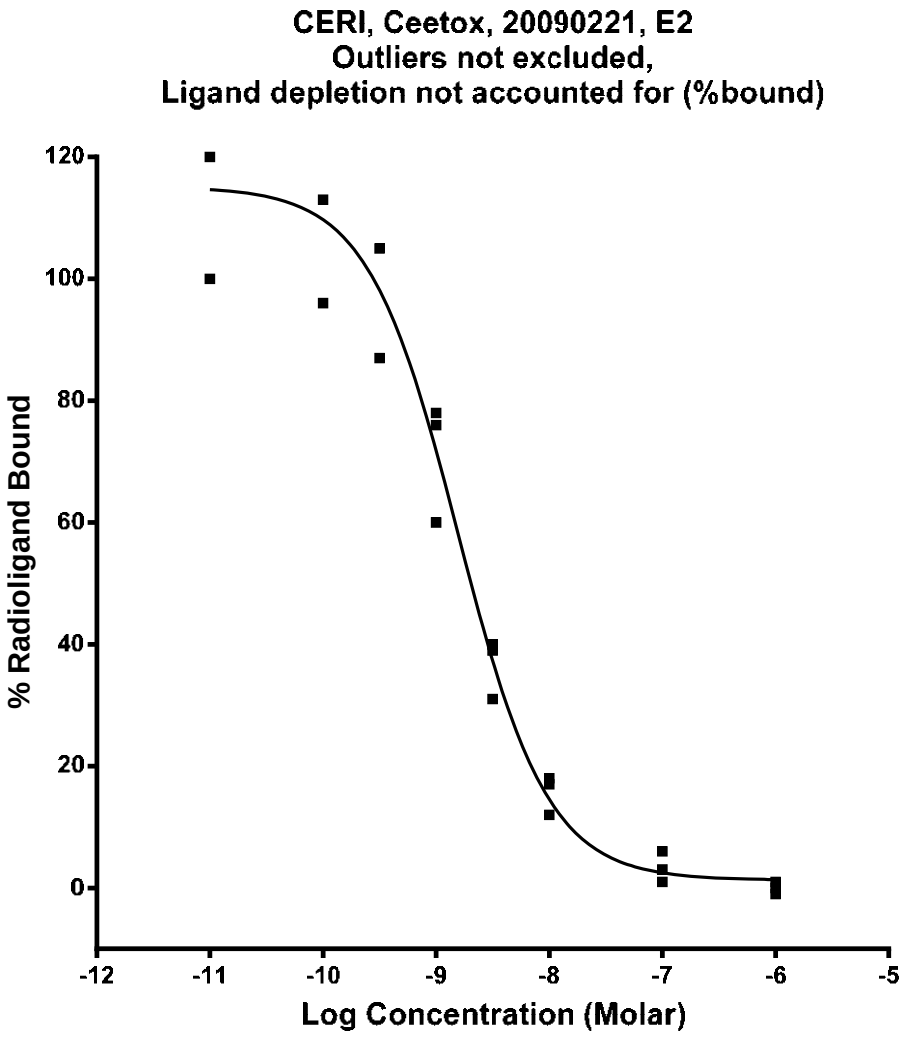
Curve not used during original analysis because EPA subjective review. However, all estimates of parameters for prism fit were w/in range of tolerance bounds.

Top: 127.4
Bottom: -1.48
Hill slope: -0.69
Log IC50: -8.62

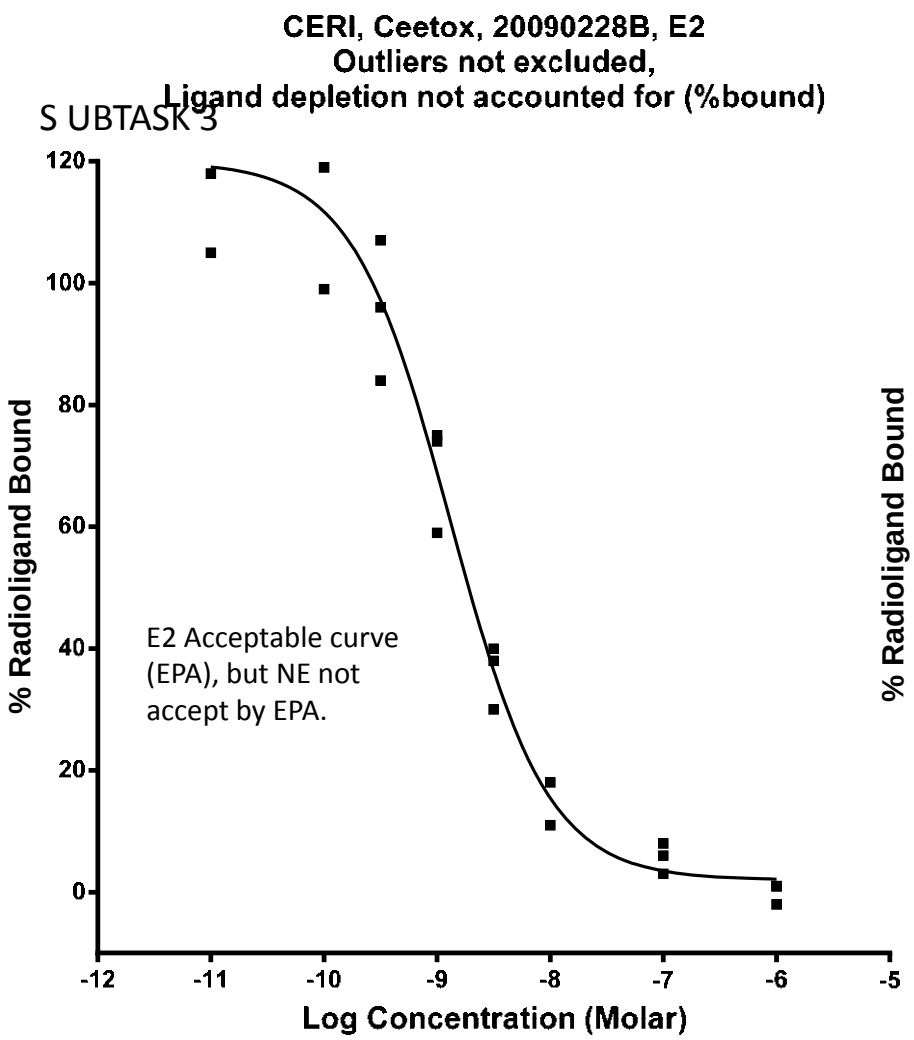
Tolerance bounds for CERI assay :
Top: avg 107.83 (range 56.34 to 159.33)
Bottom: Avg 1.05 (range -4.76 to 6.87)
HillSlope: Avg -1.2 (range -2.26 to -0.14
LogIC50: Avg -8.93 (range -9.57 to -8.28

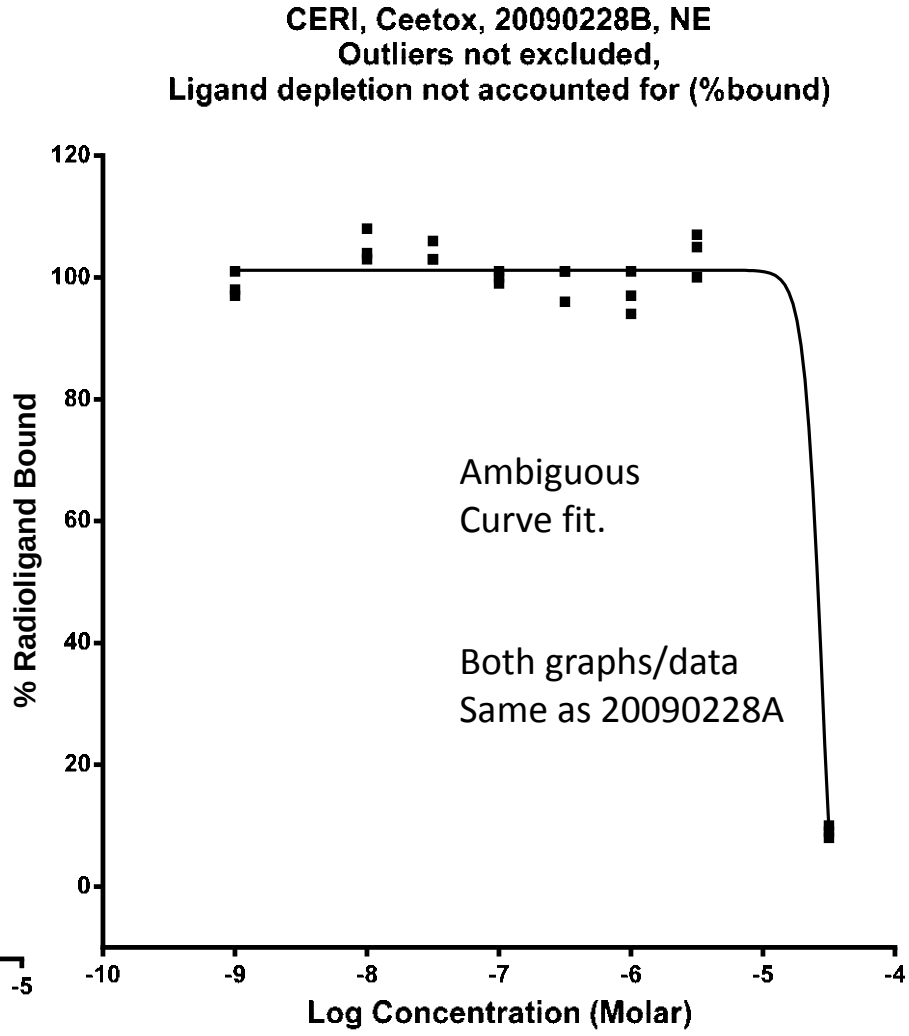
Subtask 2

Acceptable curve



S UBTASK 3

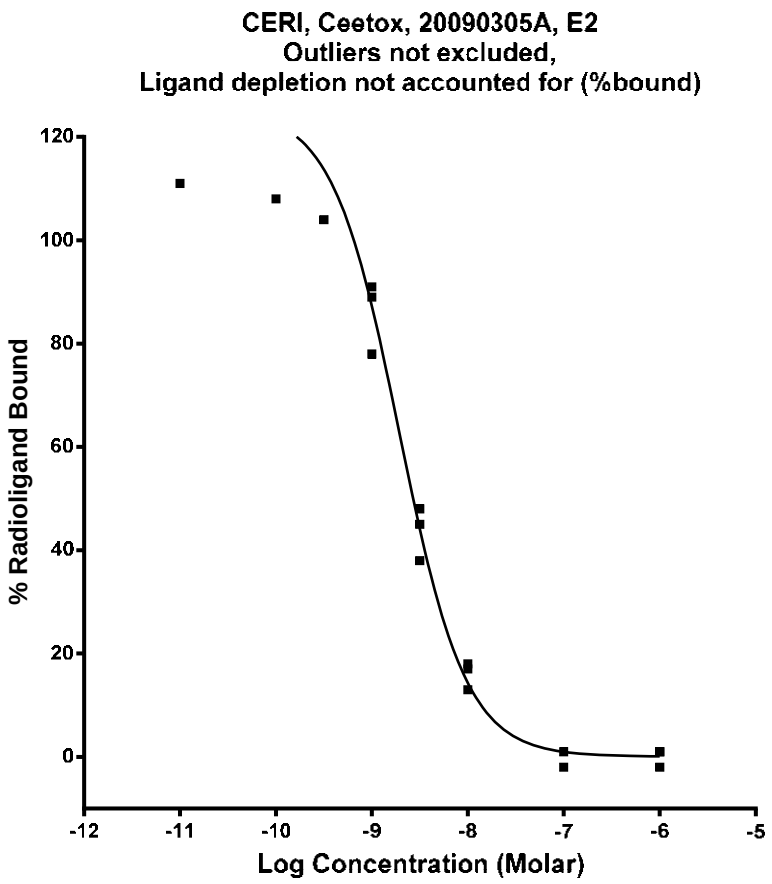

CERI, Ceetox, 20090228B, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
E2 Acceptable curve
(EPA), but NE not
accept by EPA.

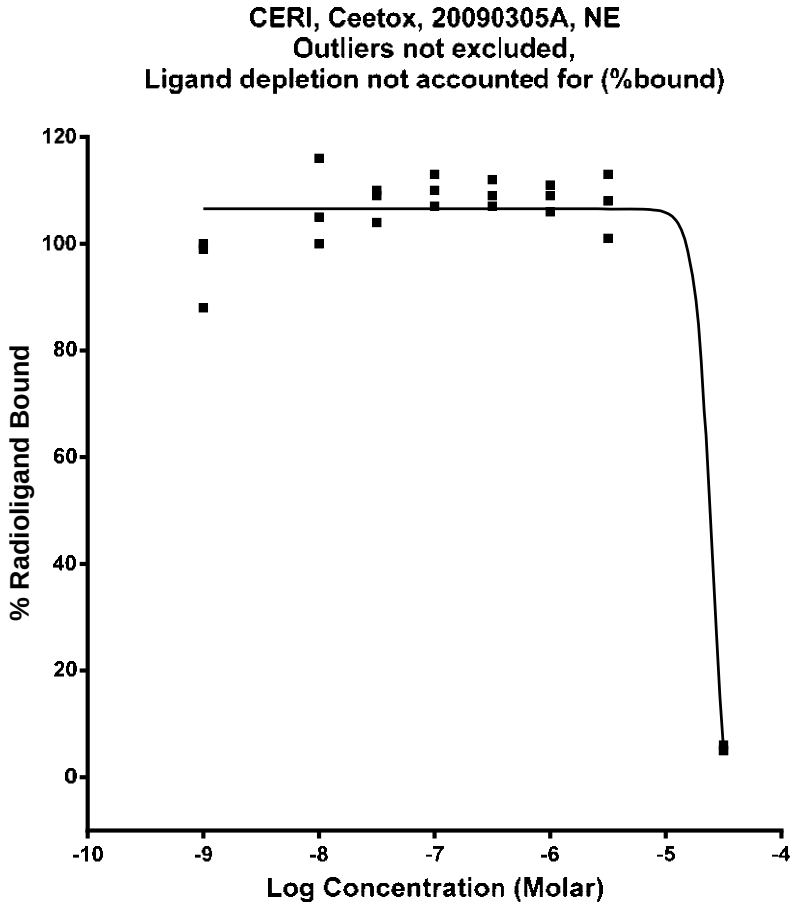

CERI, Ceetox, 20090228B, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
Ambiguous
Curve fit.
Both graphs/data
Same as 20090228A

Same data/curve as 20090305 B

Subtask 3

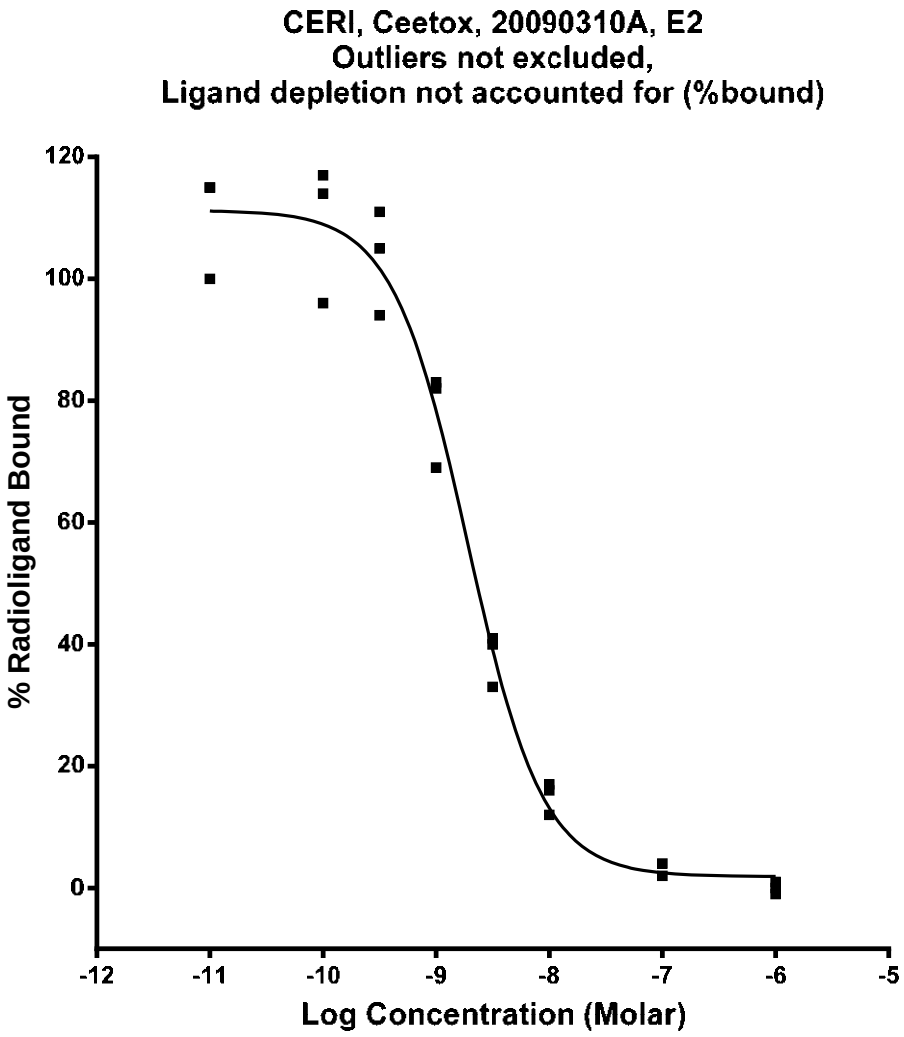
E2 curve acceptable. NE not accepted because Ambiguous fit. NE-TC Tested in same run with good curve.



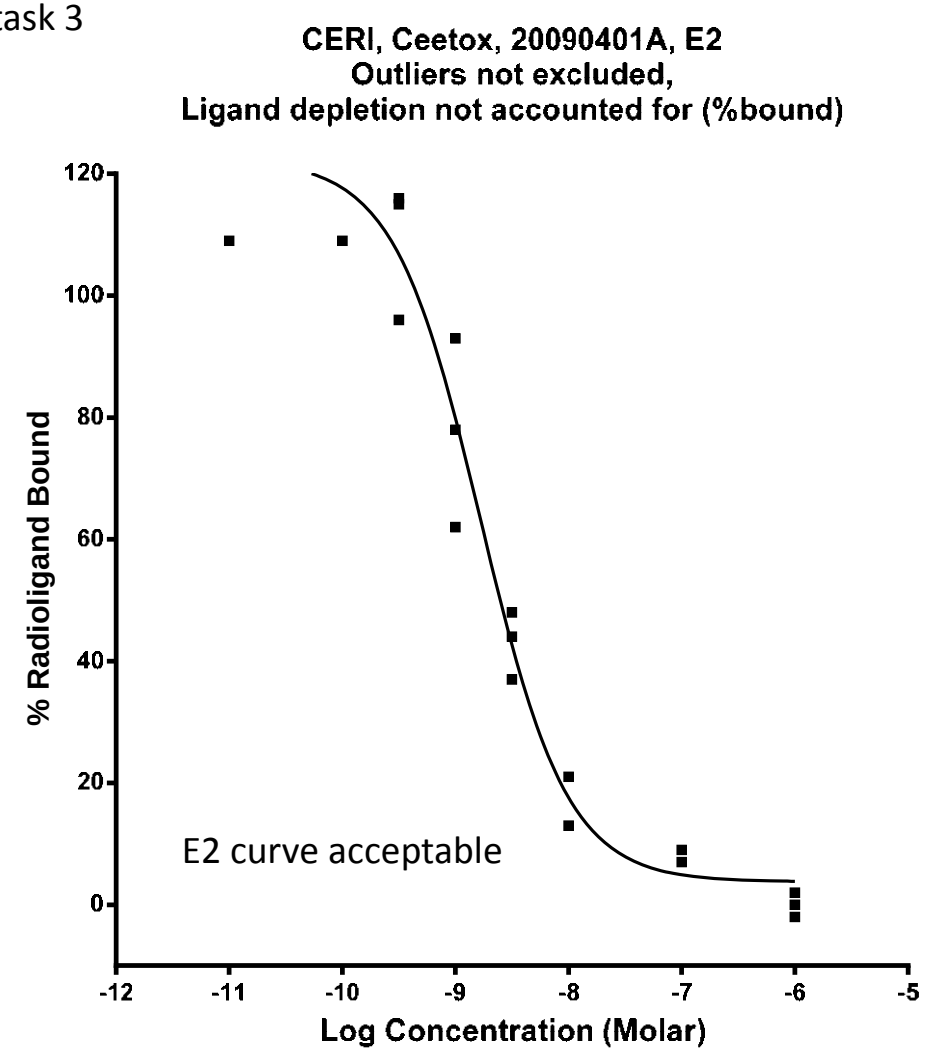


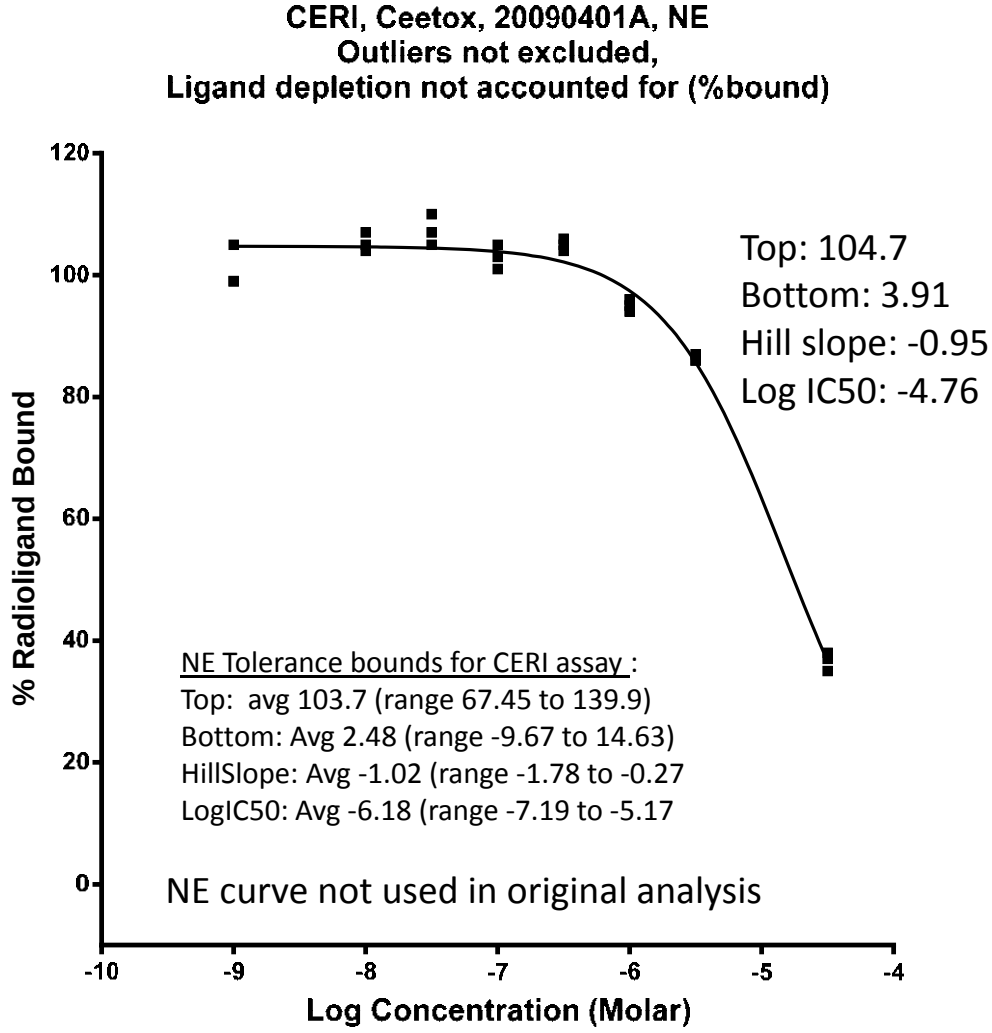
SUBTASK 3

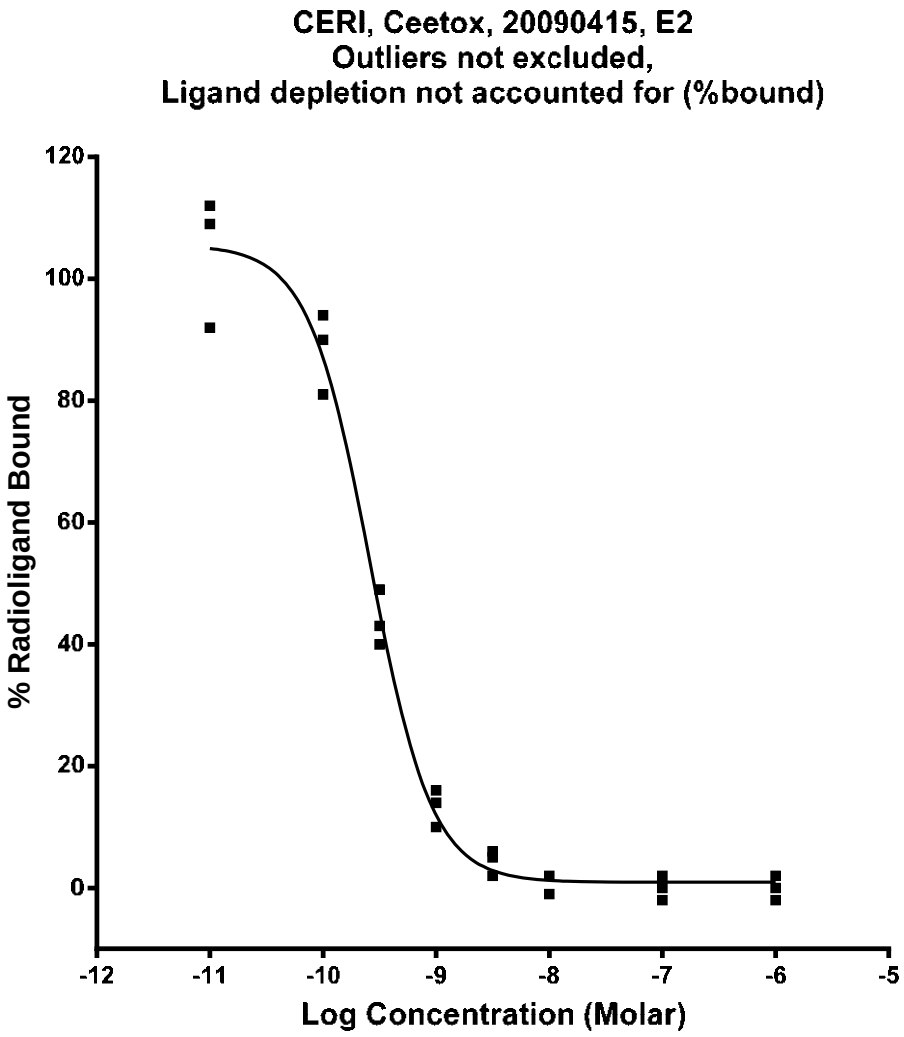
Acceptable curve (EPA), chemical Data included in original analysis



Subtask 3

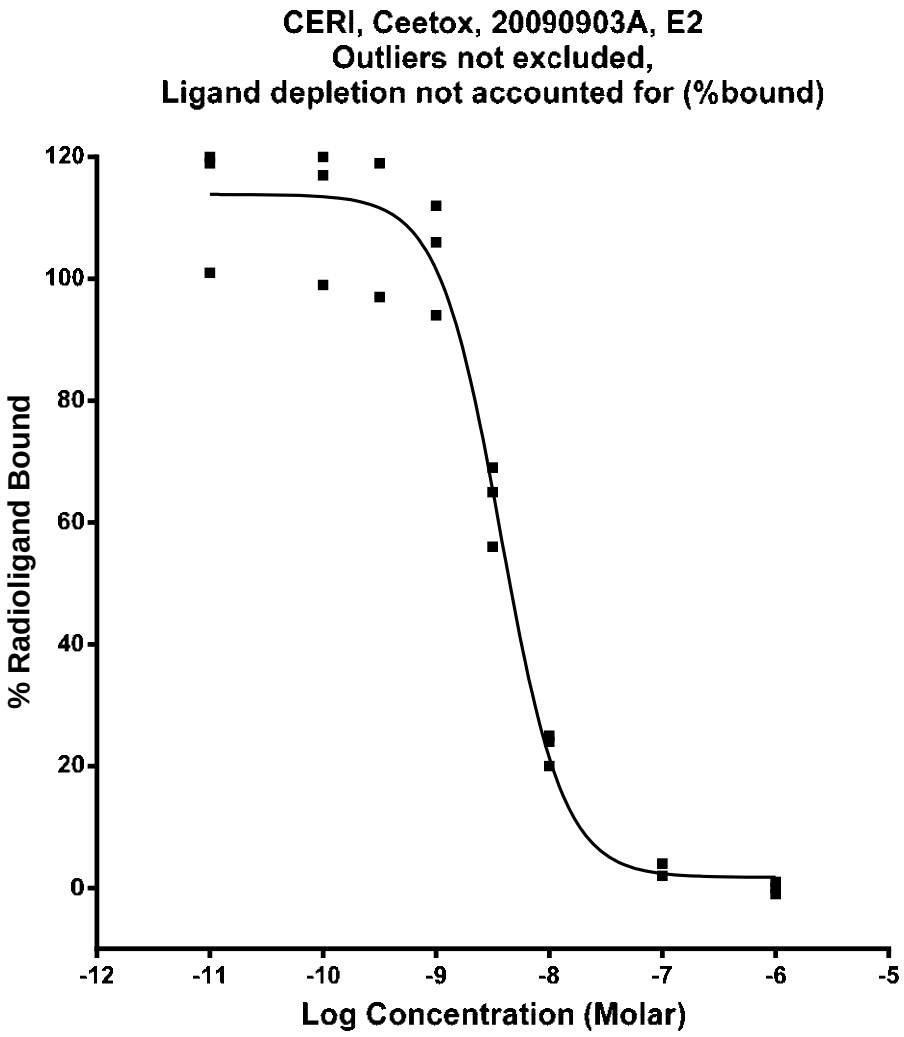

CERI, Ceetox, 20090401A, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
E2 curve acceptable

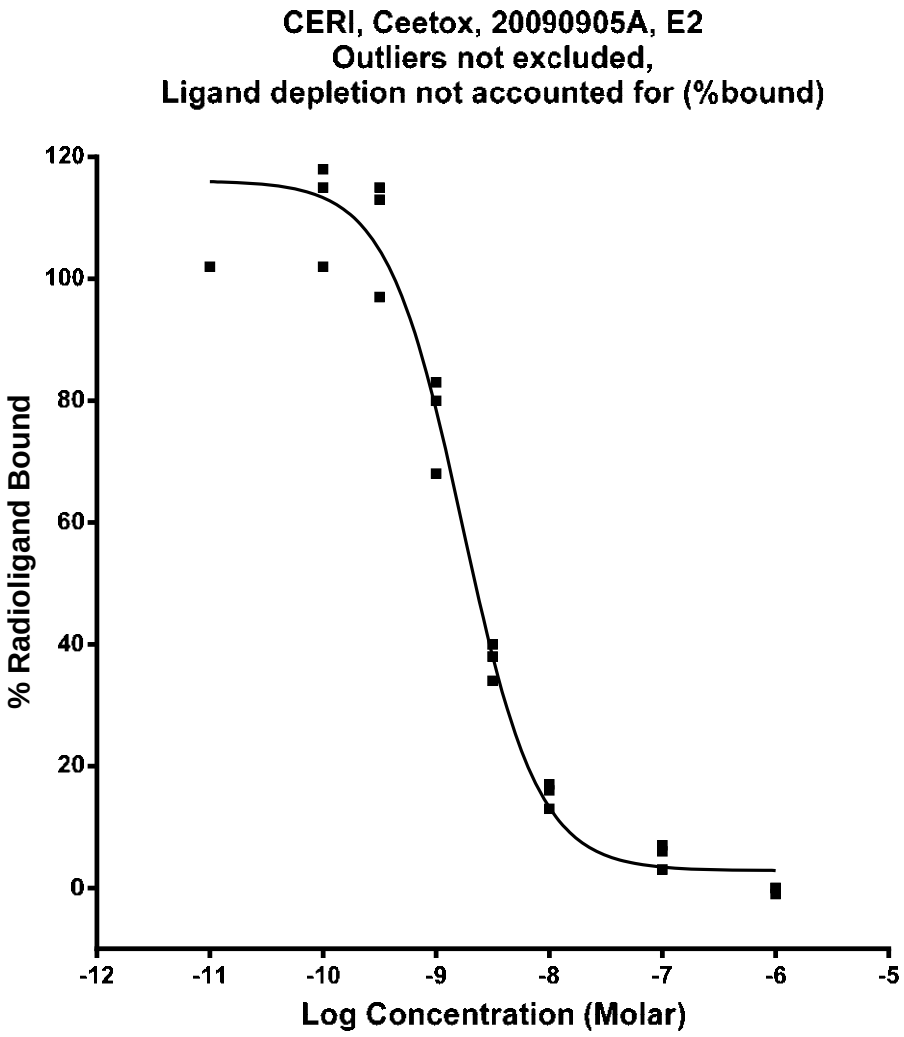

CERI, Ceetox, 20090401A, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
Top: 104.7
Bottom: 3.91
Hill slope: -0.95
Log IC50: -4.76
NE Tolerance bounds for CERI assay :
Top: avg 103.7 (range 67.45 to 139.9)
Bottom: Avg 2.48 (range -9.67 to 14.63)
HillSlope: Avg -1.02 (range -1.78 to -0.27
LogIC50: Avg -6.18 (range -7.19 to -5.17
NE curve not used in original analysis


CERI, Ceetox, 20090415, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

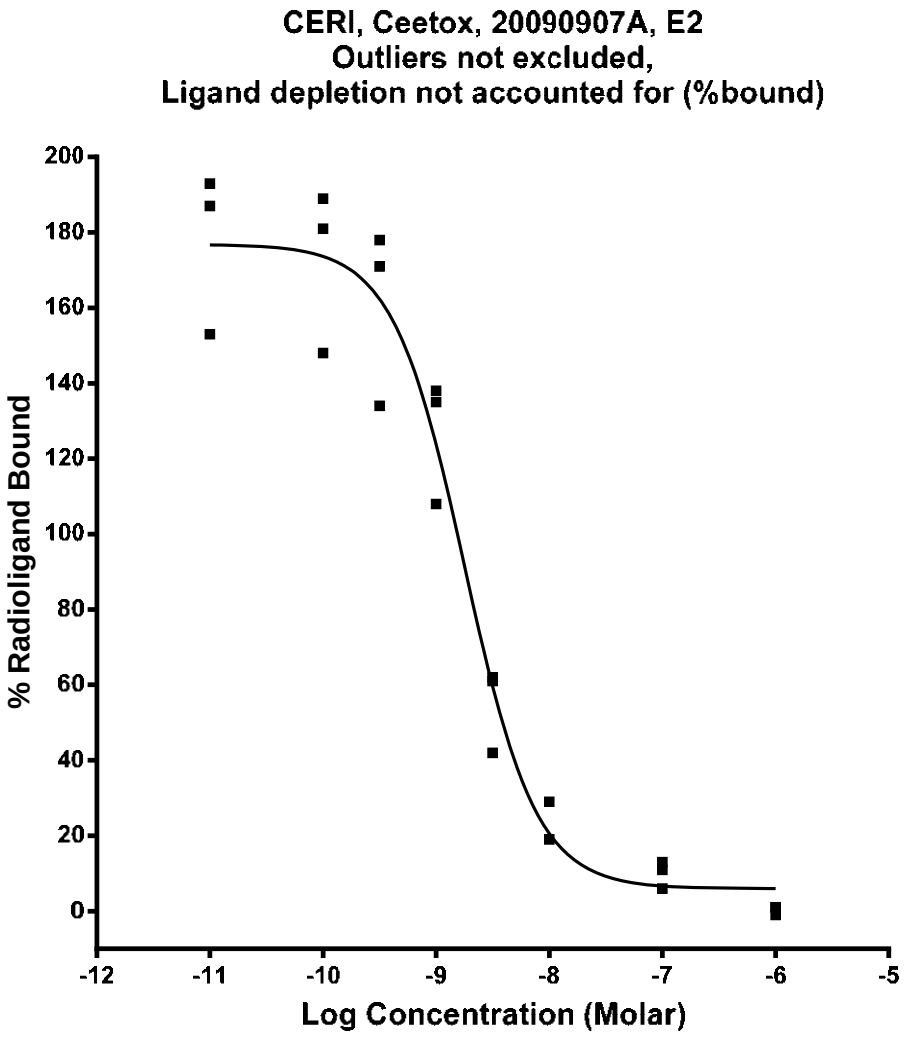
Subtask 4

Same data/curve as 20090903B

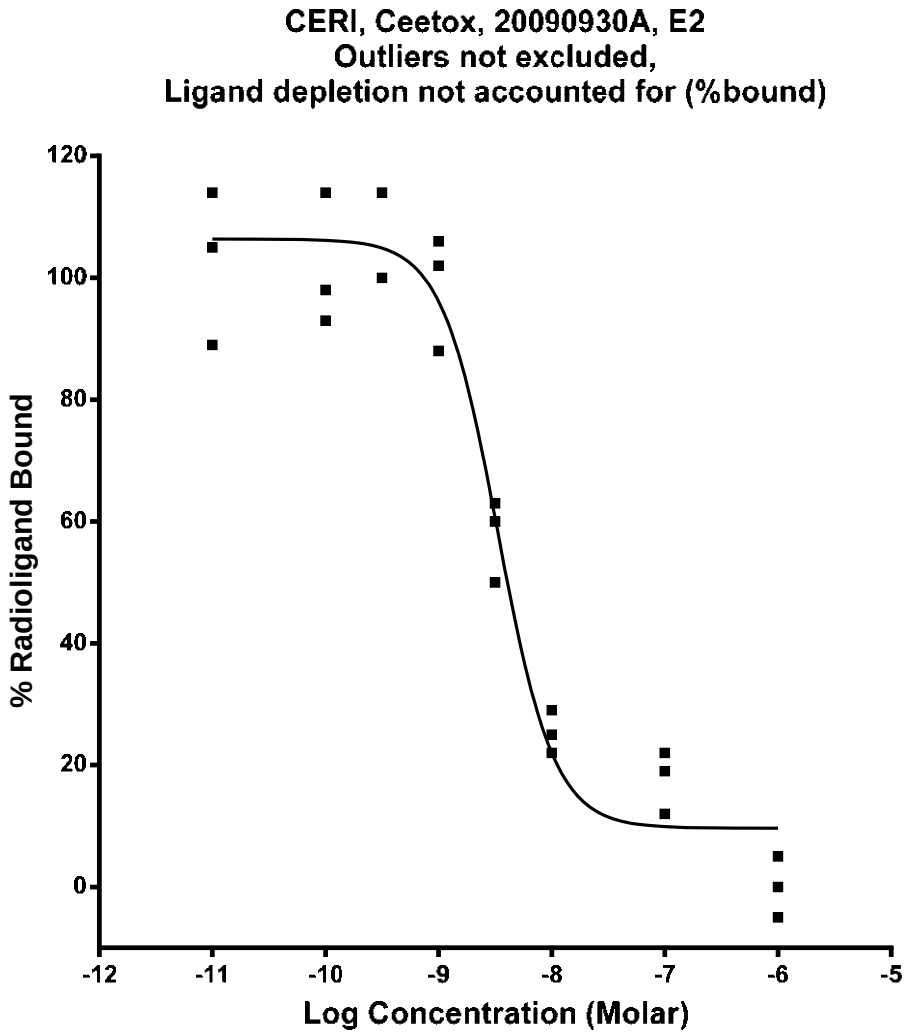


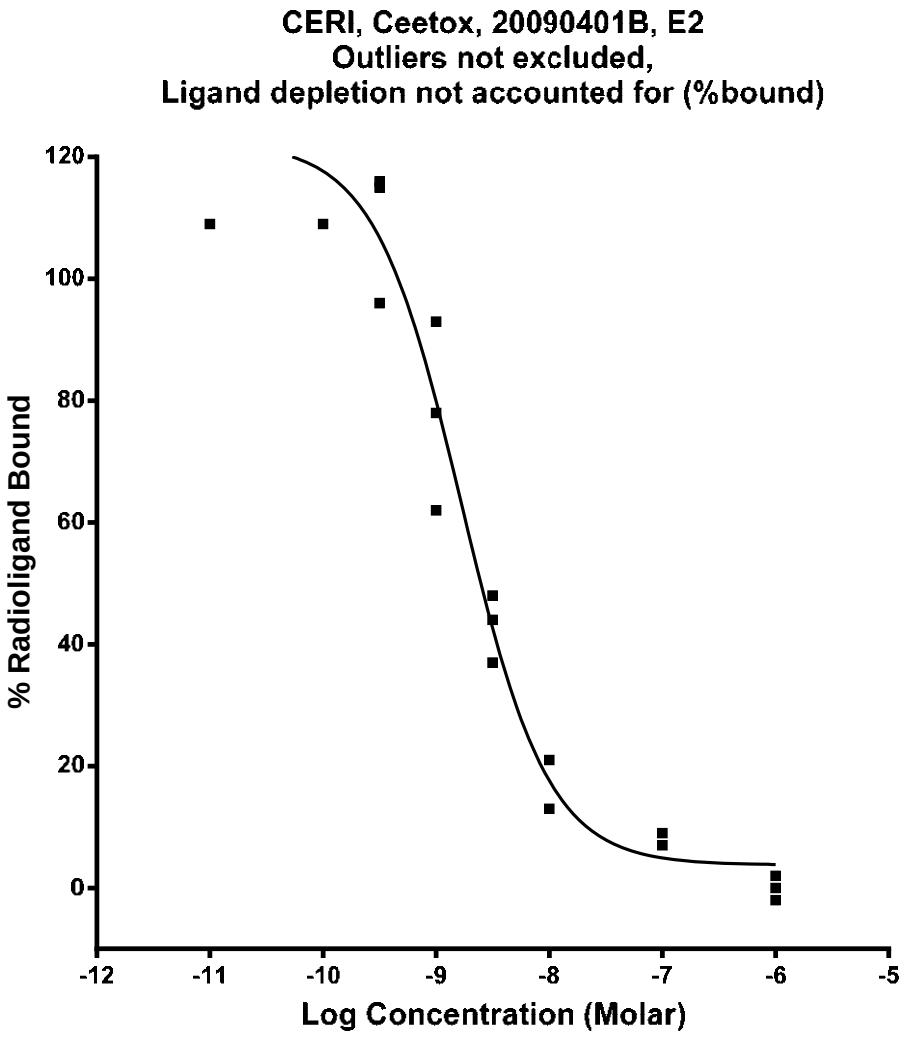

CERI, Ceetox, 20090905A, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Same data/curve as 20090907B



Same data/curve as 200090930B

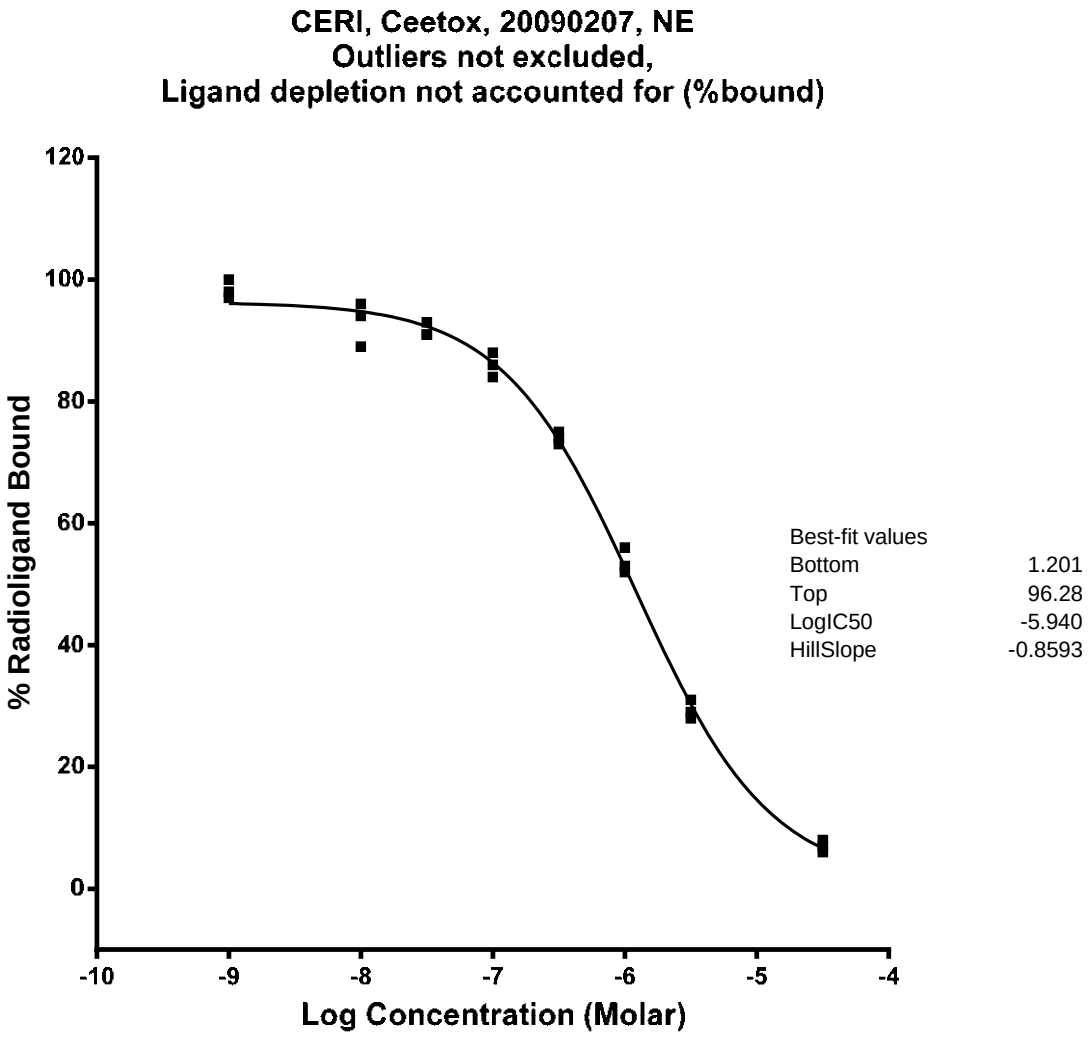



CERI, Ceetox, 20090401B, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

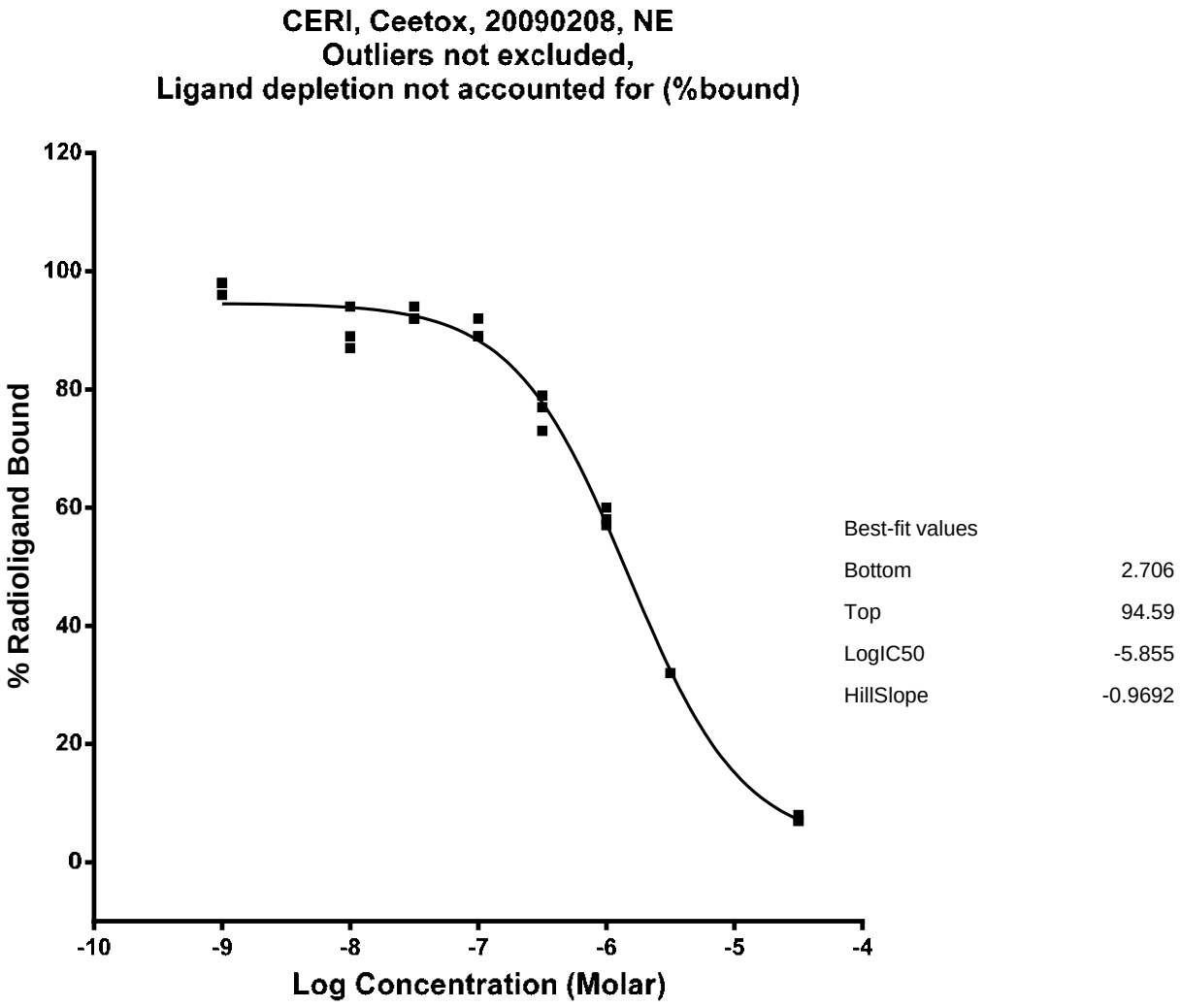
CERI ASSAY, (Ceetox Laboratory), Control Norethynodrel (NE) Curves

Begin HERE

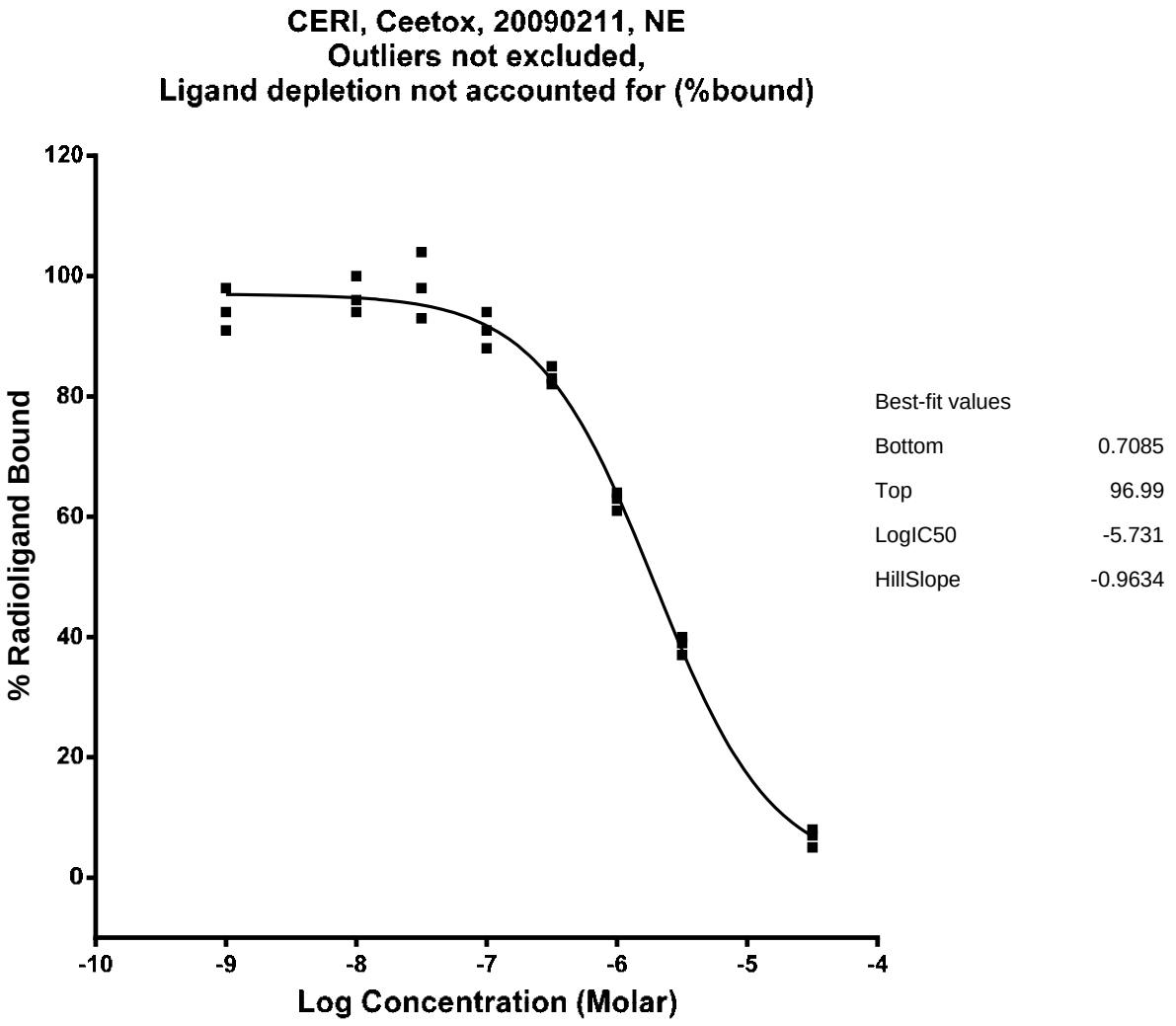
Subtask 1


CERI, Ceetox, 20090207, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Best-fit values
Bottom 1.201
Top 96.28
LogIC50 -5.940
HillSlope -0.8593

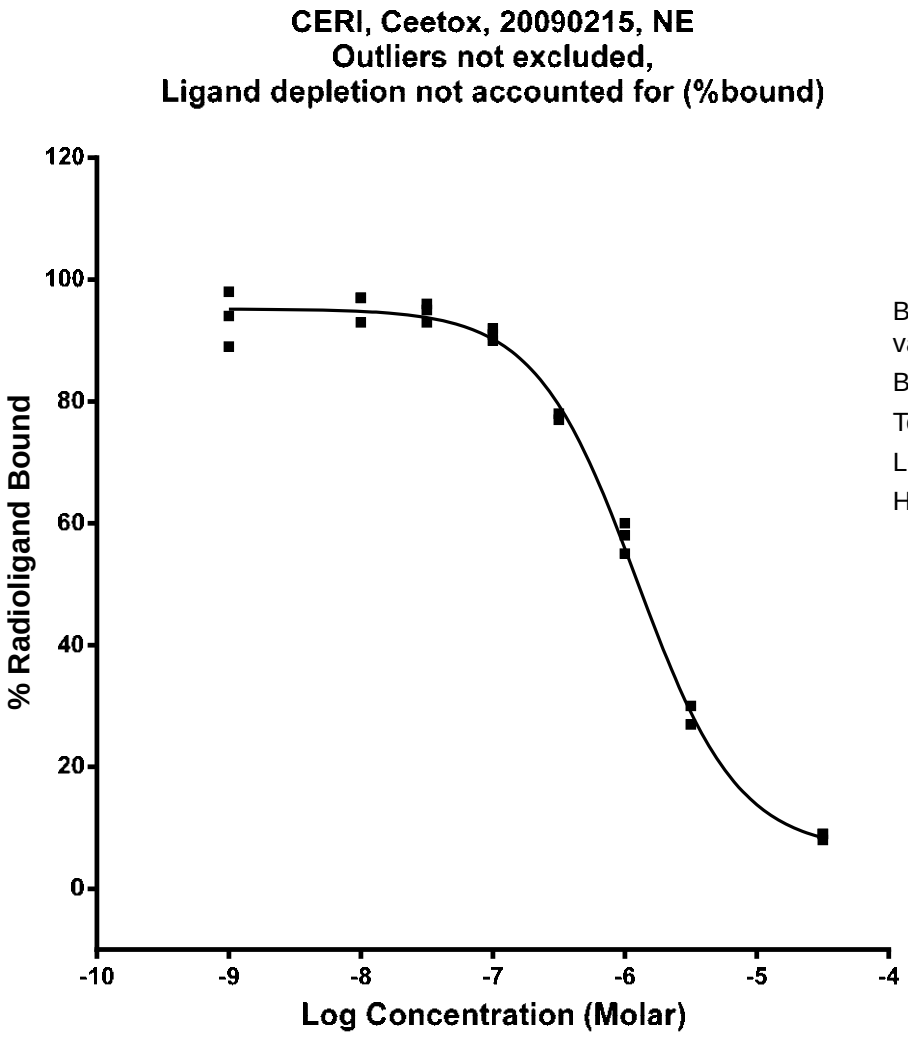
Subtask 1


CERI, Ceetox, 20090208, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log Concentration (Molar)
Best-fit values
Bottom 2.706
Top 94.59
LogIC50 -5.855
HillSlope -0.9692

Subtask 1

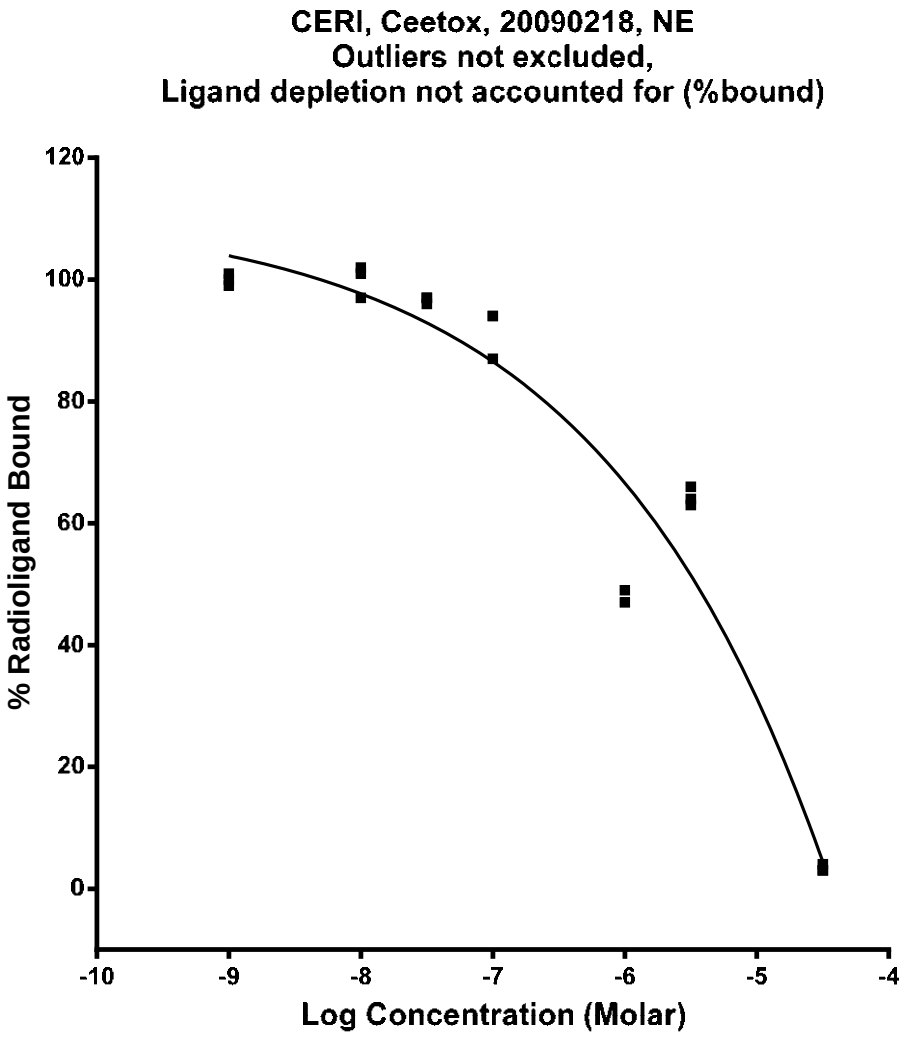

CERI, Ceetox, 20090211, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
Log Concentration (Molar)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Best-fit values
Bottom 0.7085
Top 96.99
LogIC50 -5.731
HillSlope -0.9634

Subtask 2



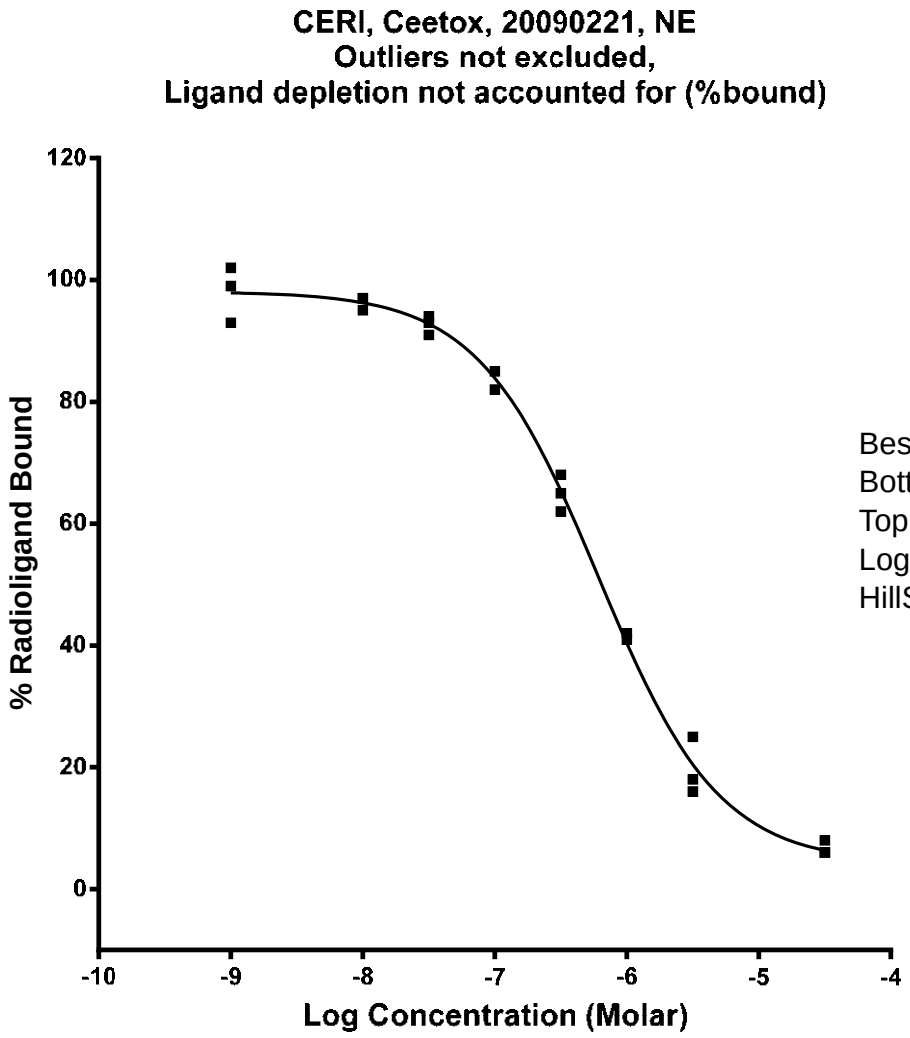
Best-fit values	
Bottom	6.145
Top	95.17
LogIC50	-5.897
HillSlope	-1.129

Subtask 2



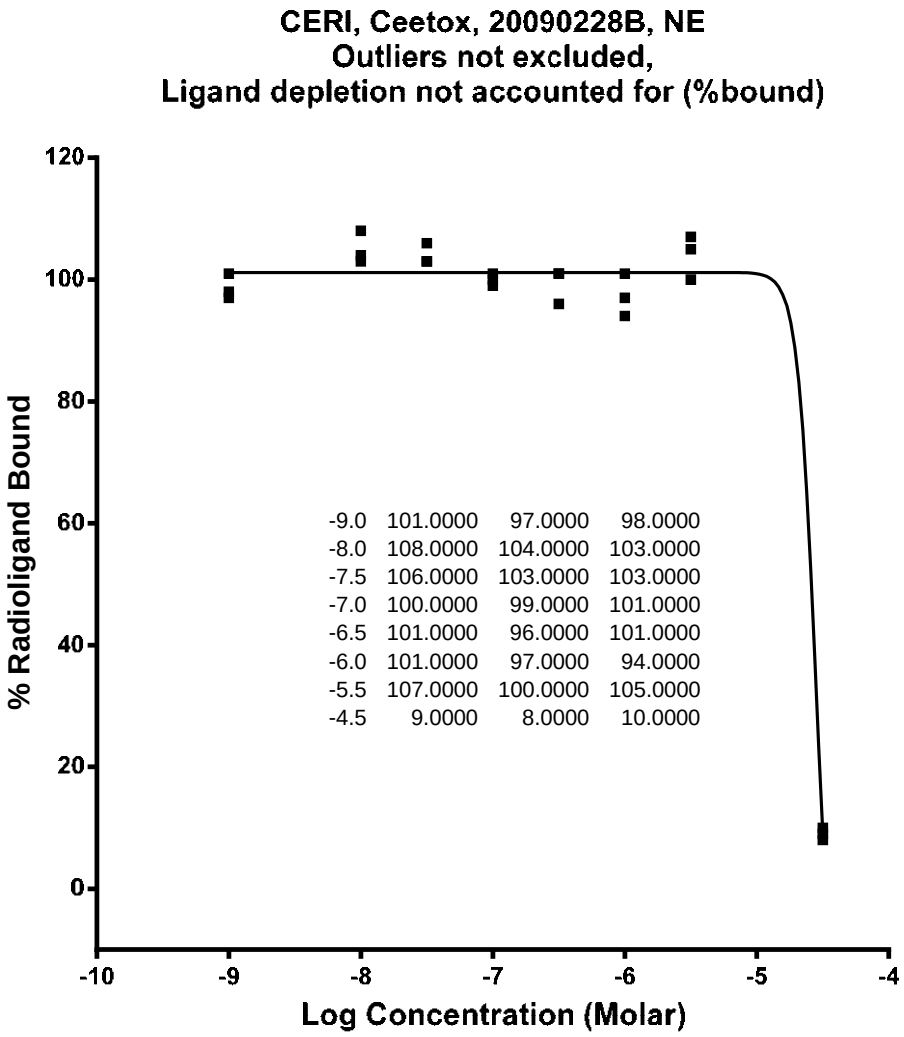
Best-fit values	
Bottom	-6366
Top	111.8
LogIC50	-5.458
HillSlope	-0.2534

Subtask 2



Best-fit values	
Bottom	4.209
Top	98.11
LogIC50	-6.190
HillSlope	-0.9511

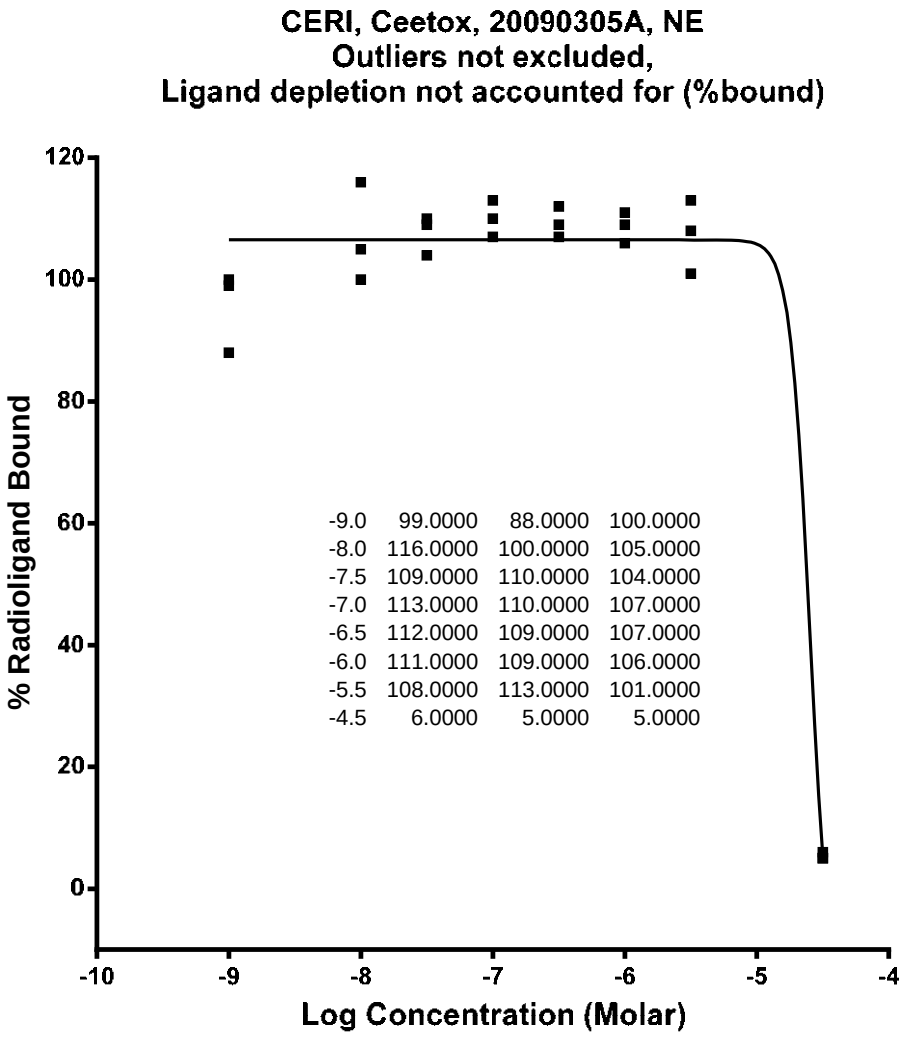
Ambiguous curve fit for NE.



logIC50	Ambiguous
Best-fit values	
Bottom	~ -40.32
Top	101.2
LogIC50	~ -4.585
HillSlope	~ -6.115
Std. Error	
Bottom	~ 1.973e+009
Top	0.9478
LogIC50	~ 1.133e+006
HillSlope	~ 1.132e+007
95% Confidence Intervals	
Bottom	(Very wide)
Top	99.21 to 103.2
LogIC50	(Very wide)
HillSlope	(Very wide)
Goodness of Fit	
Degrees of Freedom	20
R square	0.9881
Sy.x	3.669
Number of points	
Analyzed	24

Ambiguous curve fit)

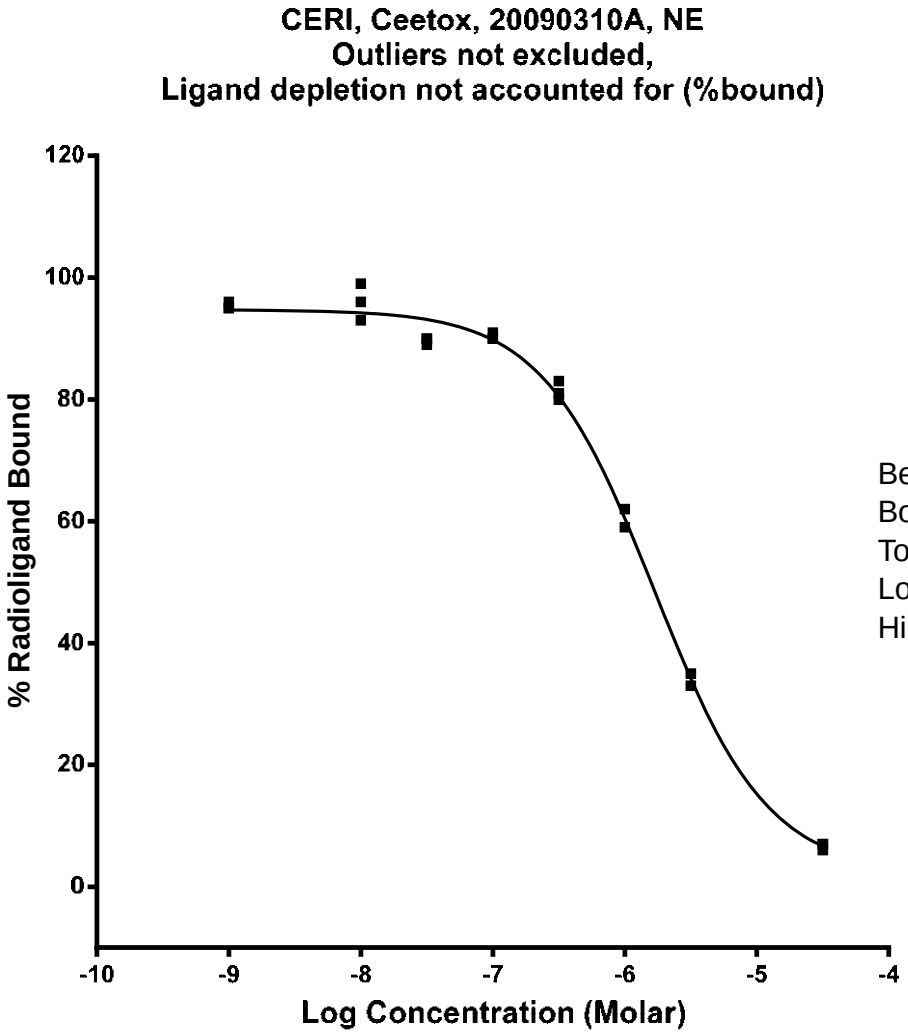
Data same for 2209305B



logIC50	Ambiguous
Best-fit values	
Bottom	~ -24.65
Top	106.5
LogIC50	~ -4.615
HillSlope	~ -5.623
Std. Error	
Bottom	~ 1.900e+008
Top	1.628
LogIC50	~ 249305
HillSlope	~ 2.124e+006
95% Confidence Intervals	
Bottom	(Very wide)
Top	103.1 to 109.9
LogIC50	(Very wide)
HillSlope	(Very wide)
Goodness of Fit	
Degrees of Freedom	20
R square	0.9713
Sy.x	6.300
Number of points Analyzed	24

Subtask 3

Acceptable Data

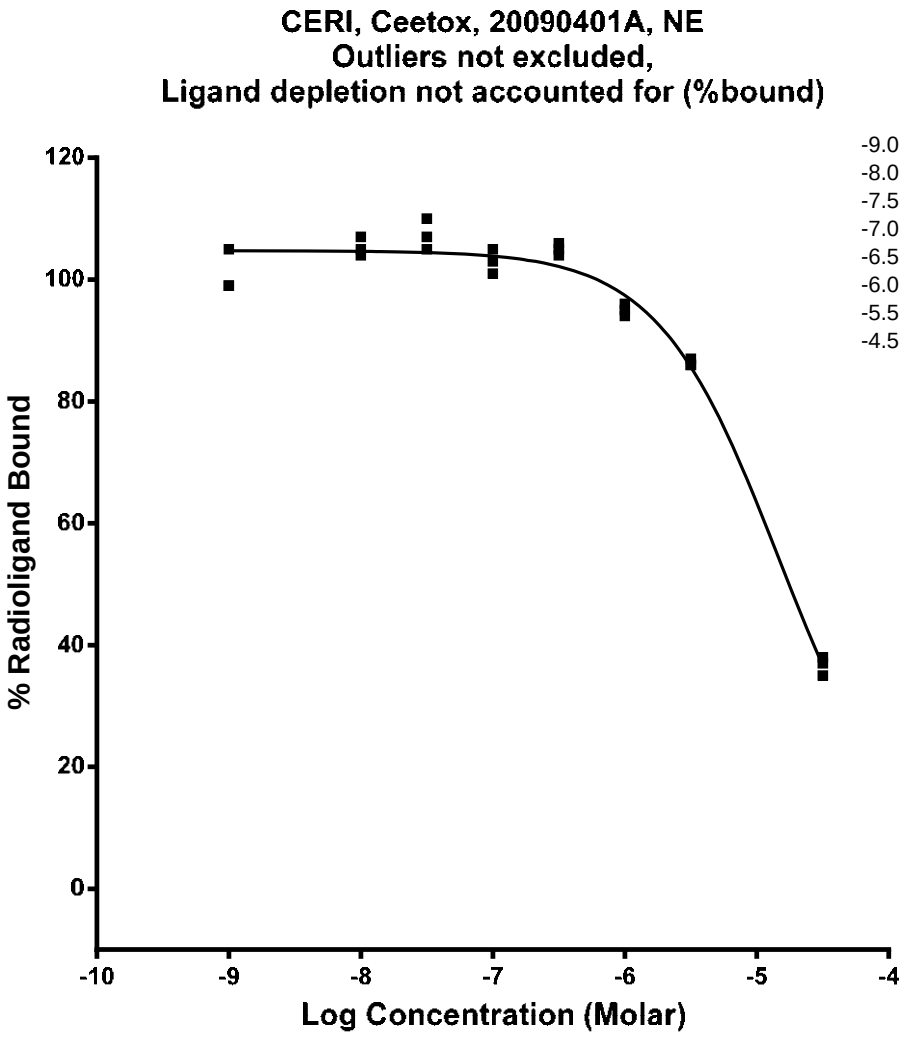


Best-fit values	
Bottom	2.213
Top	94.71
LogIC50	-5.801
HillSlope	-1.018

Curve not accepted during Initial review because lacking Bottom of curve. IC50 outside tolerance bounds, but all Other parameters ok.

Suggest accepting curve for PS analyses.

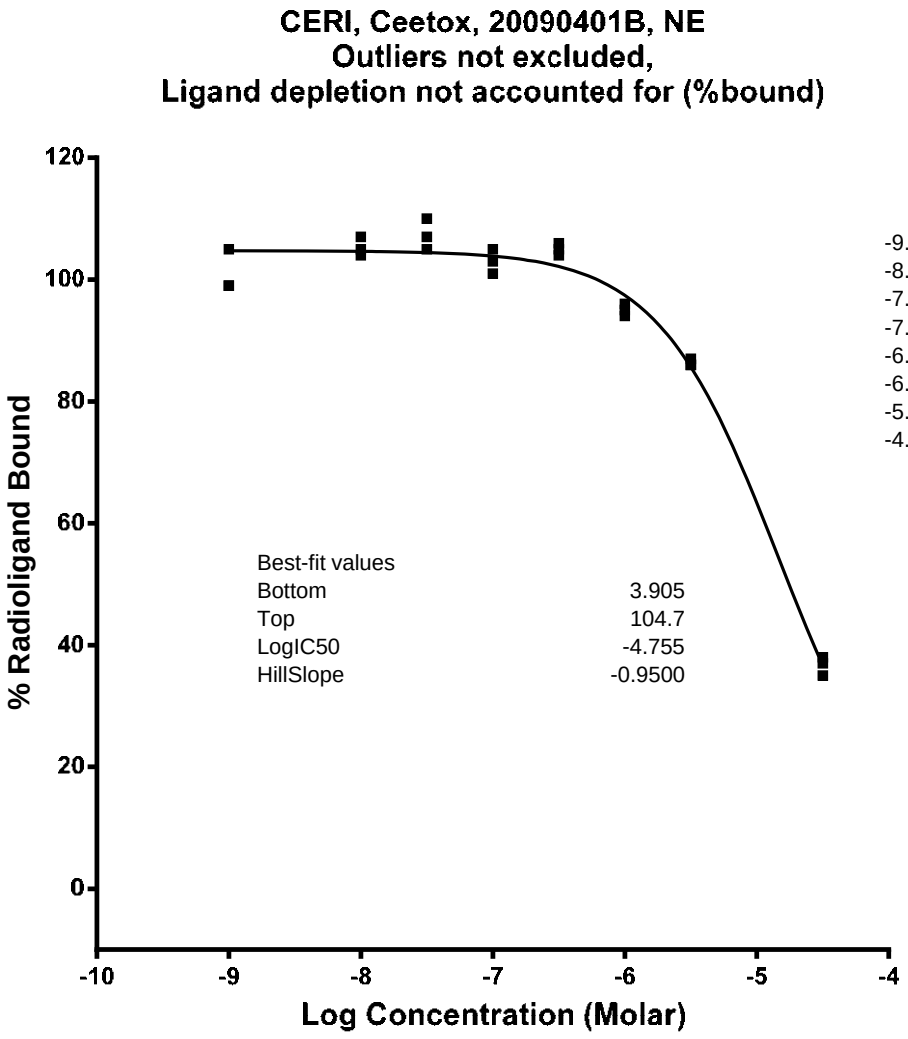
NE Tolerance bounds for CERI assay :
Top: avg 103.7 (range 67.45 to 139.9)
Bottom: Avg 2.48 (range -9.67 to 14.63)
HillSlope: Avg -1.02 (range -1.78 to -0.27
LogIC50: Avg -6.18 (range -7.19 to -5.17



-9.0	105.0000	99.0000	99.0000
-8.0	107.0000	105.0000	104.0000
-7.5	110.0000	107.0000	105.0000
-7.0	105.0000	101.0000	103.0000
-6.5	105.0000	106.0000	104.0000
-6.0	96.0000	95.0000	94.0000
-5.5	86.0000	86.0000	87.0000
-4.5	38.0000	35.0000	37.0000

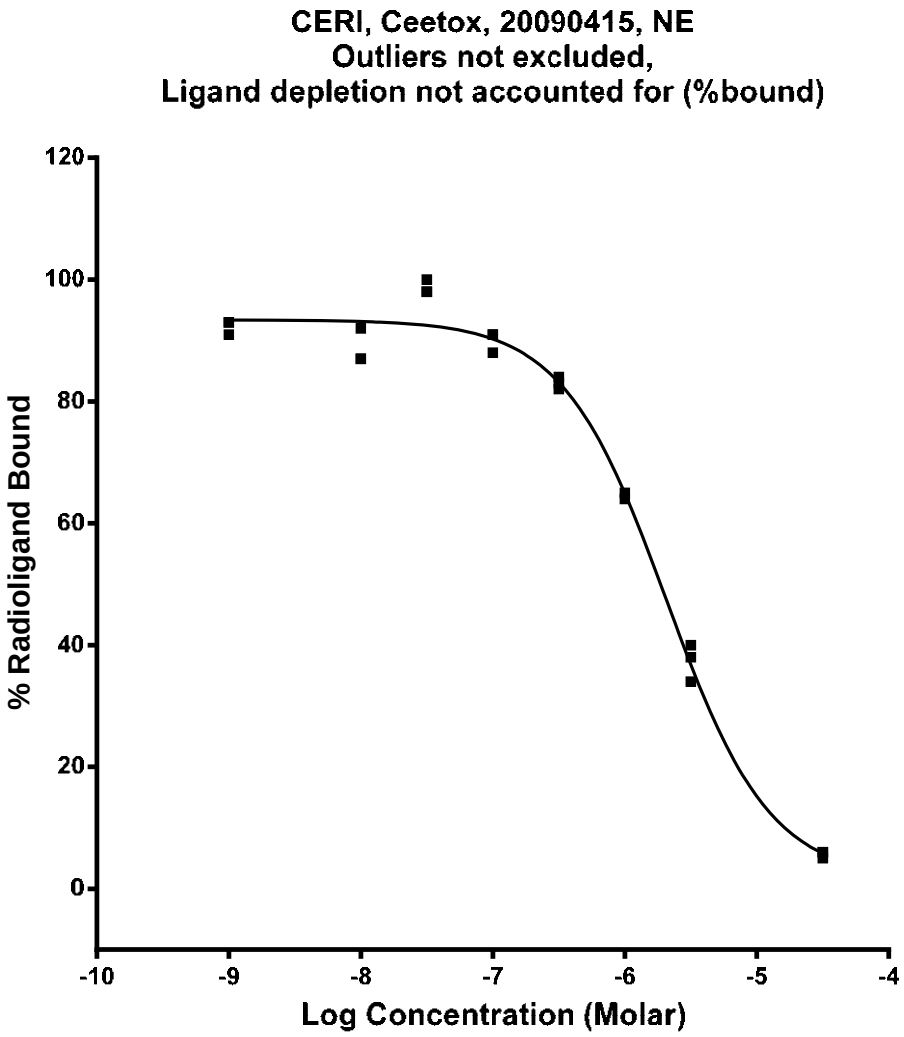
Best-fit values	
Bottom	3.905
Top	104.7
LogIC50	-4.755
HillSlope	-0.9500

This curve as same PRISM Curve fit values as 20090401A for NE.



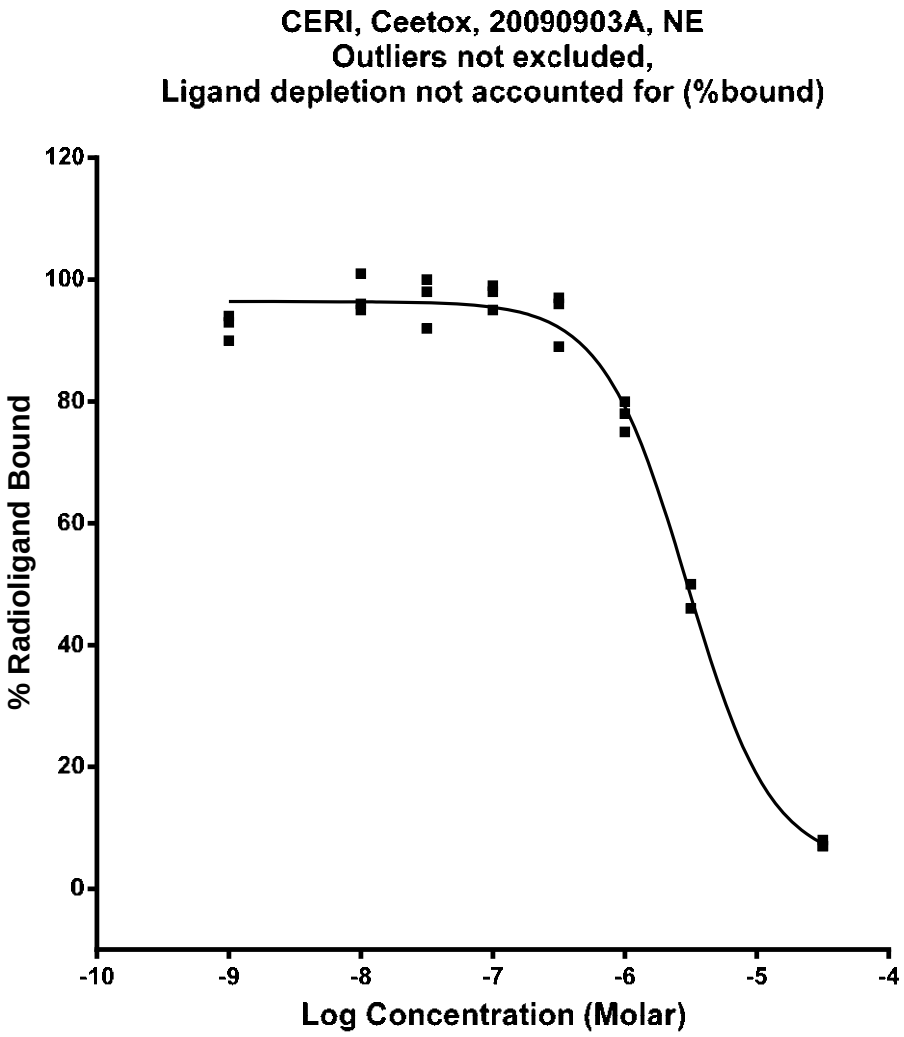
-9.0	105.0000	99.0000	99.0000
-8.0	107.0000	105.0000	104.0000
-7.5	110.0000	107.0000	105.0000
-7.0	105.0000	101.0000	103.0000
-6.5	105.0000	106.0000	104.0000
-6.0	96.0000	95.0000	94.0000
-5.5	86.0000	86.0000	87.0000
-4.5	38.0000	35.0000	37.0000

NE Tolerance bounds for CERI assay :
Top: avg 103.7 (range 67.45 to 139.9)
Bottom: Avg 2.48 (range -9.67 to 14.63)
HillSlope: Avg -1.02 (range -1.78 to -0.27
LogIC50: Avg -6.18 (range -7.19 to

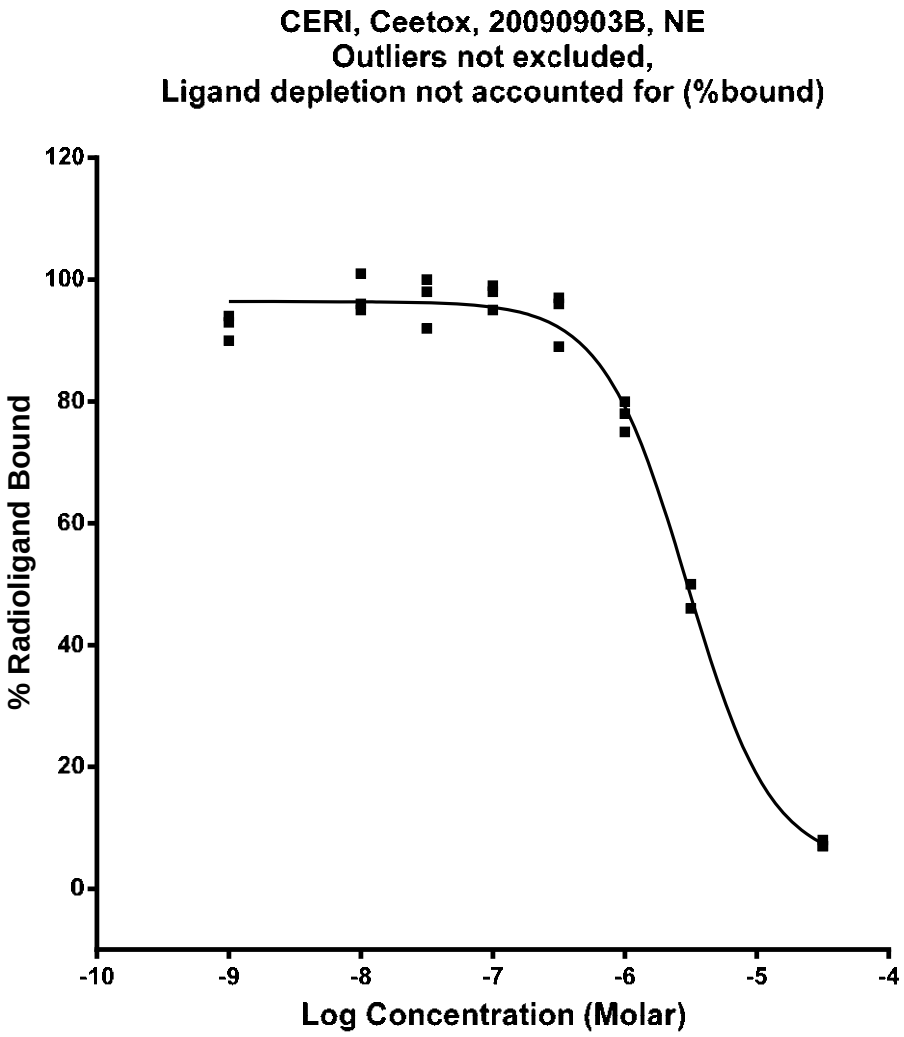


Best-fit values	
Bottom	1.509
Top	93.38
LogIC50	-5.728
HillSlope	-1.106

Subtask 4

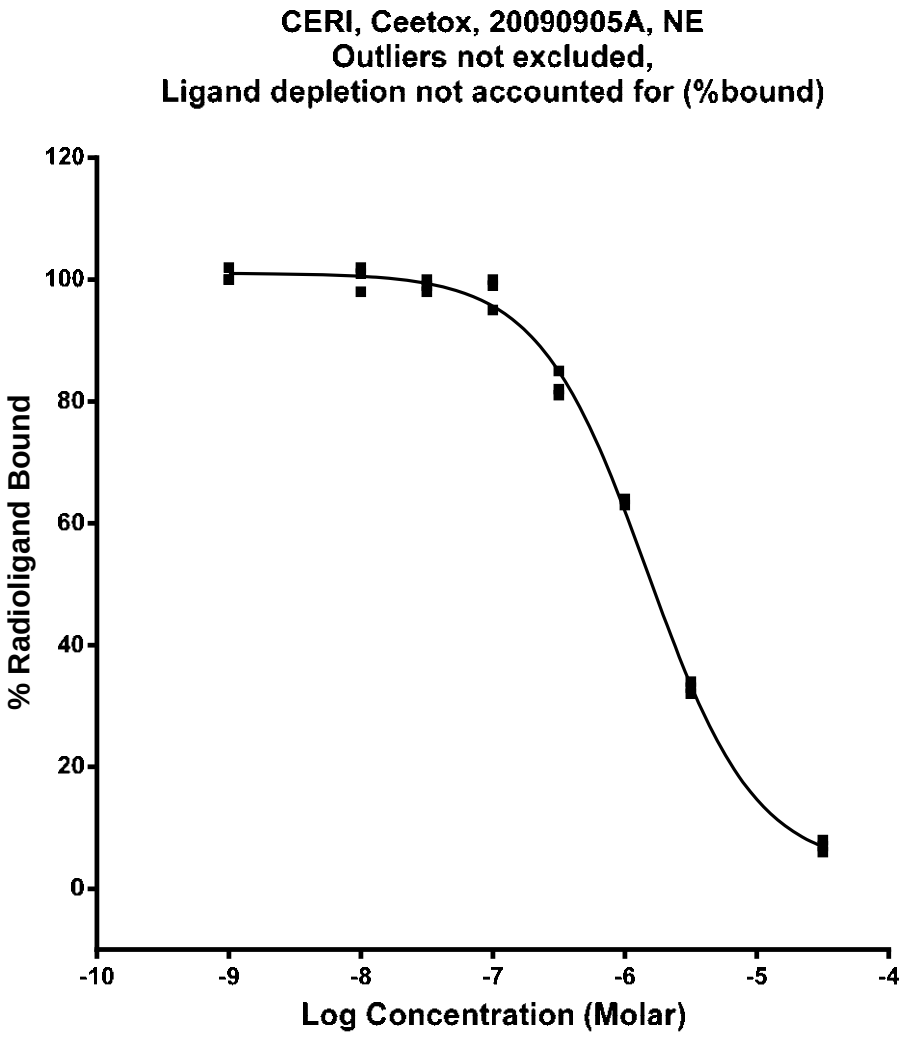


Best-fit values	
Bottom	3.846
Top	96.40
LogIC50	-5.526
HillSlope	-1.357

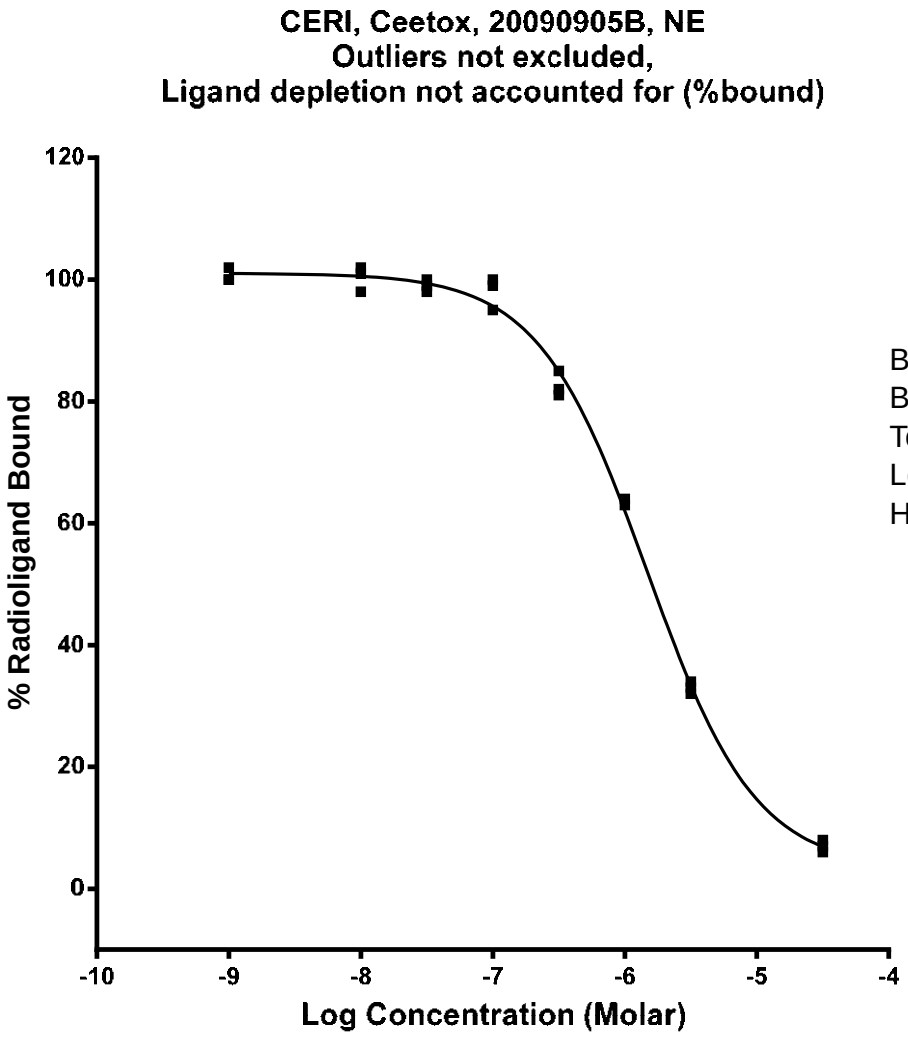


Best-fit values	
Bottom	3.846
Top	96.40
LogIC50	-5.526
HillSlope	-1.357

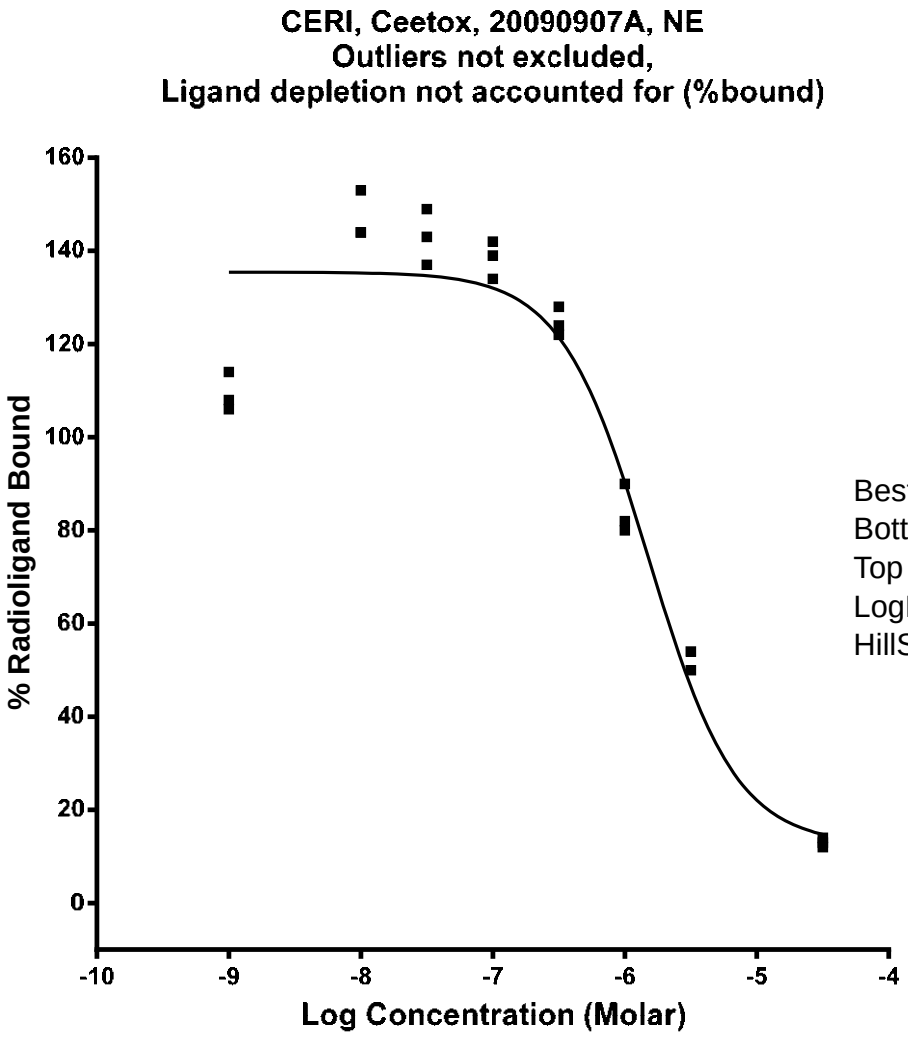
Note; same prism fit
Values as for 2009093B



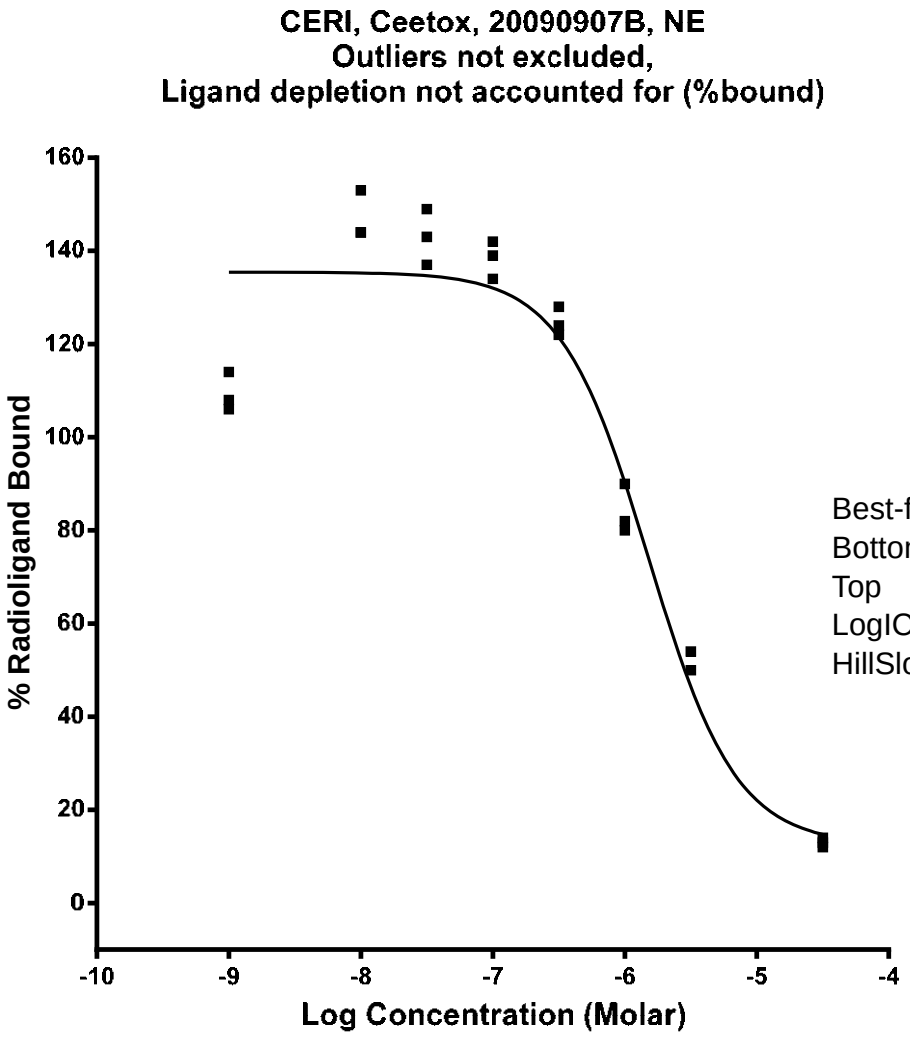
Best-fit values	
Bottom	3.176
Top	101.0
LogIC50	-5.794
HillSlope	-1.054



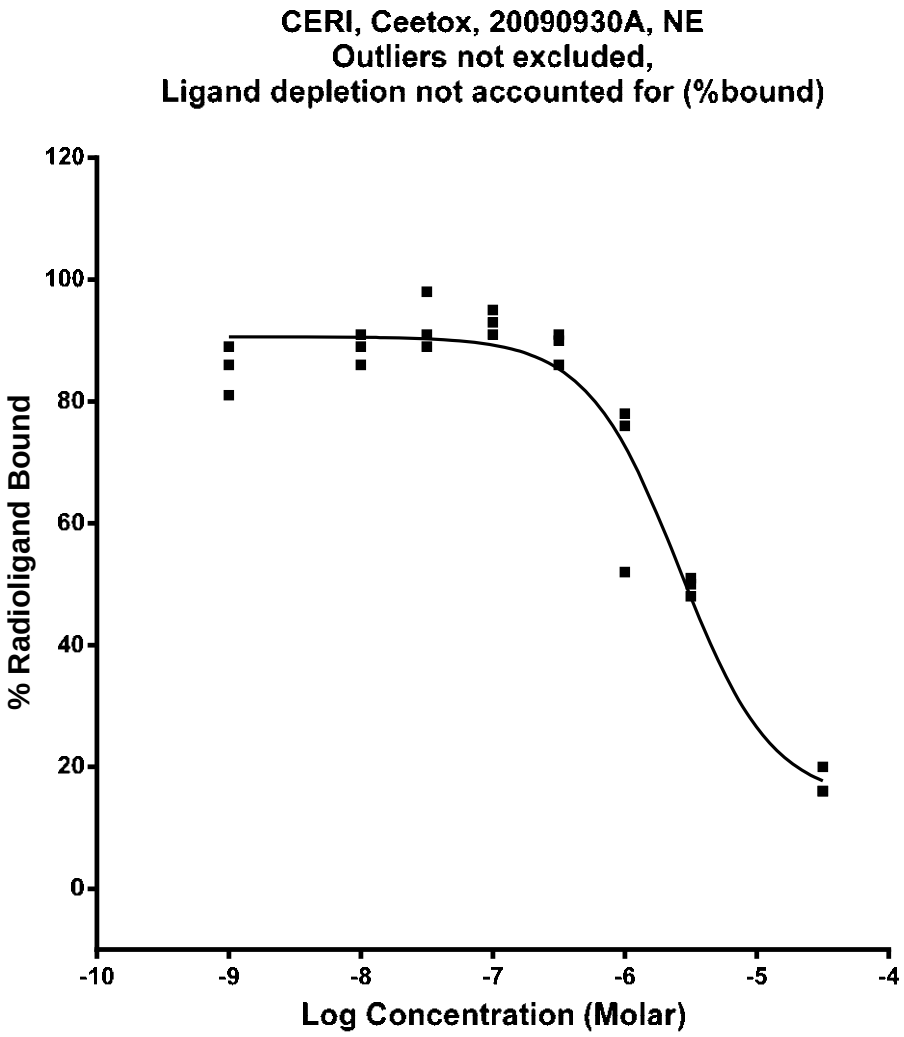
Best-fit values	
Bottom	3.176
Top	101.0
LogIC50	-5.794
HillSlope	-1.054



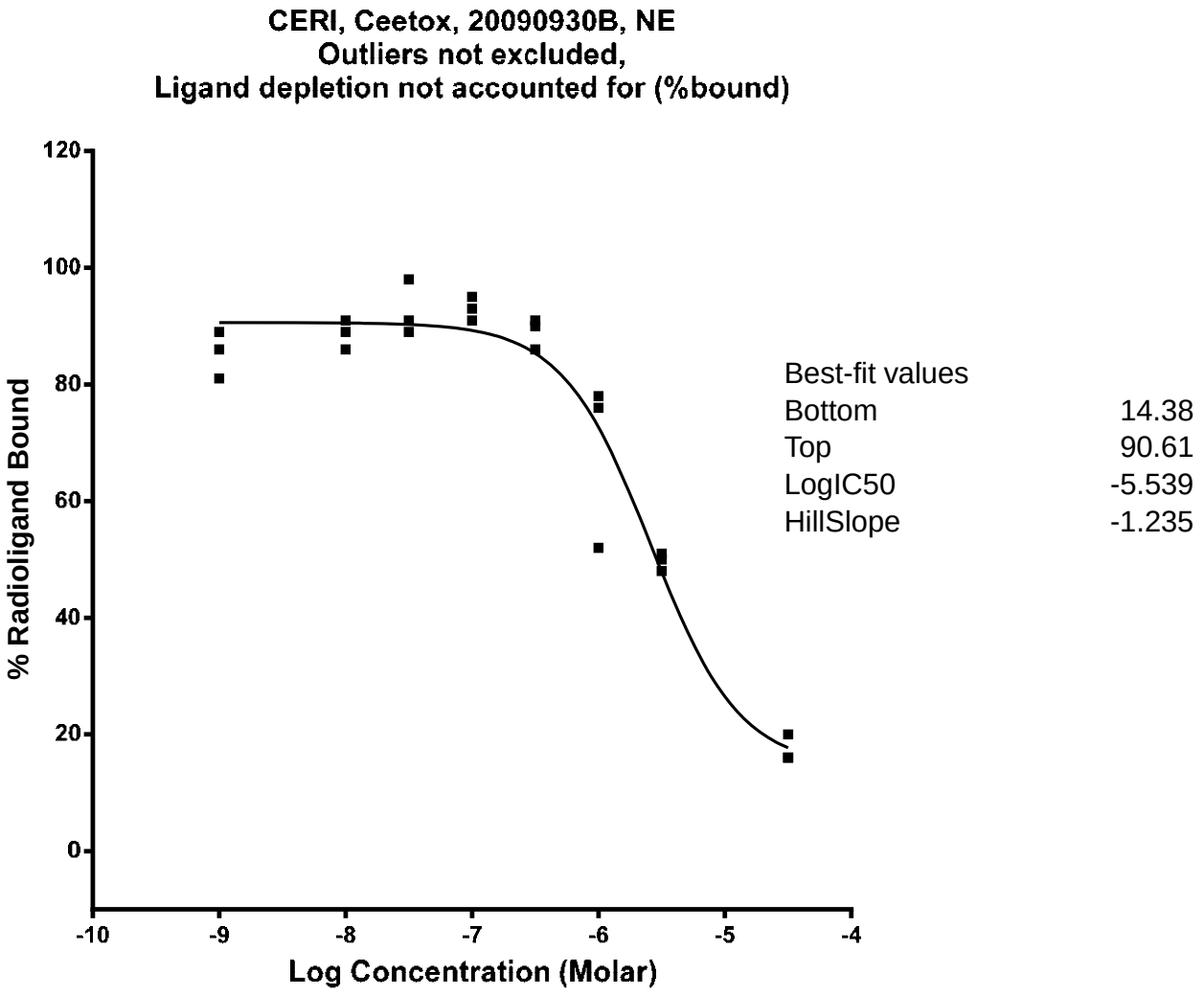
Best-fit values	
Bottom	12.50
Top	135.5
LogIC50	-5.545
HillSlope	-1.309



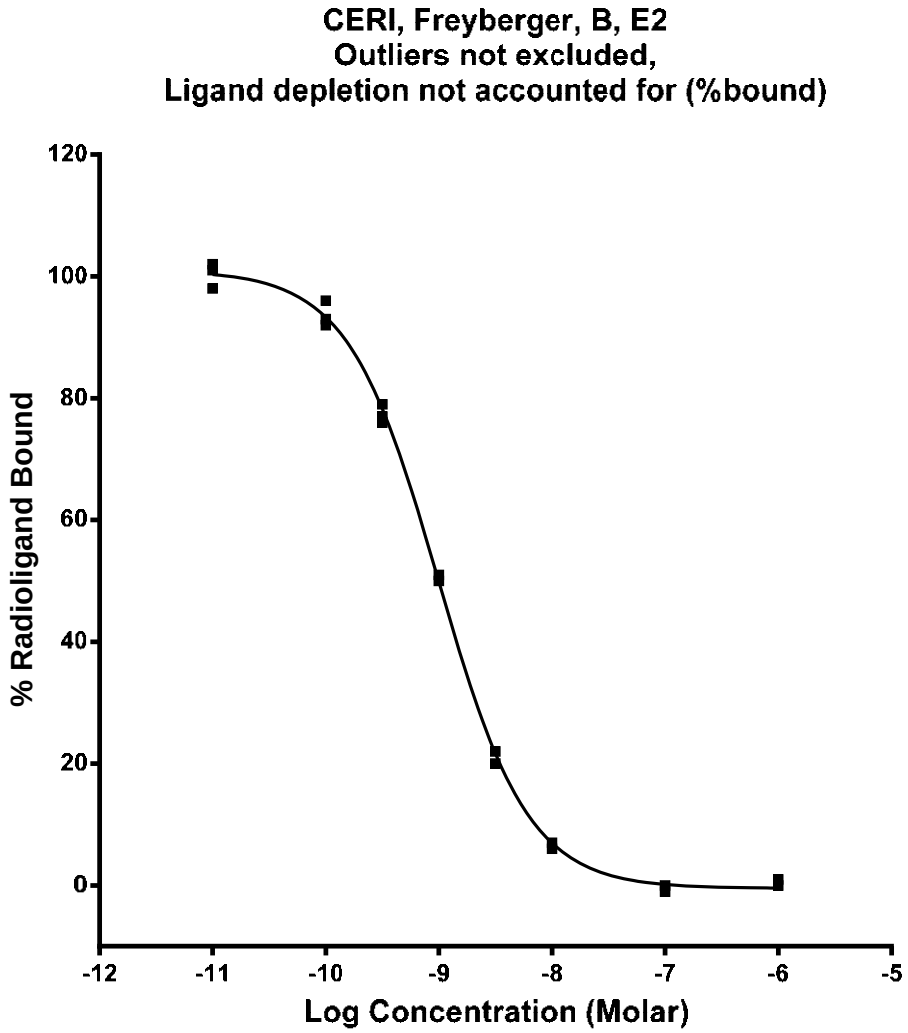
Best-fit values	
Bottom	12.50
Top	135.5
LogIC50	-5.545
HillSlope	-1.309



Best-fit values	
Bottom	14.38
Top	90.61
LogIC50	-5.539
HillSlope	-1.235

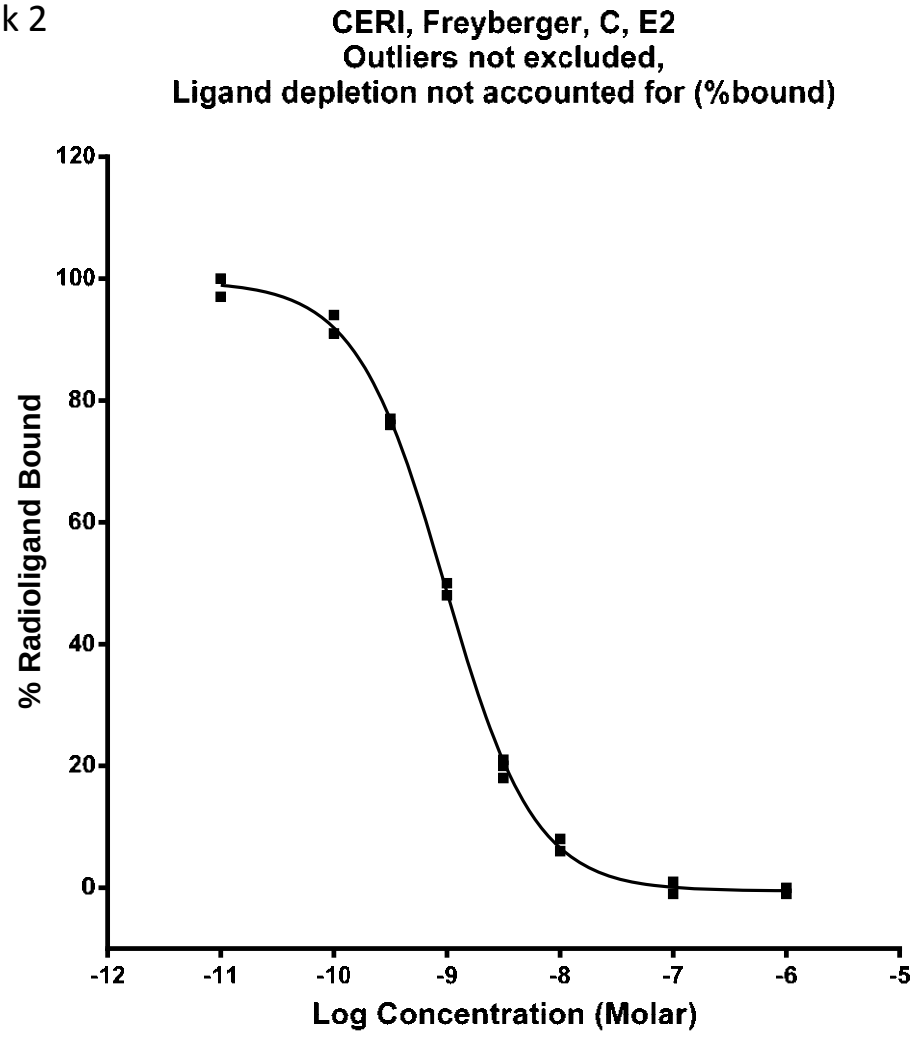

CERI, Ceetox, 20090930B, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log Concentration (Molar)
Best-fit values
Bottom 14.38
Top 90.61
LogIC50 -5.539
HillSlope -1.235

Subtask 2



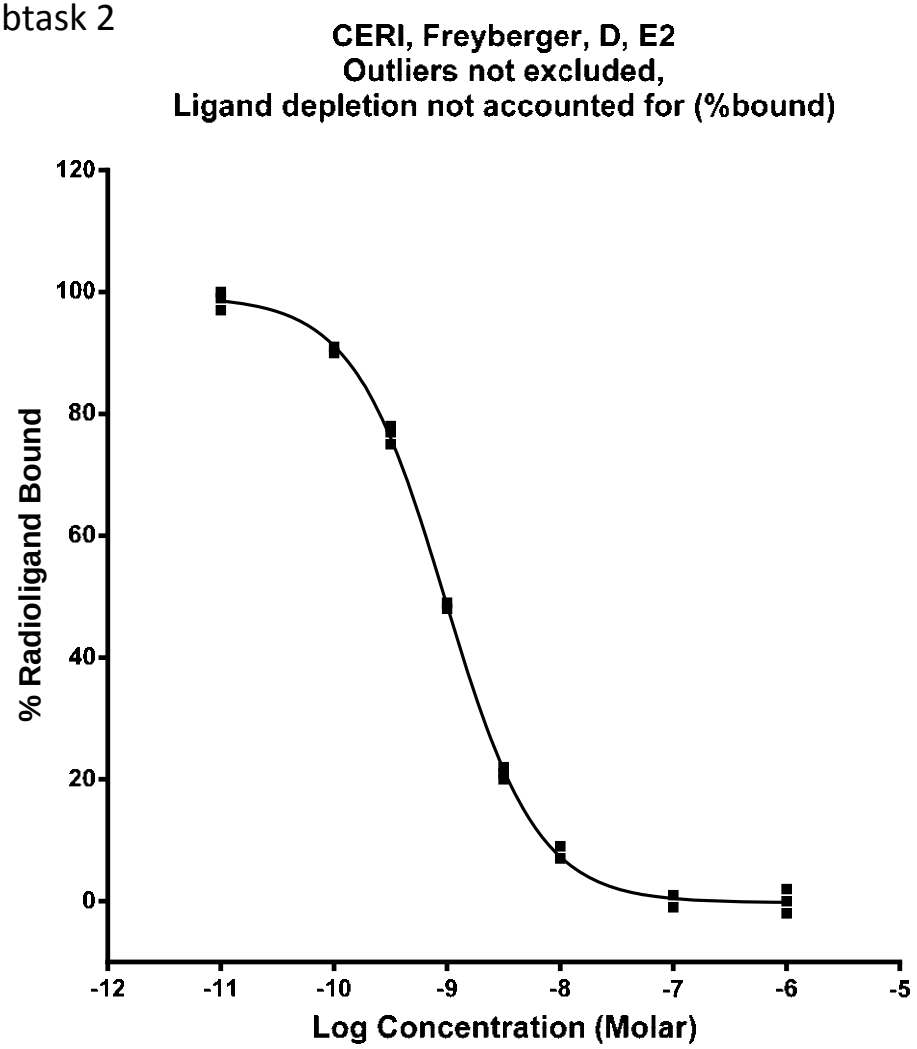
logIC50	
Best-fit values	
Bottom	-0.4851
Top	101.0
LogIC50	-9.003
HillSlope	-1.094
Std. Error	
Bottom	0.5382
Top	0.7404
LogIC50	0.008736
HillSlope	0.02938
95% Confidence Intervals	
Bottom	-1.608 to 0.6375
Top	99.42 to 102.5
LogIC50	-9.021 to -8.985
HillSlope	-1.155 to -1.033
Goodness of Fit	
Degrees of Freedom	20
R square	0.9991
Sy.x	1.332
Number of points	
Analyzed	24

Subtask 2



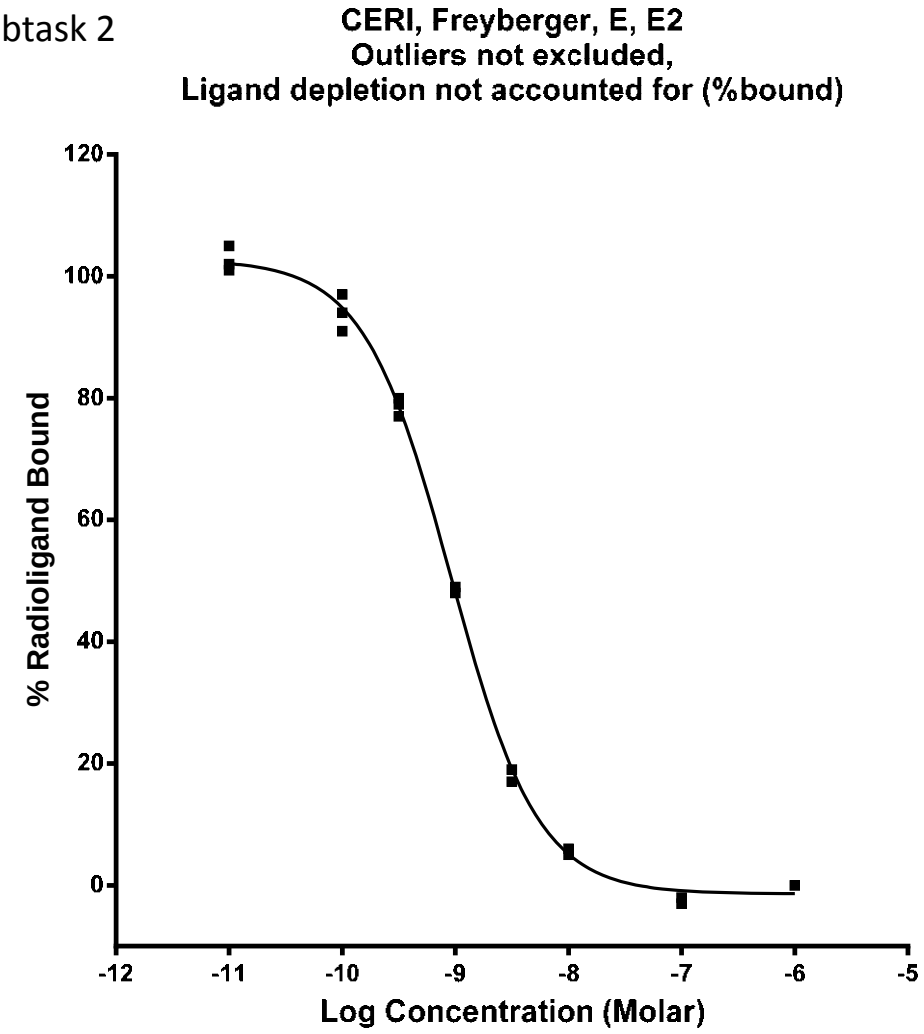
logIC50	
Best-fit values	
Bottom	-0.5349
Top	99.55
LogIC50	-9.024
HillSlope	-1.102
Std. Error	
Bottom	0.5319
Top	0.7357
LogIC50	0.008774
HillSlope	0.02981
95% Confidence Intervals	
Bottom	-1.644 to 0.5746
Top	98.02 to 101.1
LogIC50	-9.042 to -9.006
HillSlope	-1.164 to -1.040
Goodness of Fit	
Degrees of Freedom	20
R square	0.9990
Sy.x	1.323
Number of points	
Analyzed	24

Subtask 2



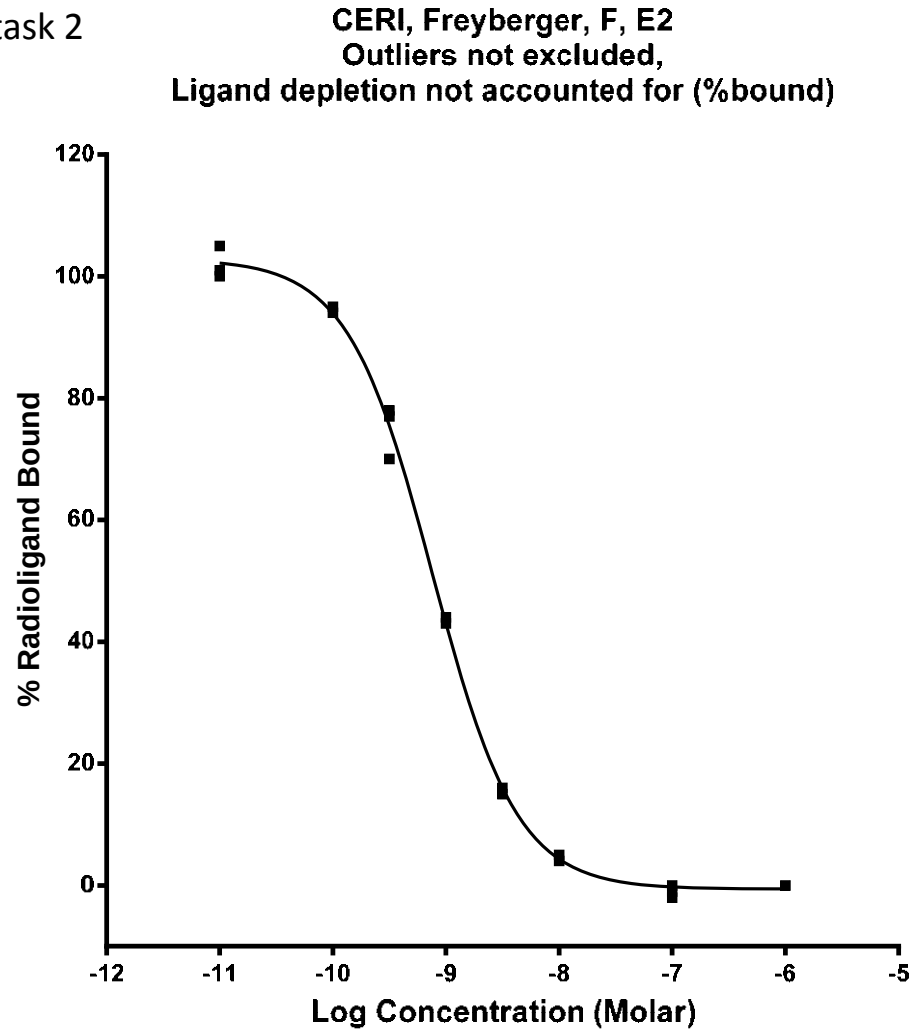
logIC50	
Best-fit values	
Bottom	-0.2586
Top	99.27
LogIC50	-9.024
HillSlope	-1.069
Std. Error	
Bottom	0.5305
Top	0.7392
LogIC50	0.008850
HillSlope	0.02870
95% Confidence Intervals	
Bottom	-1.365 to 0.8480
Top	97.73 to 100.8
LogIC50	-9.043 to -9.006
HillSlope	-1.129 to -1.010
Goodness of Fit	
Degrees of Freedom	20
R square	0.9991
Sy.x	1.305
Number of points	
Analyzed	24

Subtask 2



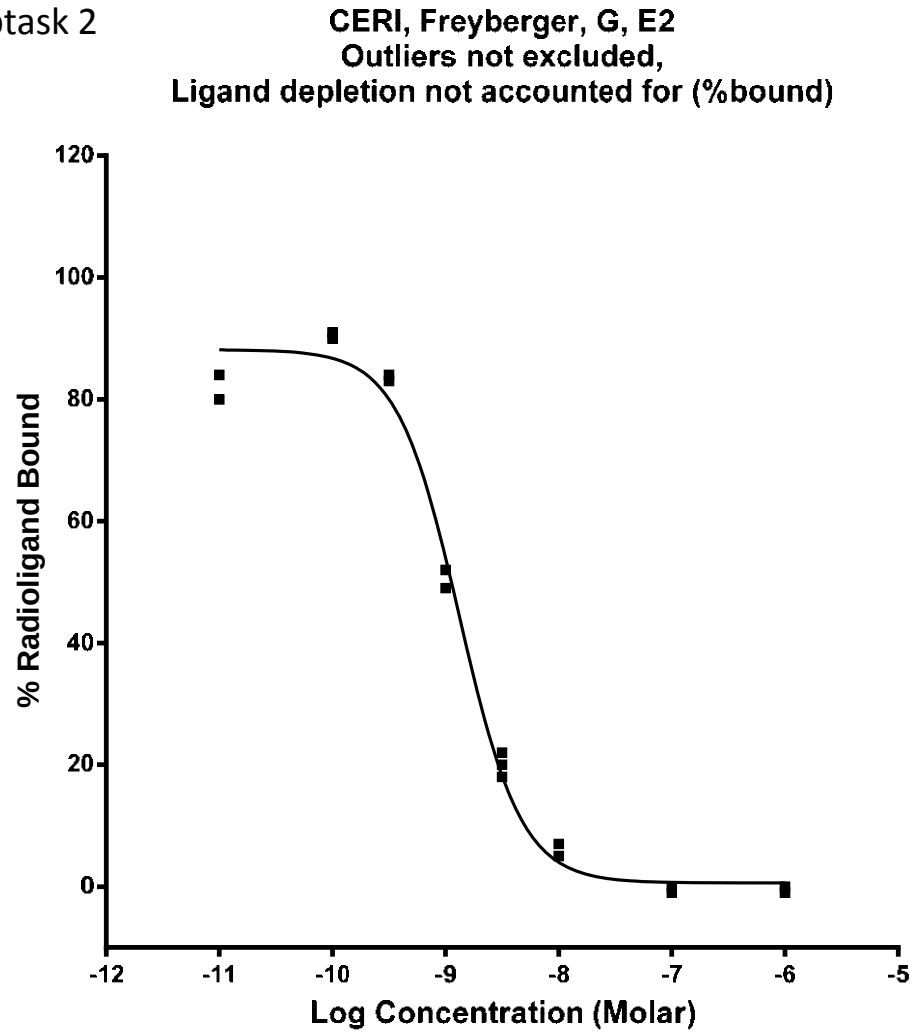
logIC50	
Best-fit values	
Bottom	-1.389
Top	102.7
LogIC50	-9.029
HillSlope	-1.131
Std. Error	
Bottom	0.6566
Top	0.9177
LogIC50	0.01041
HillSlope	0.03692
95% Confidence Intervals	
Bottom	-2.758 to -0.01897
Top	100.8 to 104.6
LogIC50	-9.051 to -9.007
HillSlope	-1.208 to -1.054
Goodness of Fit	
Degrees of Freedom	20
R square	0.9986
Sy.x	1.659
Number of points	
Analyzed	24

Subtask 2



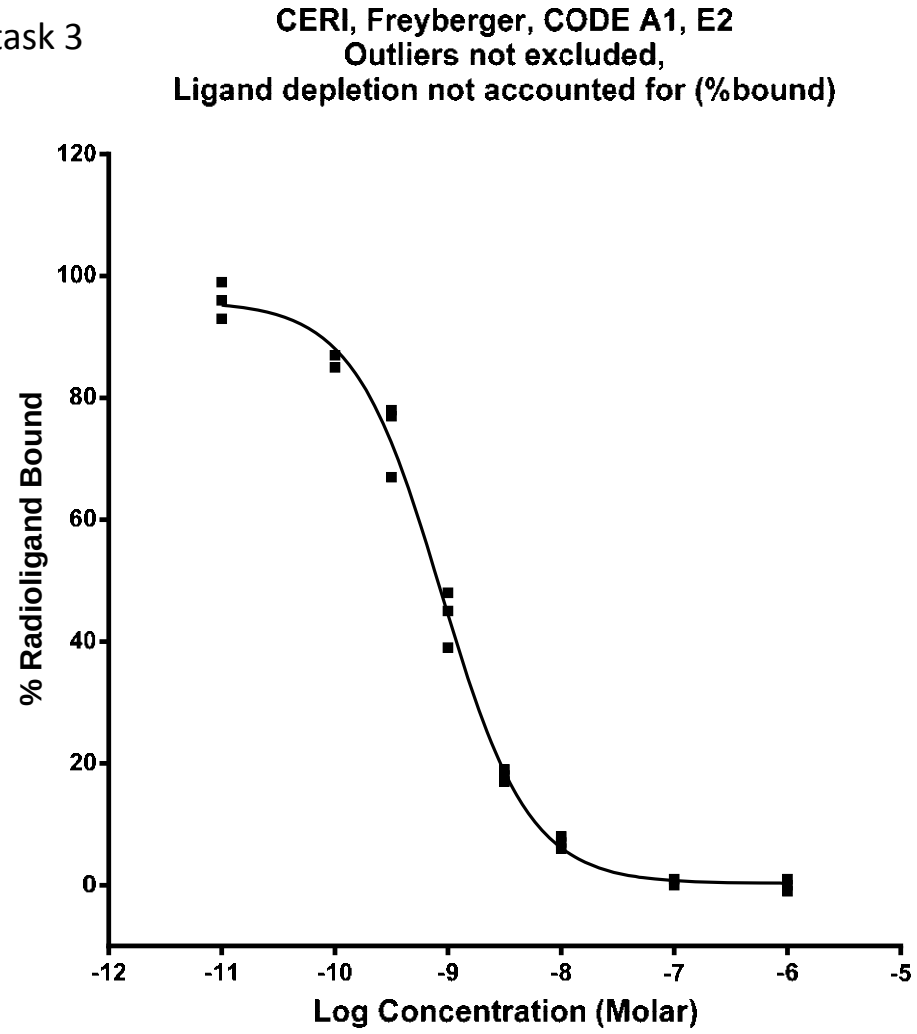
logIC50	
Best-fit values	
Bottom	-0.6075
Top	102.8
LogIC50	-9.102
HillSlope	-1.162
Std. Error	
Bottom	0.6732
Top	0.9923
LogIC50	0.01092
HillSlope	0.04058
95% Confidence Intervals	
Bottom	-2.012 to 0.7969
Top	100.8 to 104.9
LogIC50	-9.125 to -9.080
HillSlope	-1.247 to -1.077
Goodness of Fit	
Degrees of Freedom	20
R square	0.9984
Sy.x	1.755
Number of points	
Analyzed	24

Subtask 2



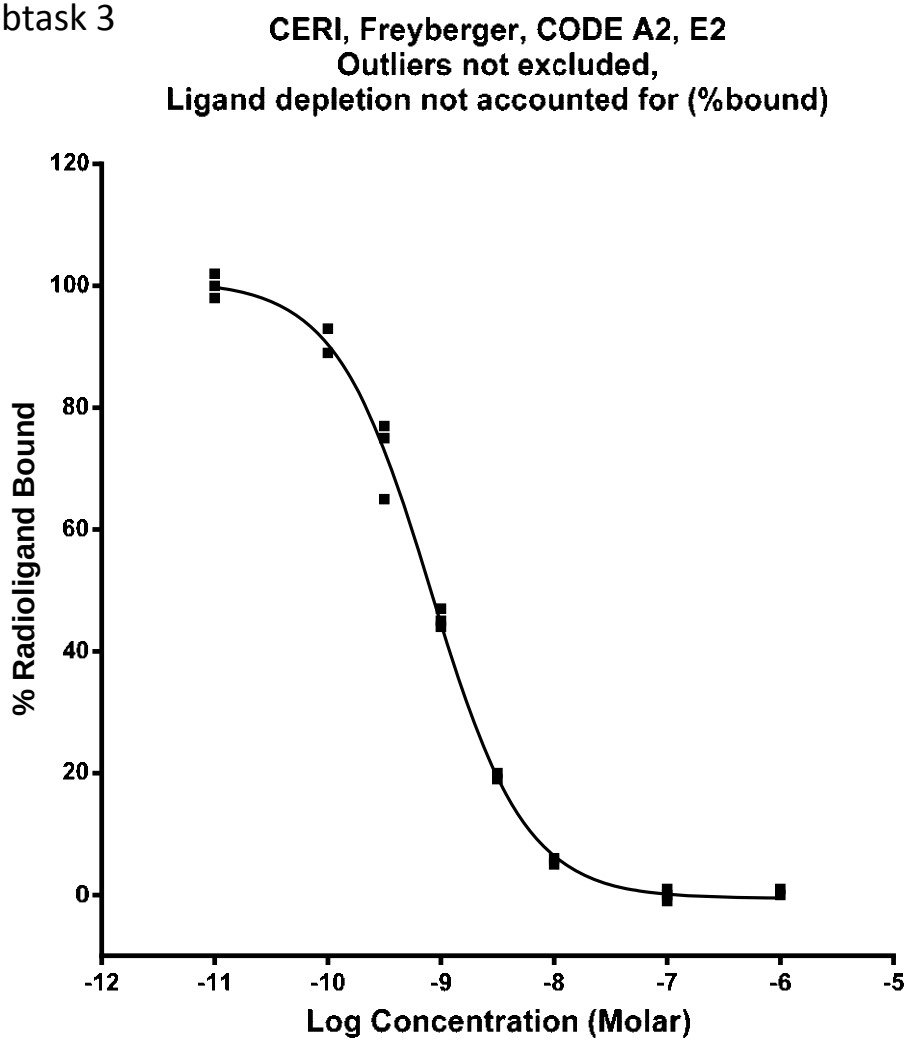
logIC50	
Best-fit values	
Bottom	0.6031
Top	88.15
LogIC50	-8.948
HillSlope	-1.590
Std. Error	
Bottom	1.328
Top	1.517
LogIC50	0.02241
HillSlope	0.1375
95% Confidence Intervals	
Bottom	-2.167 to 3.374
Top	84.99 to 91.31
LogIC50	-8.995 to -8.901
HillSlope	-1.877 to -1.303
Goodness of Fit	
Degrees of Freedom	20
R square	0.9925
Sy.x	3.549
Number of points	
Analyzed	24

Subtask 3



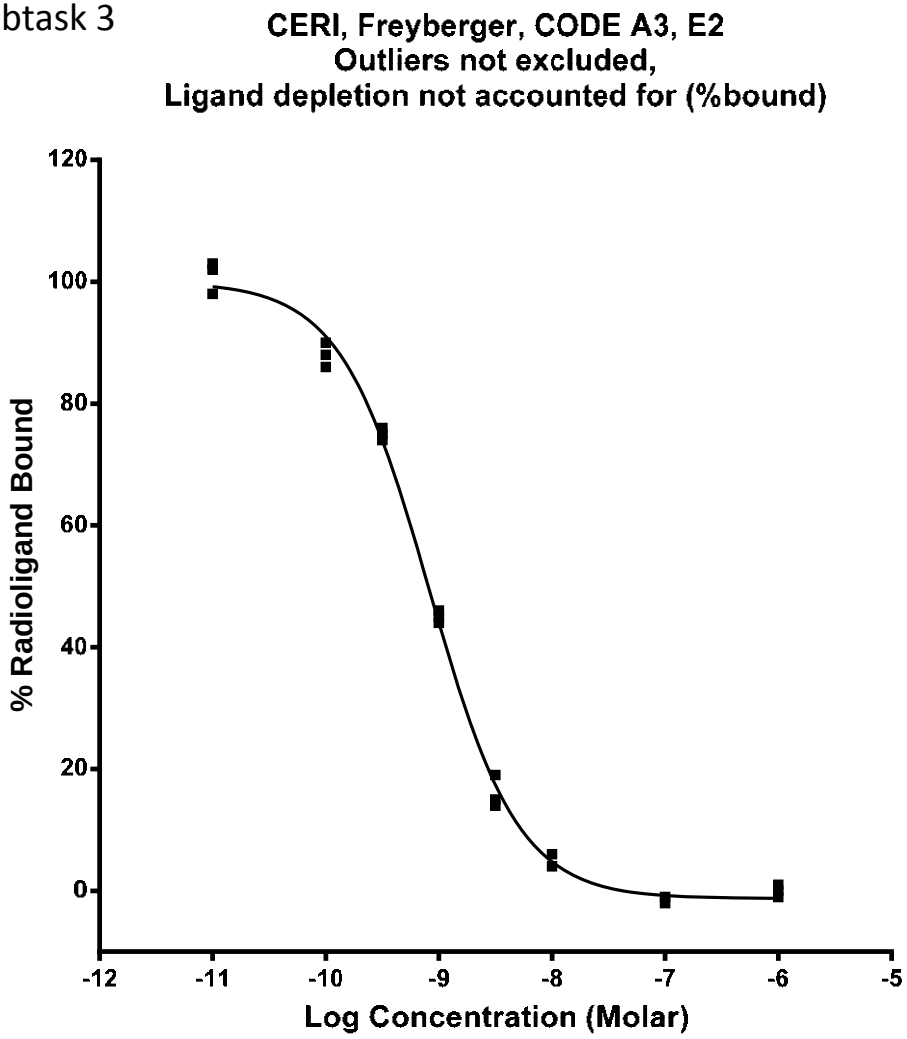
logIC50	
Best-fit values	
Bottom	0.2734
Top	95.85
LogIC50	-9.091
HillSlope	-1.120
Std. Error	
Bottom	1.132
Top	1.610
LogIC50	0.01982
HillSlope	0.06882
95% Confidence Intervals	
Bottom	-2.087 to 2.634
Top	92.49 to 99.21
LogIC50	-9.132 to -9.050
HillSlope	-1.263 to -0.9761
Goodness of Fit	
Degrees of Freedom	20
R square	0.9951
Sy.x	2.865
Number of points	
Analyzed	24

Subtask 3



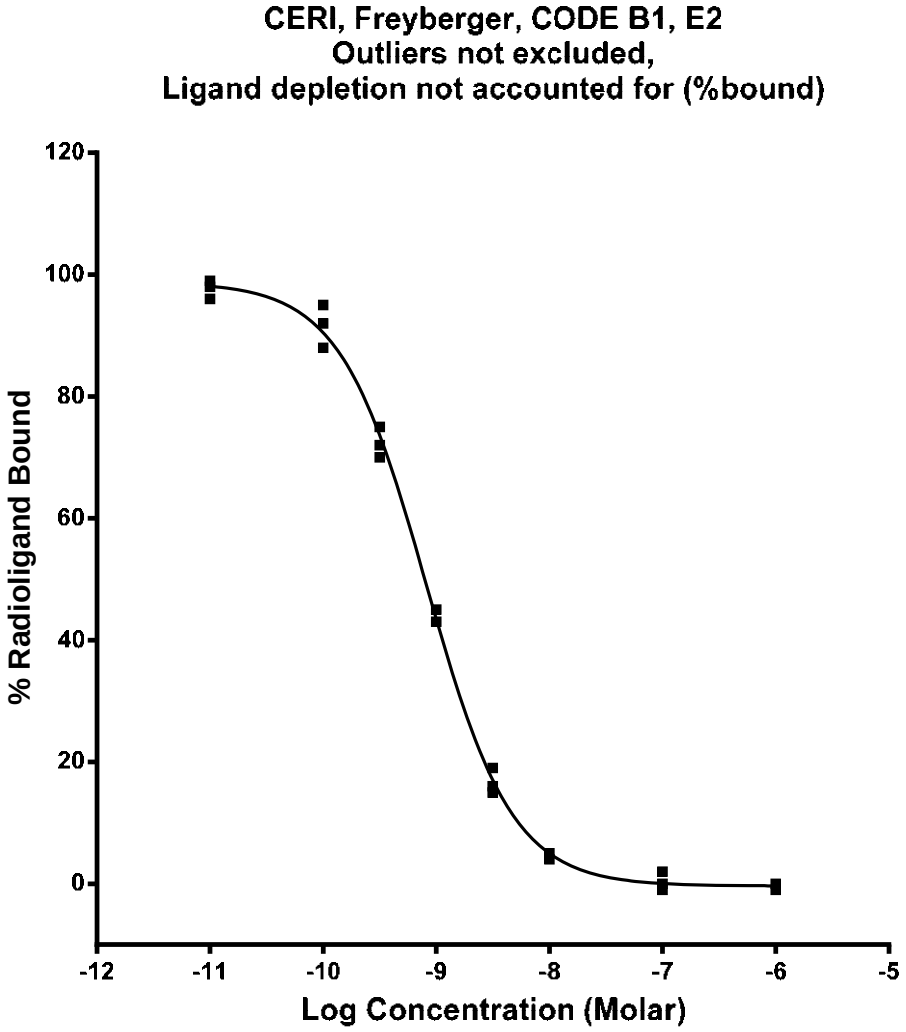
logIC50	
Best-fit values	
Bottom	-0.5603
Top	100.8
LogIC50	-9.090
HillSlope	-1.036
Std. Error	
Bottom	0.9848
Top	1.462
LogIC50	0.01653
HillSlope	0.05129
95% Confidence Intervals	
Bottom	-2.615 to 1.494
Top	97.78 to 103.9
LogIC50	-9.125 to -9.056
HillSlope	-1.143 to -0.9289
Goodness of Fit	
Degrees of Freedom	20
R square	0.9967
Sy.x	2.438
Number of points	
Analyzed	24

Subtask 3



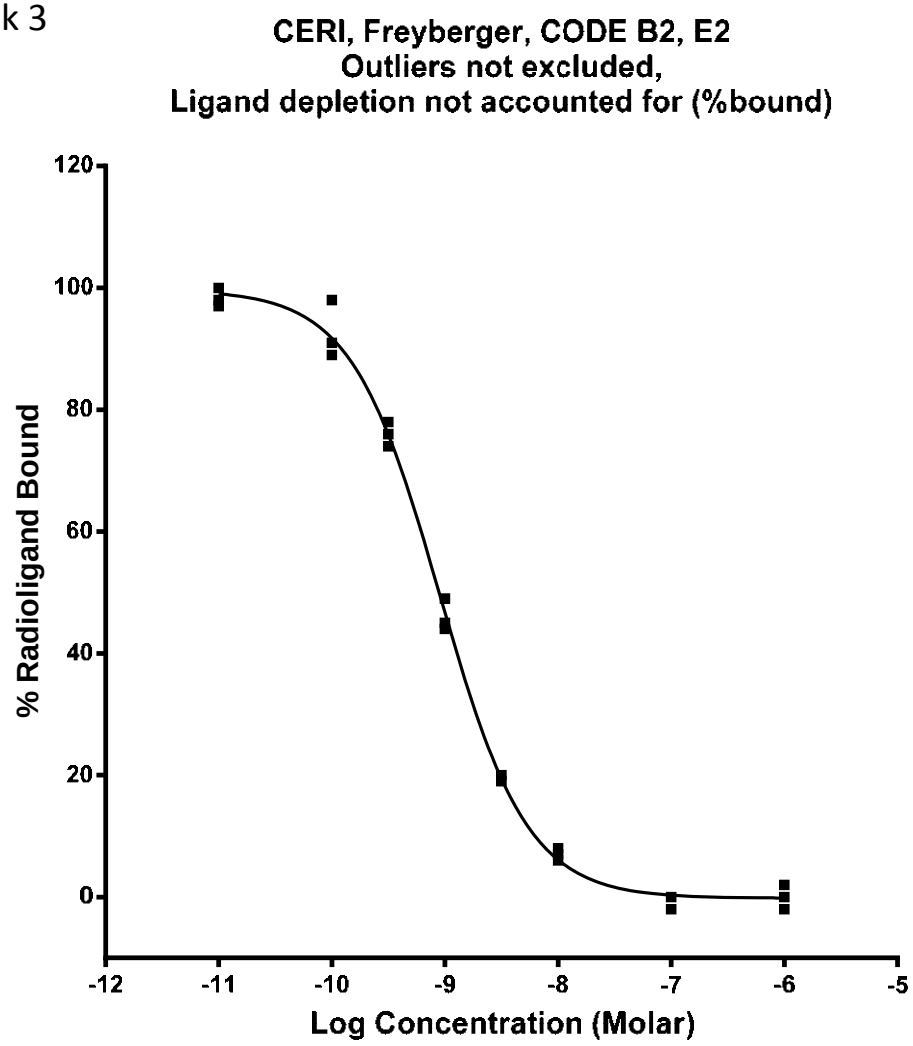
logIC50	
Best-fit values	
Bottom	-1.267
Top	99.95
LogIC50	-9.093
HillSlope	-1.106
Std. Error	
Bottom	0.8838
Top	1.283
LogIC50	0.01468
HillSlope	0.05028
95% Confidence Intervals	
Bottom	-3.111 to 0.5764
Top	97.28 to 102.6
LogIC50	-9.124 to -9.063
HillSlope	-1.211 to -1.002
Goodness of Fit	
Degrees of Freedom	20
R square	0.9973
Sy.x	2.241
Number of points	
Analyzed	24

Subtask 3



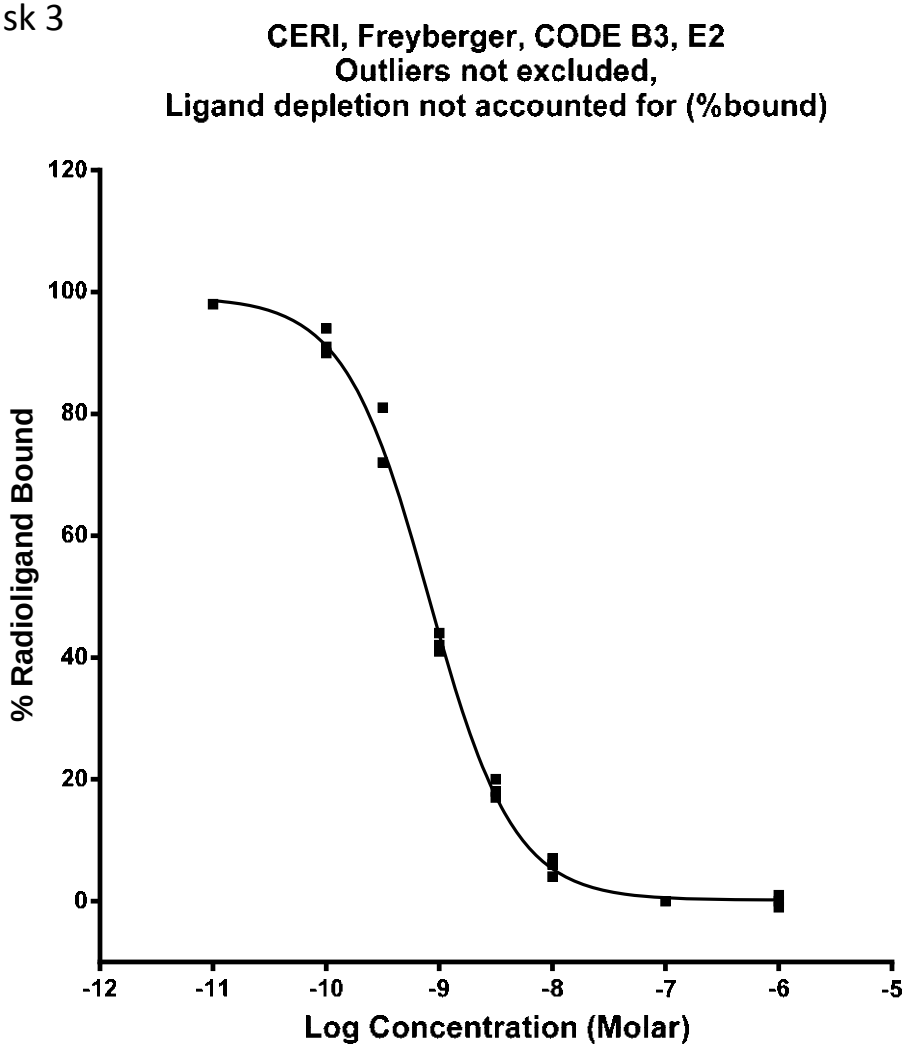
logIC50	
Best-fit values	
Bottom	-0.3734
Top	98.76
LogIC50	-9.101
HillSlope	-1.142
Std. Error	
Bottom	0.7420
Top	1.074
LogIC50	0.01253
HillSlope	0.04512
95% Confidence Intervals	
Bottom	-1.921 to 1.174
Top	96.52 to 101.0
LogIC50	-9.127 to -9.075
HillSlope	-1.236 to -1.048
Goodness of Fit	
Degrees of Freedom	20
R square	0.9980
Sy.x	1.907
Number of points	
Analyzed	24

Subtask 3



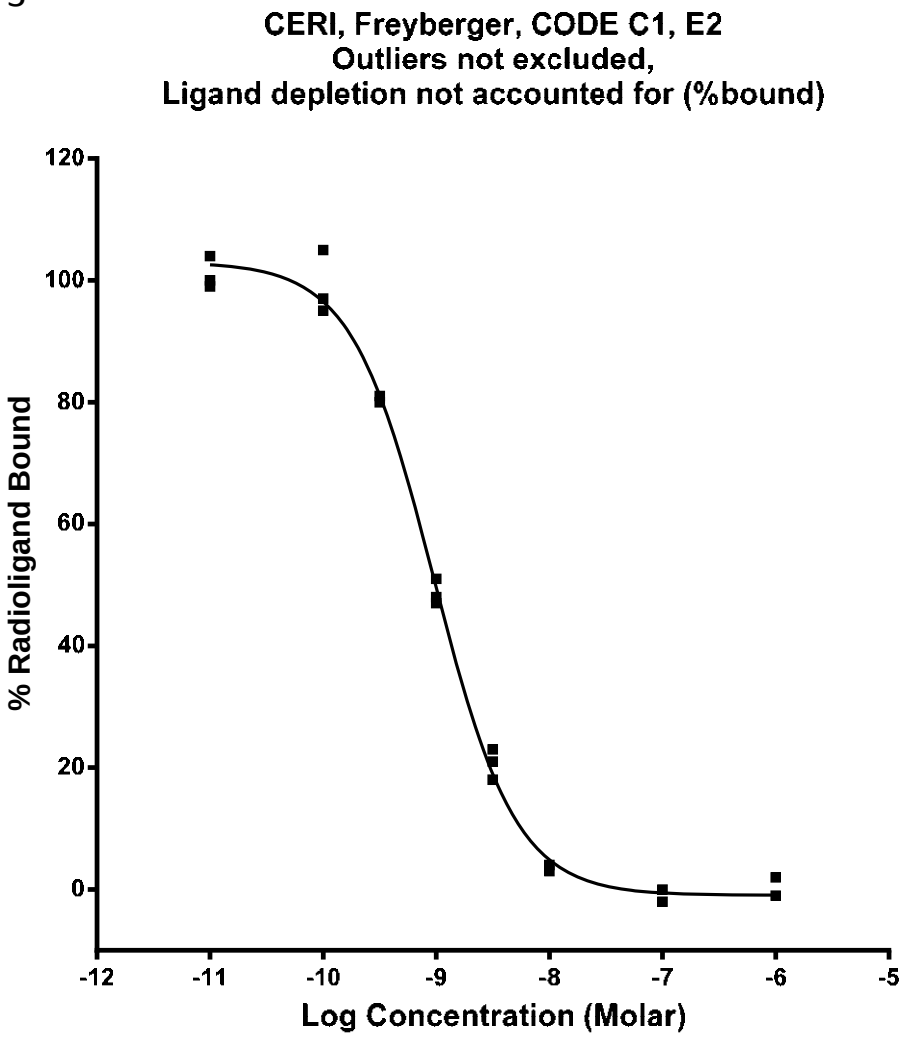
logIC50	
Best-fit values	
Bottom	-0.2106
Top	99.68
LogIC50	-9.052
HillSlope	-1.118
Std. Error	
Bottom	0.8668
Top	1.223
LogIC50	0.01440
HillSlope	0.05013
95% Confidence Intervals	
Bottom	-2.019 to 1.597
Top	97.13 to 102.2
LogIC50	-9.082 to -9.022
HillSlope	-1.222 to -1.013
Goodness of Fit	
Degrees of Freedom	20
R square	0.9974
Sy.x	2.186
Number of points	
Analyzed	24

Subtask 3



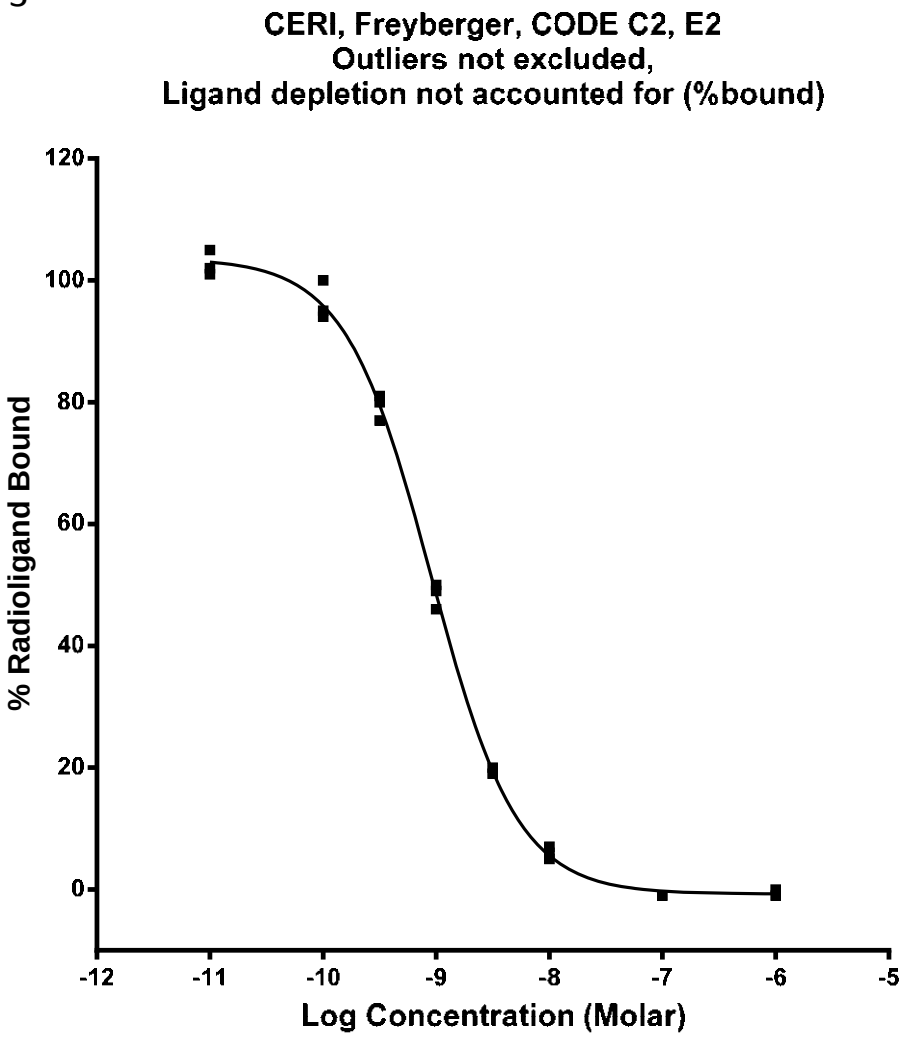
logIC50	
Best-fit values	
Bottom	0.1685
Top	99.20
LogIC50	-9.095
HillSlope	-1.158
Std. Error	
Bottom	0.8364
Top	1.209
LogIC50	0.01409
HillSlope	0.05193
95% Confidence Intervals	
Bottom	-1.576 to 1.913
Top	96.68 to 101.7
LogIC50	-9.125 to -9.066
HillSlope	-1.266 to -1.049
Goodness of Fit	
Degrees of Freedom	20
R square	0.9974
Sy.x	2.161
Number of points	
Analyzed	24

Subtask 3



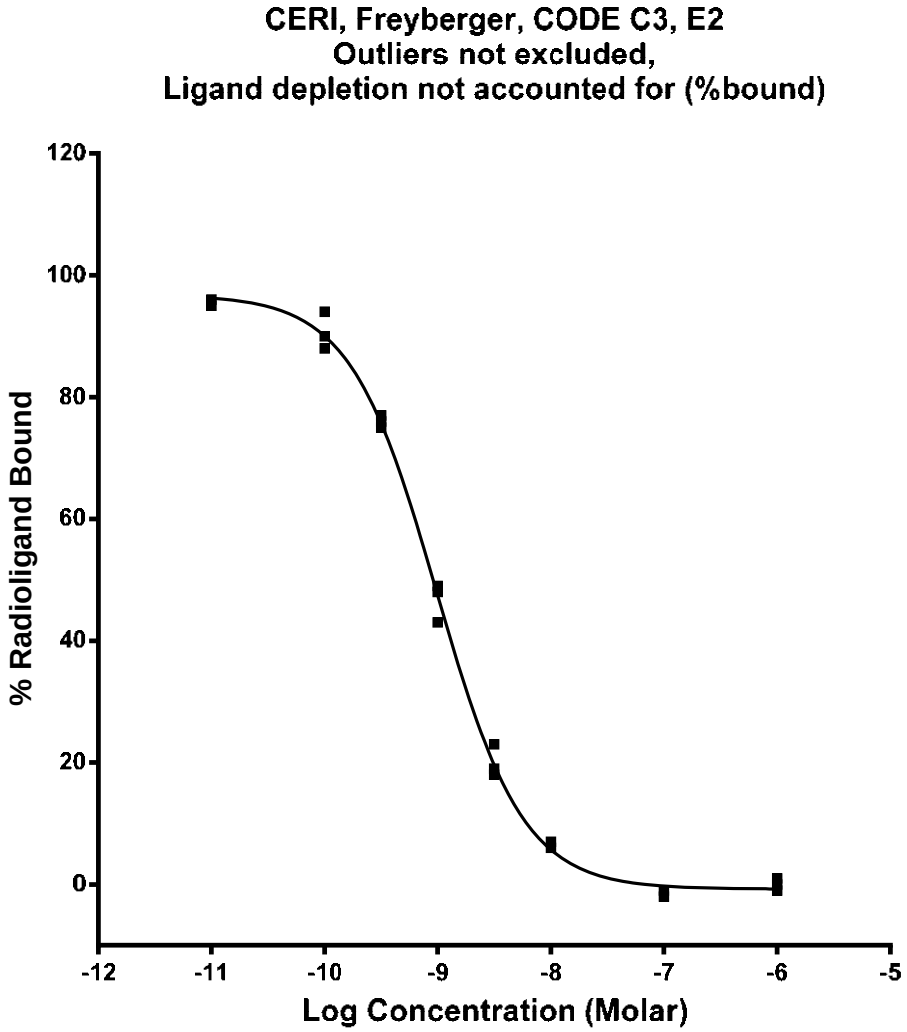
logIC50	
Best-fit values	
Bottom	-0.9596
Top	103.0
LogIC50	-9.007
HillSlope	-1.201
Std. Error	
Bottom	1.064
Top	1.448
LogIC50	0.01658
HillSlope	0.06493
95% Confidence Intervals	
Bottom	-3.179 to 1.259
Top	99.98 to 106.0
LogIC50	-9.042 to -8.972
HillSlope	-1.336 to -1.066
Goodness of Fit	
Degrees of Freedom	20
R square	0.9964
Sy.x	2.732
Number of points	
Analyzed	24

Subtask 3



logIC50	
Best-fit values	
Bottom	-0.7380
Top	103.6
LogIC50	-9.023
HillSlope	-1.144
Std. Error	
Bottom	0.6462
Top	0.9040
LogIC50	0.01021
HillSlope	0.03694
95% Confidence Intervals	
Bottom	-2.086 to 0.6100
Top	101.7 to 105.5
LogIC50	-9.044 to -9.002
HillSlope	-1.221 to -1.067
Goodness of Fit	
Degrees of Freedom	20
R square	0.9987
Sy.x	1.642
Number of points	
Analyzed	24

Subtask 3

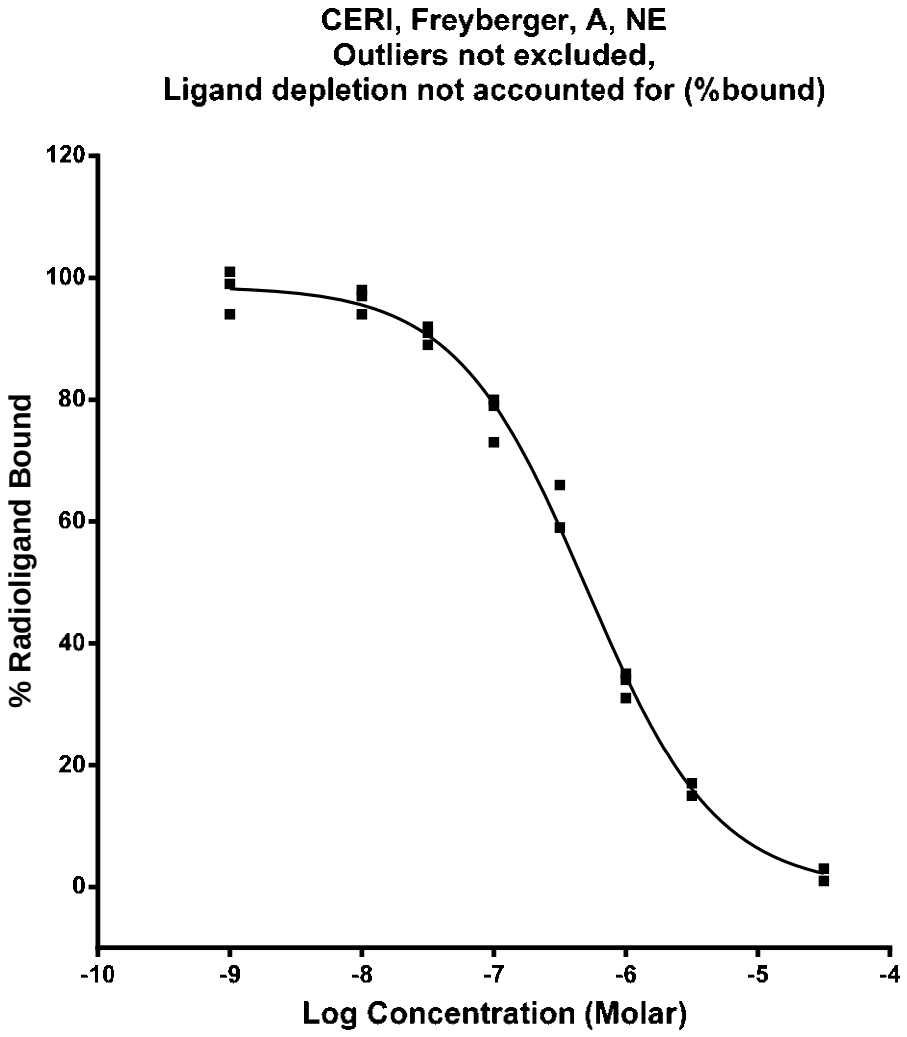


logIC50	
Best-fit values	
Bottom	-0.7737
Top	96.78
LogIC50	-9.040
HillSlope	-1.139
Std. Error	
Bottom	0.7599
Top	1.037
LogIC50	0.01281
HillSlope	0.04563
95% Confidence Intervals	
Bottom	-2.359 to 0.8115
Top	94.61 to 98.94
LogIC50	-9.067 to -9.013
HillSlope	-1.234 to -1.043
Goodness of Fit	
Degrees of Freedom	20
R square	0.9980
Sy.x	1.910
Number of points	
Analyzed	24

CERI ASSAY
(Freyberger Laboratory)
Control Norethynodrel (NE)

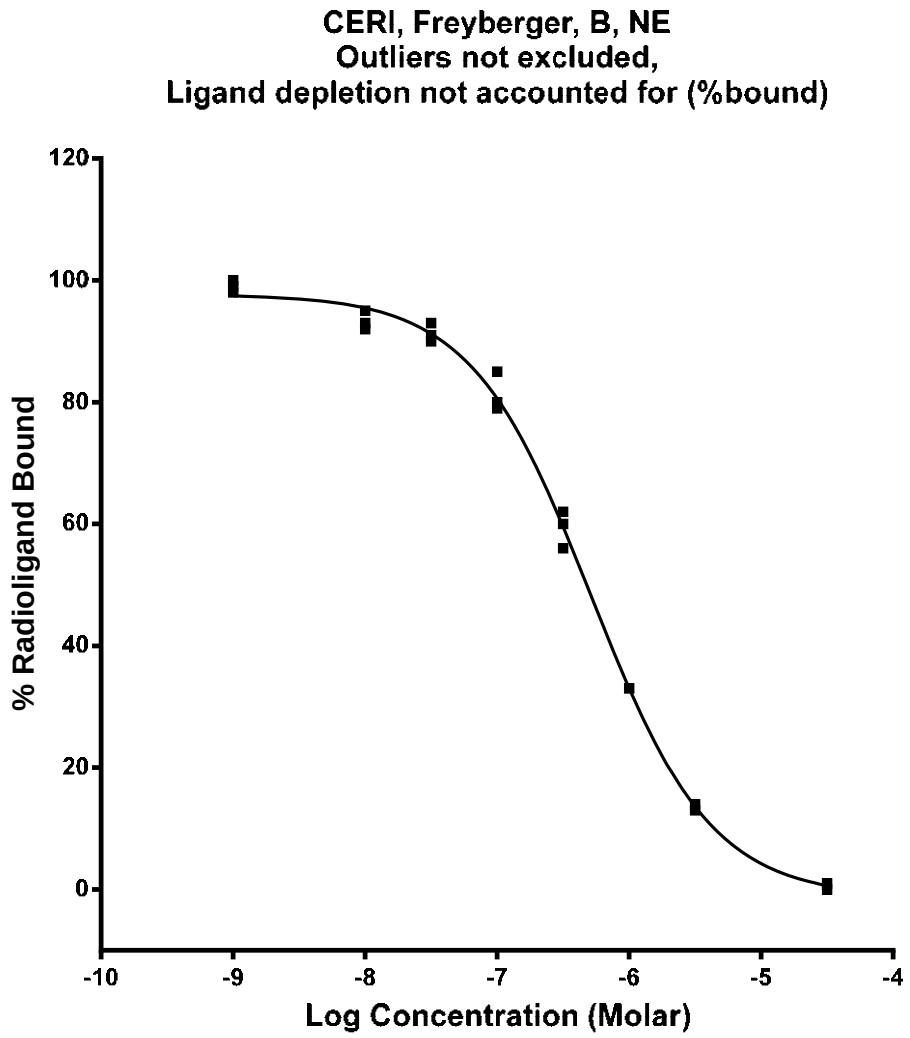
Begin Here

Subtask 2



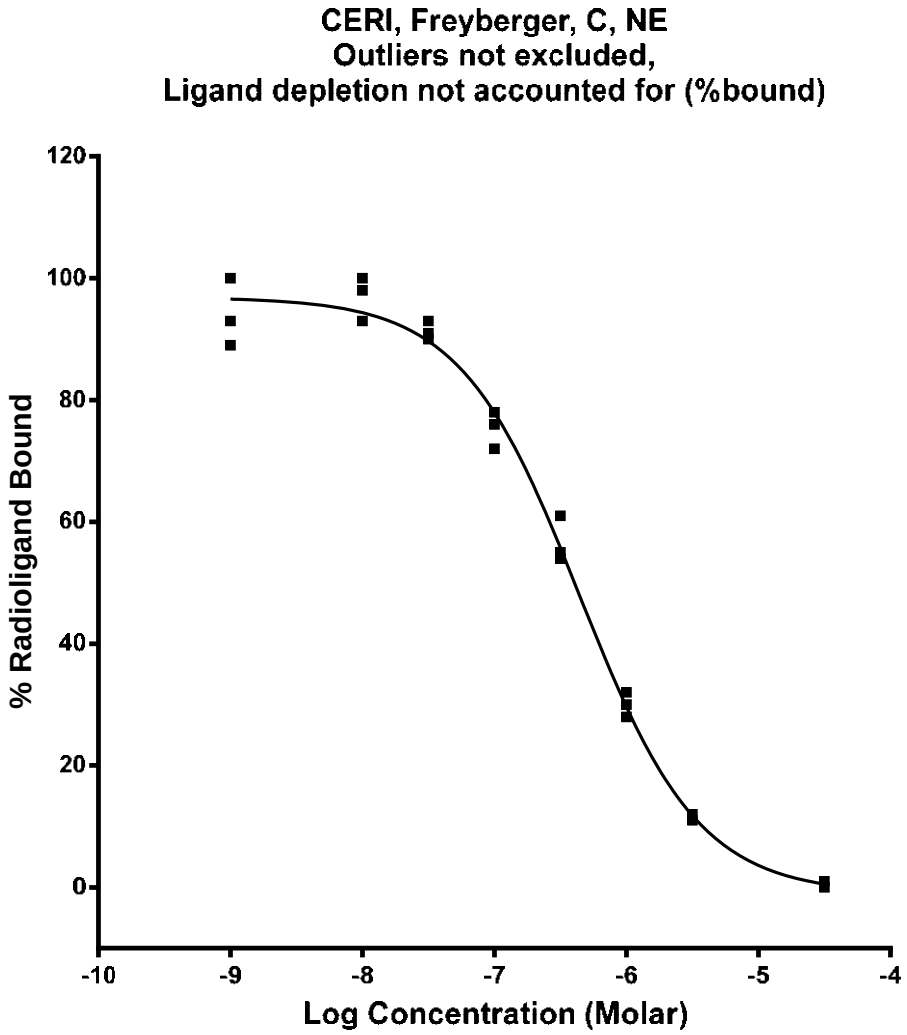
logIC50	
Best-fit values	
Bottom	-0.3280
Top	98.63
LogIC50	-6.316
HillSlope	-0.8769
Std. Error	
Bottom	1.983
Top	1.309
LogIC50	0.02109
HillSlope	0.05508
95% Confidence Intervals	
Bottom	-4.465 to 3.809
Top	95.90 to 101.4
LogIC50	-6.360 to -6.272
HillSlope	-0.9918 to -0.7620
Goodness of Fit	
Degrees of Freedom	20
R square	0.9950
Sy.x	2.732
Number of points	
Analyzed	24

Subtask 2



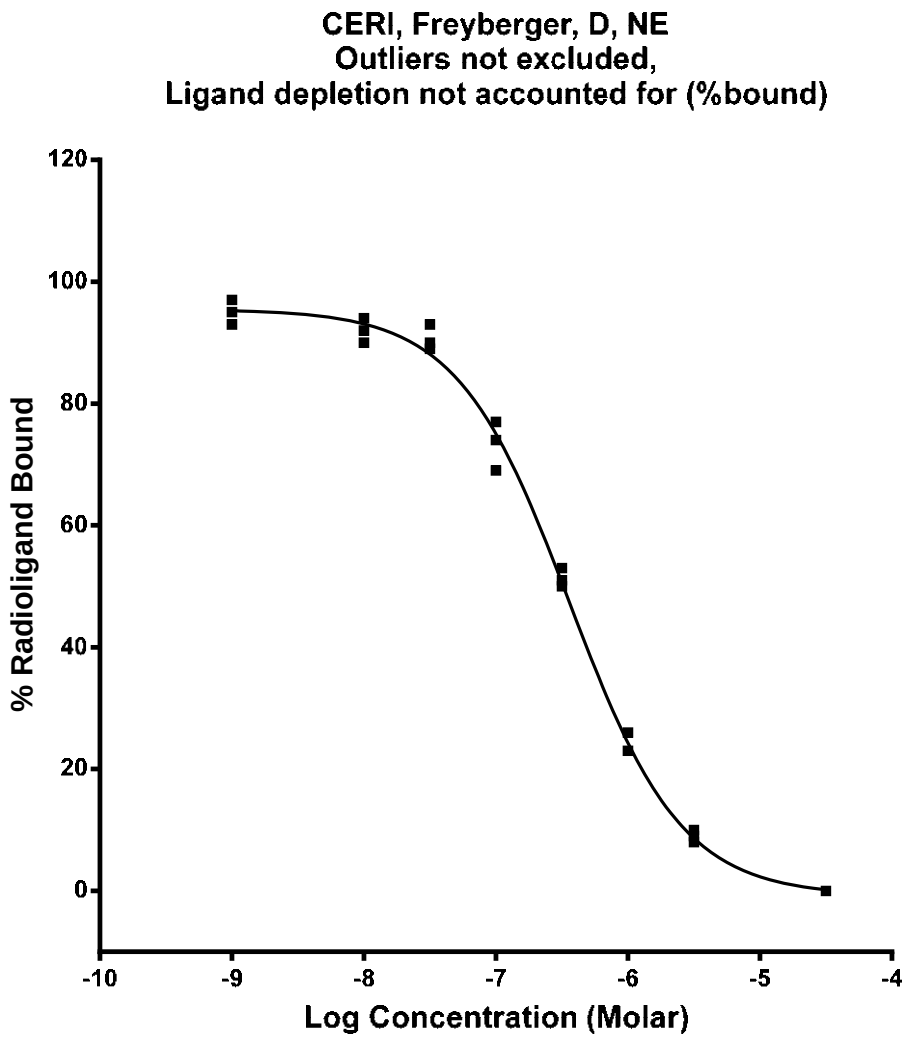
logIC50	
Best-fit values	
Bottom	-1.259
Top	97.70
LogIC50	-6.320
HillSlope	-0.9546
Std. Error	
Bottom	1.311
Top	0.8614
LogIC50	0.01415
HillSlope	0.04117
95% Confidence Intervals	
Bottom	-3.994 to 1.475
Top	95.90 to 99.49
LogIC50	-6.350 to -6.291
HillSlope	-1.040 to -0.8688
Goodness of Fit	
Degrees of Freedom	20
R square	0.9976
Sy.x	1.931
Number of points	
Analyzed	24

Subtask 2



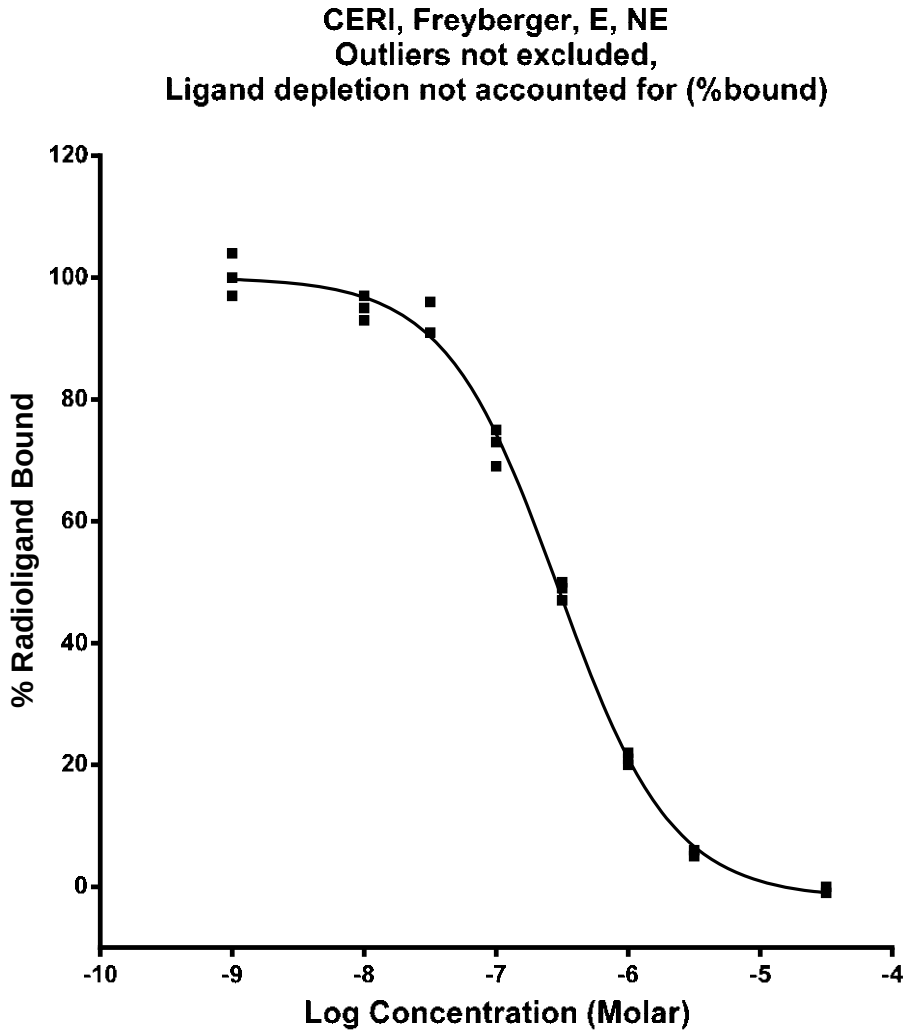
logIC50	
Best-fit values	
Bottom	-1.047
Top	96.84
LogIC50	-6.392
HillSlope	-0.9619
Std. Error	
Bottom	2.179
Top	1.518
LogIC50	0.02458
HillSlope	0.07178
95% Confidence Intervals	
Bottom	-5.593 to 3.498
Top	93.67 to 100.0
LogIC50	-6.444 to -6.341
HillSlope	-1.112 to -0.8122
Goodness of Fit	
Degrees of Freedom	20
R square	0.9930
Sy.x	3.336
Number of points	
Analyzed	24

Subtask 2



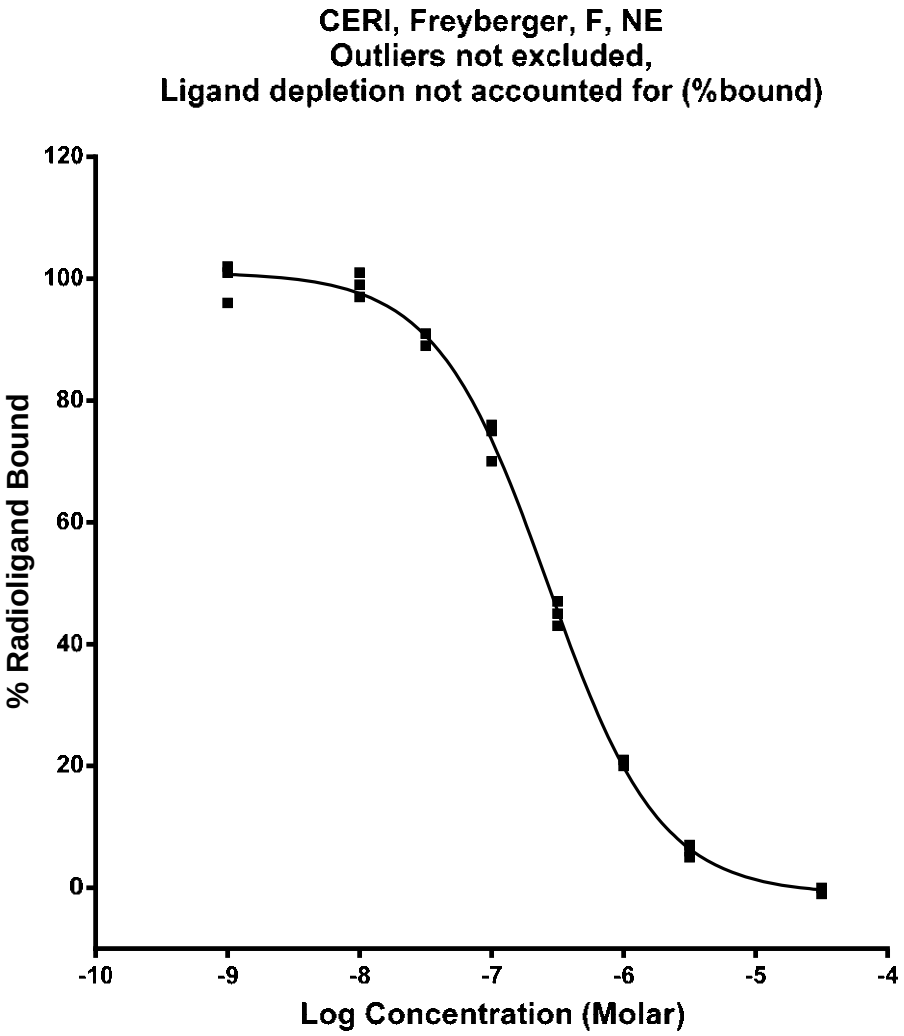
logIC50	
Best-fit values	
Bottom	-0.8012
Top	95.48
LogIC50	-6.489
HillSlope	-1.022
Std. Error	
Bottom	1.371
Top	1.035
LogIC50	0.01657
HillSlope	0.05225
95% Confidence Intervals	
Bottom	-3.661 to 2.058
Top	93.32 to 97.64
LogIC50	-6.523 to -6.454
HillSlope	-1.131 to -0.9132
Goodness of Fit	
Degrees of Freedom	20
R square	0.9967
Sy.x	2.288
Number of points	
Analyzed	24

Subtask 2



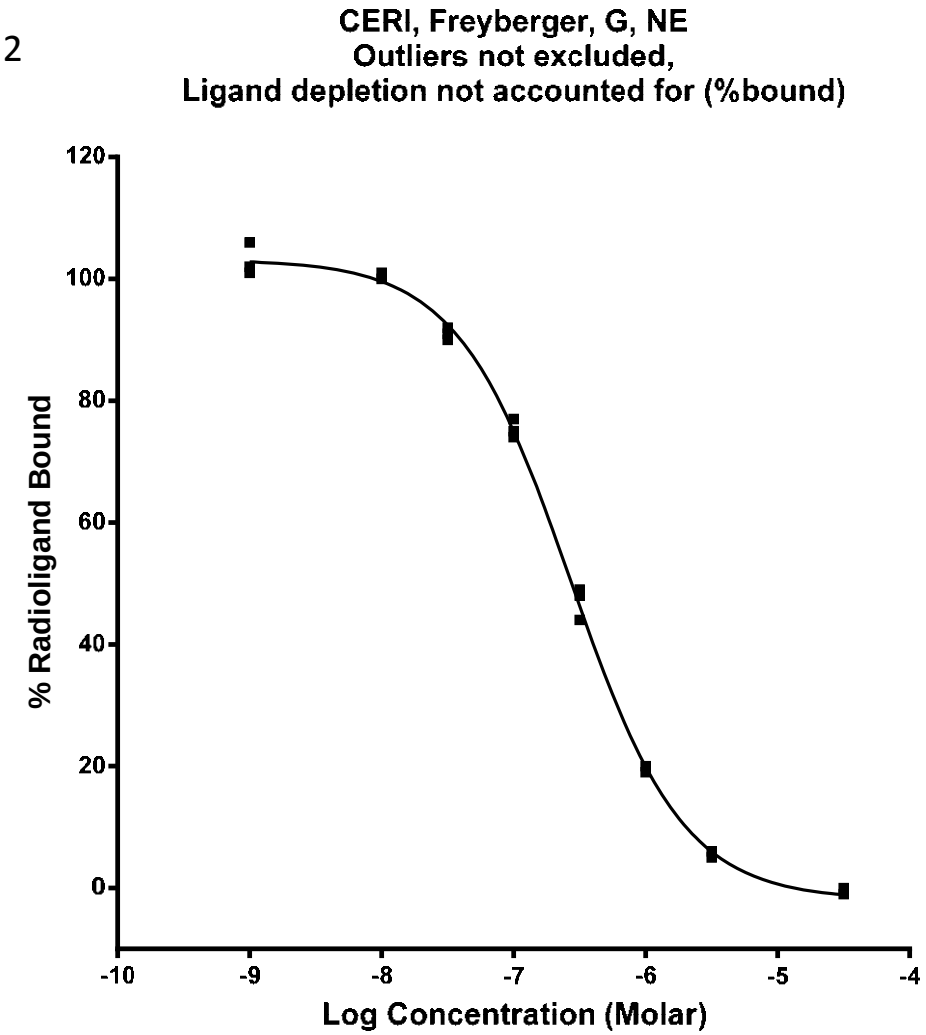
logIC50	
Best-fit values	
Bottom	-1.892
Top	100.0
LogIC50	-6.546
HillSlope	-1.009
Std. Error	
Bottom	1.454
Top	1.188
LogIC50	0.01724
HillSlope	0.05340
95% Confidence Intervals	
Bottom	-4.926 to 1.142
Top	97.54 to 102.5
LogIC50	-6.582 to -6.510
HillSlope	-1.121 to -0.8980
Goodness of Fit	
Degrees of Freedom	20
R square	0.9965
Sy.x	2.509
Number of points	
Analyzed	24

Subtask 2



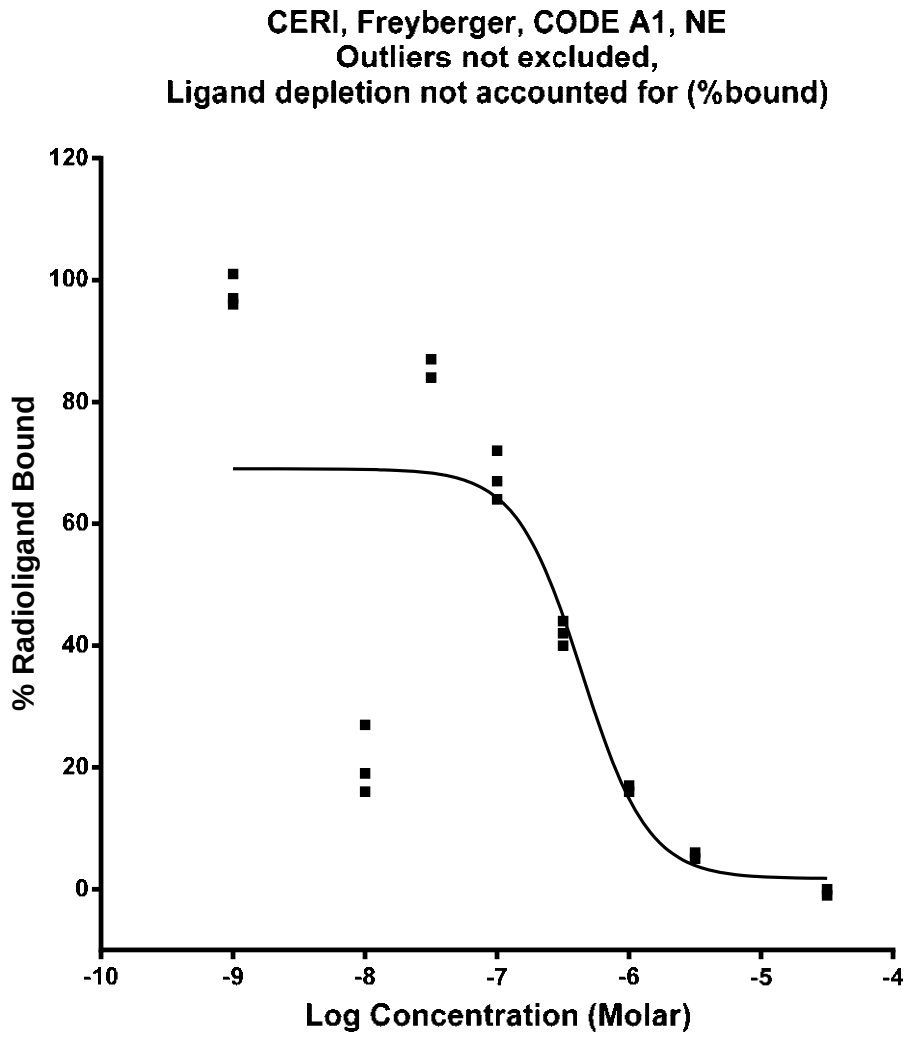
logIC50	
Best-fit values	
Bottom	-1.106
Top	101.0
LogIC50	-6.575
HillSlope	-1.023
Std. Error	
Bottom	1.096
Top	0.9325
LogIC50	0.01326
HillSlope	0.04183
95% Confidence Intervals	
Bottom	-3.391 to 1.180
Top	99.08 to 103.0
LogIC50	-6.603 to -6.547
HillSlope	-1.110 to -0.9359
Goodness of Fit	
Degrees of Freedom	20
R square	0.9979
Sy.x	1.949
Number of points	
Analyzed	24

Subtask 2



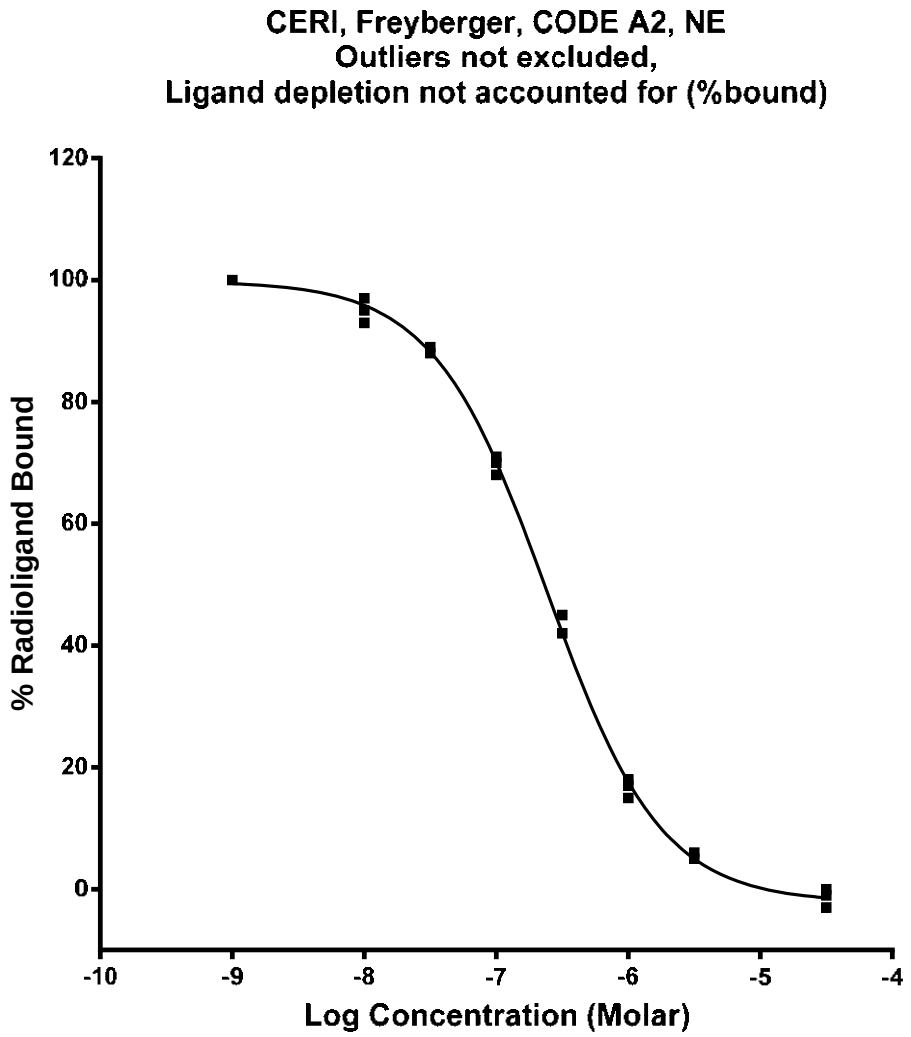
logIC50	
Best-fit values	
Bottom	-1.942
Top	103.1
LogIC50	-6.560
HillSlope	-1.017
Std. Error	
Bottom	0.8883
Top	0.7529
LogIC50	0.01042
HillSlope	0.03260
95% Confidence Intervals	
Bottom	-3.795 to -0.08885
Top	101.6 to 104.7
LogIC50	-6.582 to -6.538
HillSlope	-1.085 to -0.9493
Goodness of Fit	
Degrees of Freedom	20
R square	0.9987
Sy.x	1.570
Number of points	
Analyzed	24

Subtask 3



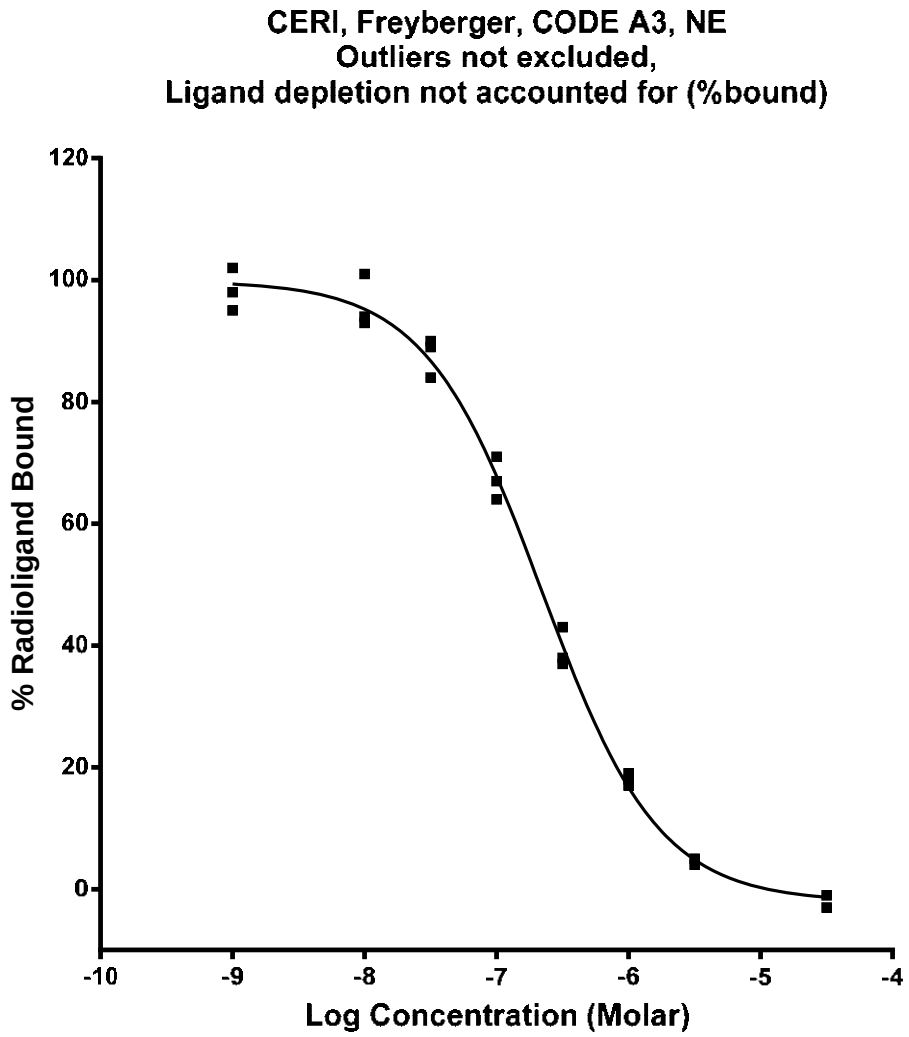
logIC50	
Best-fit values	
Bottom	1.745
Top	69.01
LogIC50	-6.588
HillSlope	-1.734
Std. Error	
Bottom	11.05
Top	7.757
LogIC50	0.2222
HillSlope	1.314
95% Confidence Intervals	
Bottom	-21.30 to 24.79
Top	52.83 to 85.19
LogIC50	-7.051 to -6.124
HillSlope	-4.475 to 1.007
Goodness of Fit	
Degrees of Freedom	20
R square	0.6448
Sy.x	23.09
Number of points	
Analyzed	24

Subtask 3



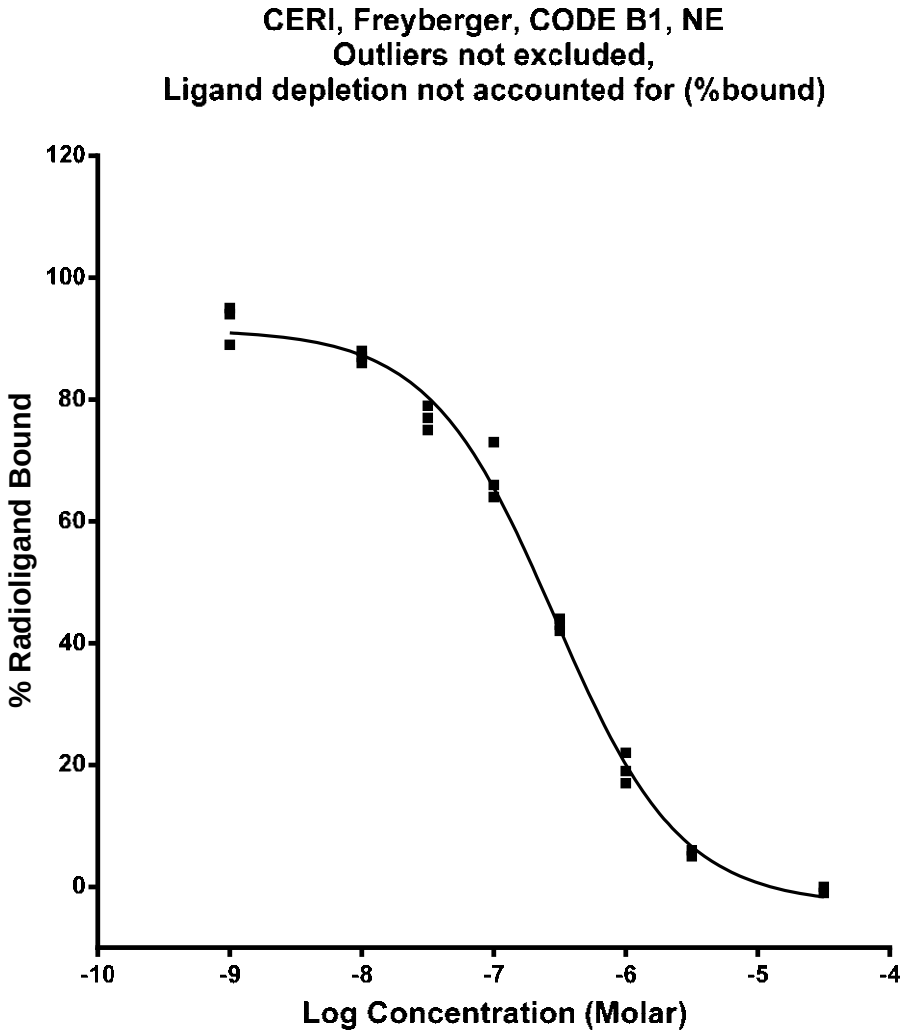
logIC50	
Best-fit values	
Bottom	-2.172
Top	99.82
LogIC50	-6.634
HillSlope	-1.006
Std. Error	
Bottom	0.7855
Top	0.6935
LogIC50	0.009684
HillSlope	0.02979
95% Confidence Intervals	
Bottom	-3.810 to -0.5335
Top	98.38 to 101.3
LogIC50	-6.655 to -6.614
HillSlope	-1.068 to -0.9441
Goodness of Fit	
Degrees of Freedom	20
R square	0.9989
Sy.x	1.407
Number of points	
Analyzed	24

Subtask 3



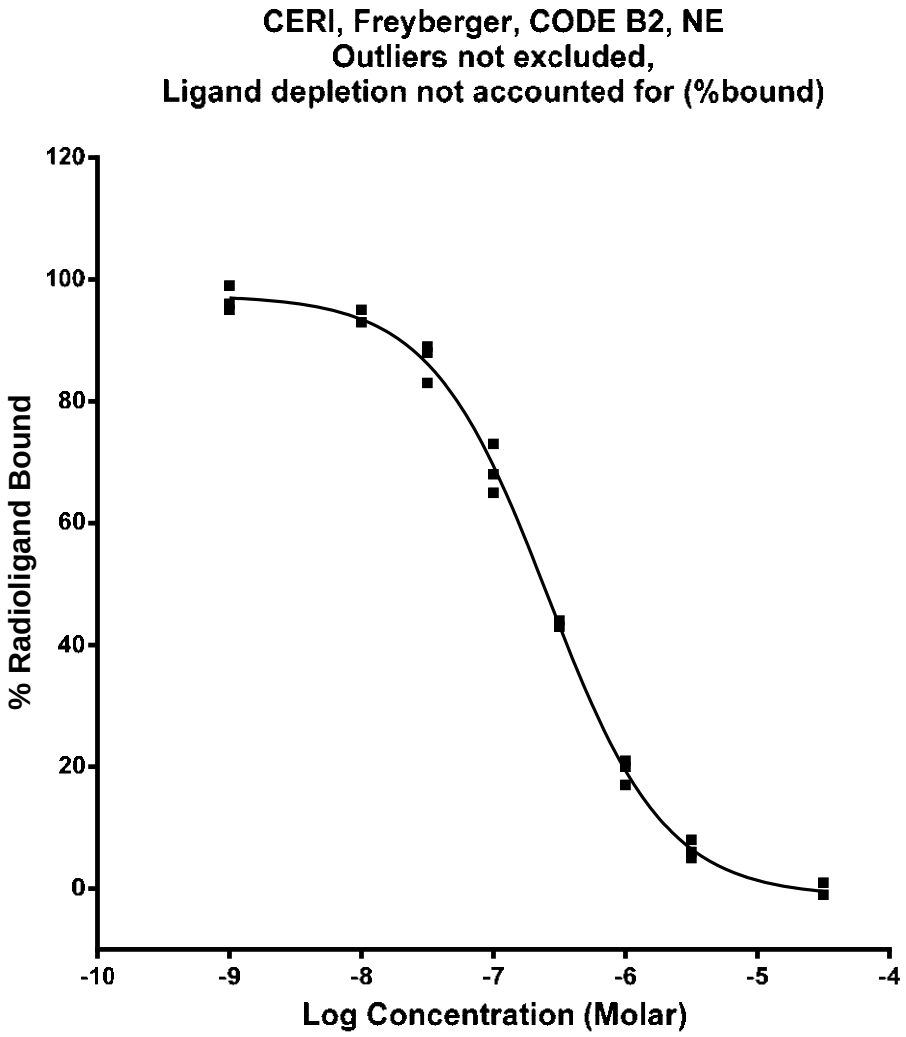
logIC50	
Best-fit values	
Bottom	-2.069
Top	99.85
LogIC50	-6.674
HillSlope	-0.9847
Std. Error	
Bottom	1.517
Top	1.389
LogIC50	0.01902
HillSlope	0.05669
95% Confidence Intervals	
Bottom	-5.233 to 1.095
Top	96.95 to 102.7
LogIC50	-6.713 to -6.634
HillSlope	-1.103 to -0.8664
Goodness of Fit	
Degrees of Freedom	20
R square	0.9959
Sy.x	2.727
Number of points	
Analyzed	24

Subtask 3



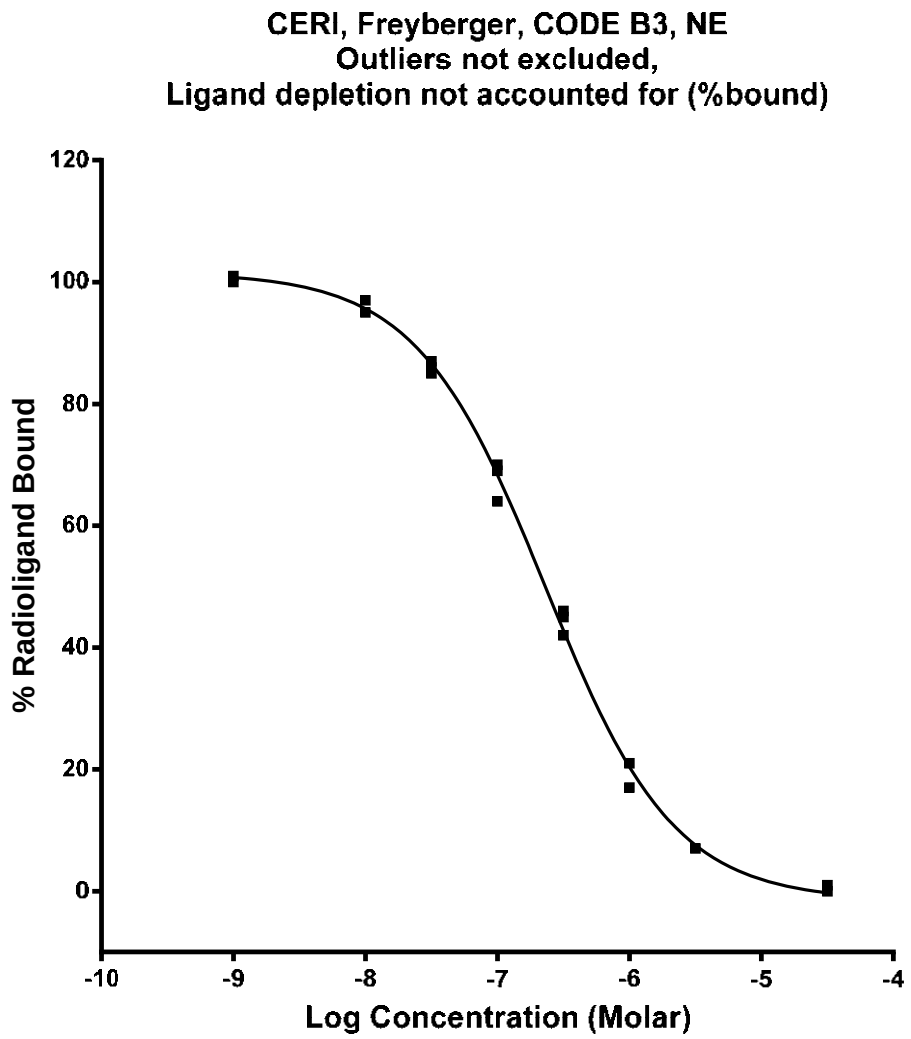
logIC50	
Best-fit values	
Bottom	-2.938
Top	91.46
LogIC50	-6.654
HillSlope	-0.9126
Std. Error	
Bottom	1.751
Top	1.440
LogIC50	0.02260
HillSlope	0.05968
95% Confidence Intervals	
Bottom	-6.590 to 0.7134
Top	88.45 to 94.46
LogIC50	-6.701 to -6.607
HillSlope	-1.037 to -0.7881
Goodness of Fit	
Degrees of Freedom	20
R square	0.9946
Sy.x	2.812
Number of points	
Analyzed	24

Subtask 3



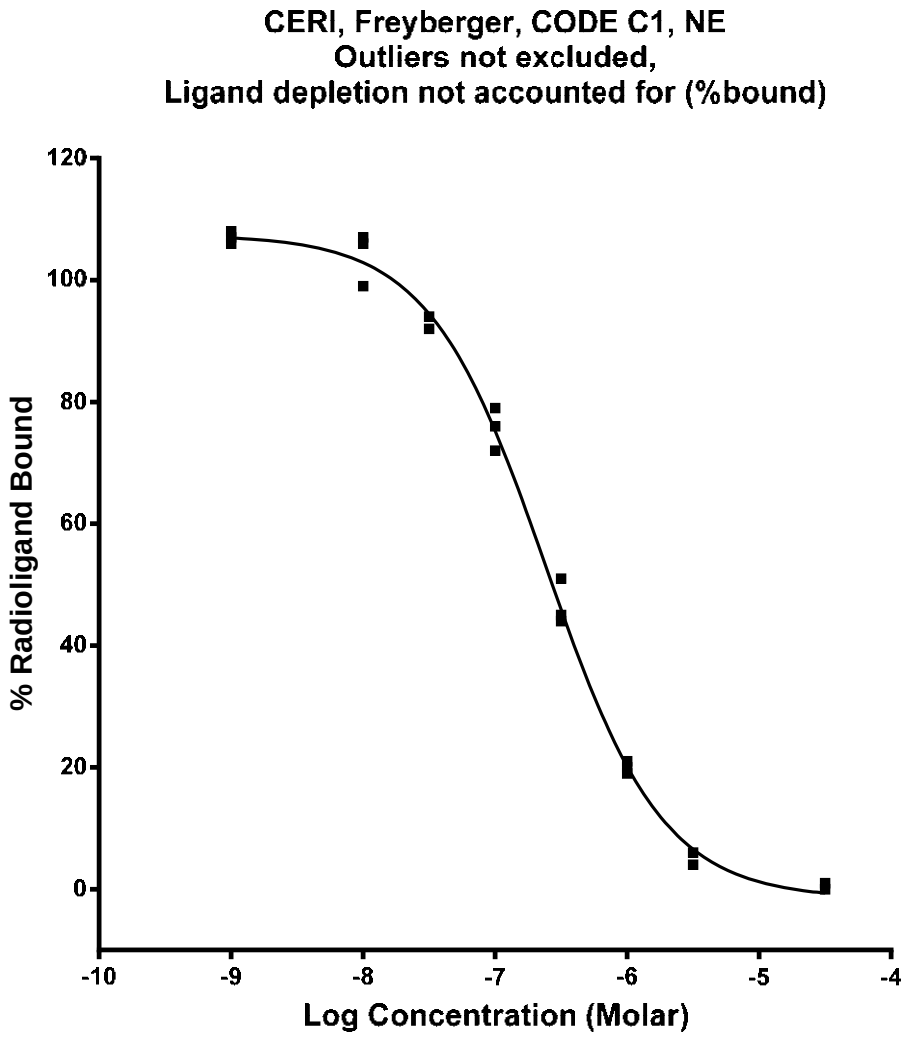
logIC50	
Best-fit values	
Bottom	-1.332
Top	97.39
LogIC50	-6.623
HillSlope	-0.9780
Std. Error	
Bottom	1.190
Top	1.025
LogIC50	0.01493
HillSlope	0.04408
95% Confidence Intervals	
Bottom	-3.814 to 1.149
Top	95.25 to 99.53
LogIC50	-6.654 to -6.592
HillSlope	-1.070 to -0.8861
Goodness of Fit	
Degrees of Freedom	20
R square	0.9975
Sy.x	2.063
Number of points	
Analyzed	24

Subtask 3



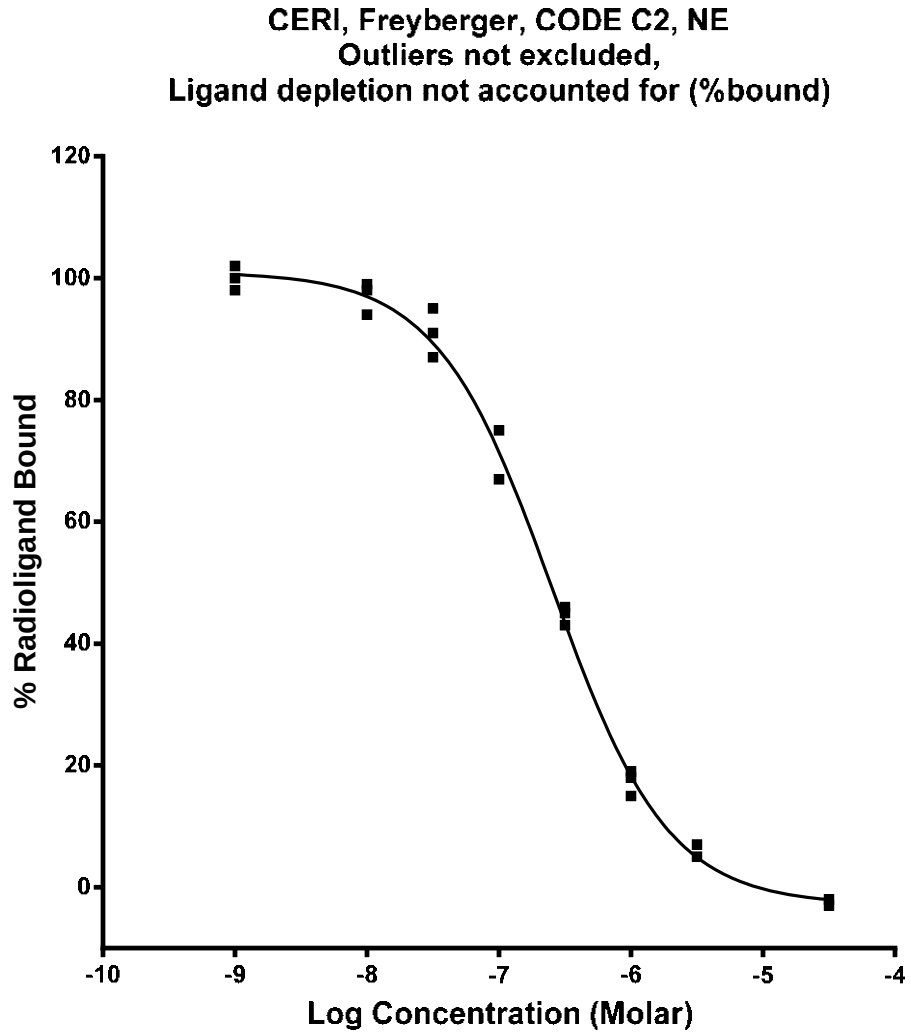
logIC50	
Best-fit values	
Bottom	-1.520
Top	101.6
LogIC50	-6.636
HillSlope	-0.8916
Std. Error	
Bottom	1.054
Top	0.9491
LogIC50	0.01273
HillSlope	0.03318
95% Confidence Intervals	
Bottom	-3.719 to 0.6781
Top	99.57 to 103.5
LogIC50	-6.663 to -6.610
HillSlope	-0.9608 to -0.8223
Goodness of Fit	
Degrees of Freedom	20
R square	0.9982
Sy.x	1.741
Number of points	
Analyzed	24

Subtask 3



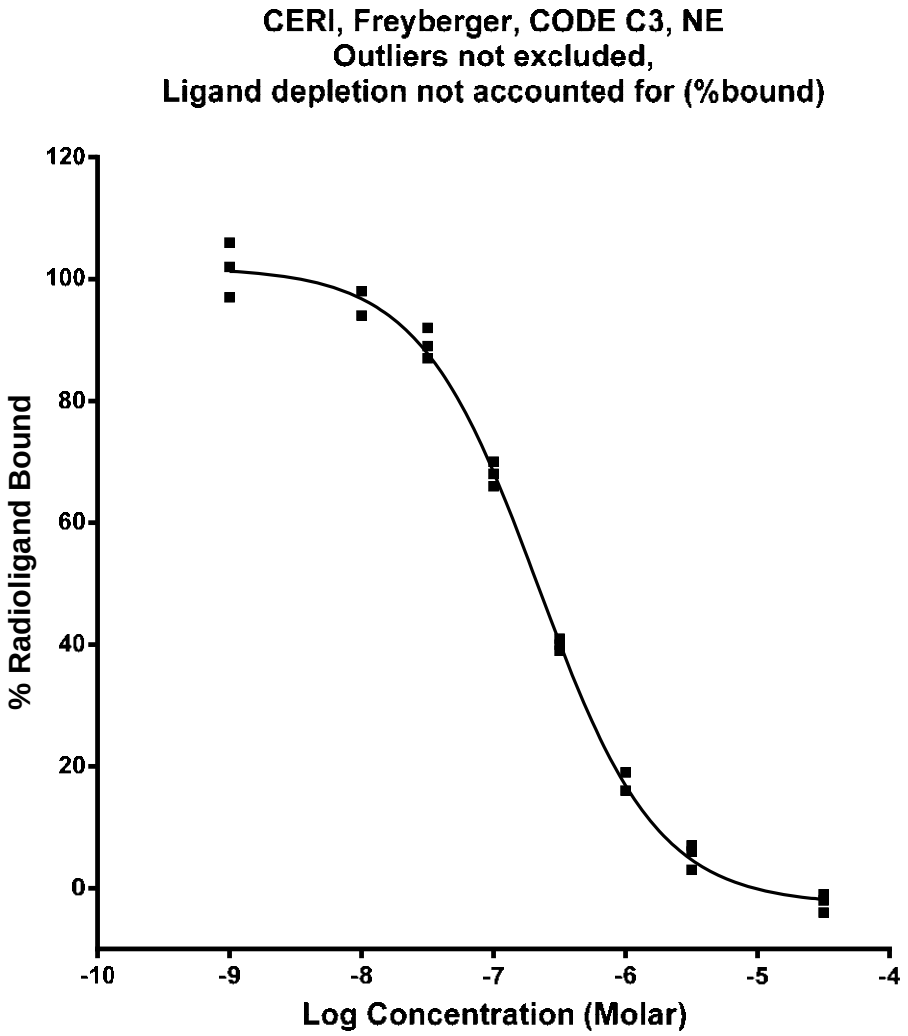
logIC50	
Best-fit values	
Bottom	-1.530
Top	107.4
LogIC50	-6.566
HillSlope	-0.9828
Std. Error	
Bottom	1.416
Top	1.250
LogIC50	0.01638
HillSlope	0.04847
95% Confidence Intervals	
Bottom	-4.483 to 1.423
Top	104.8 to 110.0
LogIC50	-6.601 to -6.532
HillSlope	-1.084 to -0.8817
Goodness of Fit	
Degrees of Freedom	20
R square	0.9970
Sy.x	2.494
Number of points	
Analyzed	24

Subtask 3



logIC50	
Best-fit values	
Bottom	-2.878
Top	101.0
LogIC50	-6.612
HillSlope	-0.9943
Std. Error	
Bottom	1.513
Top	1.313
LogIC50	0.01813
HillSlope	0.05486
95% Confidence Intervals	
Bottom	-6.035 to 0.2779
Top	98.26 to 103.7
LogIC50	-6.649 to -6.574
HillSlope	-1.109 to -0.8799
Goodness of Fit	
Degrees of Freedom	20
R square	0.9962
Sy.x	2.665
Number of points	
Analyzed	24

Subtask 3



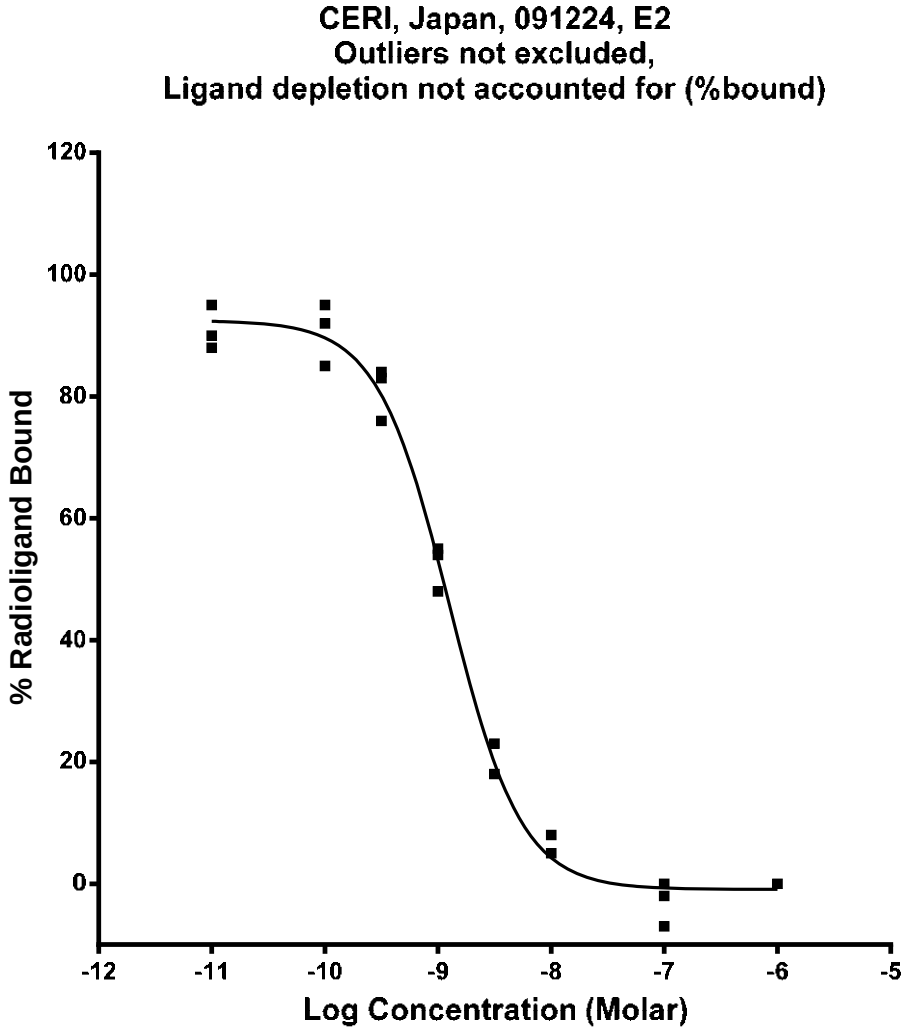
logIC50	
Best-fit values	
Bottom	-2.695
Top	101.9
LogIC50	-6.668
HillSlope	-0.9640
Std. Error	
Bottom	1.319
Top	1.215
LogIC50	0.01612
HillSlope	0.04665
95% Confidence Intervals	
Bottom	-5.447 to 0.05666
Top	99.32 to 104.4
LogIC50	-6.702 to -6.634
HillSlope	-1.061 to -0.8667
Goodness of Fit	
Degrees of Freedom	20
R square	0.9971
Sy.x	2.342
Number of points	
Analyzed	24

CERI ASSAY (JapanCERI Lab)

Control E2 and Norethynodrel (NE) Curves

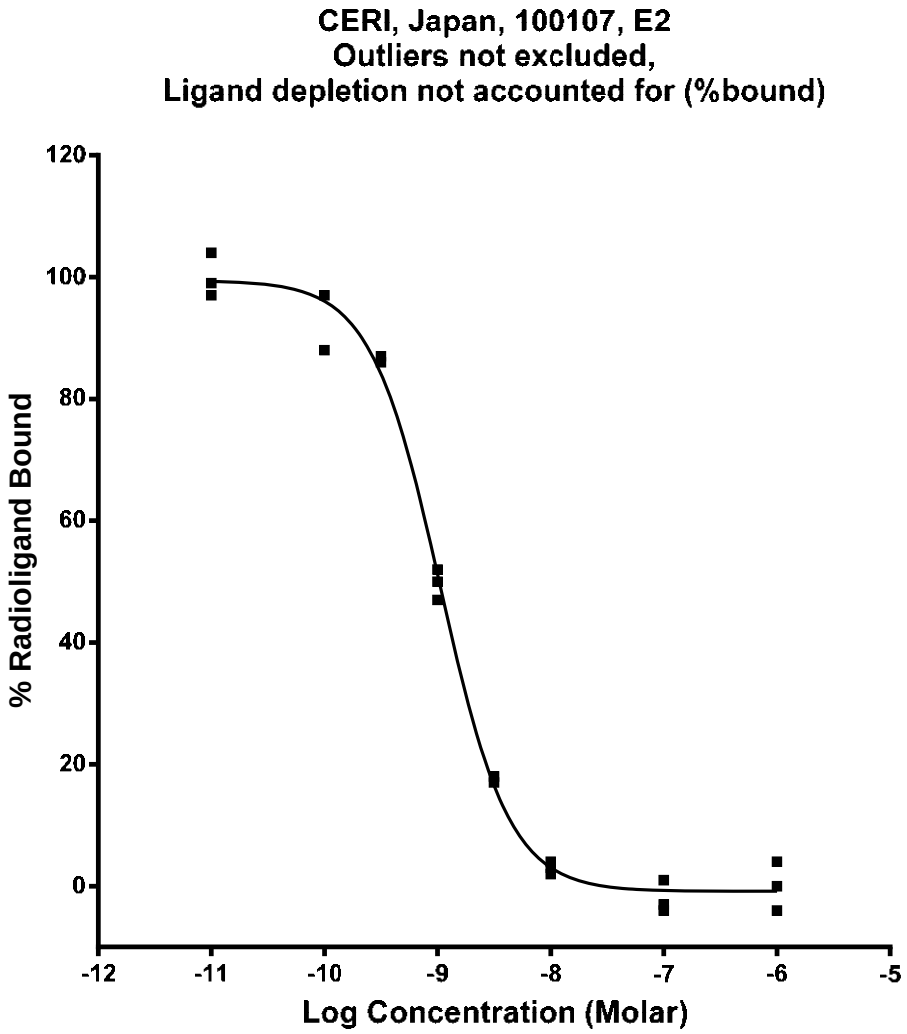
Note: All controls E2 and NE were acceptable for initial data analysis. Thus, all runs for the test chemicals were used in initial data analysis.

Subtask 1



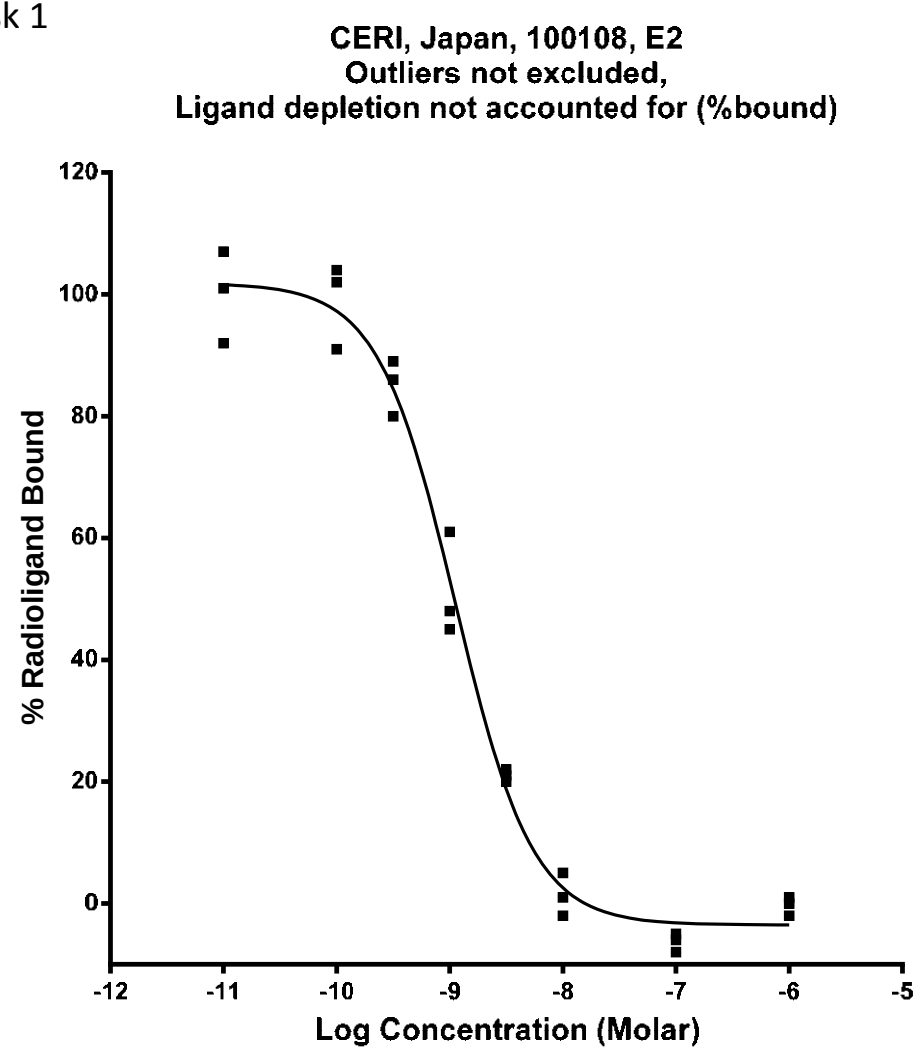
logIC50	
Best-fit values	
Bottom	-0.9330
Top	92.45
LogIC50	-8.957
HillSlope	-1.367
Std. Error	
Bottom	1.322
Top	1.591
LogIC50	0.02182
HillSlope	0.1040
95% Confidence Intervals	
Bottom	-3.691 to 1.825
Top	89.13 to 95.77
LogIC50	-9.003 to -8.912
HillSlope	-1.584 to -1.150
Goodness of Fit	
Degrees of Freedom	20
R square	0.9935
Sy.x	3.418
Number of points	
Analyzed	24

Subtask 1



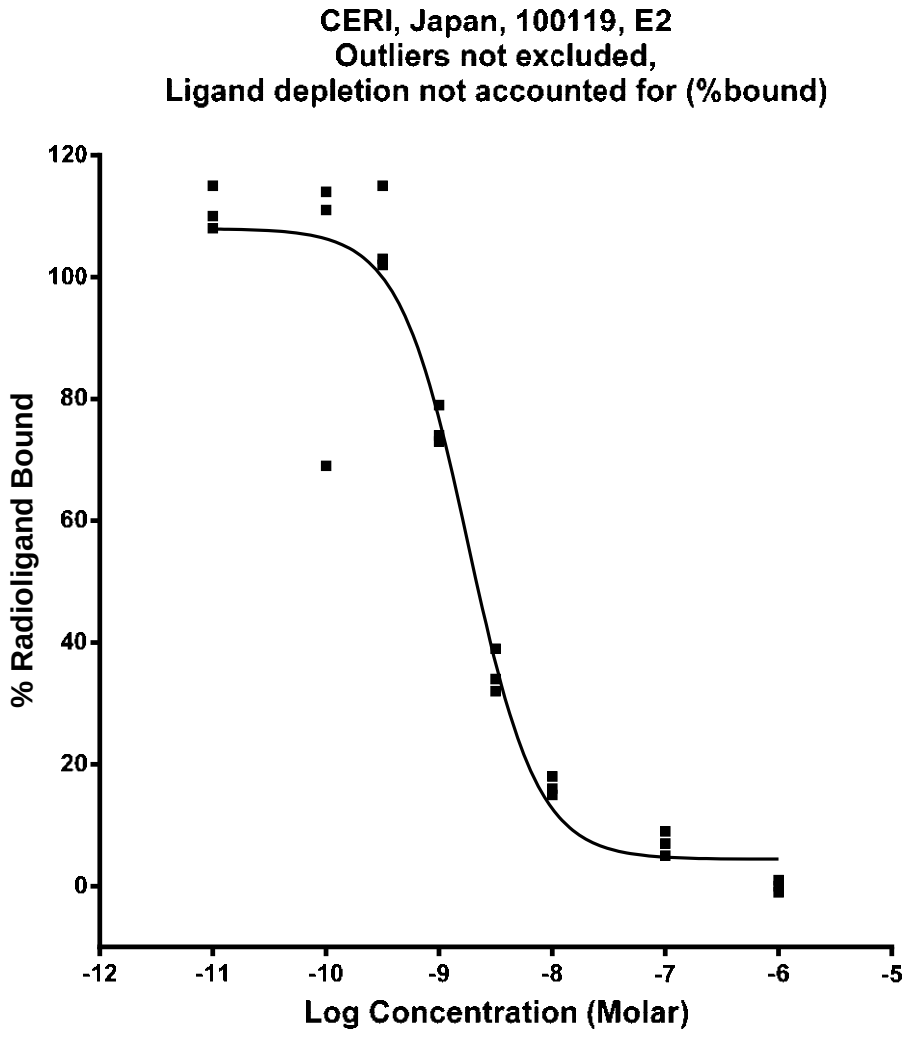
logIC50	
Best-fit values	
Bottom	-0.8592
Top	99.42
LogIC50	-8.986
HillSlope	-1.424
Std. Error	
Bottom	1.149
Top	1.459
LogIC50	0.01757
HillSlope	0.09269
95% Confidence Intervals	
Bottom	-3.257 to 1.538
Top	96.38 to 102.5
LogIC50	-9.022 to -8.949
HillSlope	-1.617 to -1.231
Goodness of Fit	
Degrees of Freedom	20
R square	0.9955
Sy.x	3.072
Number of points	
Analyzed	24

Subtask 1



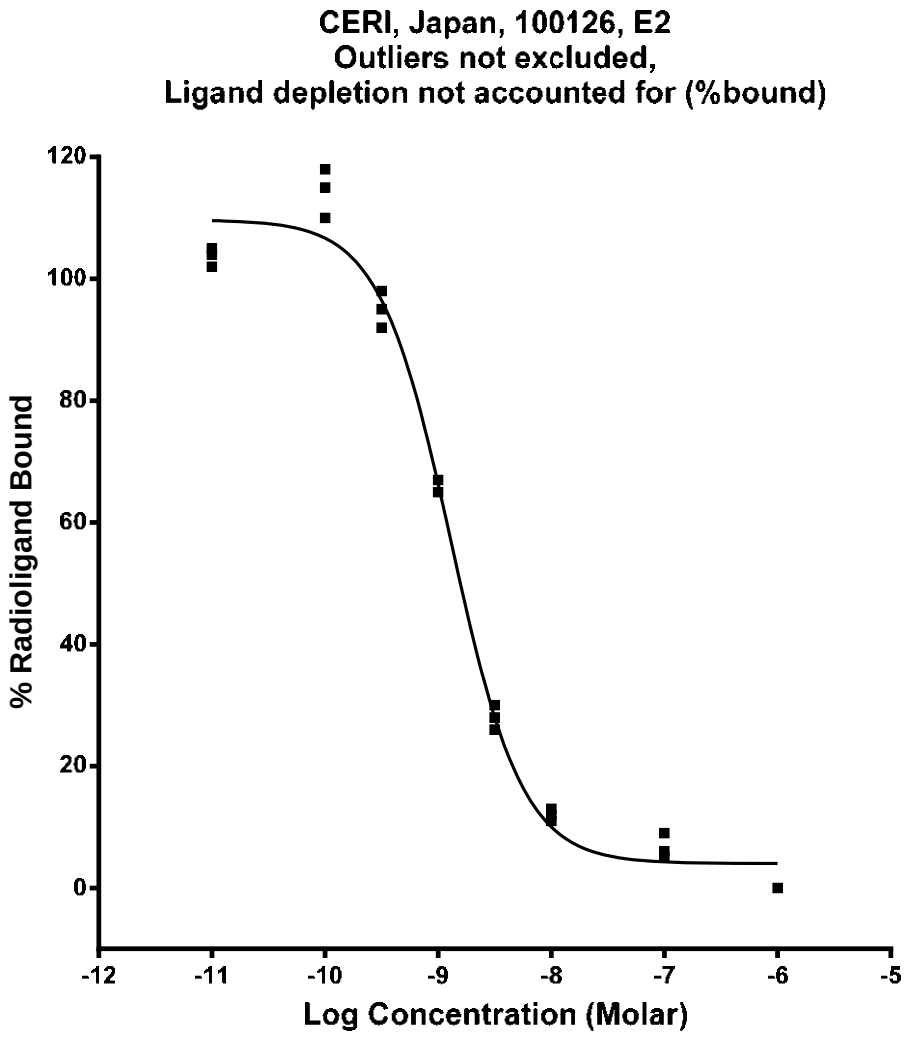
logIC50	
Best-fit values	
Bottom	-3.529
Top	101.8
LogIC50	-8.959
HillSlope	-1.277
Std. Error	
Bottom	2.028
Top	2.576
LogIC50	0.03017
HillSlope	0.1304
95% Confidence Intervals	
Bottom	-7.758 to 0.7007
Top	96.45 to 107.2
LogIC50	-9.022 to -8.896
HillSlope	-1.549 to -1.005
Goodness of Fit	
Degrees of Freedom	20
R square	0.9879
Sy.x	5.207
Number of points	
Analyzed	24

Subtask 2



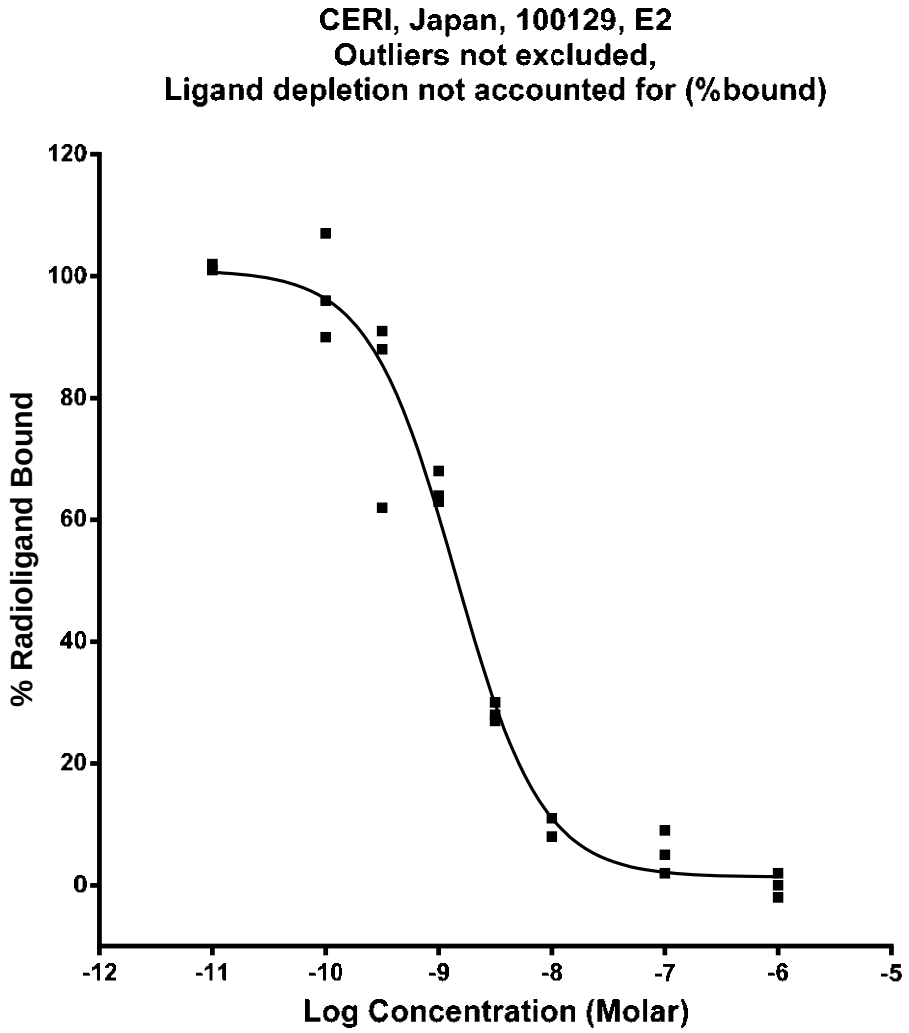
logIC50	
Best-fit values	
Bottom	4.423
Top	108.0
LogIC50	-8.670
HillSlope	-1.423
Std. Error	
Bottom	3.925
Top	4.198
LogIC50	0.05590
HillSlope	0.2779
95% Confidence Intervals	
Bottom	-3.764 to 12.61
Top	99.20 to 116.7
LogIC50	-8.787 to -8.554
HillSlope	-2.003 to -0.8436
Goodness of Fit	
Degrees of Freedom	20
R square	0.9587
Sy.x	9.886
Number of points	
Analyzed	24

Subtask 2



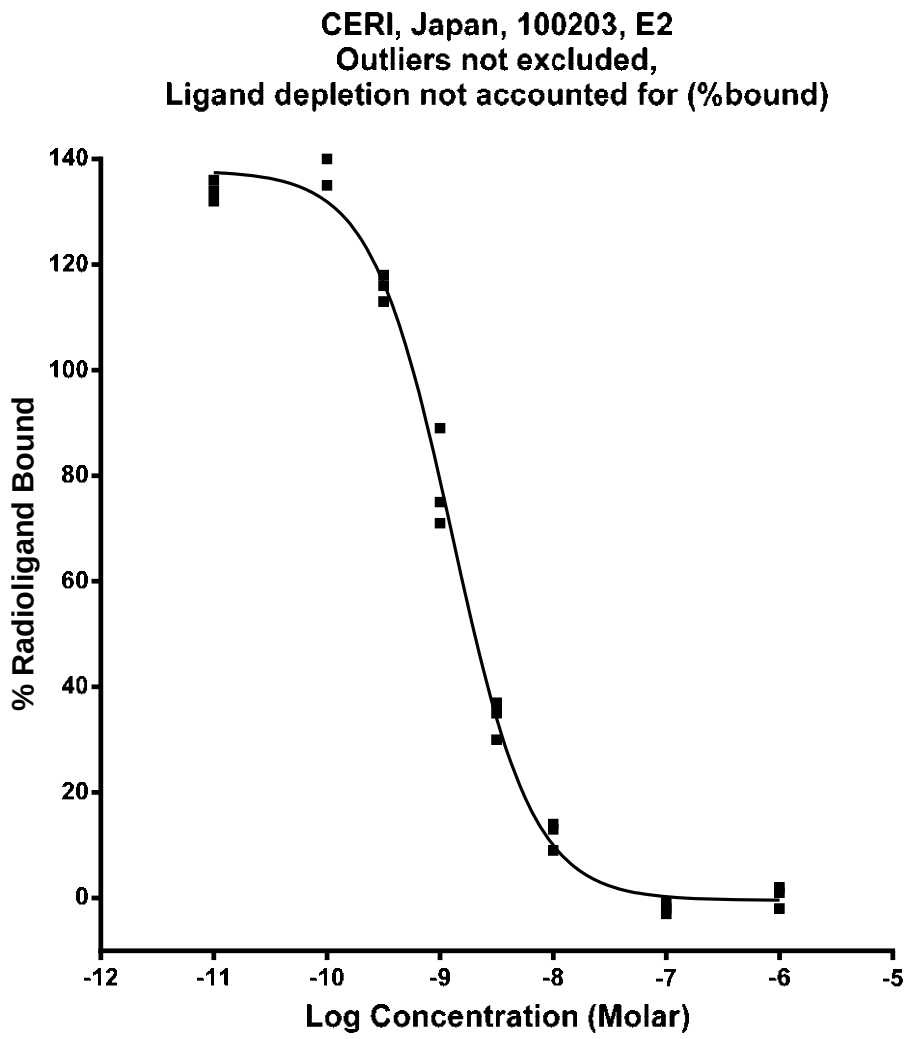
logIC50	
Best-fit values	
Bottom	4.007
Top	109.7
LogIC50	-8.804
HillSlope	-1.377
Std. Error	
Bottom	1.820
Top	2.165
LogIC50	0.02659
HillSlope	0.1271
95% Confidence Intervals	
Bottom	0.2107 to 7.803
Top	105.2 to 114.2
LogIC50	-8.859 to -8.748
HillSlope	-1.642 to -1.112
Goodness of Fit	
Degrees of Freedom	20
R square	0.9905
Sy.x	4.699
Number of points	
Analyzed	24

Subtask 2



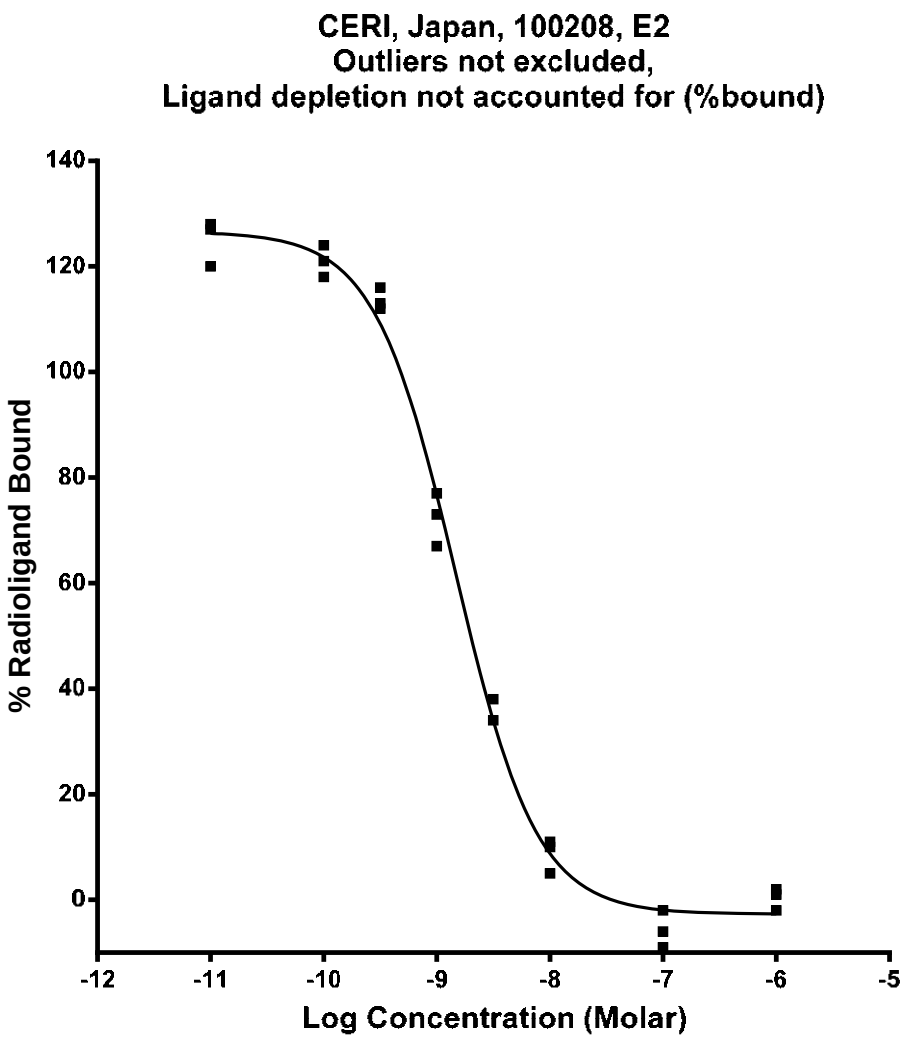
logIC50	
Best-fit values	
Bottom	1.344
Top	101.0
LogIC50	-8.835
HillSlope	-1.136
Std. Error	
Bottom	2.803
Top	3.419
LogIC50	0.04417
HillSlope	0.1569
95% Confidence Intervals	
Bottom	-4.503 to 7.190
Top	93.86 to 108.1
LogIC50	-8.927 to -8.742
HillSlope	-1.463 to -0.8084
Goodness of Fit	
Degrees of Freedom	20
R square	0.9764
Sy.x	6.768
Number of points	
Analyzed	24

Subtask 3



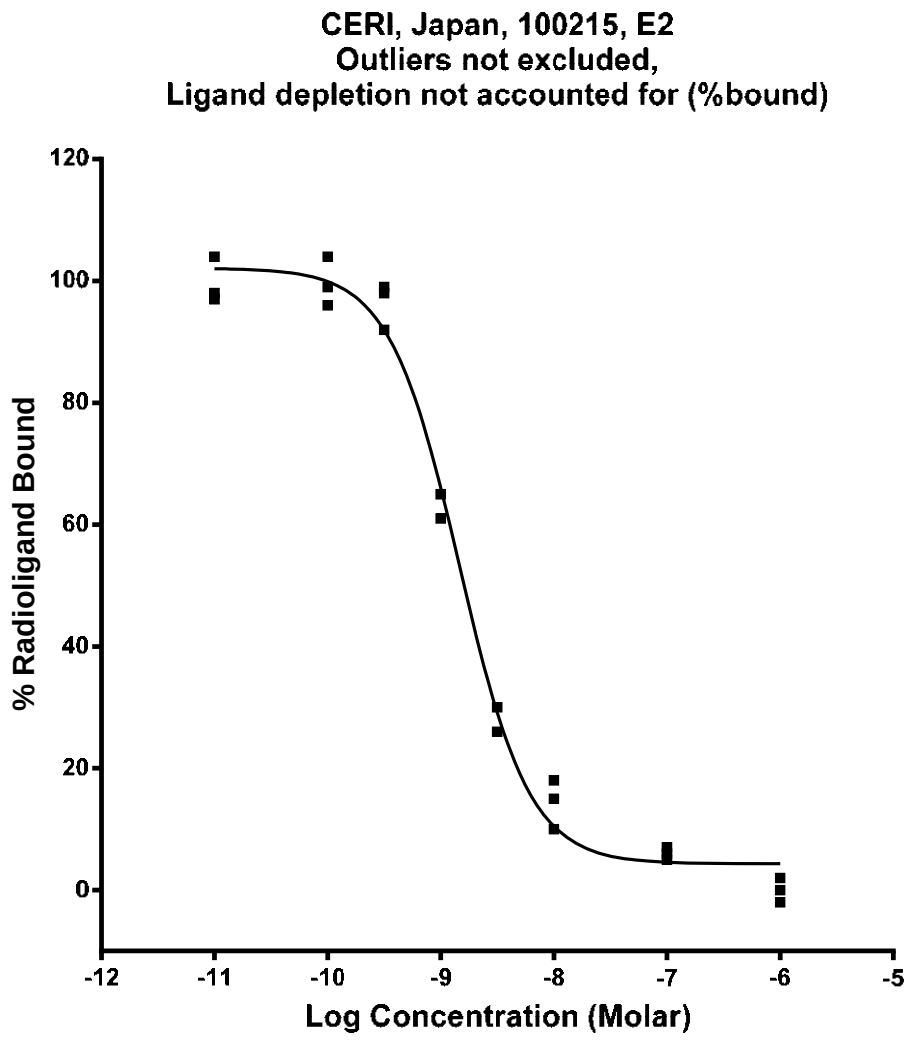
logIC50	
Best-fit values	
Bottom	-0.4119
Top	137.8
LogIC50	-8.695
HillSlope	-1.220
Std. Error	
Bottom	1.798
Top	2.214
LogIC50	0.02244
HillSlope	0.08099
95% Confidence Intervals	
Bottom	-4.162 to 3.338
Top	133.2 to 142.4
LogIC50	-8.742 to -8.648
HillSlope	-1.389 to -1.051
Goodness of Fit	
Degrees of Freedom	20
R square	0.9947
Sy.x	4.488
Number of points	
Analyzed	24

Subtask 3



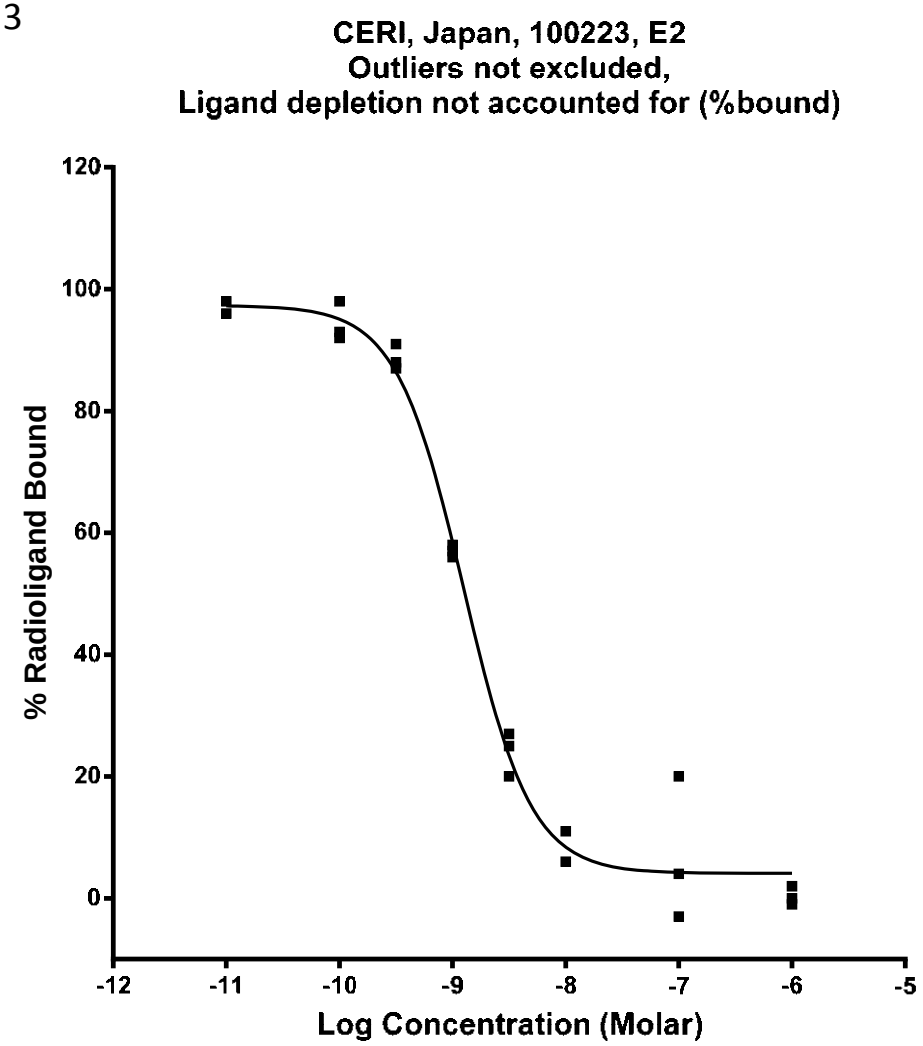
logIC50	
Best-fit values	
Bottom	-2.682
Top	126.6
LogIC50	-8.700
HillSlope	-1.212
Std. Error	
Bottom	1.814
Top	2.146
LogIC50	0.02265
HillSlope	0.08501
95% Confidence Intervals	
Bottom	-6.466 to 1.102
Top	122.1 to 131.0
LogIC50	-8.747 to -8.652
HillSlope	-1.389 to -1.034
Goodness of Fit	
Degrees of Freedom	20
R square	0.9940
Sy.x	4.455
Number of points	
Analyzed	24

Subtask 3



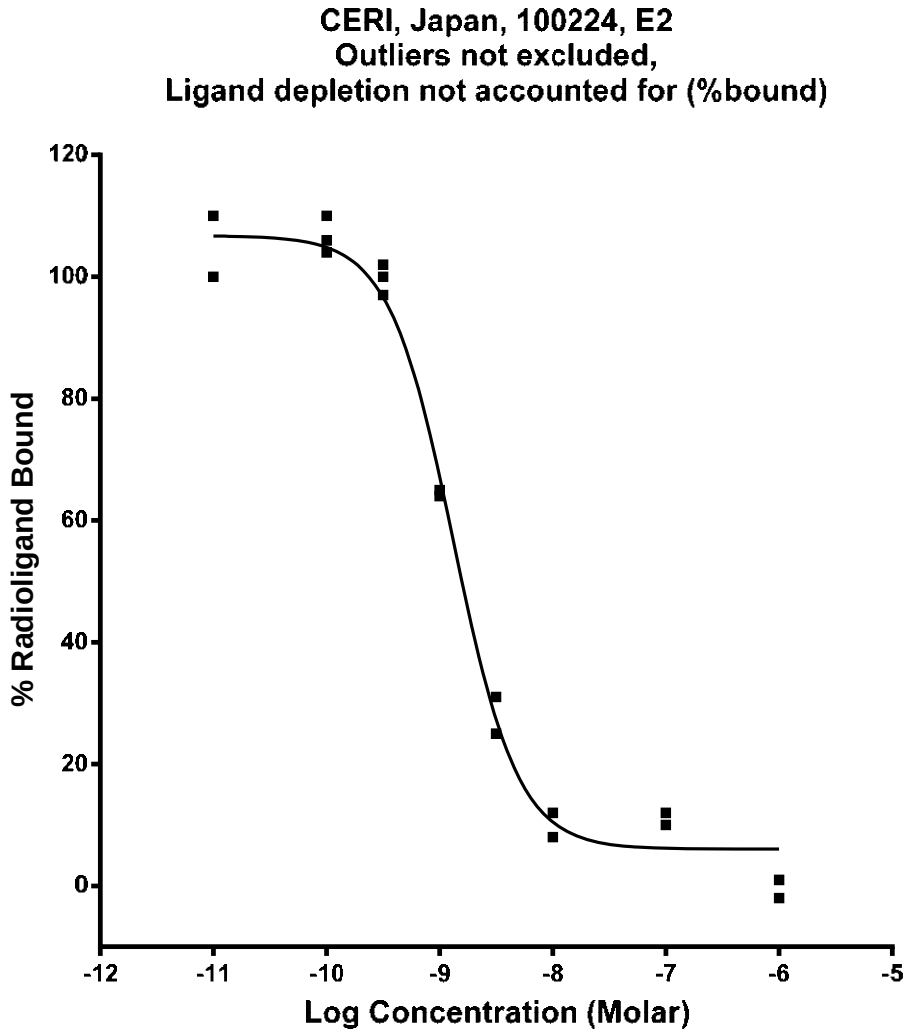
logIC50	
Best-fit values	
Bottom	4.303
Top	102.1
LogIC50	-8.793
HillSlope	-1.405
Std. Error	
Bottom	1.638
Top	1.866
LogIC50	0.02497
HillSlope	0.1244
95% Confidence Intervals	
Bottom	0.8854 to 7.720
Top	98.21 to 106.0
LogIC50	-8.845 to -8.741
HillSlope	-1.665 to -1.146
Goodness of Fit	
Degrees of Freedom	20
R square	0.9913
Sy.x	4.198
Number of points	
Analyzed	24

Subtask 3



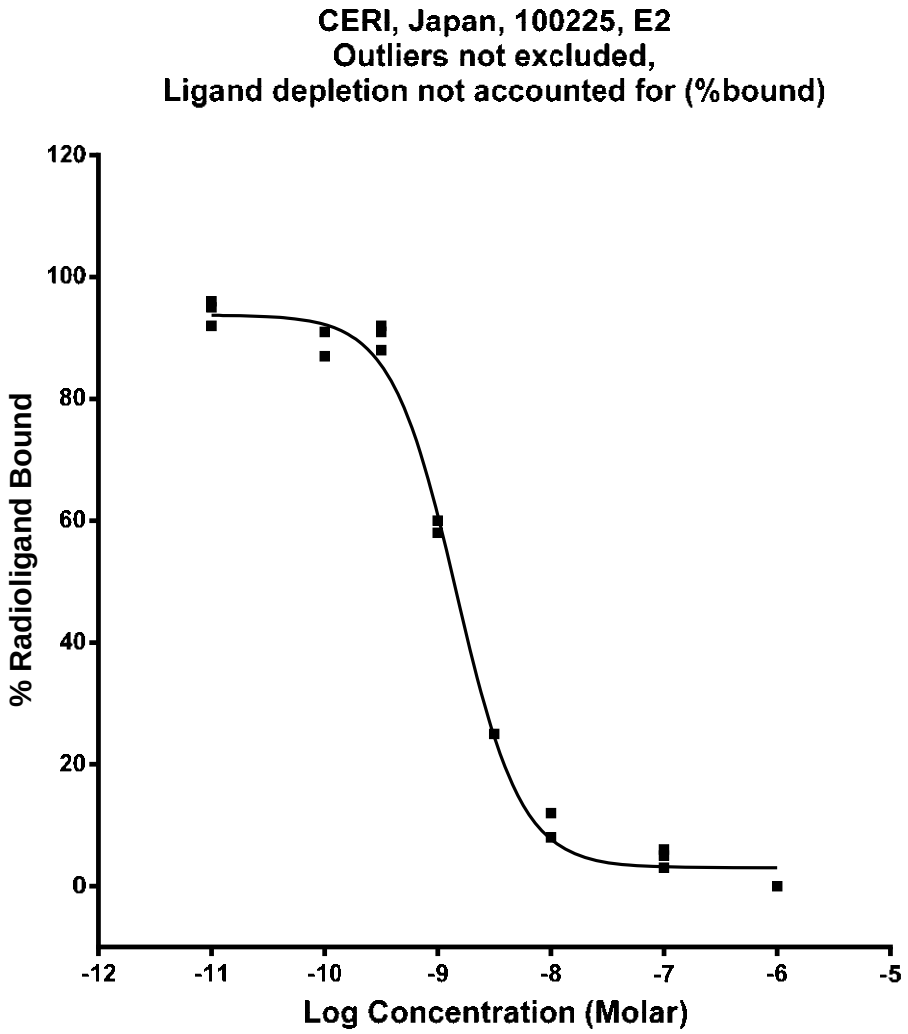
logIC50	
Best-fit values	
Bottom	4.079
Top	97.33
LogIC50	-8.891
HillSlope	-1.459
Std. Error	
Bottom	1.809
Top	2.145
LogIC50	0.02889
HillSlope	0.1569
95% Confidence Intervals	
Bottom	0.3050 to 7.852
Top	92.86 to 101.8
LogIC50	-8.951 to -8.830
HillSlope	-1.786 to -1.132
Goodness of Fit	
Degrees of Freedom	20
R square	0.9878
Sy.x	4.760
Number of points	
Analyzed	24

Subtask 3



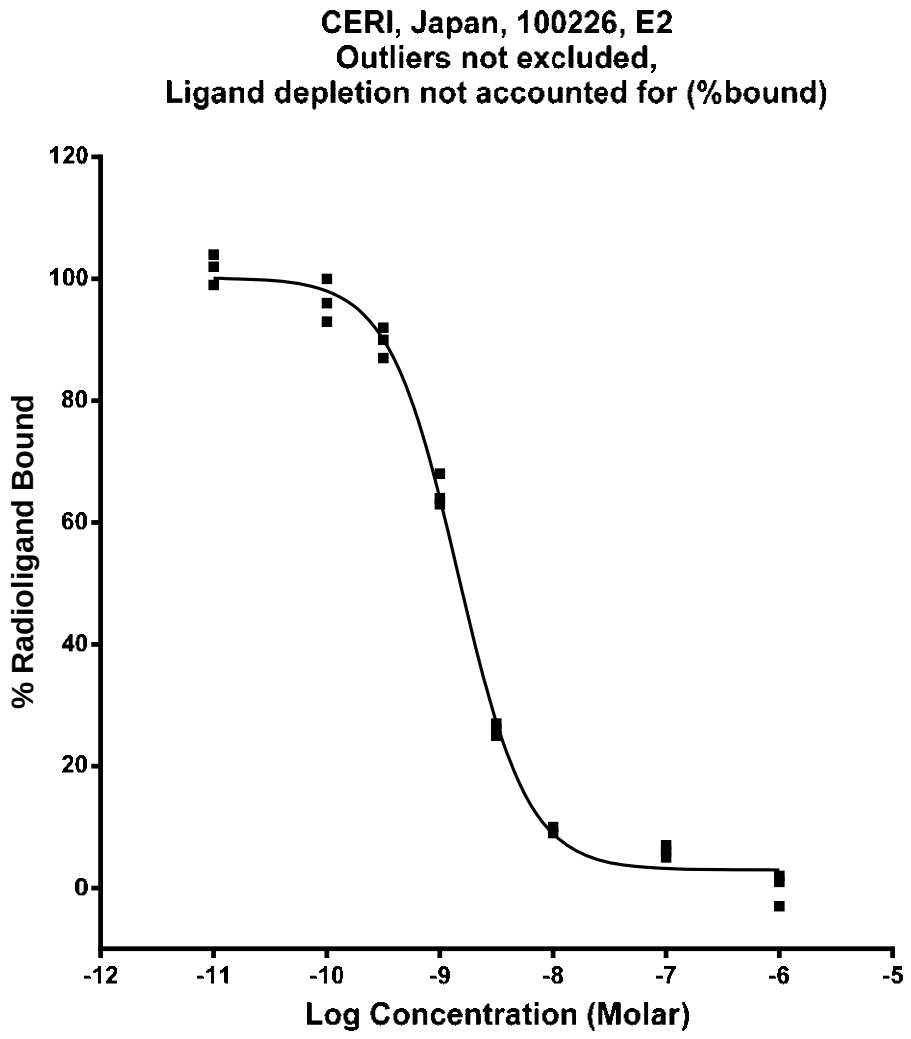
logIC50	
Best-fit values	
Bottom	6.039
Top	106.7
LogIC50	-8.803
HillSlope	-1.527
Std. Error	
Bottom	1.755
Top	2.021
LogIC50	0.02614
HillSlope	0.1487
95% Confidence Intervals	
Bottom	2.377 to 9.701
Top	102.5 to 111.0
LogIC50	-8.858 to -8.749
HillSlope	-1.837 to -1.217
Goodness of Fit	
Degrees of Freedom	20
R square	0.9902
Sy.x	4.643
Number of points	
Analyzed	24

Subtask 3



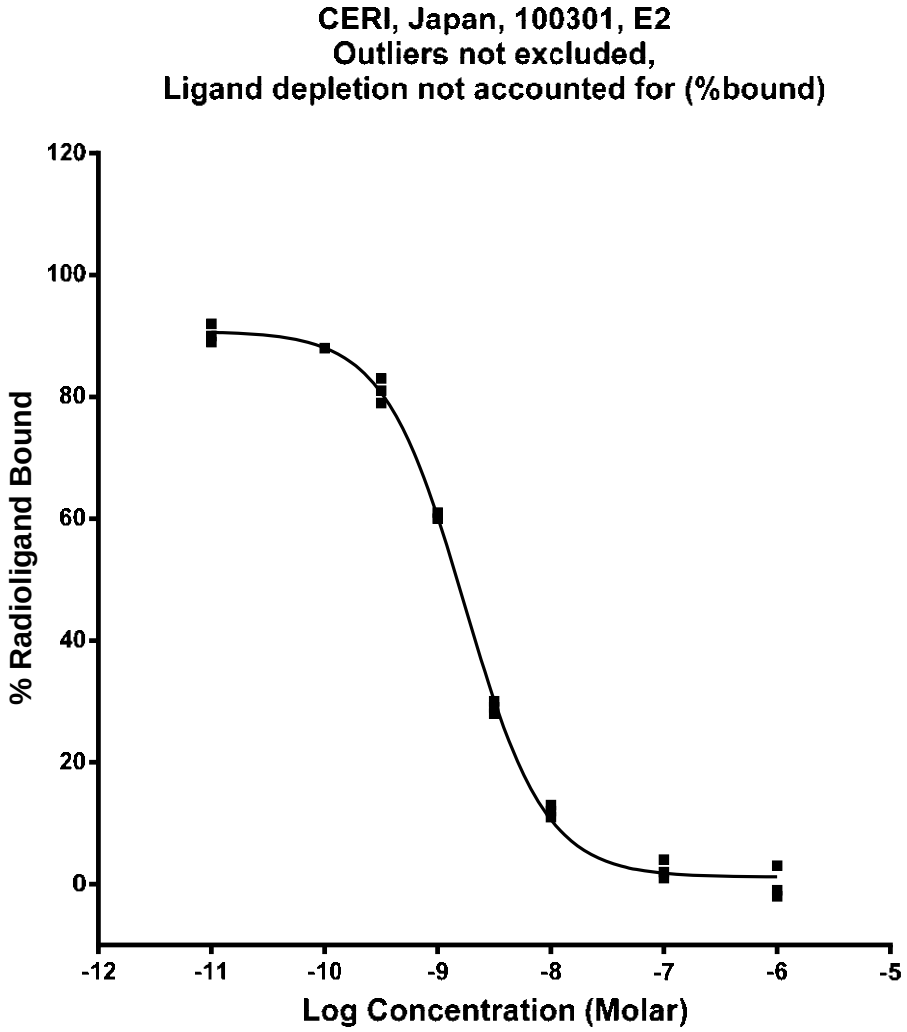
logIC50	
Best-fit values	
Bottom	3.021
Top	93.76
LogIC50	-8.858
HillSlope	-1.510
Std. Error	
Bottom	1.267
Top	1.422
LogIC50	0.02036
HillSlope	0.1147
95% Confidence Intervals	
Bottom	0.3789 to 5.663
Top	90.79 to 96.73
LogIC50	-8.900 to -8.815
HillSlope	-1.750 to -1.271
Goodness of Fit	
Degrees of Freedom	20
R square	0.9939
Sy.x	3.310
Number of points	
Analyzed	24

Subtask 3



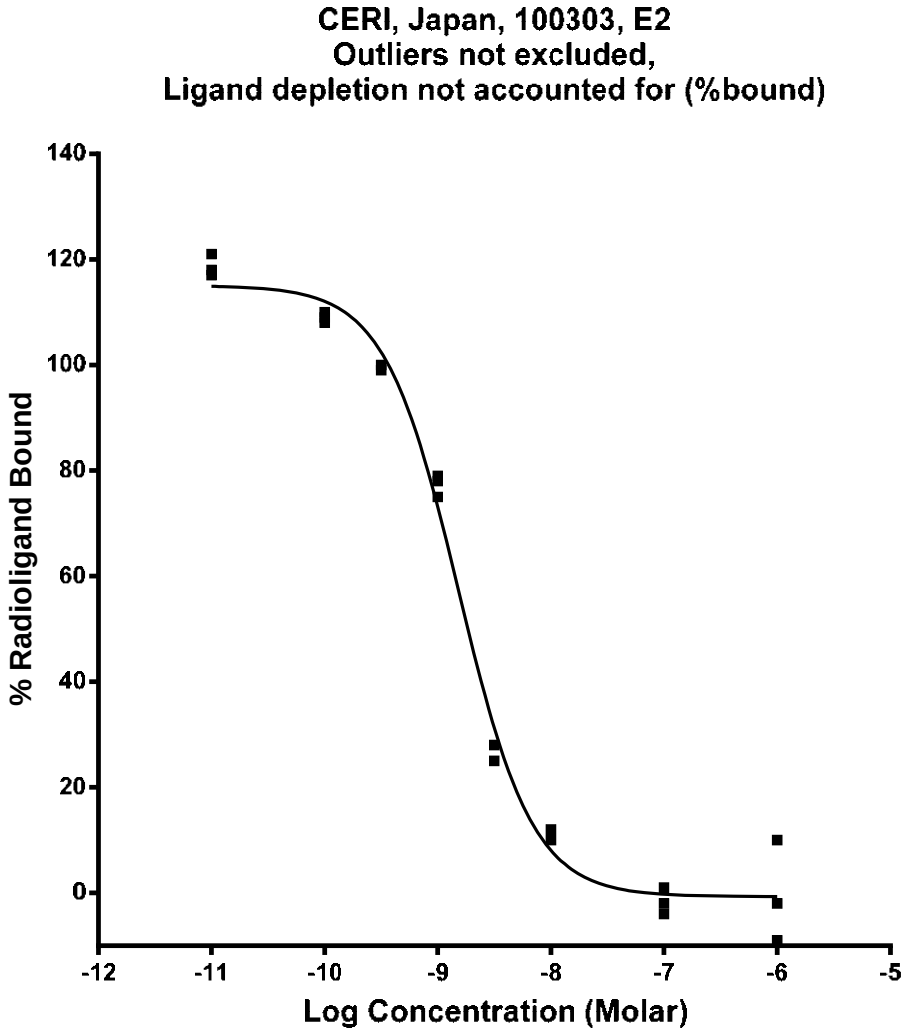
logIC50	
Best-fit values	
Bottom	2.946
Top	100.2
LogIC50	-8.820
HillSlope	-1.413
Std. Error	
Bottom	1.092
Top	1.247
LogIC50	0.01667
HillSlope	0.08423
95% Confidence Intervals	
Bottom	0.6673 to 5.224
Top	97.58 to 102.8
LogIC50	-8.854 to -8.785
HillSlope	-1.589 to -1.237
Goodness of Fit	
Degrees of Freedom	20
R square	0.9960
Sy.x	2.807
Number of points	
Analyzed	24

Subtask 3



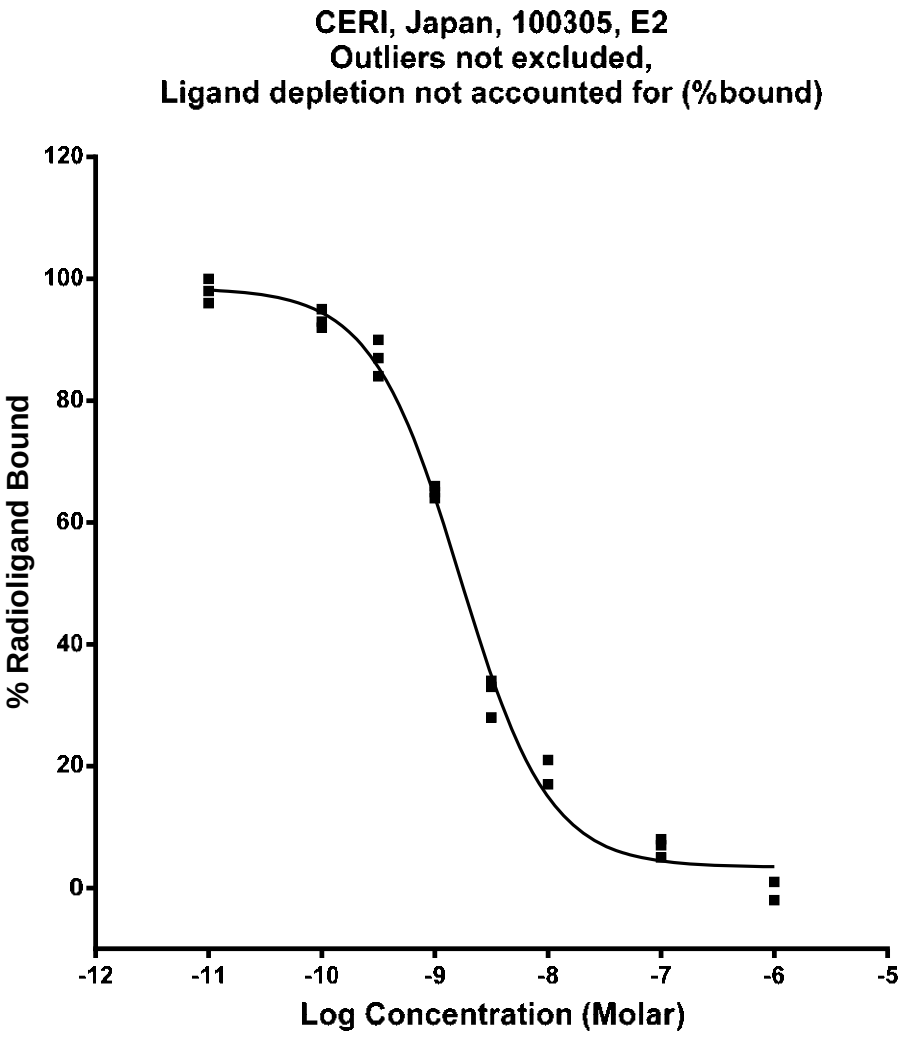
logIC50	
Best-fit values	
Bottom	1.150
Top	90.77
LogIC50	-8.828
HillSlope	-1.217
Std. Error	
Bottom	0.6660
Top	0.7501
LogIC50	0.01144
HillSlope	0.04446
95% Confidence Intervals	
Bottom	-0.2391 to 2.539
Top	89.21 to 92.33
LogIC50	-8.852 to -8.804
HillSlope	-1.310 to -1.124
Goodness of Fit	
Degrees of Freedom	20
R square	0.9984
Sy.x	1.611
Number of points	
Analyzed	24

Subtask 3



logIC50	
Best-fit values	
Bottom	-0.6877
Top	115.1
LogIC50	-8.734
HillSlope	-1.332
Std. Error	
Bottom	1.913
Top	2.179
LogIC50	0.02525
HillSlope	0.1133
95% Confidence Intervals	
Bottom	-4.678 to 3.303
Top	110.5 to 119.6
LogIC50	-8.786 to -8.681
HillSlope	-1.569 to -1.096
Goodness of Fit	
Degrees of Freedom	20
R square	0.9917
Sy.x	4.810
Number of points	
Analyzed	24

Subtask 3



logIC50	
Best-fit values	
Bottom	3.412
Top	98.48
LogIC50	-8.761
HillSlope	-1.106
Std. Error	
Bottom	1.359
Top	1.584
LogIC50	0.02216
HillSlope	0.07571
95% Confidence Intervals	
Bottom	0.5777 to 6.246
Top	95.17 to 101.8
LogIC50	-8.807 to -8.714
HillSlope	-1.264 to -0.9485
Goodness of Fit	
Degrees of Freedom	20
R square	0.9941
Sy.x	3.193
Number of points	
Analyzed	24

CERI ASSAY, (JapanCERI Laboratory) /2ntrol Norethynodrel (NE) /dzNJØŜă

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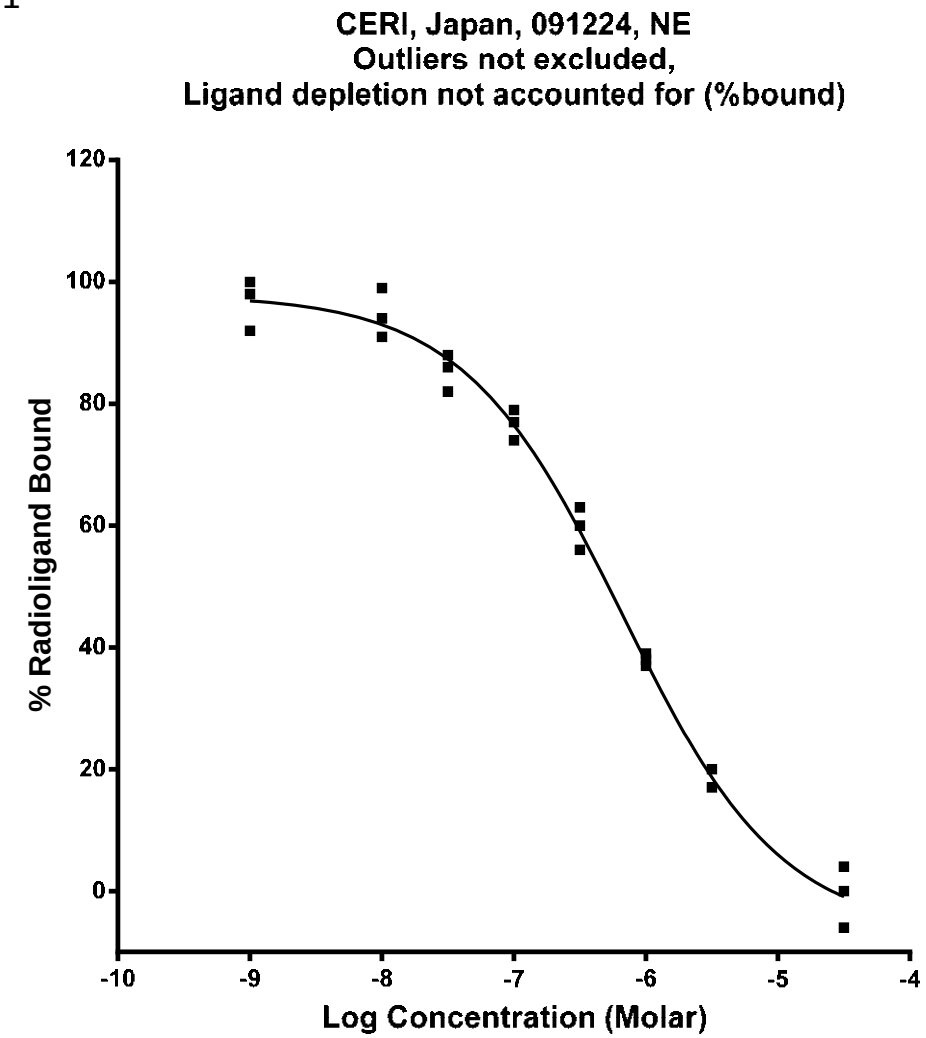
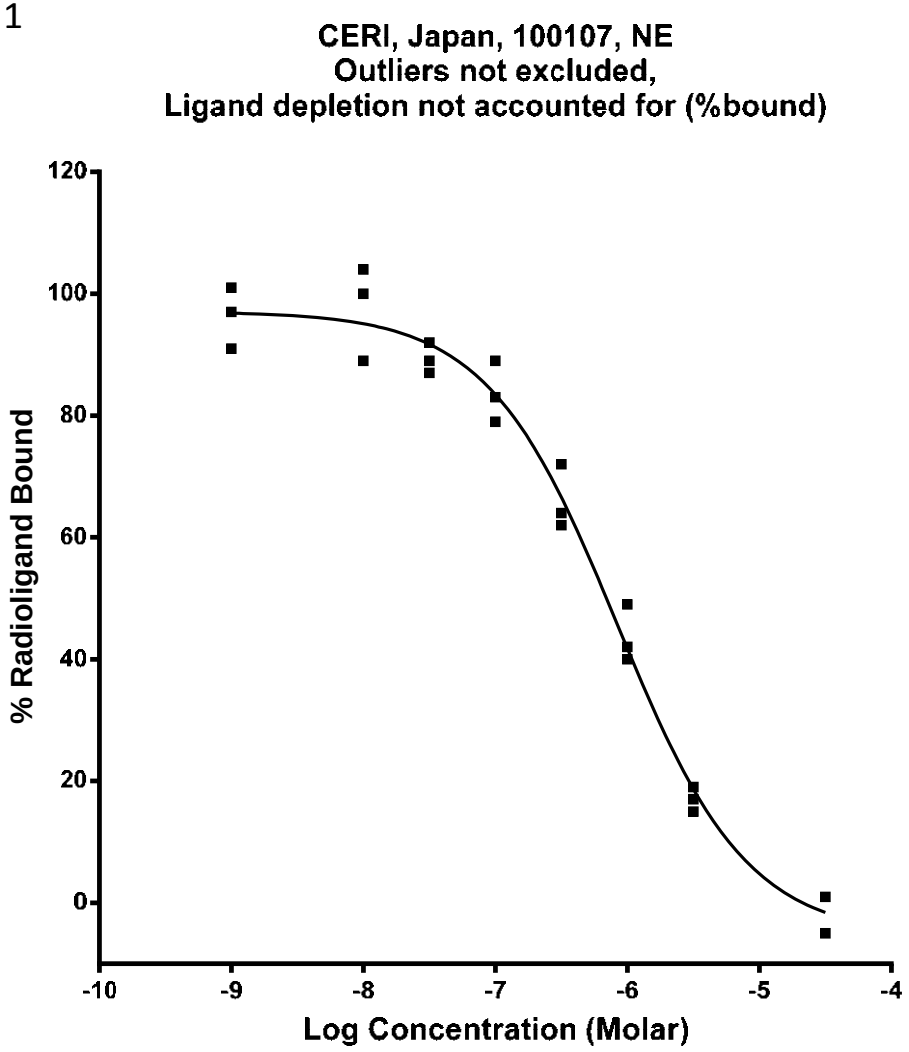
Subtask 1



logIC50	
Best-fit values	
Bottom	-7.019
Top	97.85
LogIC50	-6.283
HillSlope	-0.7210
Std. Error	
Bottom	3.122
Top	1.770
LogIC50	0.02669
HillSlope	0.05781
95% Confidence Intervals	
Bottom	-13.53 to -0.5058
Top	94.16 to 101.5
LogIC50	-6.339 to -6.228
HillSlope	-0.8416 to -0.6004
Goodness of Fit	
Degrees of Freedom	20
R square	0.9927
Sy.x	3.205
Number of points	
Analyzed	24

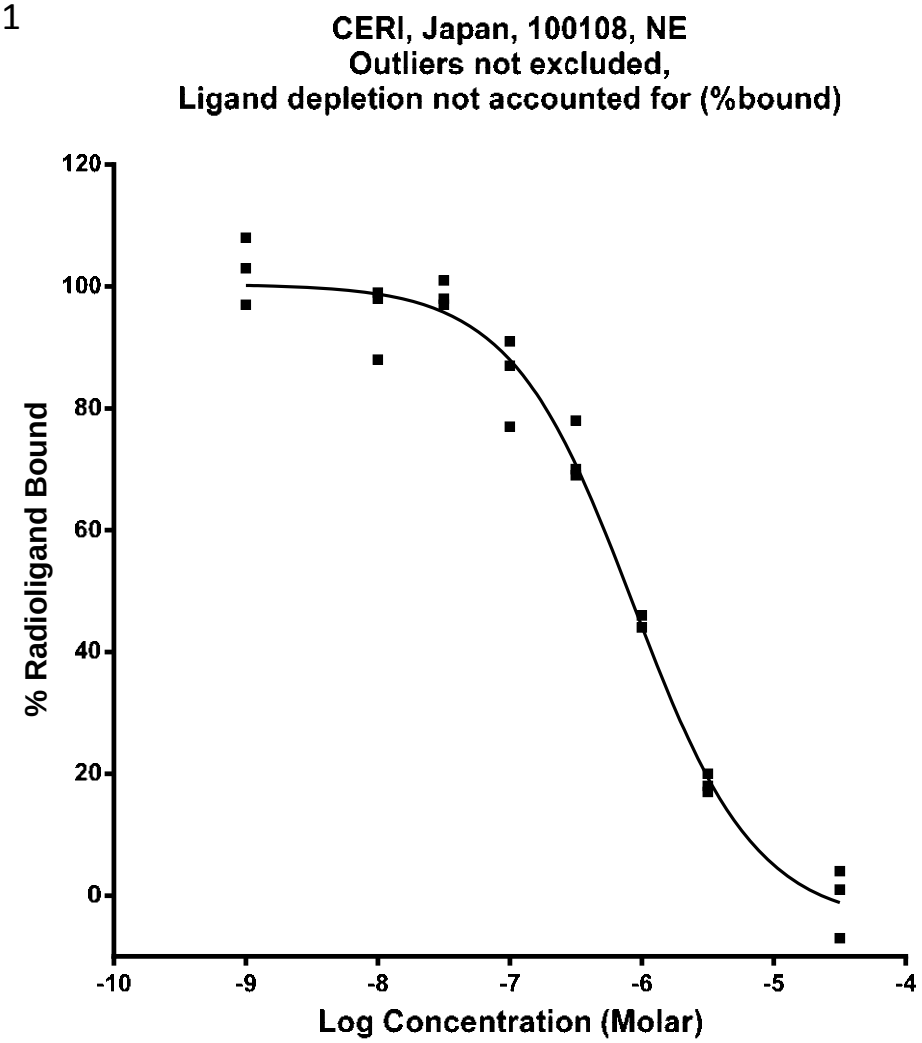
Subtask 1



logIC50	
Best-fit values	
Bottom	-5.558
Top	97.09
LogIC50	-6.158
HillSlope	-0.8831
Std. Error	
Bottom	3.791
Top	2.030
LogIC50	0.03453
HillSlope	0.09485
95% Confidence Intervals	
Bottom	-13.47 to 2.351
Top	92.86 to 101.3
LogIC50	-6.230 to -6.086
HillSlope	-1.081 to -0.6853
Goodness of Fit	
Degrees of Freedom	20
R square	0.9860
Sy.x	4.619
Number of points	
Analyzed	24

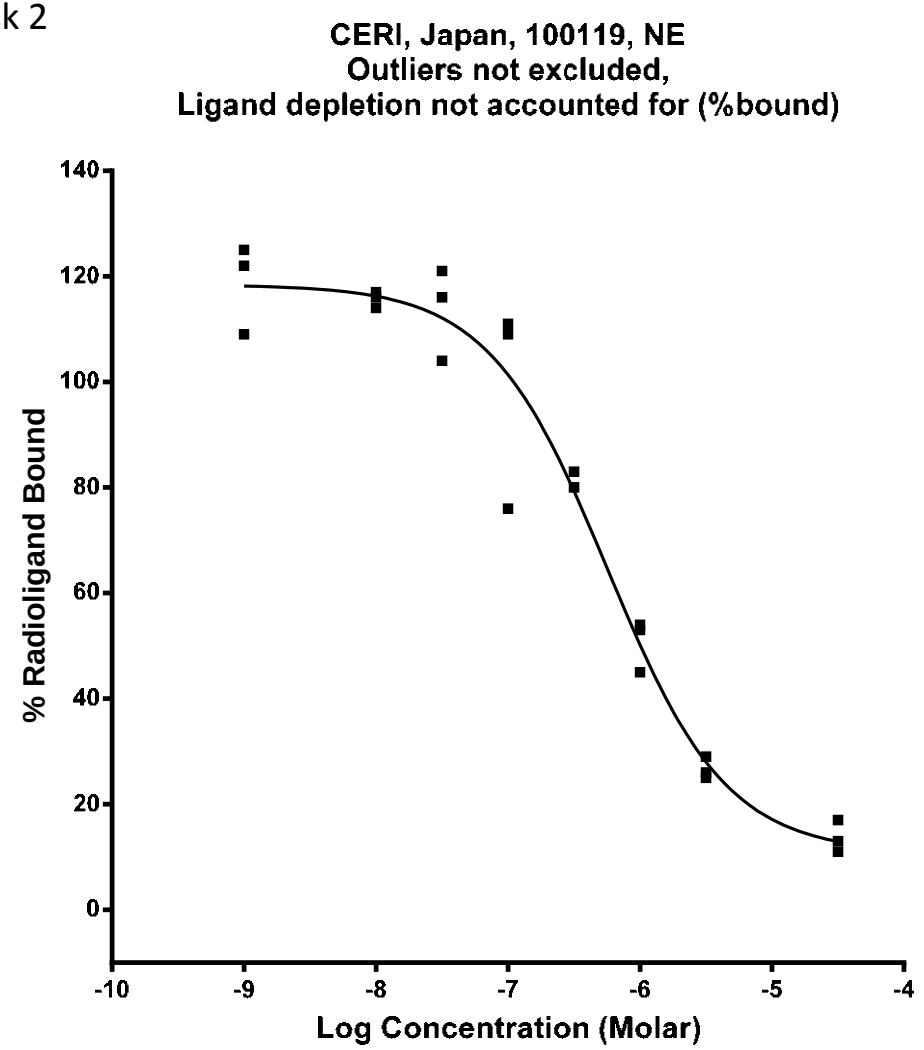
3

Subtask 1



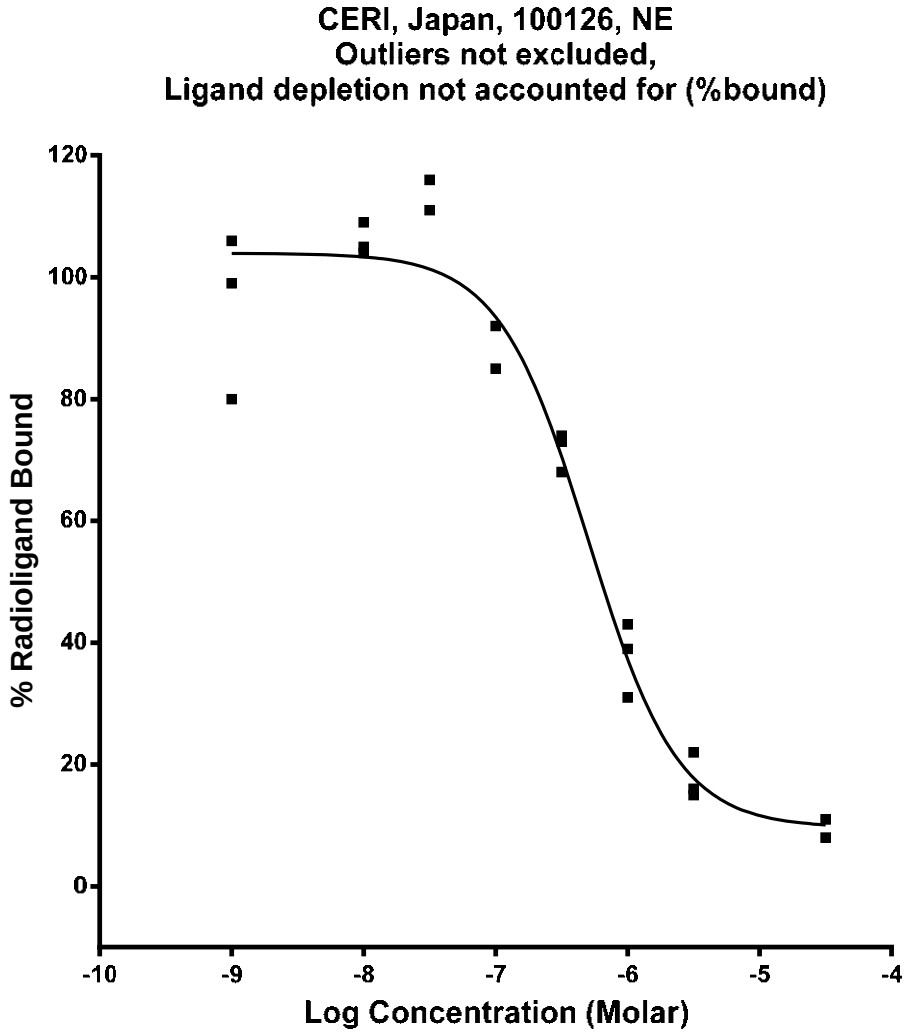
logIC50	
Best-fit values	
Bottom	-4.651
Top	100.4
LogIC50	-6.102
HillSlope	-0.9335
Std. Error	
Bottom	3.879
Top	2.078
LogIC50	0.03502
HillSlope	0.1029
95% Confidence Intervals	
Bottom	-12.74 to 3.440
Top	96.03 to 104.7
LogIC50	-6.175 to -6.029
HillSlope	-1.148 to -0.7188
Goodness of Fit	
Degrees of Freedom	20
R square	0.9850
Sy.x	4.961
Number of points	
Analyzed	24

Subtask 2



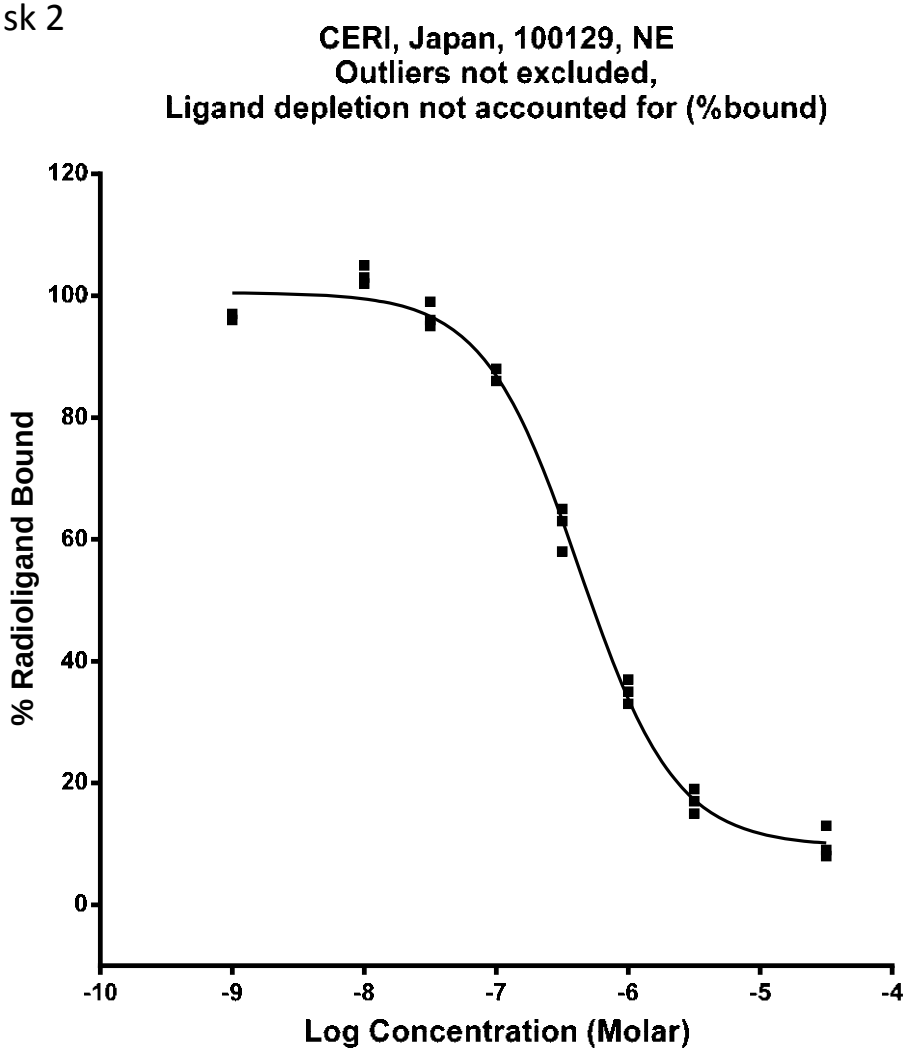
logIC50	
Best-fit values	
Bottom	10.77
Top	118.4
LogIC50	-5.995
HillSlope	-0.9635
Std. Error	
Bottom	5.338
Top	3.382
LogIC50	0.05999
HillSlope	0.1538
95% Confidence Intervals	
Bottom	-0.3605 to 21.91
Top	111.4 to 125.5
LogIC50	-6.120 to -5.870
HillSlope	-1.284 to -0.6426
Goodness of Fit	
Degrees of Freedom	20
R square	0.9684
Sy.x	7.757
Number of points	
Analyzed	24

Subtask 2



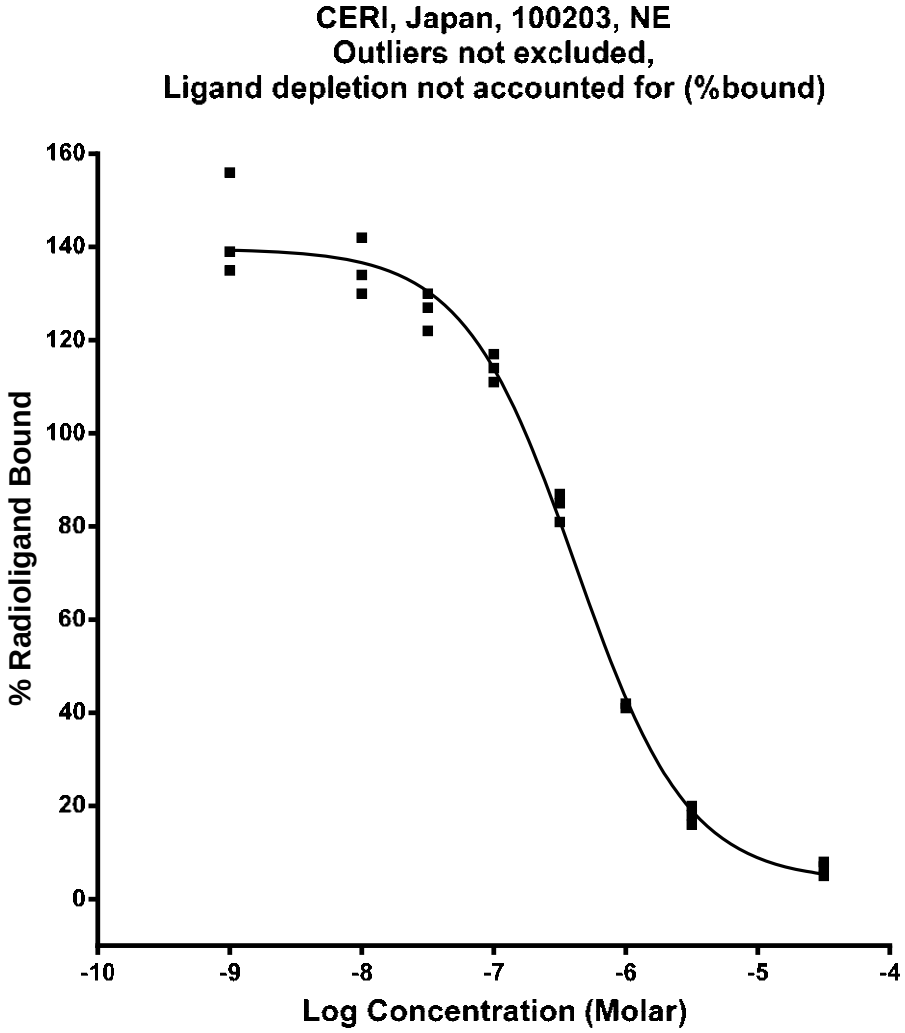
logIC50	
Best-fit values	
Bottom	9.629
Top	104.0
LogIC50	-6.198
HillSlope	-1.285
Std. Error	
Bottom	4.537
Top	3.026
LogIC50	0.05471
HillSlope	0.2343
95% Confidence Intervals	
Bottom	0.1638 to 19.09
Top	97.64 to 110.3
LogIC50	-6.312 to -6.084
HillSlope	-1.773 to -0.7960
Goodness of Fit	
Degrees of Freedom	20
R square	0.9620
Sy.x	8.116
Number of points	
Analyzed	24

Subtask 2



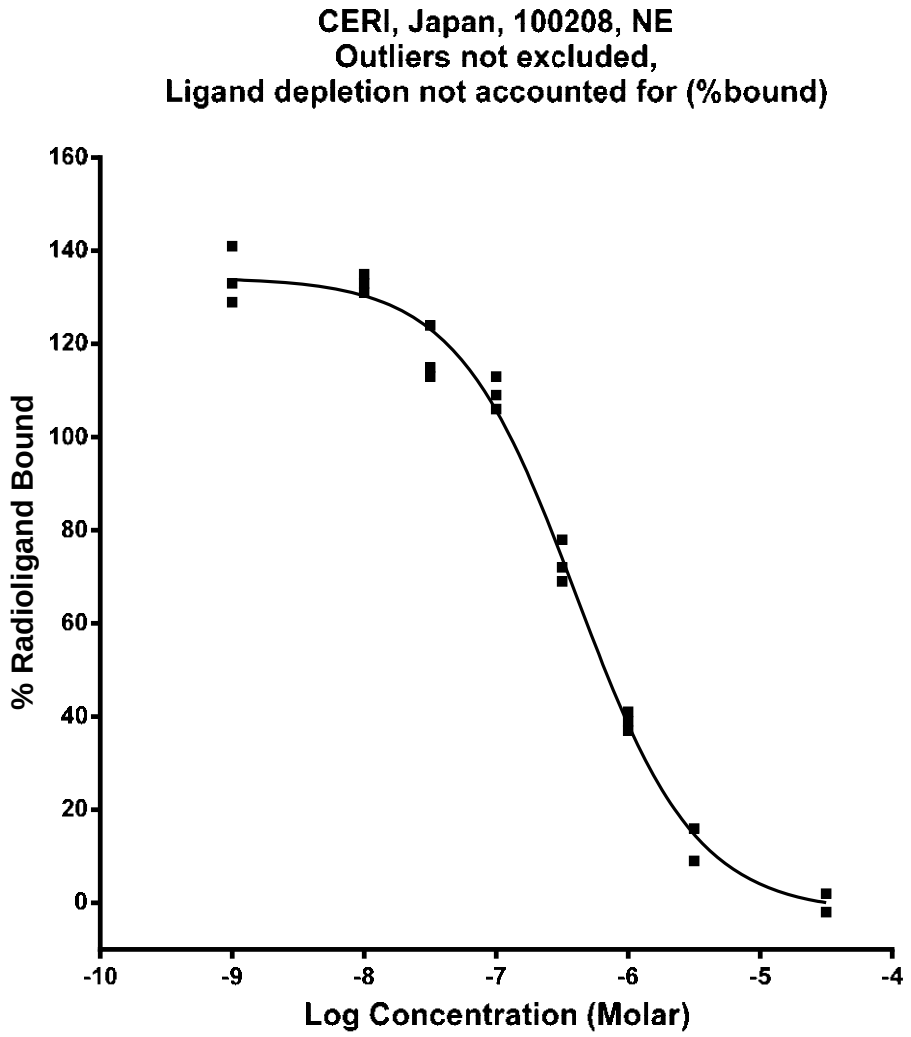
logIC50	
Best-fit values	
Bottom	9.638
Top	100.5
LogIC50	-6.287
HillSlope	-1.194
Std. Error	
Bottom	1.636
Top	1.158
LogIC50	0.02087
HillSlope	0.08114
95% Confidence Intervals	
Bottom	6.225 to 13.05
Top	98.09 to 102.9
LogIC50	-6.330 to -6.243
HillSlope	-1.363 to -1.024
Goodness of Fit	
Degrees of Freedom	20
R square	0.9945
Sy.x	2.906
Number of points	
Analyzed	24

Subtask 3



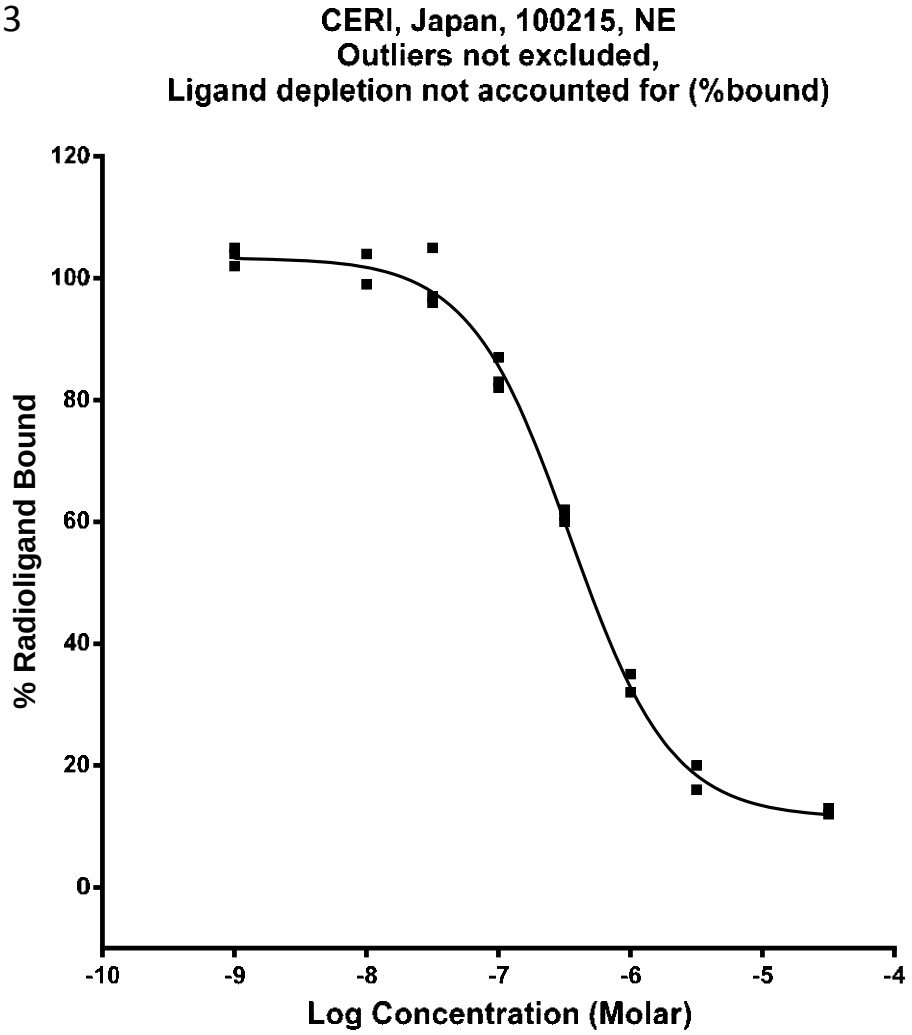
logIC50	
Best-fit values	
Bottom	3.755
Top	139.6
LogIC50	-6.098
HillSlope	-1.019
Std. Error	
Bottom	3.270
Top	2.335
LogIC50	0.03202
HillSlope	0.08563
95% Confidence Intervals	
Bottom	-3.066 to 10.58
Top	134.7 to 144.5
LogIC50	-6.164 to -6.031
HillSlope	-1.198 to -0.8408
Goodness of Fit	
Degrees of Freedom	20
R square	0.9912
Sy.x	5.290
Number of points	
Analyzed	24

Subtask 3



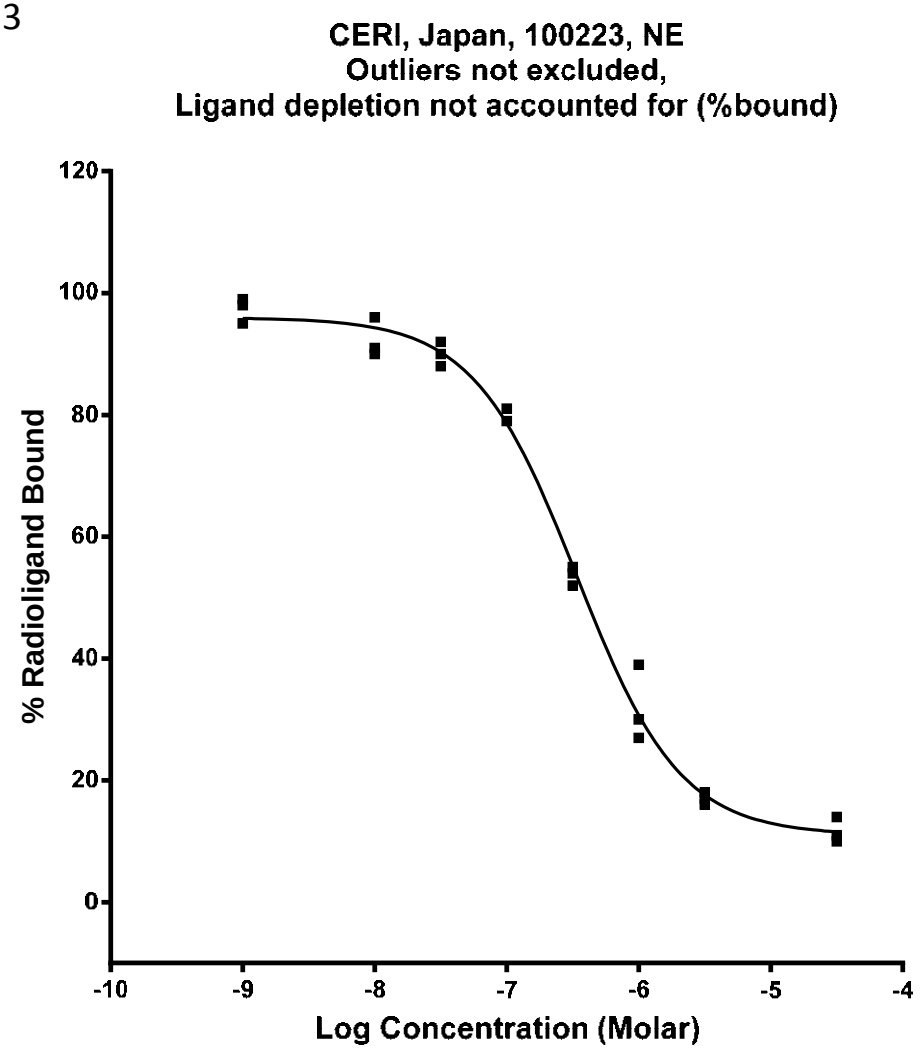
logIC50	
Best-fit values	
Bottom	-2.033
Top	134.2
LogIC50	-6.174
HillSlope	-0.9541
Std. Error	
Bottom	3.030
Top	2.188
LogIC50	0.02770
HillSlope	0.07199
95% Confidence Intervals	
Bottom	-8.354 to 4.287
Top	129.7 to 138.8
LogIC50	-6.232 to -6.117
HillSlope	-1.104 to -0.8039
Goodness of Fit	
Degrees of Freedom	20
R square	0.9928
Sy.x	4.701
Number of points	
Analyzed	24

Subtask 3



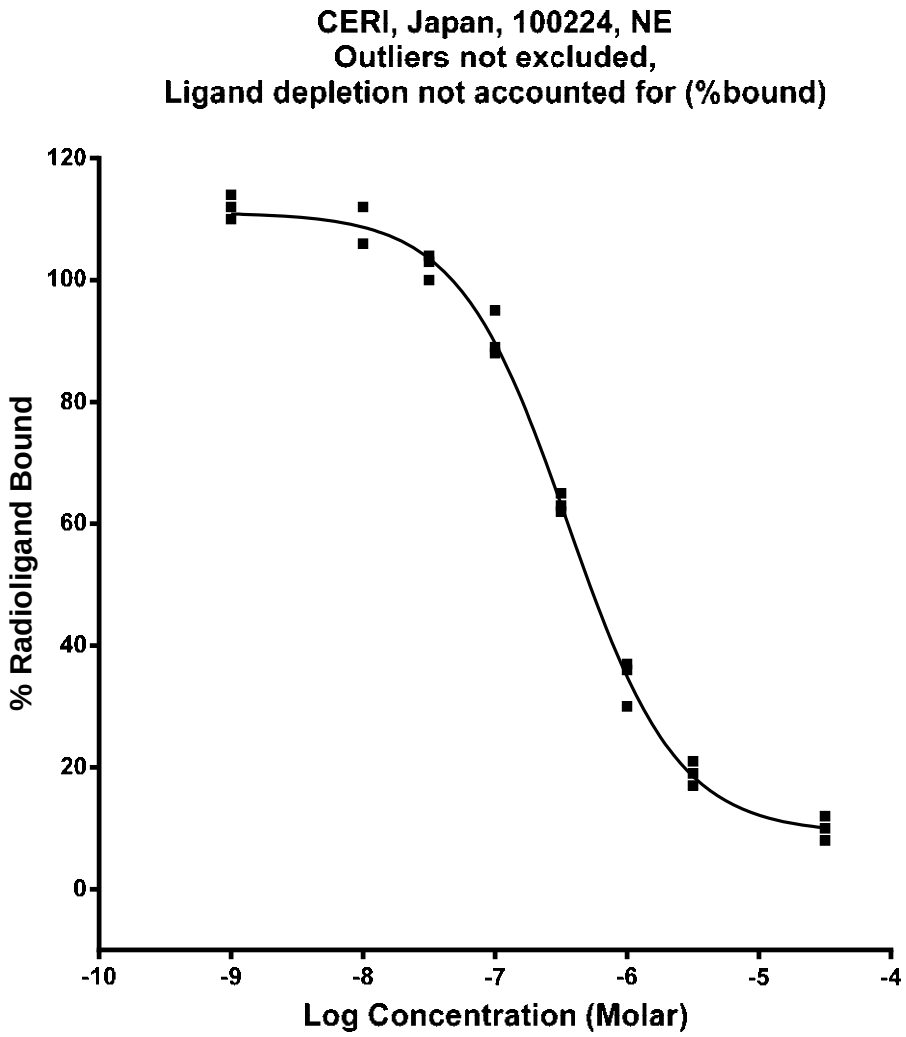
logIC50	
Best-fit values	
Bottom	11.40
Top	103.4
LogIC50	-6.331
HillSlope	-1.135
Std. Error	
Bottom	1.418
Top	1.083
LogIC50	0.01898
HillSlope	0.06695
95% Confidence Intervals	
Bottom	8.443 to 14.36
Top	101.1 to 105.6
LogIC50	-6.370 to -6.291
HillSlope	-1.275 to -0.9957
Goodness of Fit	
Degrees of Freedom	20
R square	0.9958
Sy.x	2.546
Number of points	
Analyzed	24

Subtask 3



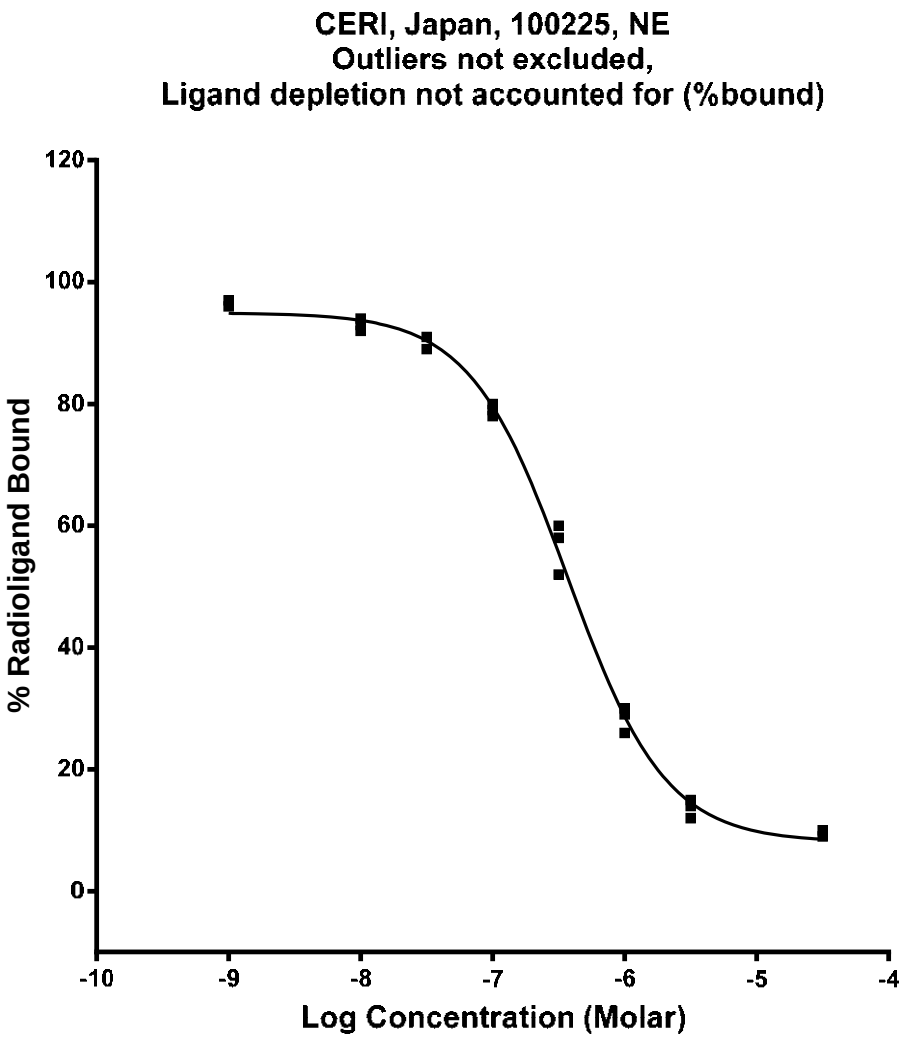
logIC50	
Best-fit values	
Bottom	11.01
Top	95.98
LogIC50	-6.403
HillSlope	-1.110
Std. Error	
Bottom	1.655
Top	1.279
LogIC50	0.02335
HillSlope	0.08215
95% Confidence Intervals	
Bottom	7.560 to 14.47
Top	93.31 to 98.65
LogIC50	-6.452 to -6.354
HillSlope	-1.281 to -0.9384
Goodness of Fit	
Degrees of Freedom	20
R square	0.9934
Sy.x	2.951
Number of points	
Analyzed	24

Subtask 3



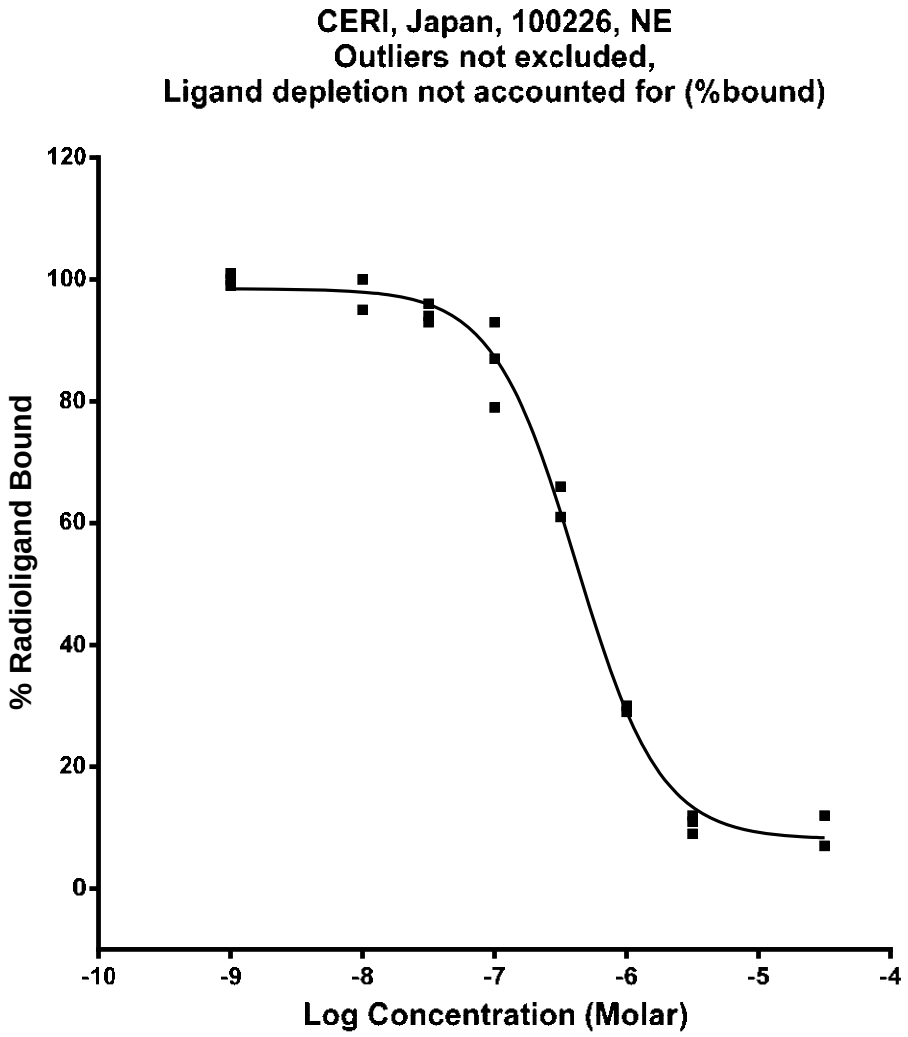
logIC50	
Best-fit values	
Bottom	9.151
Top	111.1
LogIC50	-6.282
HillSlope	-1.046
Std. Error	
Bottom	1.517
Top	1.152
LogIC50	0.01863
HillSlope	0.05678
95% Confidence Intervals	
Bottom	5.987 to 12.31
Top	108.7 to 113.5
LogIC50	-6.321 to -6.243
HillSlope	-1.165 to -0.9279
Goodness of Fit	
Degrees of Freedom	20
R square	0.9964
Sy.x	2.580
Number of points	
Analyzed	24

Subtask 3



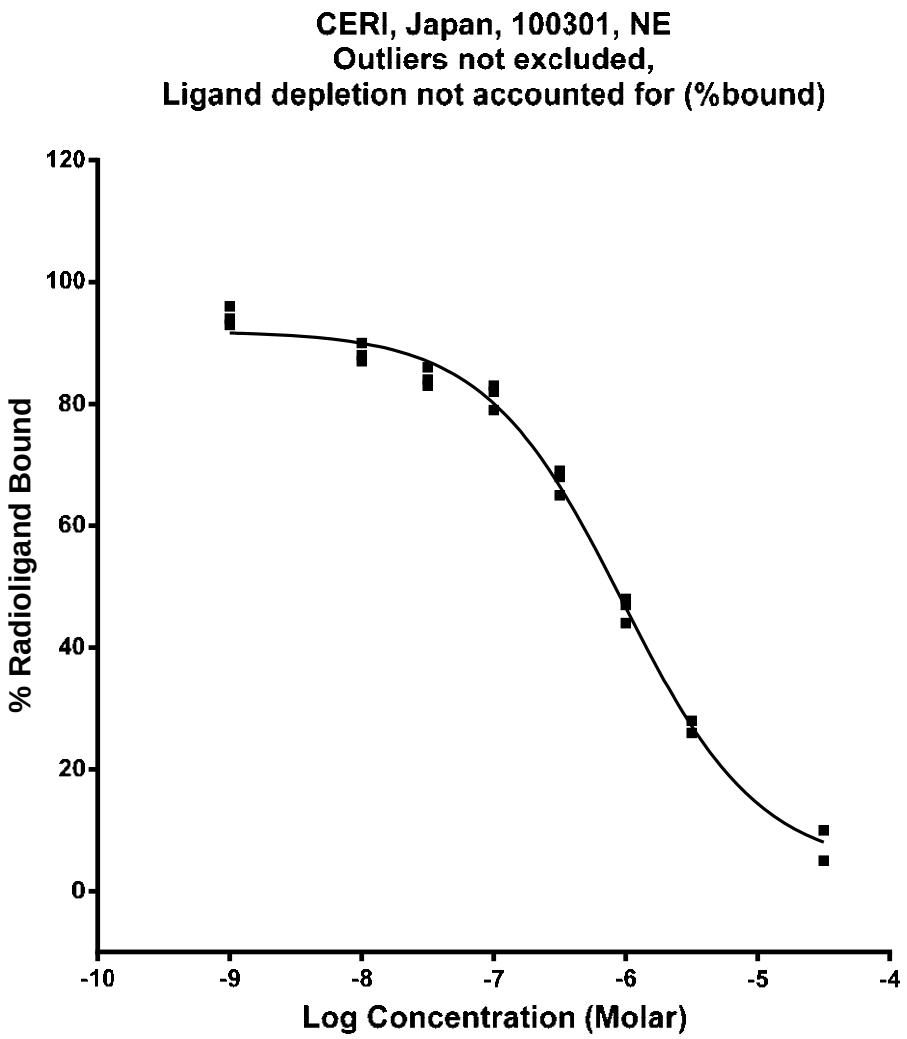
logIC50	
Best-fit values	
Bottom	8.046
Top	94.95
LogIC50	-6.405
HillSlope	-1.173
Std. Error	
Bottom	1.070
Top	0.7996
LogIC50	0.01436
HillSlope	0.05567
95% Confidence Intervals	
Bottom	5.814 to 10.28
Top	93.29 to 96.62
LogIC50	-6.435 to -6.375
HillSlope	-1.290 to -1.057
Goodness of Fit	
Degrees of Freedom	20
R square	0.9973
Sy.x	1.937
Number of points	
Analyzed	24

Subtask 3



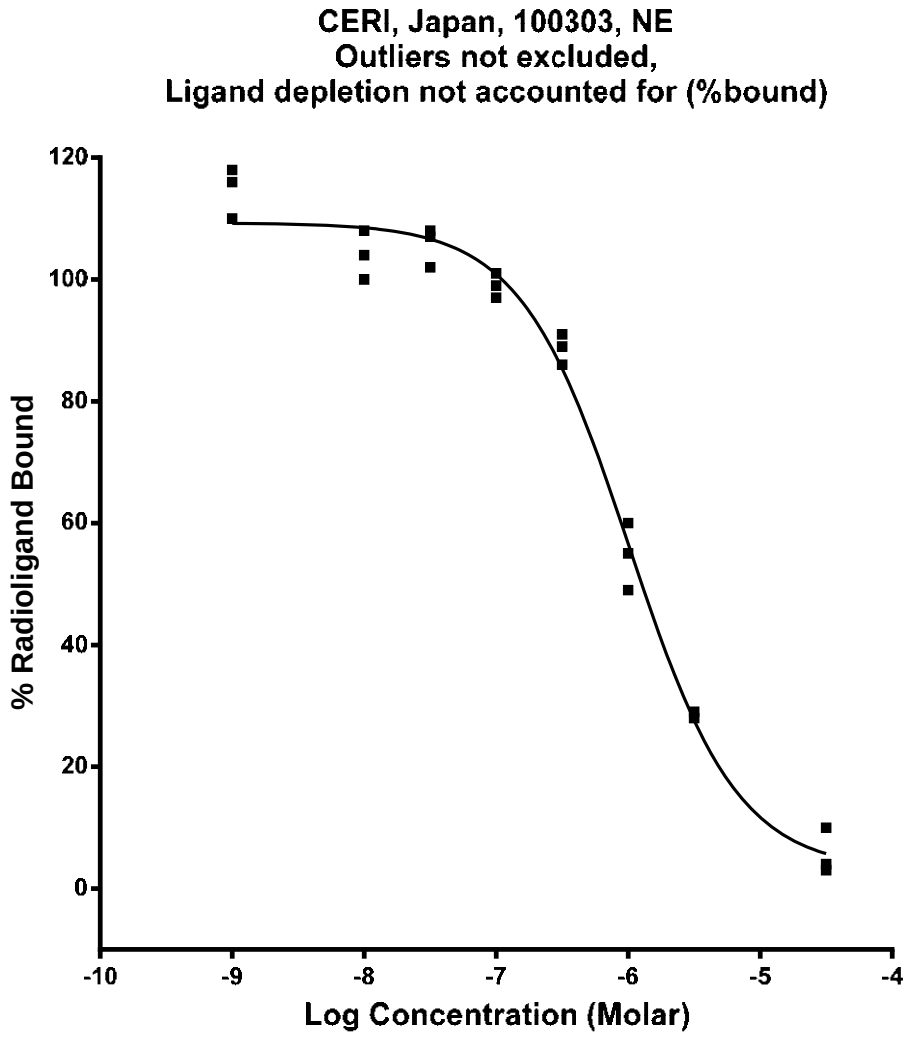
logIC50	
Best-fit values	
Bottom	8.090
Top	98.47
LogIC50	-6.332
HillSlope	-1.370
Std. Error	
Bottom	1.717
Top	1.227
LogIC50	0.02171
HillSlope	0.1069
95% Confidence Intervals	
Bottom	4.508 to 11.67
Top	95.91 to 101.0
LogIC50	-6.377 to -6.287
HillSlope	-1.593 to -1.147
Goodness of Fit	
Degrees of Freedom	20
R square	0.9933
Sy.x	3.290
Number of points	
Analyzed	24

Subtask 3



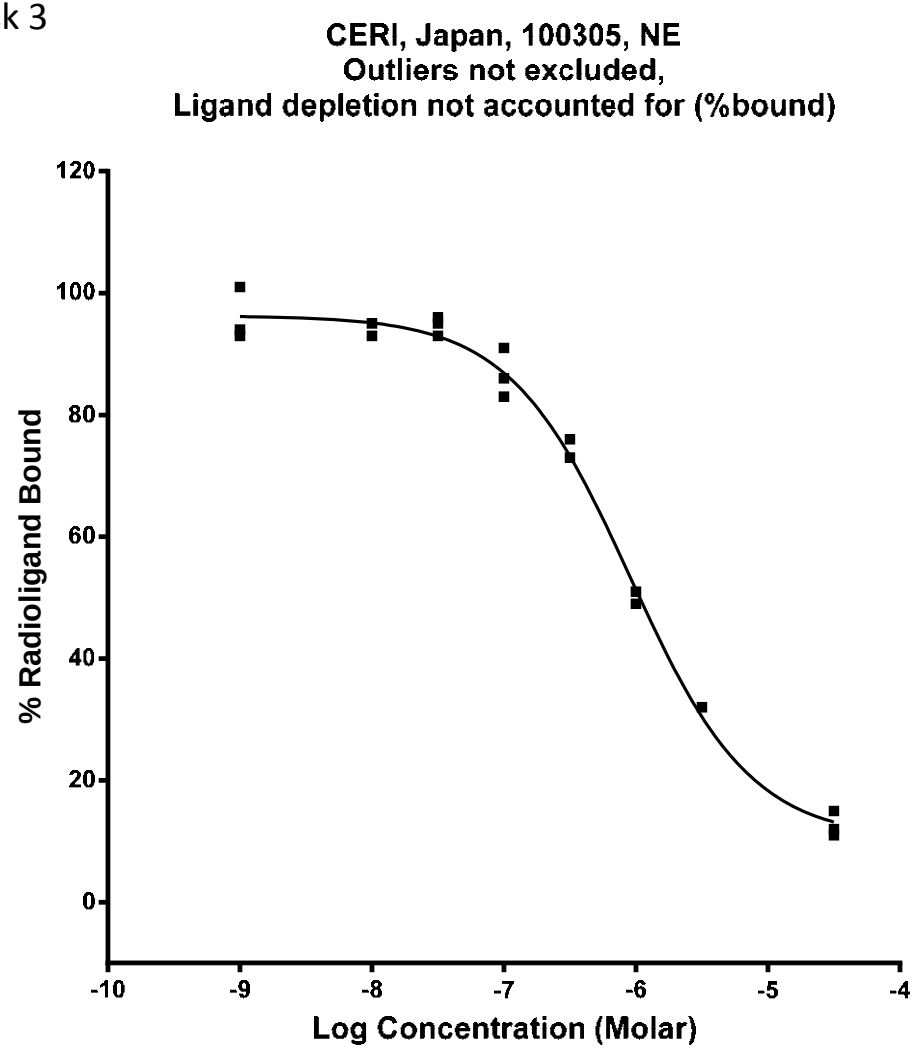
logIC50	
Best-fit values	
Bottom	3.591
Top	91.93
LogIC50	-6.080
HillSlope	-0.8333
Std. Error	
Bottom	2.202
Top	1.105
LogIC50	0.02210
HillSlope	0.05743
95% Confidence Intervals	
Bottom	-1.004 to 8.185
Top	89.63 to 94.24
LogIC50	-6.126 to -6.034
HillSlope	-0.9531 to -0.7135
Goodness of Fit	
Degrees of Freedom	20
R square	0.9944
Sy.x	2.429
Number of points	
Analyzed	24

Subtask 3



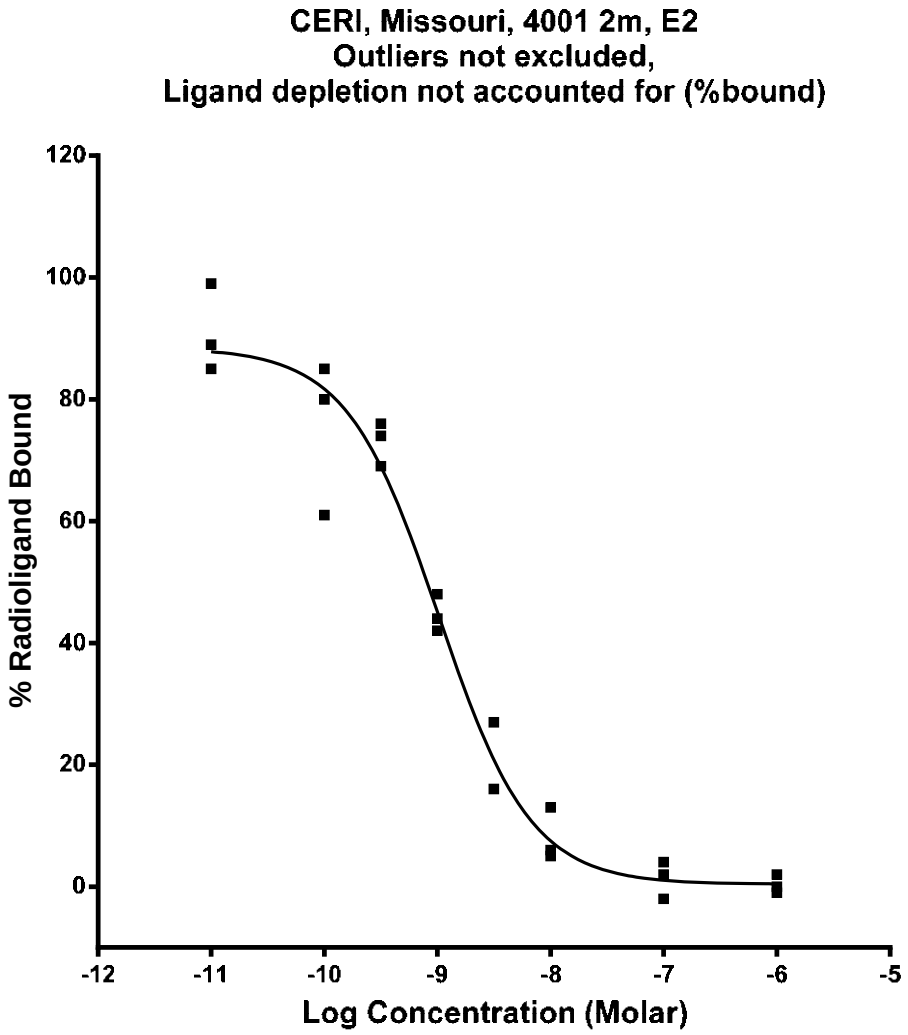
logIC50	
Best-fit values	
Bottom	2.993
Top	109.3
LogIC50	-5.896
HillSlope	-1.057
Std. Error	
Bottom	3.292
Top	1.693
LogIC50	0.03084
HillSlope	0.1022
95% Confidence Intervals	
Bottom	-3.874 to 9.860
Top	105.7 to 112.8
LogIC50	-5.961 to -5.832
HillSlope	-1.271 to -0.8442
Goodness of Fit	
Degrees of Freedom	20
R square	0.9883
Sy.x	4.511
Number of points	
Analyzed	24

Subtask 3



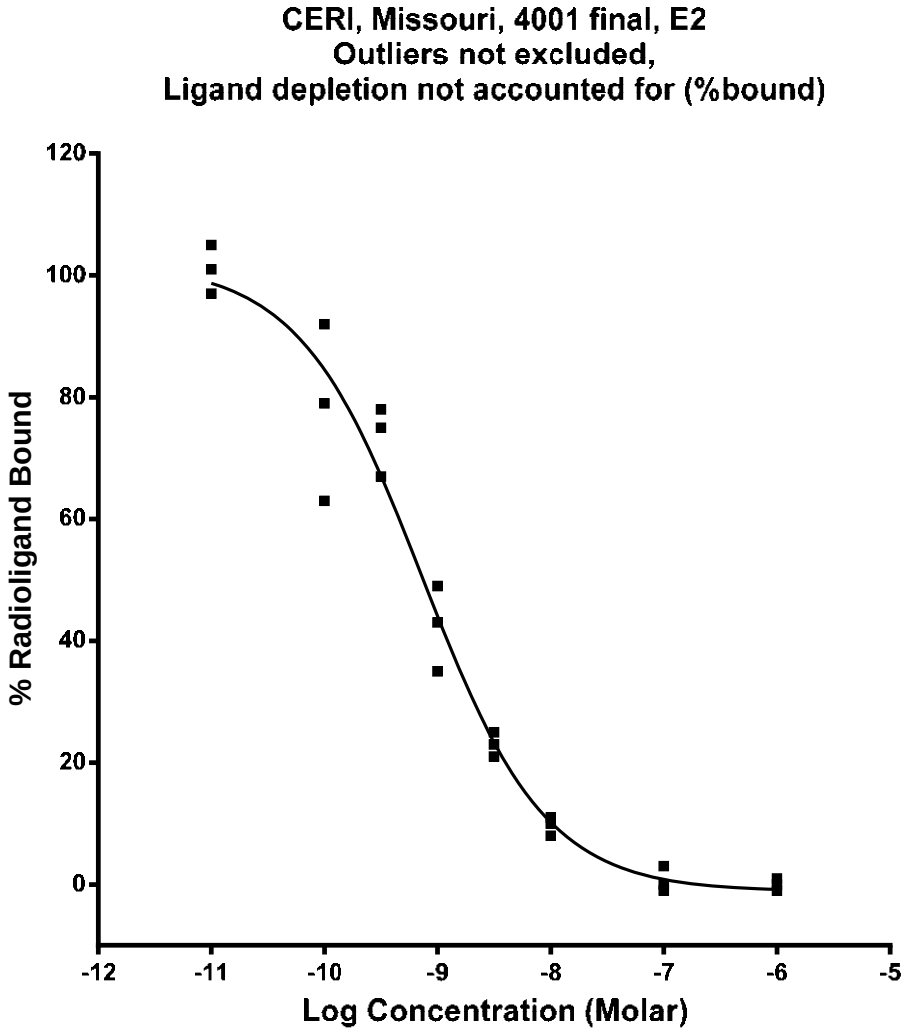
logIC50	
Best-fit values	
Bottom	10.33
Top	96.27
LogIC50	-5.970
HillSlope	-0.9508
Std. Error	
Bottom	1.984
Top	1.044
LogIC50	0.02257
HillSlope	0.06570
95% Confidence Intervals	
Bottom	6.192 to 14.47
Top	94.10 to 98.45
LogIC50	-6.017 to -5.923
HillSlope	-1.088 to -0.8137
Goodness of Fit	
Degrees of Freedom	20
R square	0.9940
Sy.x	2.546
Number of points	
Analyzed	24

Subtask 1



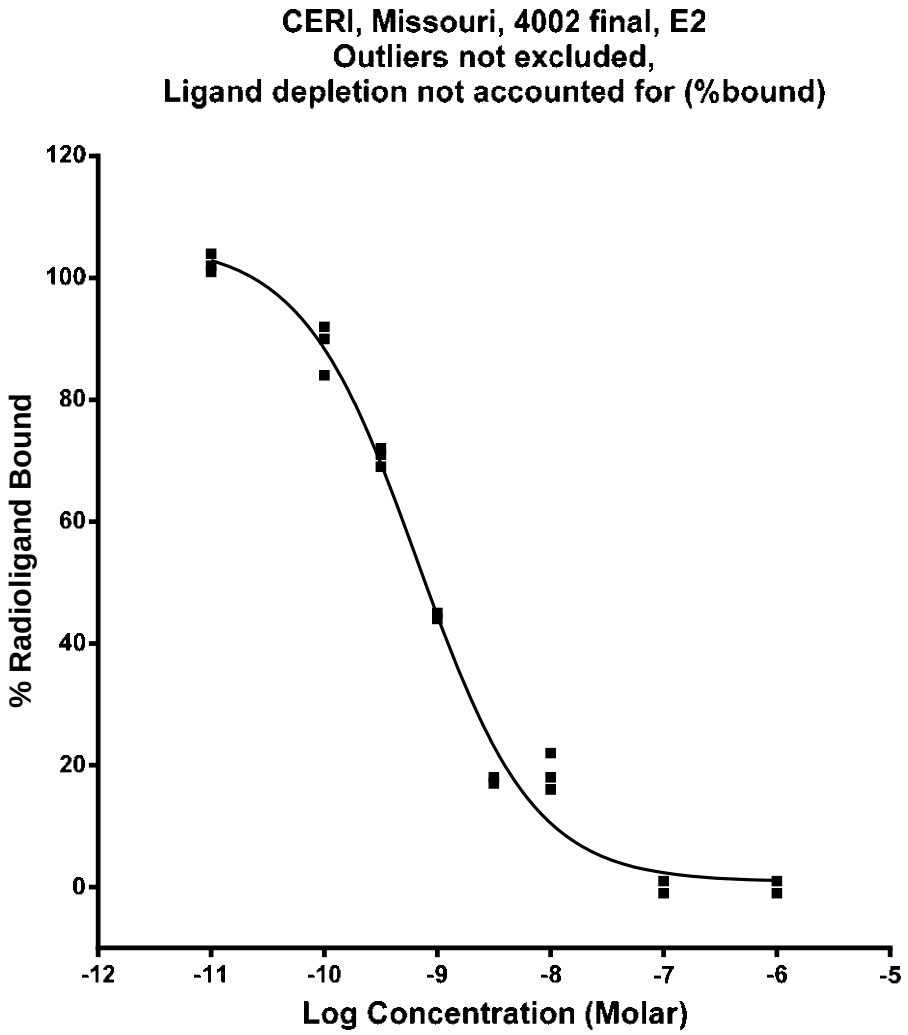
logIC50	
Best-fit values	
Bottom	0.3780
Top	88.43
LogIC50	-9.091
HillSlope	-1.068
Std. Error	
Bottom	2.618
Top	3.574
LogIC50	0.05069
HillSlope	0.1585
95% Confidence Intervals	
Bottom	-5.084 to 5.840
Top	80.97 to 95.89
LogIC50	-9.197 to -8.986
HillSlope	-1.399 to -0.7374
Goodness of Fit	
Degrees of Freedom	20
R square	0.9718
Sy.x	6.390
Number of points	
Analyzed	24

Subtask 1



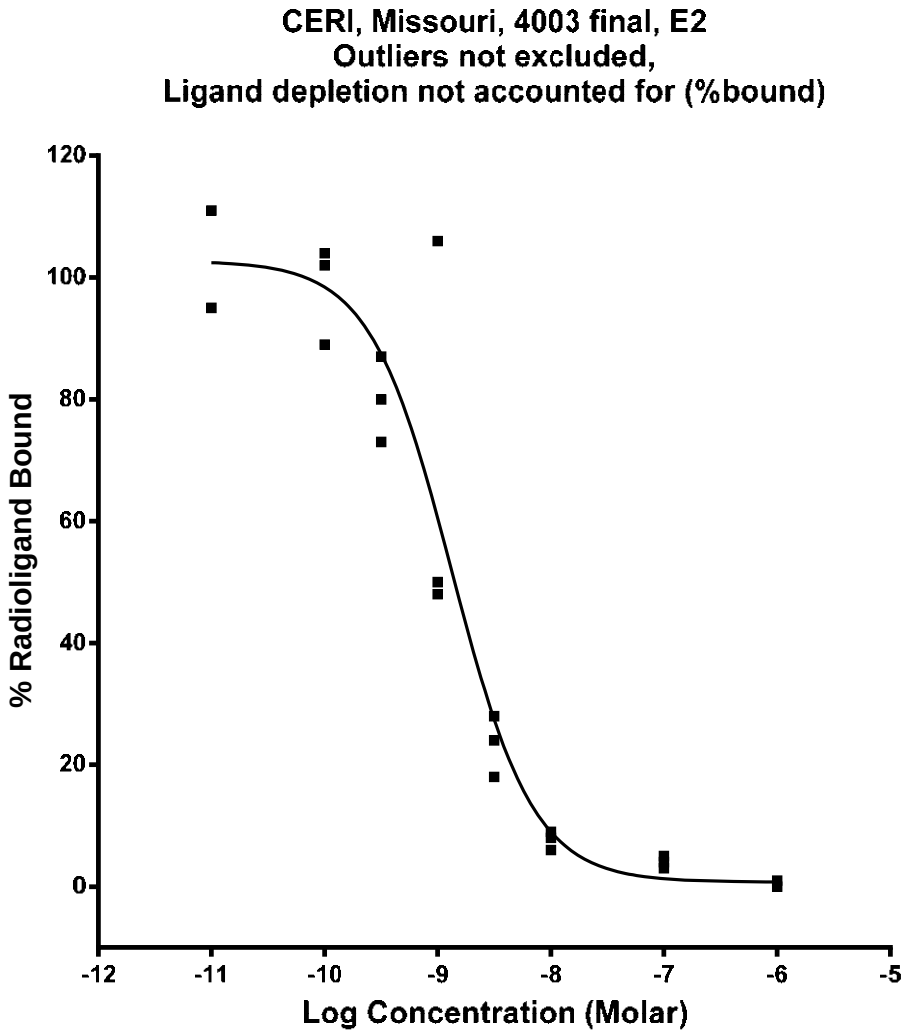
logIC50	
Best-fit values	
Bottom	-1.150
Top	101.9
LogIC50	-9.126
HillSlope	-0.7993
Std. Error	
Bottom	3.084
Top	5.124
LogIC50	0.05279
HillSlope	0.1164
95% Confidence Intervals	
Bottom	-7.584 to 5.284
Top	91.25 to 112.6
LogIC50	-9.236 to -9.016
HillSlope	-1.042 to -0.5564
Goodness of Fit	
Degrees of Freedom	20
R square	0.9720
Sy.x	6.754
Number of points	
Analyzed	24

Subtask 1



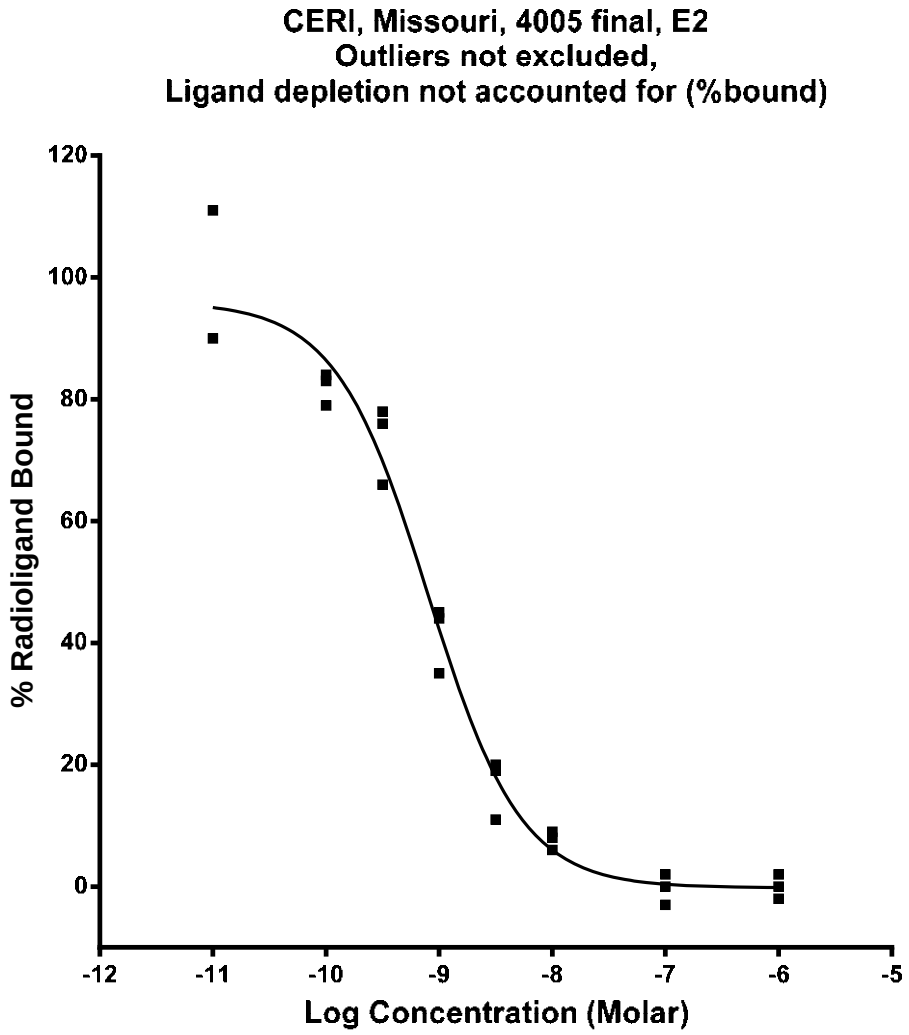
logIC50	
Best-fit values	
Bottom	0.8615
Top	105.7
LogIC50	-9.108
HillSlope	-0.8488
Std. Error	
Bottom	1.931
Top	3.230
LogIC50	0.03267
HillSlope	0.07793
95% Confidence Intervals	
Bottom	-3.167 to 4.890
Top	99.00 to 112.5
LogIC50	-9.176 to -9.040
HillSlope	-1.011 to -0.6863
Goodness of Fit	
Degrees of Freedom	20
R square	0.9885
Sy.x	4.425
Number of points	
Analyzed	24

Subtask 1



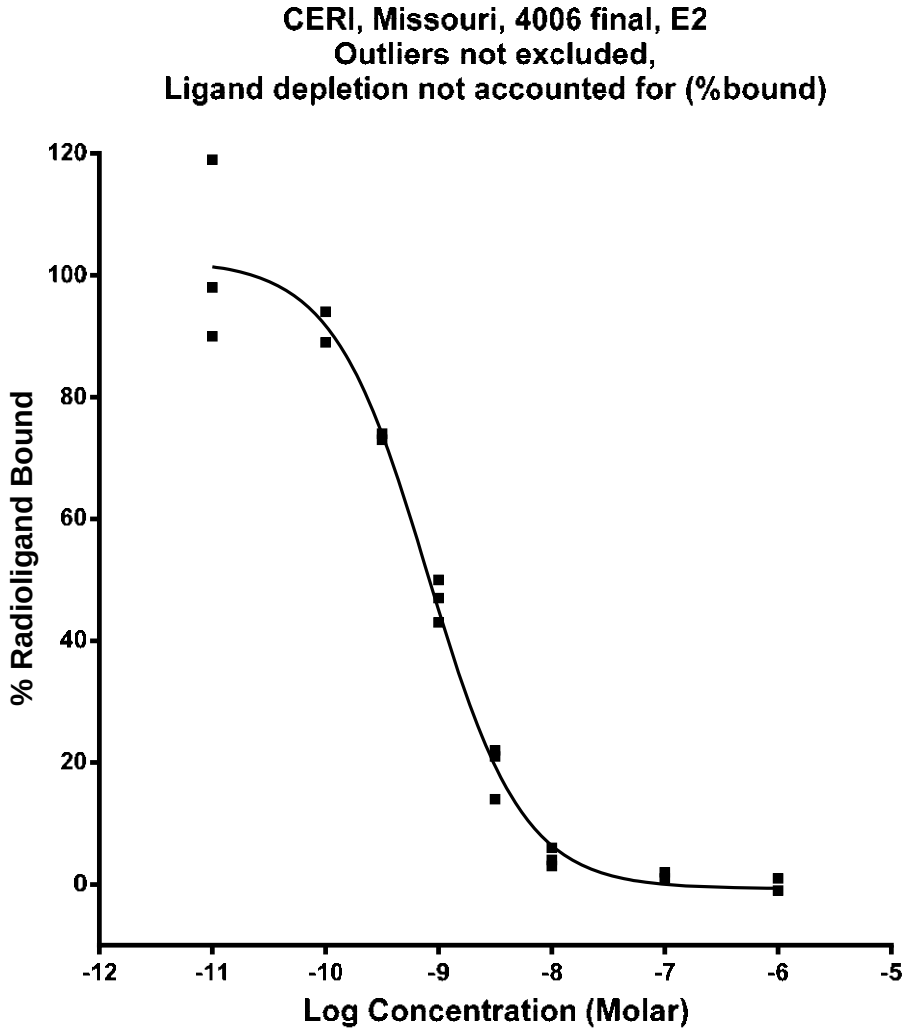
logIC50	
Best-fit values	
Bottom	0.7419
Top	102.7
LogIC50	-8.850
HillSlope	-1.206
Std. Error	
Bottom	5.003
Top	6.100
LogIC50	0.07644
HillSlope	0.2989
95% Confidence Intervals	
Bottom	-9.694 to 11.18
Top	90.00 to 115.4
LogIC50	-9.009 to -8.690
HillSlope	-1.830 to -0.5826
Goodness of Fit	
Degrees of Freedom	20
R square	0.9299
Sy.x	12.39
Number of points	
Analyzed	24

Subtask 1



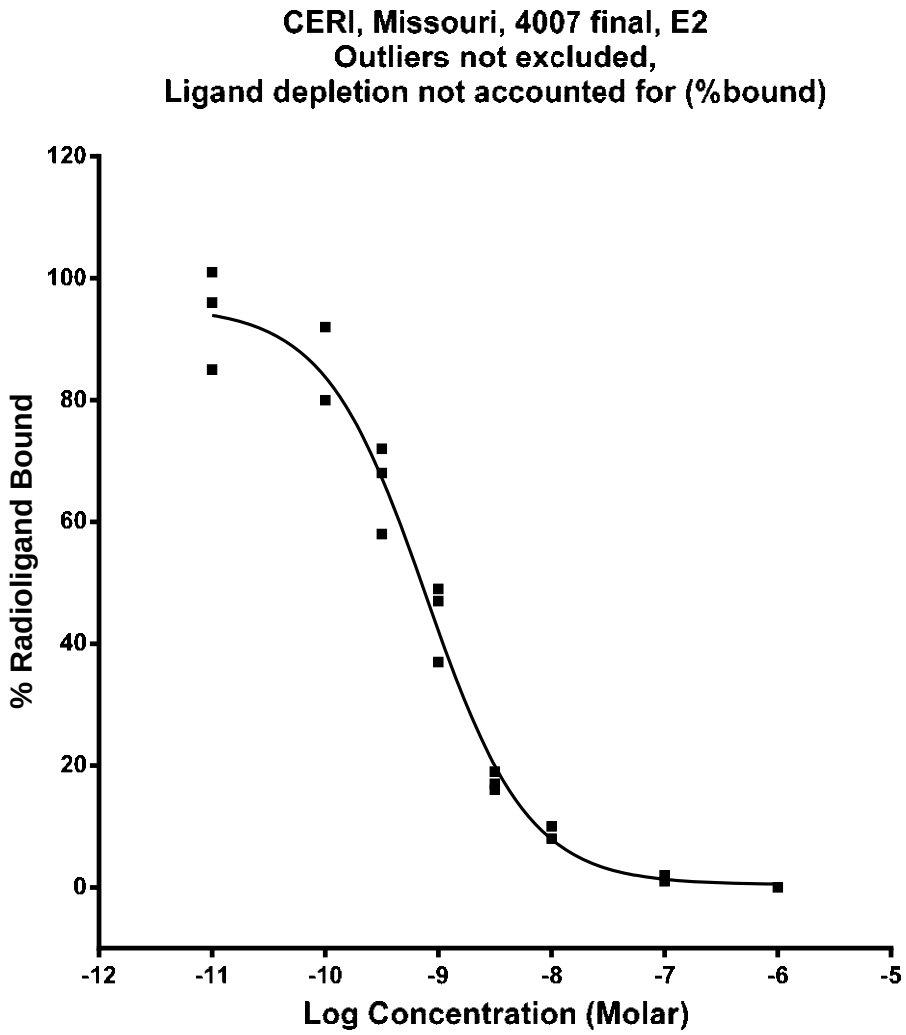
logIC50	
Best-fit values	
Bottom	-0.2007
Top	95.96
LogIC50	-9.133
HillSlope	-1.060
Std. Error	
Bottom	2.279
Top	3.374
LogIC50	0.04053
HillSlope	0.1292
95% Confidence Intervals	
Bottom	-4.955 to 4.553
Top	88.93 to 103.0
LogIC50	-9.217 to -9.048
HillSlope	-1.329 to -0.7903
Goodness of Fit	
Degrees of Freedom	20
R square	0.9805
Sy.x	5.701
Number of points	
Analyzed	24

Subtask 1



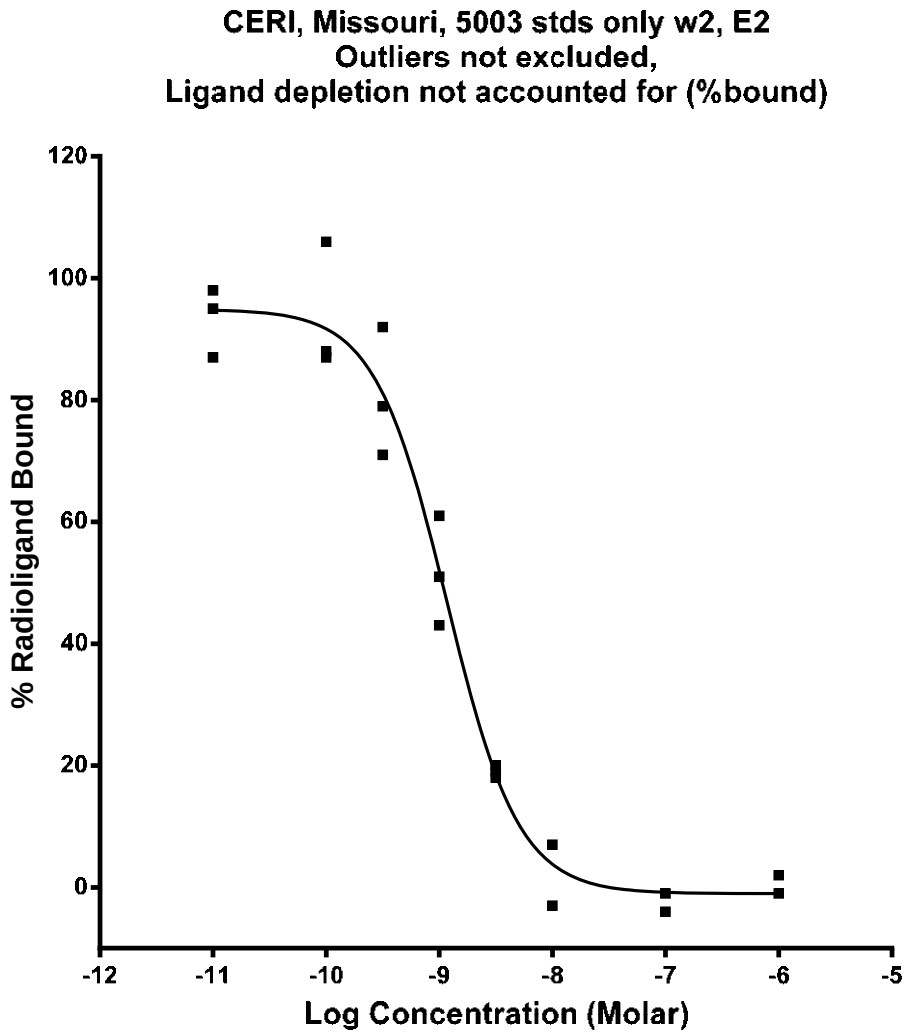
logIC50	
Best-fit values	
Bottom	-0.6895
Top	102.5
LogIC50	-9.081
HillSlope	-1.034
Std. Error	
Bottom	2.158
Top	3.212
LogIC50	0.03560
HillSlope	0.1102
95% Confidence Intervals	
Bottom	-5.190 to 3.811
Top	95.81 to 109.2
LogIC50	-9.156 to -9.007
HillSlope	-1.264 to -0.8039
Goodness of Fit	
Degrees of Freedom	20
R square	0.9849
Sy.x	5.342
Number of points	
Analyzed	24

Subtask 1



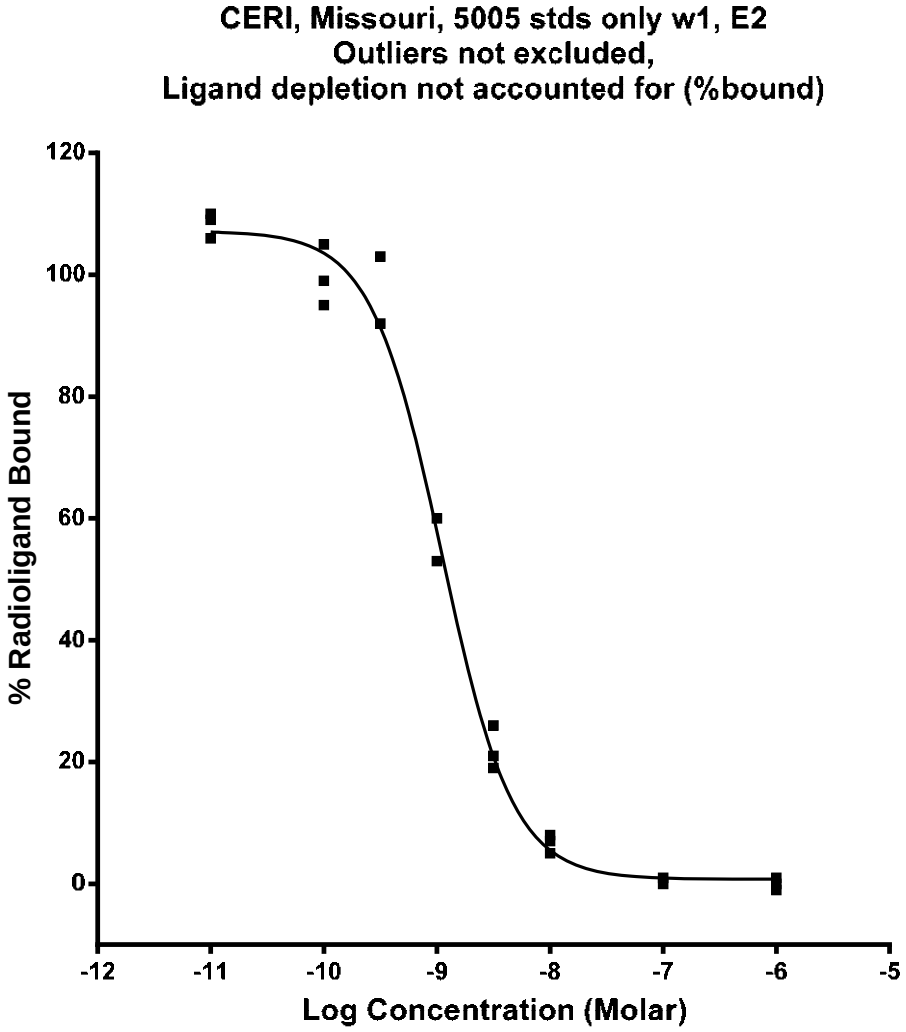
logIC50	
Best-fit values	
Bottom	0.4471
Top	95.29
LogIC50	-9.150
HillSlope	-0.9647
Std. Error	
Bottom	2.015
Top	3.086
LogIC50	0.03703
HillSlope	0.1028
95% Confidence Intervals	
Bottom	-3.756 to 4.650
Top	88.86 to 101.7
LogIC50	-9.227 to -9.073
HillSlope	-1.179 to -0.7503
Goodness of Fit	
Degrees of Freedom	20
R square	0.9846
Sy.x	4.856
Number of points	
Analyzed	24

Subtask 1



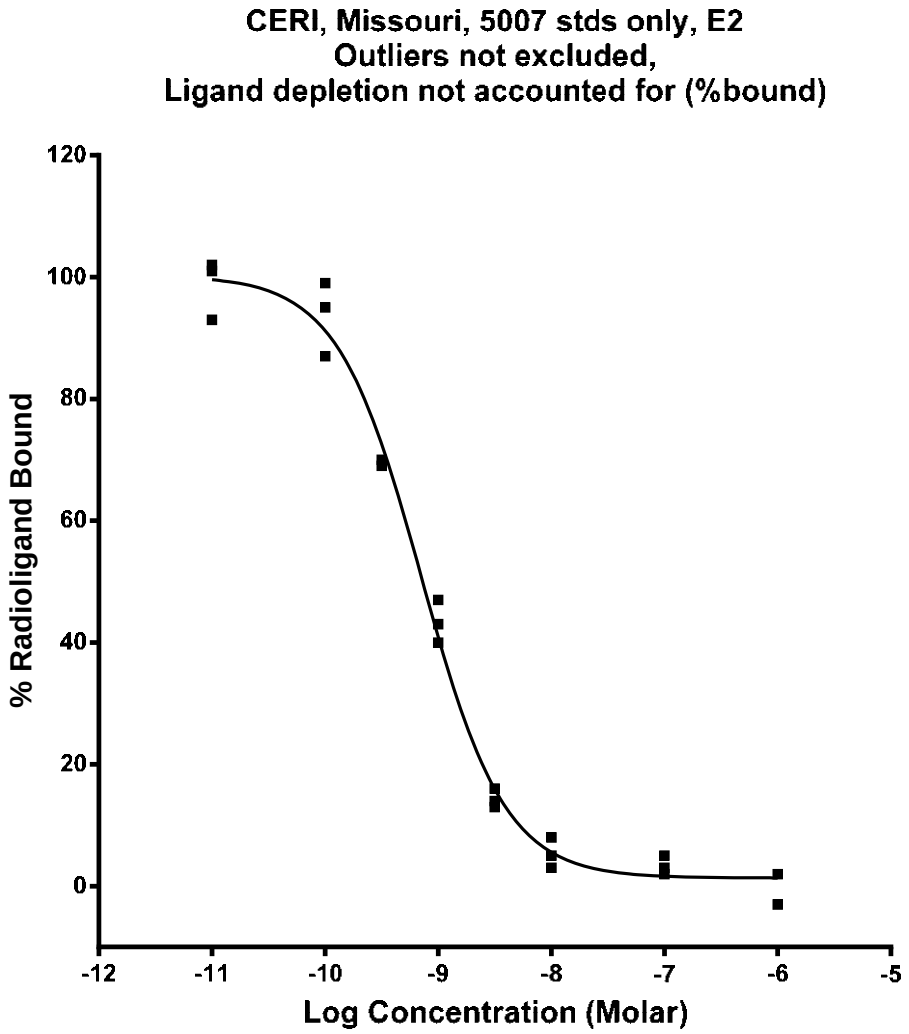
logIC50	
Best-fit values	
Bottom	-1.036
Top	94.90
LogIC50	-8.974
HillSlope	-1.369
Std. Error	
Bottom	2.406
Top	2.972
LogIC50	0.03870
HillSlope	0.1872
95% Confidence Intervals	
Bottom	-6.054 to 3.982
Top	88.70 to 101.1
LogIC50	-9.054 to -8.893
HillSlope	-1.759 to -0.9781
Goodness of Fit	
Degrees of Freedom	20
R square	0.9795
Sy.x	6.278
Number of points	
Analyzed	24

Subtask 1



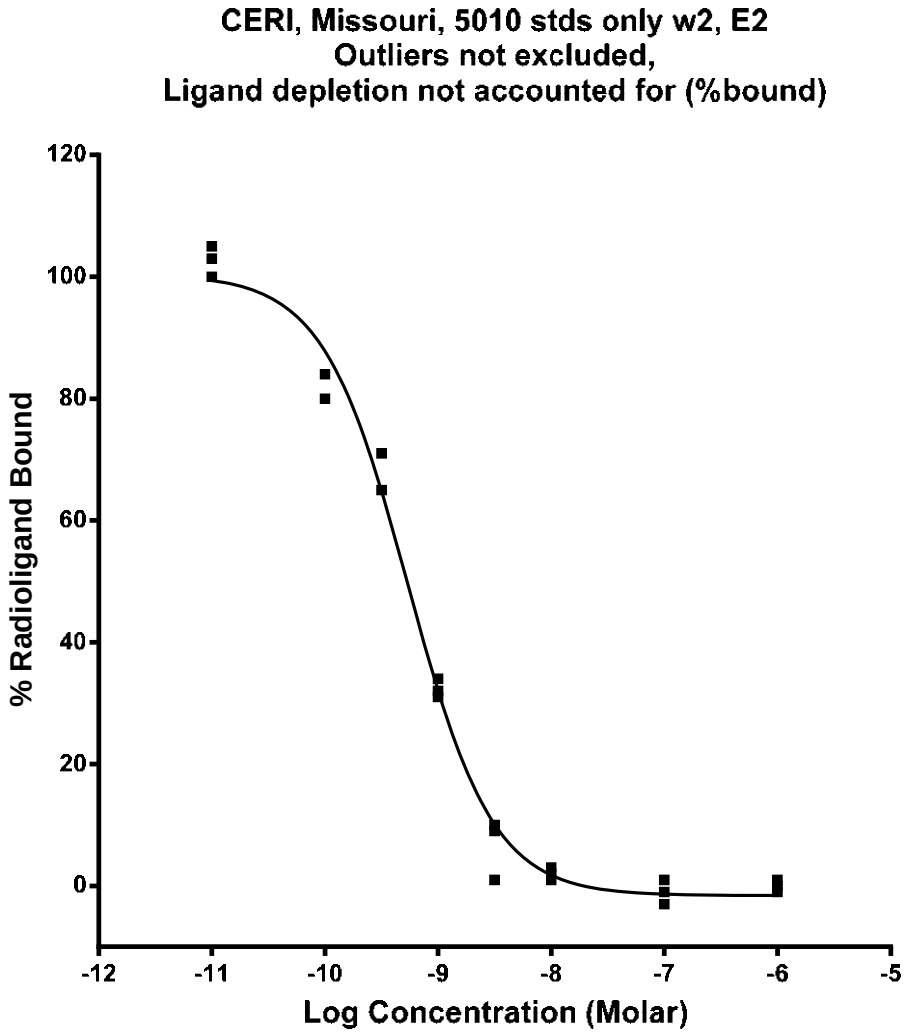
logIC50	
Best-fit values	
Bottom	0.7431
Top	107.2
LogIC50	-8.908
HillSlope	-1.392
Std. Error	
Bottom	1.613
Top	2.010
LogIC50	0.02356
HillSlope	0.1230
95% Confidence Intervals	
Bottom	-2.632 to 4.119
Top	102.9 to 111.4
LogIC50	-8.958 to -8.859
HillSlope	-1.649 to -1.134
Goodness of Fit	
Degrees of Freedom	19
R square	0.9924
Sy.x	4.234
Number of points	
Analyzed	23

Subtask 1



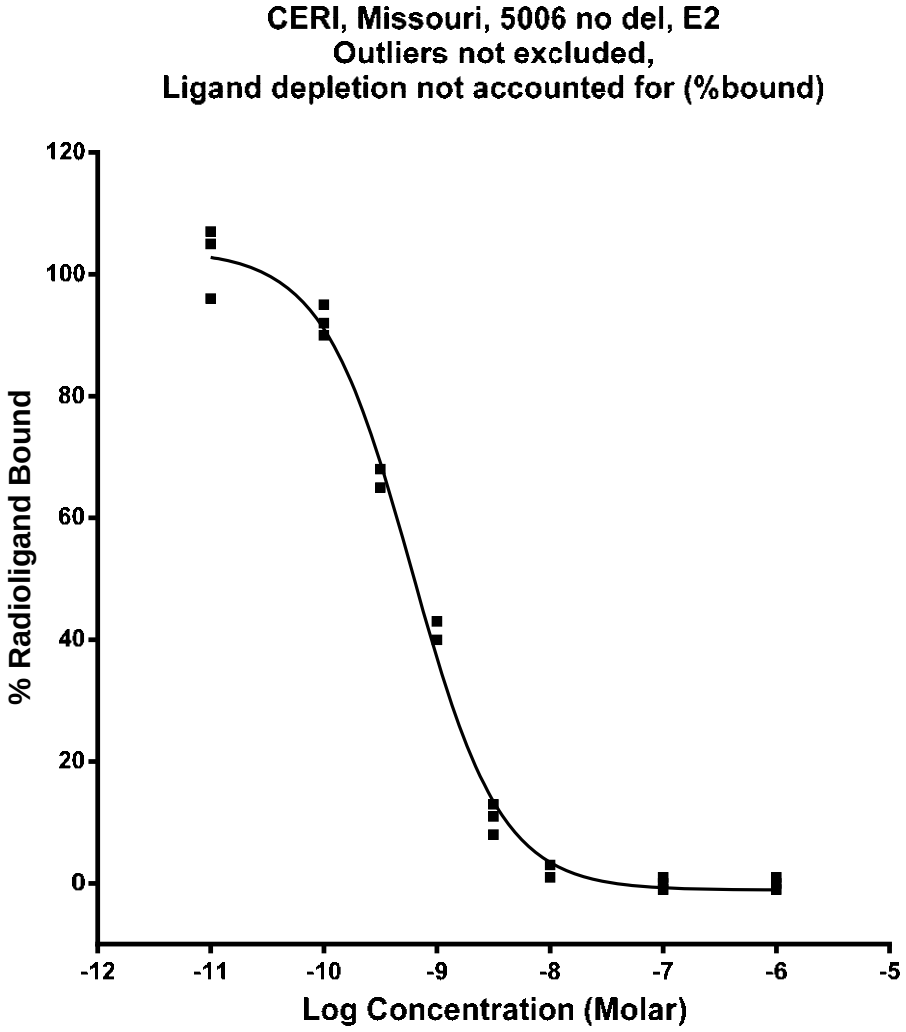
logIC50	
Best-fit values	
Bottom	1.330
Top	100.2
LogIC50	-9.138
HillSlope	-1.173
Std. Error	
Bottom	1.394
Top	2.099
LogIC50	0.02382
HillSlope	0.08994
95% Confidence Intervals	
Bottom	-1.577 to 4.238
Top	95.86 to 104.6
LogIC50	-9.188 to -9.089
HillSlope	-1.361 to -0.9856
Goodness of Fit	
Degrees of Freedom	20
R square	0.9925
Sy.x	3.677
Number of points	
Analyzed	24

Subtask 1



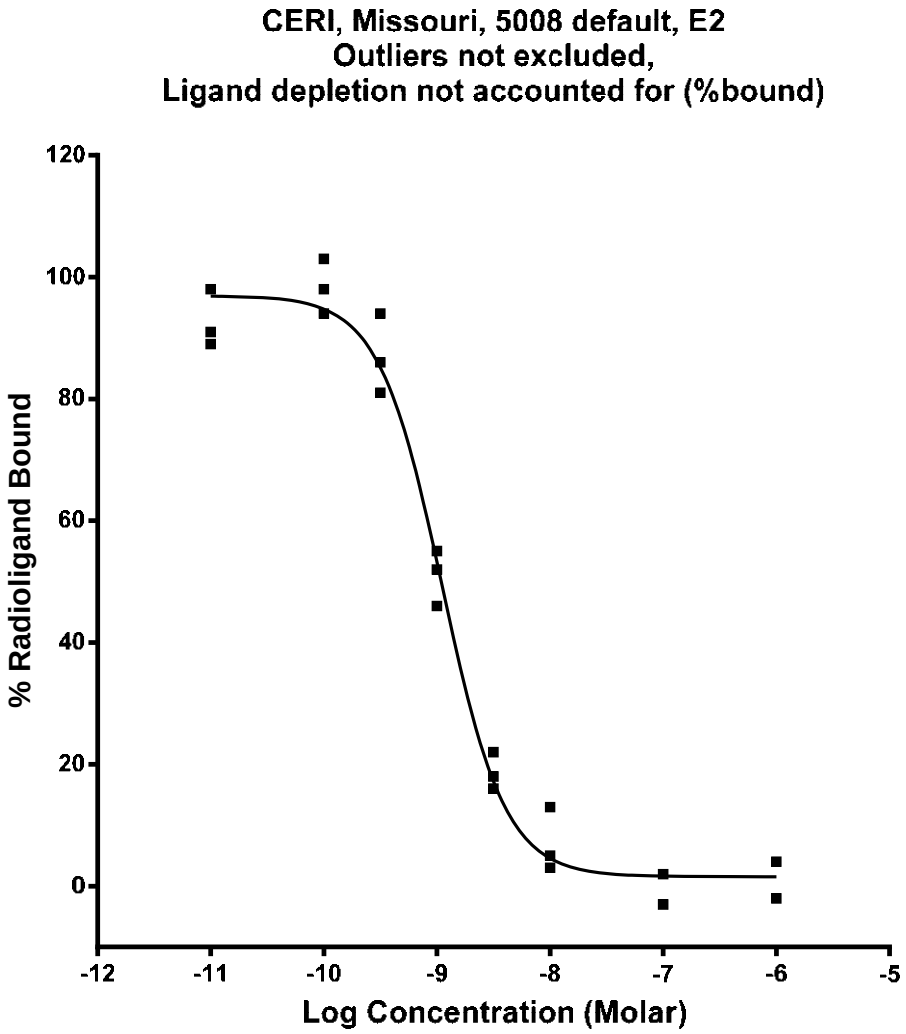
logIC50	
Best-fit values	
Bottom	-1.597
Top	100.4
LogIC50	-9.274
HillSlope	-1.159
Std. Error	
Bottom	1.578
Top	2.590
LogIC50	0.02714
HillSlope	0.1010
95% Confidence Intervals	
Bottom	-4.889 to 1.694
Top	94.97 to 105.8
LogIC50	-9.331 to -9.218
HillSlope	-1.370 to -0.9486
Goodness of Fit	
Degrees of Freedom	20
R square	0.9901
Sy.x	4.275
Number of points	
Analyzed	24

Subtask 2



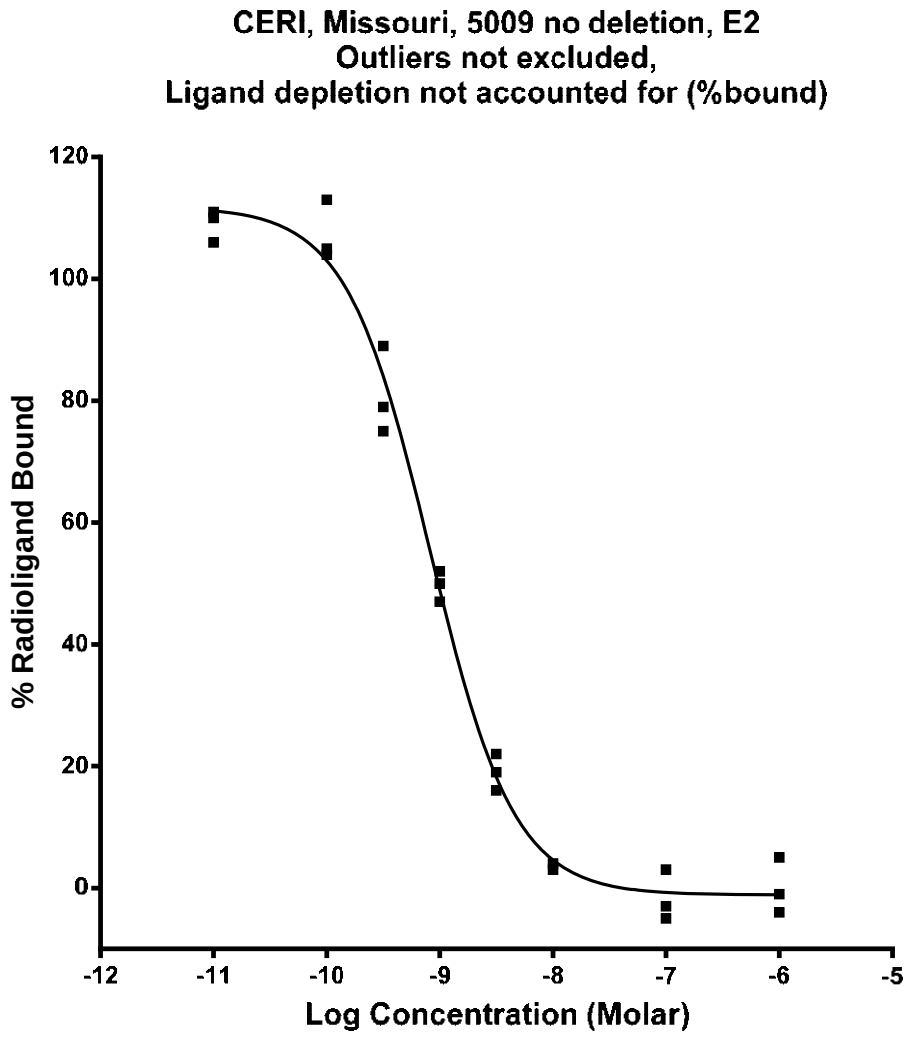
logIC50	
Best-fit values	
Bottom	-1.100
Top	103.9
LogIC50	-9.201
HillSlope	-1.099
Std. Error	
Bottom	1.284
Top	2.065
LogIC50	0.02133
HillSlope	0.07305
95% Confidence Intervals	
Bottom	-3.778 to 1.579
Top	99.60 to 108.2
LogIC50	-9.246 to -9.157
HillSlope	-1.251 to -0.9464
Goodness of Fit	
Degrees of Freedom	20
R square	0.9941
Sy.x	3.367
Number of points	
Analyzed	24

Subtask 2



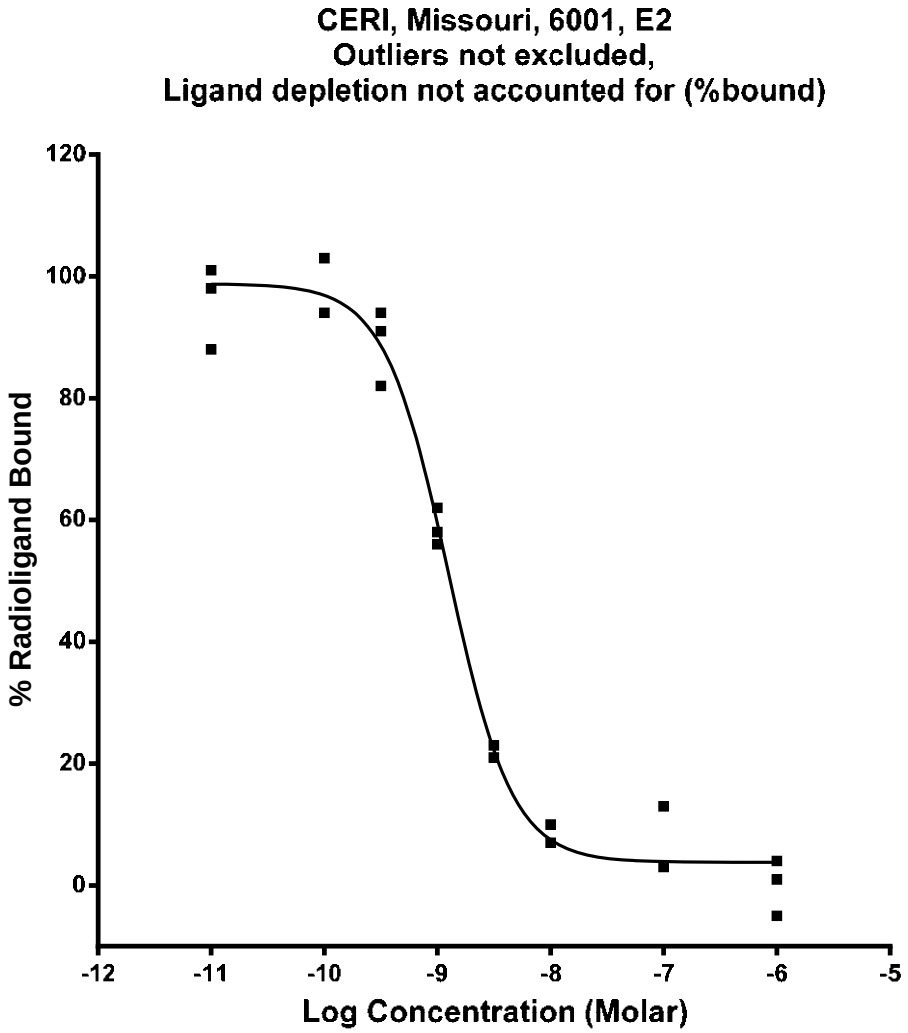
logIC50	
Best-fit values	
Bottom	1.561
Top	96.94
LogIC50	-8.962
HillSlope	-1.560
Std. Error	
Bottom	1.805
Top	2.206
LogIC50	0.02798
HillSlope	0.1751
95% Confidence Intervals	
Bottom	-2.204 to 5.326
Top	92.33 to 101.5
LogIC50	-9.021 to -8.904
HillSlope	-1.925 to -1.194
Goodness of Fit	
Degrees of Freedom	20
R square	0.9878
Sy.x	4.906
Number of points	
Analyzed	24

Subtask 2



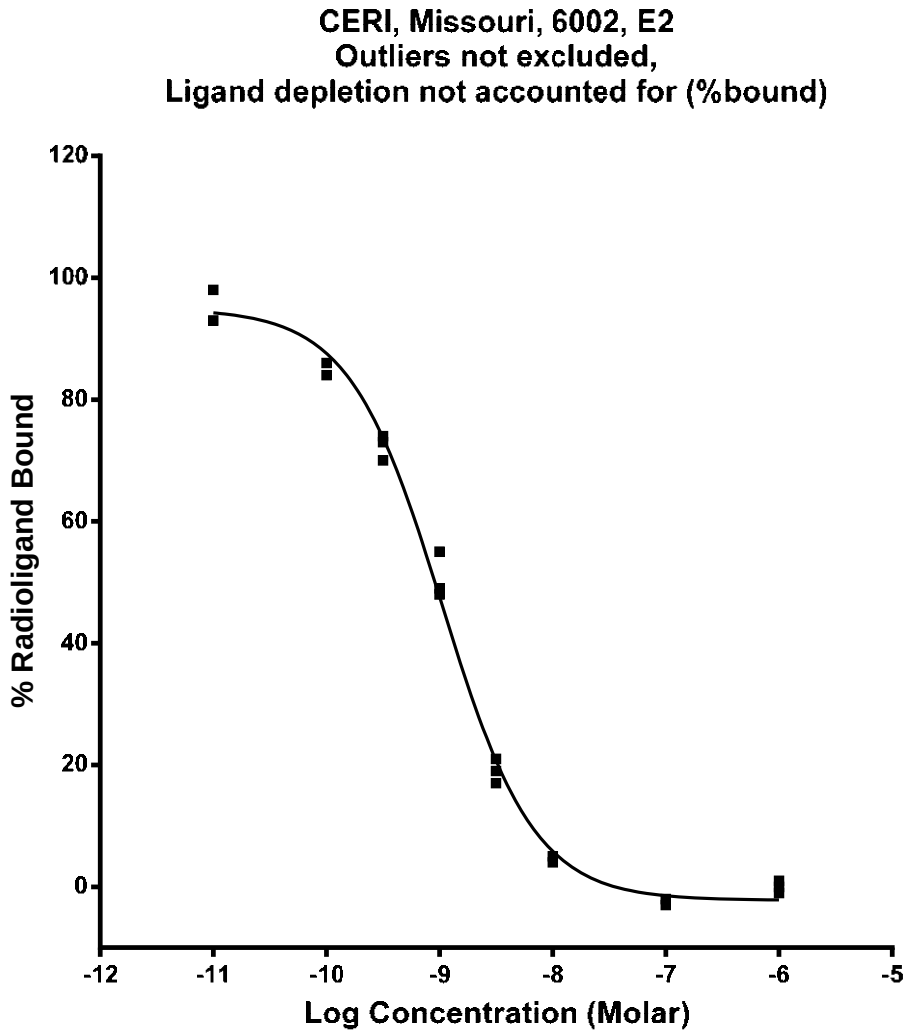
logIC50	
Best-fit values	
Bottom	-1.141
Top	111.8
LogIC50	-9.015
HillSlope	-1.172
Std. Error	
Bottom	1.694
Top	2.432
LogIC50	0.02509
HillSlope	0.09370
95% Confidence Intervals	
Bottom	-4.675 to 2.393
Top	106.7 to 116.9
LogIC50	-9.067 to -8.962
HillSlope	-1.367 to -0.9764
Goodness of Fit	
Degrees of Freedom	20
R square	0.9920
Sy.x	4.390
Number of points	
Analyzed	24

Subtask 3



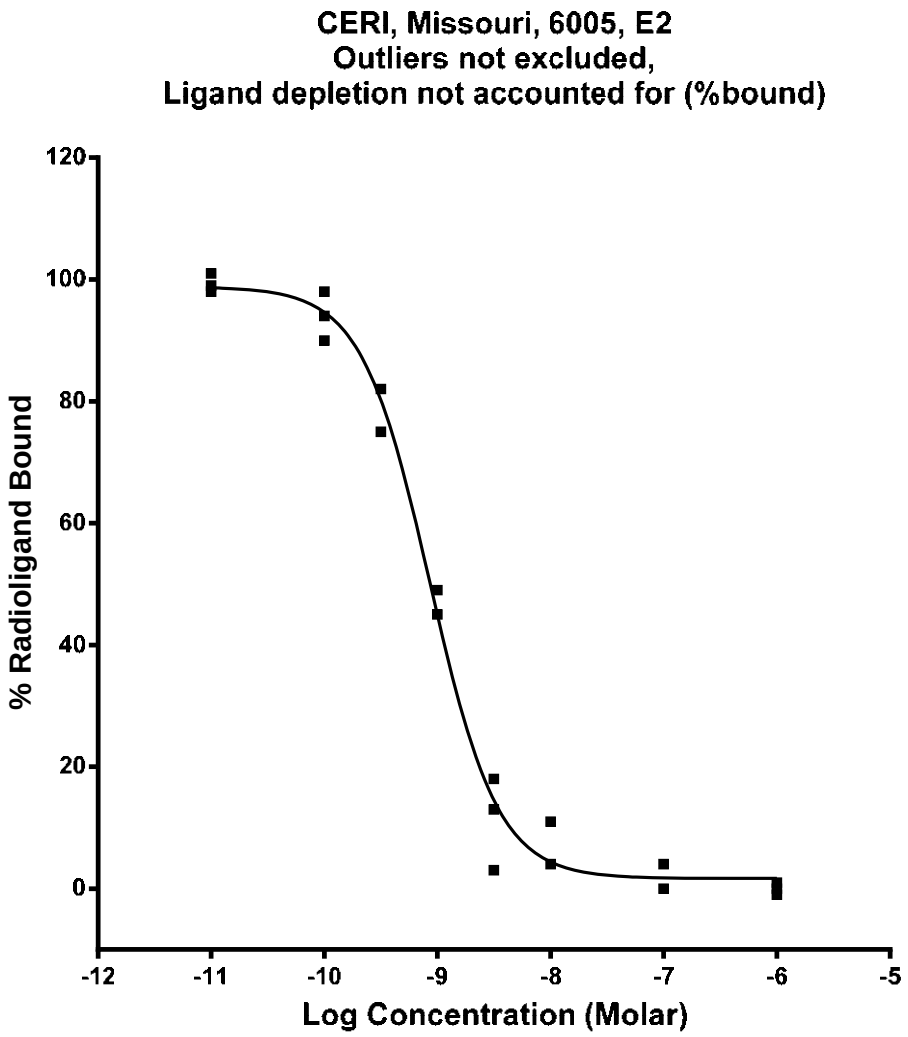
logIC50	
Best-fit values	
Bottom	3.797
Top	98.78
LogIC50	-8.887
HillSlope	-1.542
Std. Error	
Bottom	1.849
Top	2.170
LogIC50	0.02856
HillSlope	0.1714
95% Confidence Intervals	
Bottom	-0.06075 to 7.655
Top	94.25 to 103.3
LogIC50	-8.947 to -8.828
HillSlope	-1.900 to -1.184
Goodness of Fit	
Degrees of Freedom	20
R square	0.9876
Sy.x	4.938
Number of points	
Analyzed	24

Subtask 3



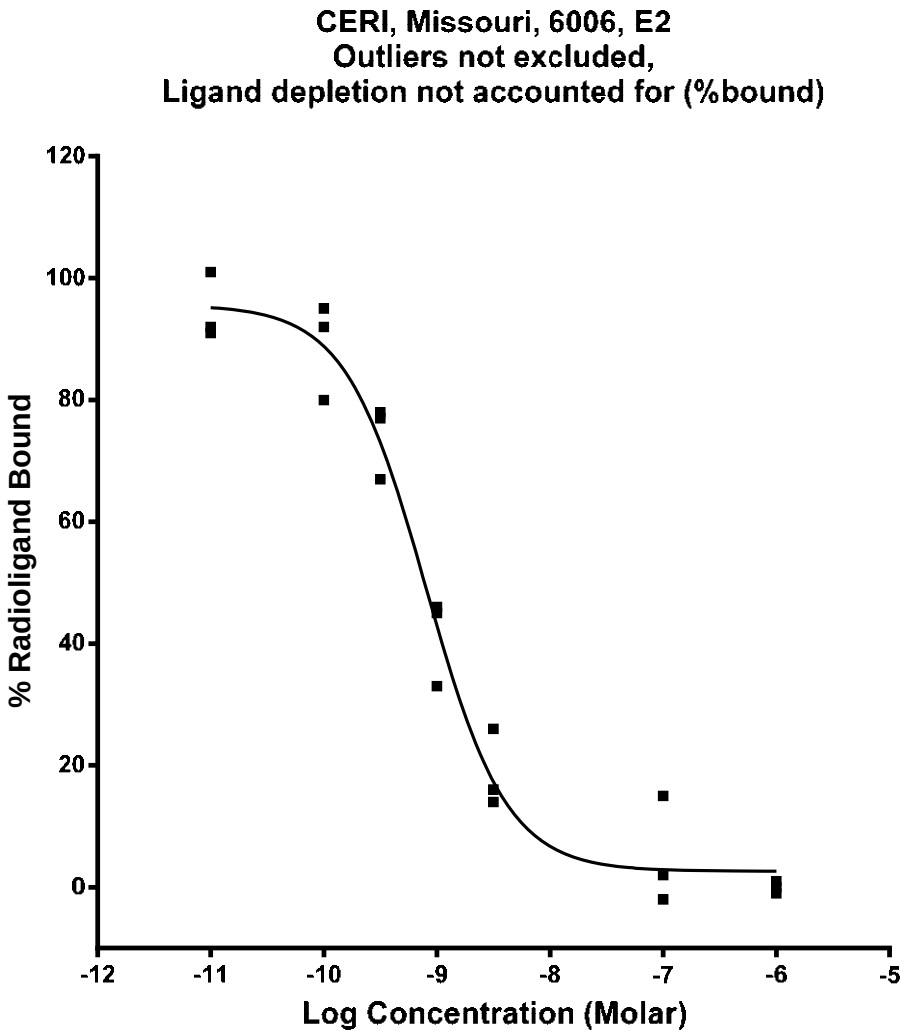
logIC50	
Best-fit values	
Bottom	-2.216
Top	94.92
LogIC50	-9.041
HillSlope	-1.066
Std. Error	
Bottom	1.160
Top	1.576
LogIC50	0.01993
HillSlope	0.06335
95% Confidence Intervals	
Bottom	-4.637 to 0.2048
Top	91.63 to 98.21
LogIC50	-9.083 to -8.999
HillSlope	-1.198 to -0.9336
Goodness of Fit	
Degrees of Freedom	20
R square	0.9954
Sy.x	2.825
Number of points	
Analyzed	24

Subtask 3



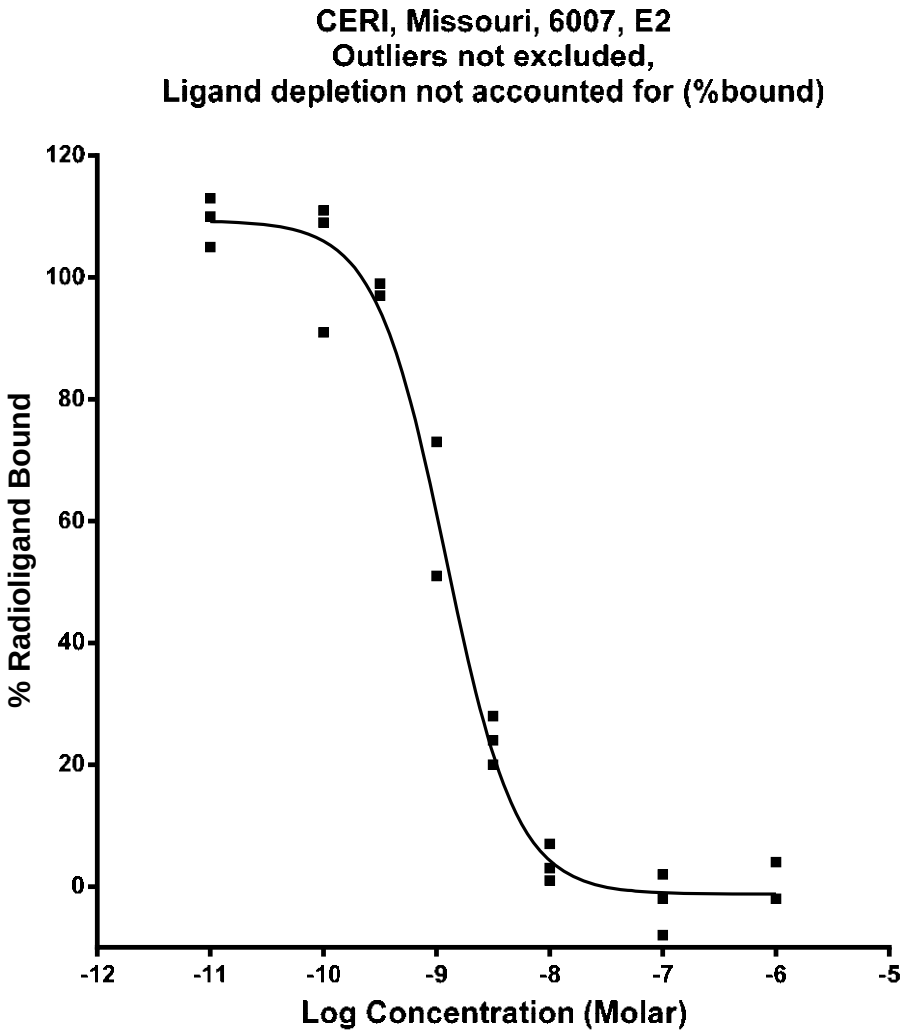
logIC50	
Best-fit values	
Bottom	1.657
Top	98.80
LogIC50	-9.063
HillSlope	-1.443
Std. Error	
Bottom	1.505
Top	2.057
LogIC50	0.02438
HillSlope	0.1320
95% Confidence Intervals	
Bottom	-1.483 to 4.797
Top	94.51 to 103.1
LogIC50	-9.114 to -9.013
HillSlope	-1.719 to -1.168
Goodness of Fit	
Degrees of Freedom	20
R square	0.9911
Sy.x	4.156
Number of points	
Analyzed	24

Subtask 3[4]



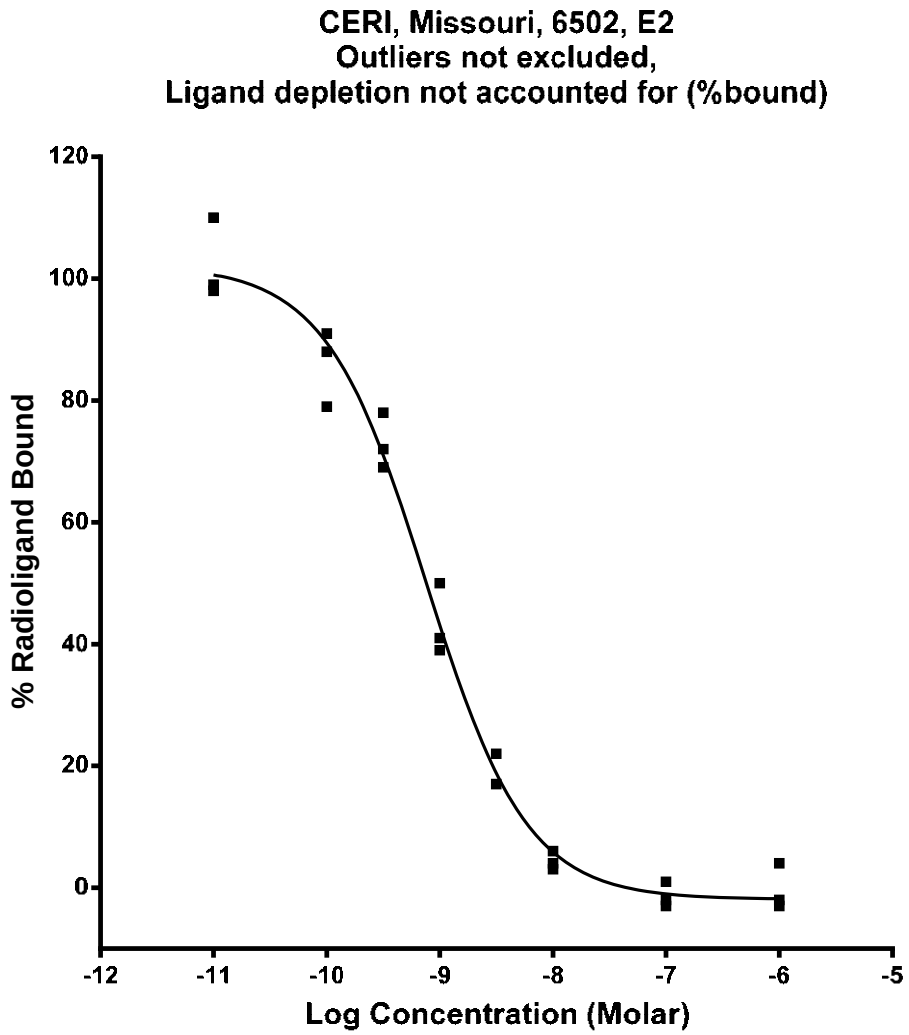
logIC50	
Best-fit values	
Bottom	2.622
Top	95.60
LogIC50	-9.109
HillSlope	-1.220
Std. Error	
Bottom	2.549
Top	3.414
LogIC50	0.04206
HillSlope	0.1720
95% Confidence Intervals	
Bottom	-2.756 to 8.000
Top	88.39 to 102.8
LogIC50	-9.198 to -9.021
HillSlope	-1.583 to -0.8573
Goodness of Fit	
Degrees of Freedom	17
R square	0.9775
Sy.x	6.227
Number of points	
Analyzed	21

Subtask 3



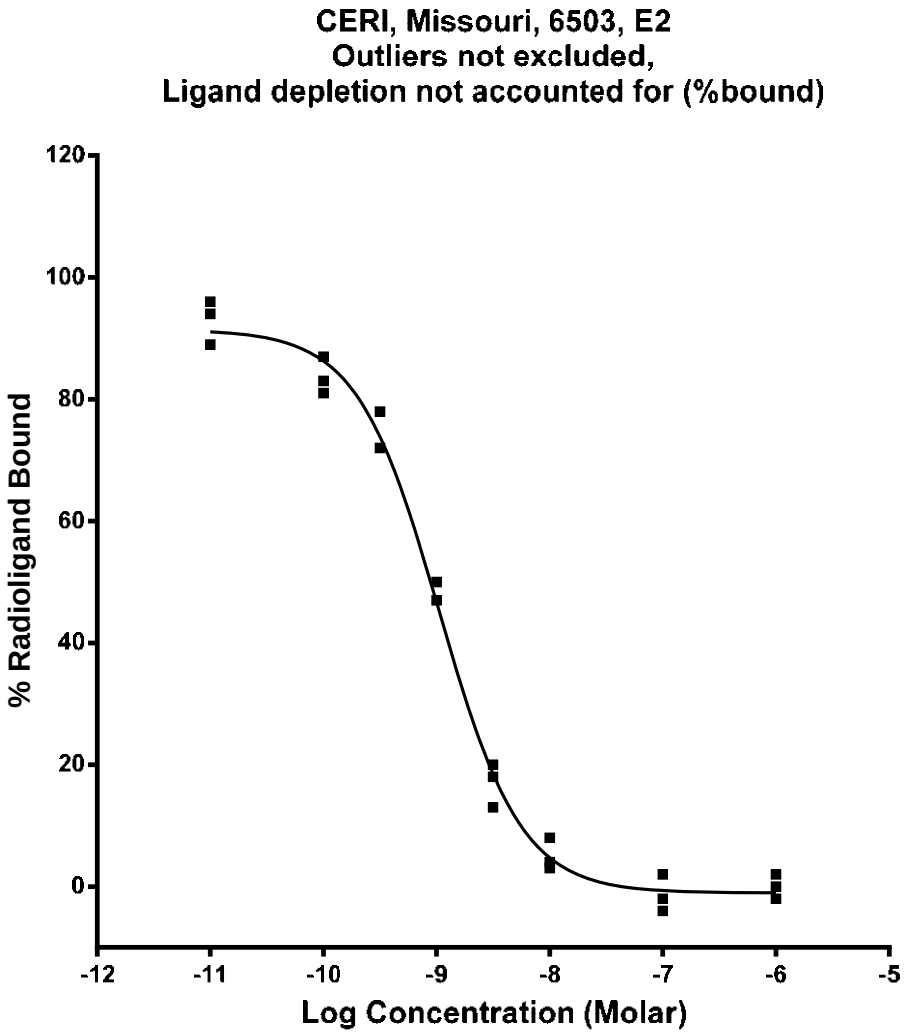
logIC50	
Best-fit values	
Bottom	-1.248
Top	109.3
LogIC50	-8.872
HillSlope	-1.393
Std. Error	
Bottom	2.484
Top	3.020
LogIC50	0.03427
HillSlope	0.1711
95% Confidence Intervals	
Bottom	-6.430 to 3.933
Top	103.0 to 115.6
LogIC50	-8.944 to -8.801
HillSlope	-1.750 to -1.036
Goodness of Fit	
Degrees of Freedom	20
R square	0.9836
Sy.x	6.488
Number of points	
Analyzed	24

Subtask 3



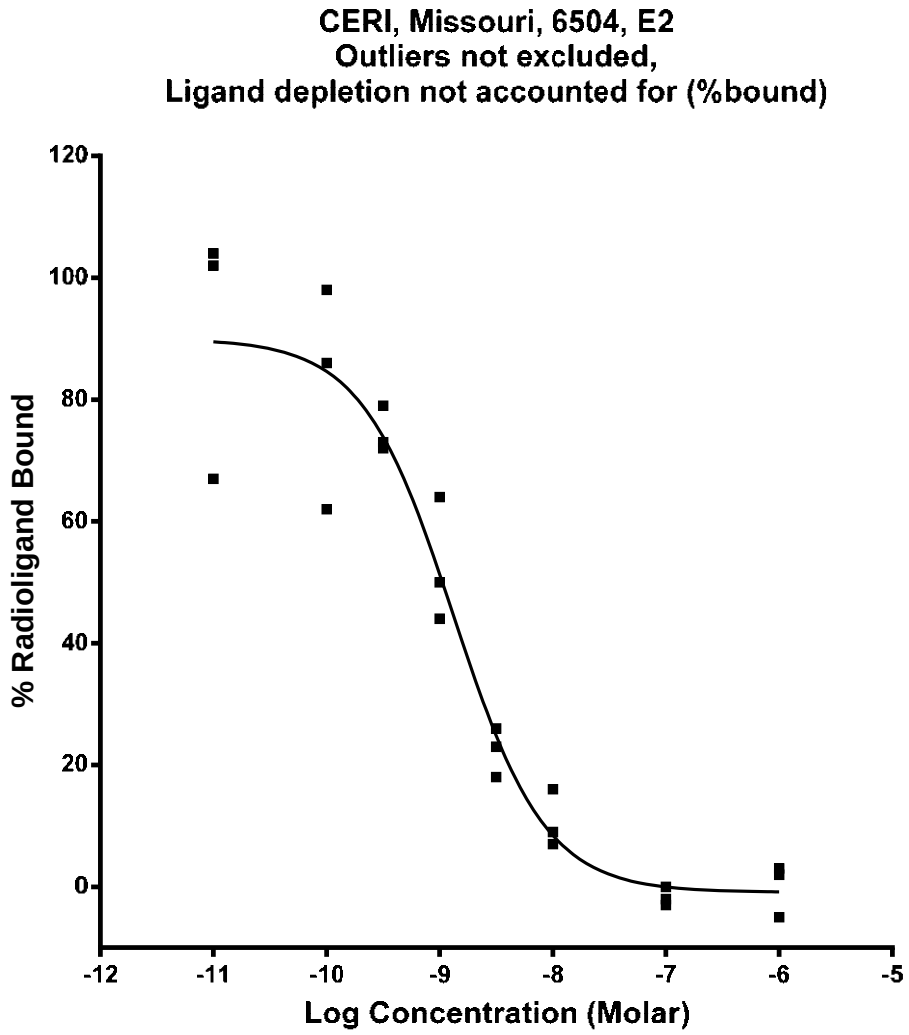
logIC50	
Best-fit values	
Bottom	-1.905
Top	102.1
LogIC50	-9.119
HillSlope	-0.9759
Std. Error	
Bottom	1.855
Top	2.855
LogIC50	0.03087
HillSlope	0.08792
95% Confidence Intervals	
Bottom	-5.775 to 1.966
Top	96.16 to 108.1
LogIC50	-9.183 to -9.054
HillSlope	-1.159 to -0.7925
Goodness of Fit	
Degrees of Freedom	20
R square	0.9890
Sy.x	4.507
Number of points	
Analyzed	24

Subtask 3



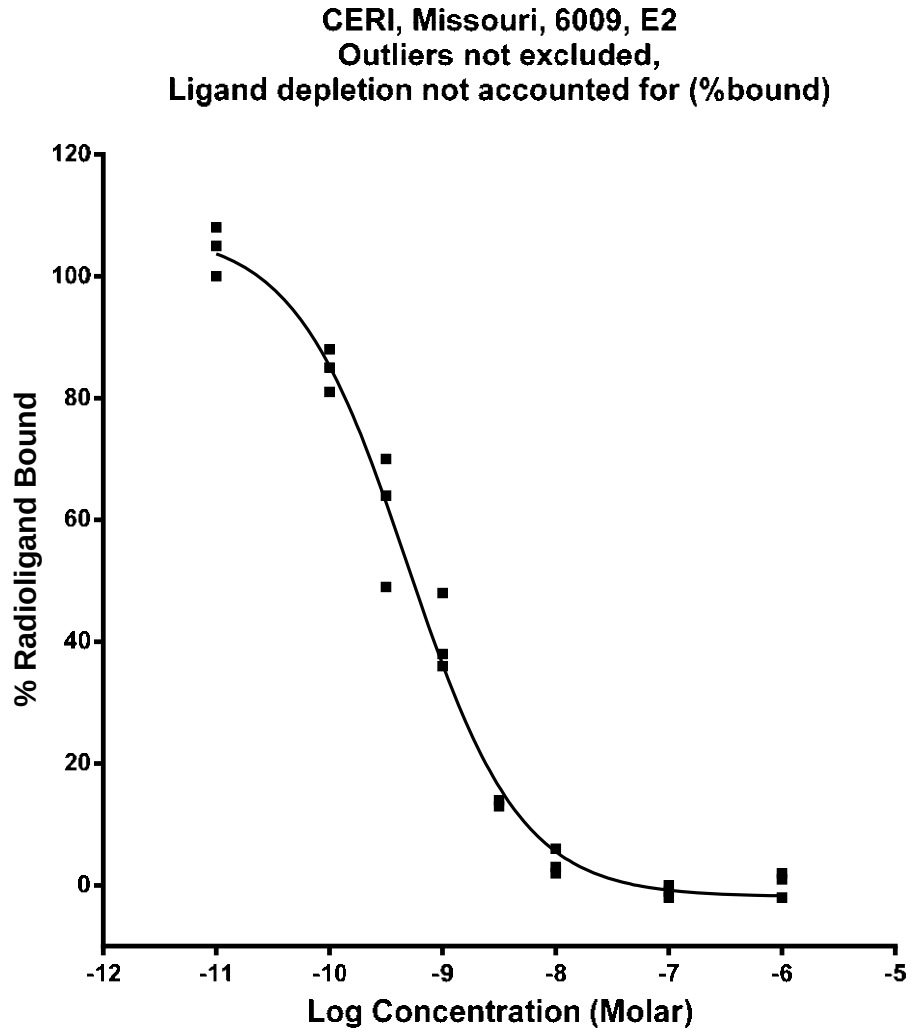
logIC50	
Best-fit values	
Bottom	-1.051
Top	91.40
LogIC50	-9.052
HillSlope	-1.203
Std. Error	
Bottom	1.211
Top	1.594
LogIC50	0.02148
HillSlope	0.08217
95% Confidence Intervals	
Bottom	-3.578 to 1.476
Top	88.08 to 94.72
LogIC50	-9.097 to -9.007
HillSlope	-1.374 to -1.032
Goodness of Fit	
Degrees of Freedom	20
R square	0.9943
Sy.x	3.076
Number of points	
Analyzed	24

Subtask 38



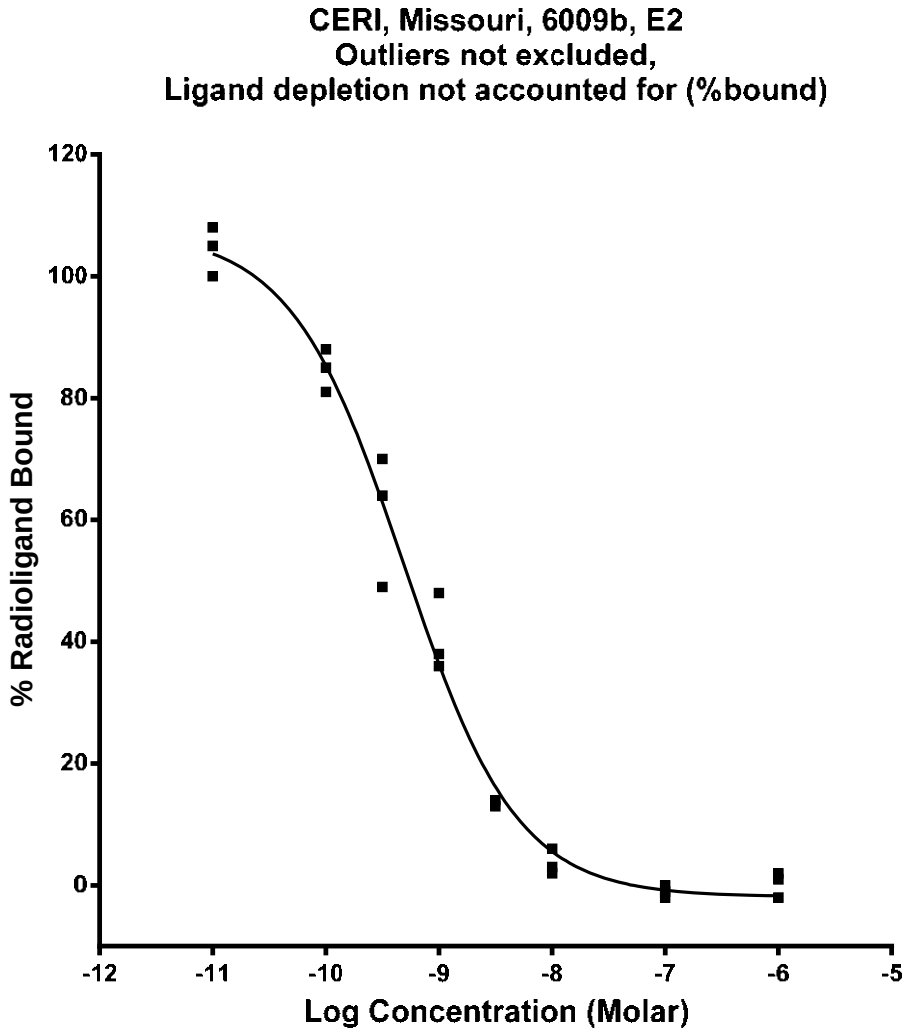
logIC50	
Best-fit values	
Bottom	-0.8957
Top	89.96
LogIC50	-8.976
HillSlope	-1.072
Std. Error	
Bottom	4.176
Top	5.267
LogIC50	0.07577
HillSlope	0.2390
95% Confidence Intervals	
Bottom	-9.606 to 7.815
Top	78.97 to 100.9
LogIC50	-9.134 to -8.818
HillSlope	-1.571 to -0.5736
Goodness of Fit	
Degrees of Freedom	20
R square	0.9393
Sy.x	9.936
Number of points	
Analyzed	24

Subtask 3



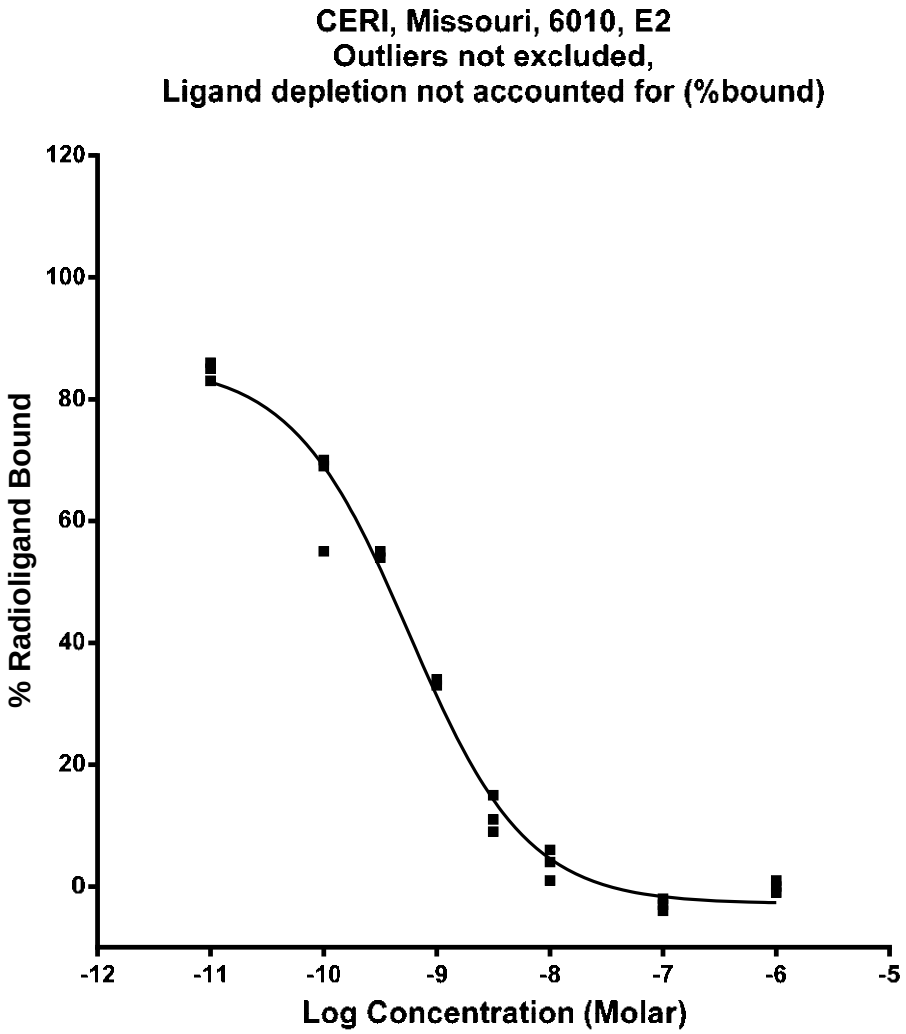
logIC50	
Best-fit values	
Bottom	-1.868
Top	107.3
LogIC50	-9.261
HillSlope	-0.8675
Std. Error	
Bottom	2.103
Top	3.887
LogIC50	0.03543
HillSlope	0.08874
95% Confidence Intervals	
Bottom	-6.255 to 2.519
Top	99.20 to 115.4
LogIC50	-9.335 to -9.187
HillSlope	-1.053 to -0.6823
Goodness of Fit	
Degrees of Freedom	20
R square	0.9859
Sy.x	5.035
Number of points	
Analyzed	24

Subtask 3



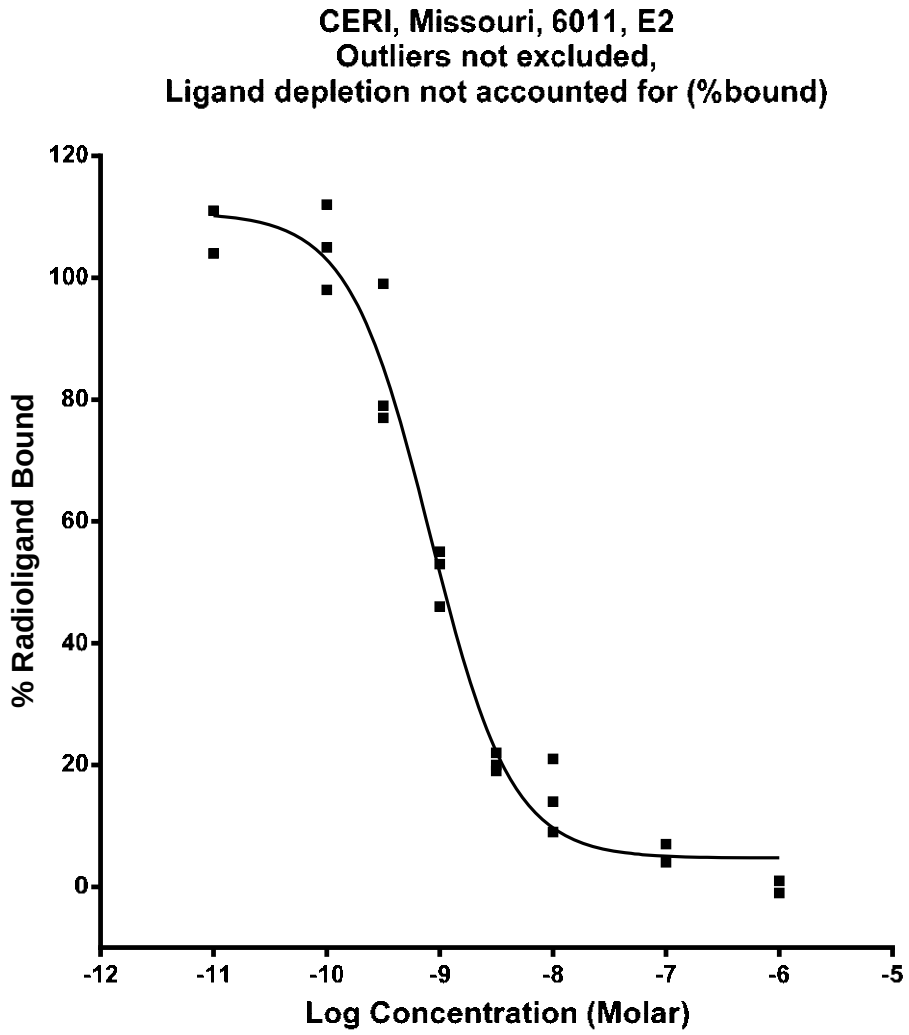
logIC50	
Best-fit values	
Bottom	-1.868
Top	107.3
LogIC50	-9.261
HillSlope	-0.8675
Std. Error	
Bottom	2.103
Top	3.887
LogIC50	0.03543
HillSlope	0.08874
95% Confidence Intervals	
Bottom	-6.255 to 2.519
Top	99.20 to 115.4
LogIC50	-9.335 to -9.187
HillSlope	-1.053 to -0.6823
Goodness of Fit	
Degrees of Freedom	20
R square	0.9859
Sy.x	5.035
Number of points	
Analyzed	24

Subtask 3[1]



logIC50	
Best-fit values	
Bottom	-2.831
Top	85.70
LogIC50	-9.447
HillSlope	-0.8367
Std. Error	
Bottom	1.756
Top	3.120
LogIC50	0.03986
HillSlope	0.08497
95% Confidence Intervals	
Bottom	-6.493 to 0.8321
Top	79.19 to 92.21
LogIC50	-9.530 to -9.364
HillSlope	-1.014 to -0.6595
Goodness of Fit	
Degrees of Freedom	20
R square	0.9861
Sy.x	4.055
Number of points	
Analyzed	24

Subtask 3

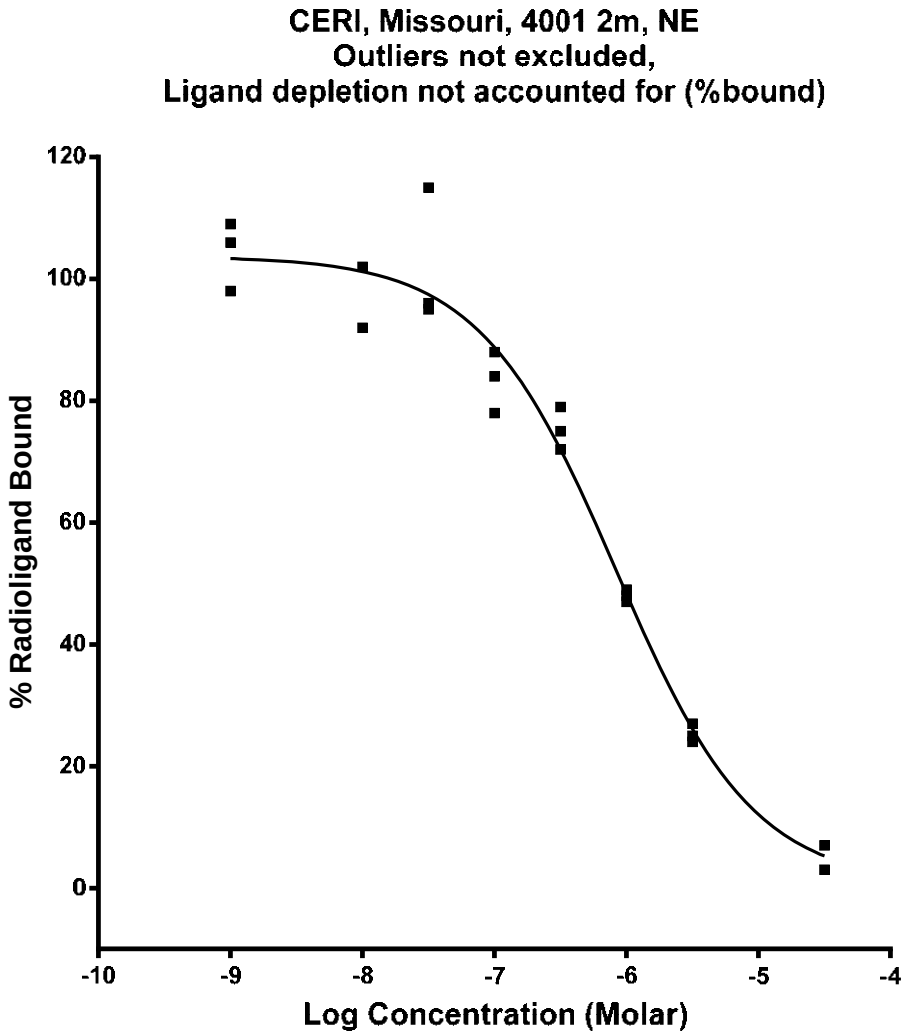


logIC50	
Best-fit values	
Bottom	4.716
Top	110.7
LogIC50	-8.979
HillSlope	-1.207
Std. Error	
Bottom	2.517
Top	3.328
LogIC50	0.03724
HillSlope	0.1457
95% Confidence Intervals	
Bottom	-0.5522 to 9.983
Top	103.7 to 117.7
LogIC50	-9.057 to -8.901
HillSlope	-1.512 to -0.9020
Goodness of Fit	
Degrees of Freedom	19
R square	0.9828
Sy.x	6.095
Number of points	
Analyzed	23

CERI ASSAYS, (Missouri Laboratory), Control Norethynodrel (NE)

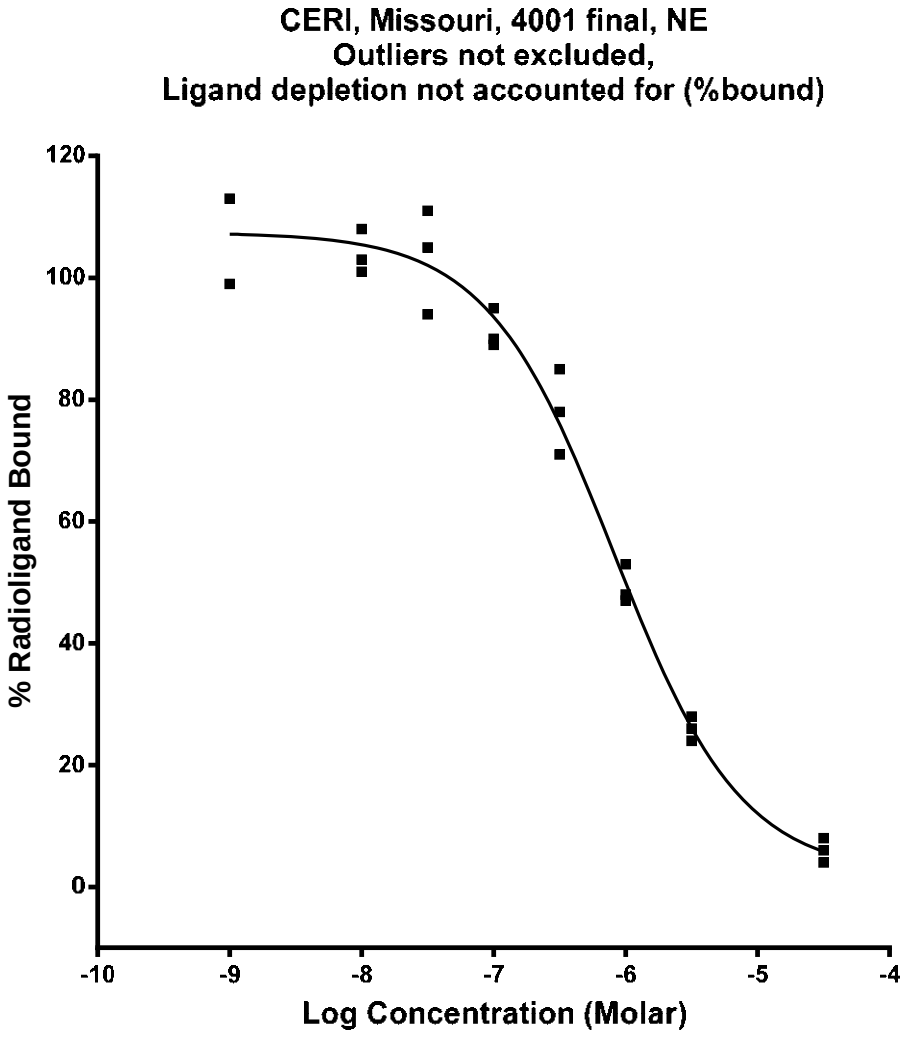
Begin Here

Subtask 1



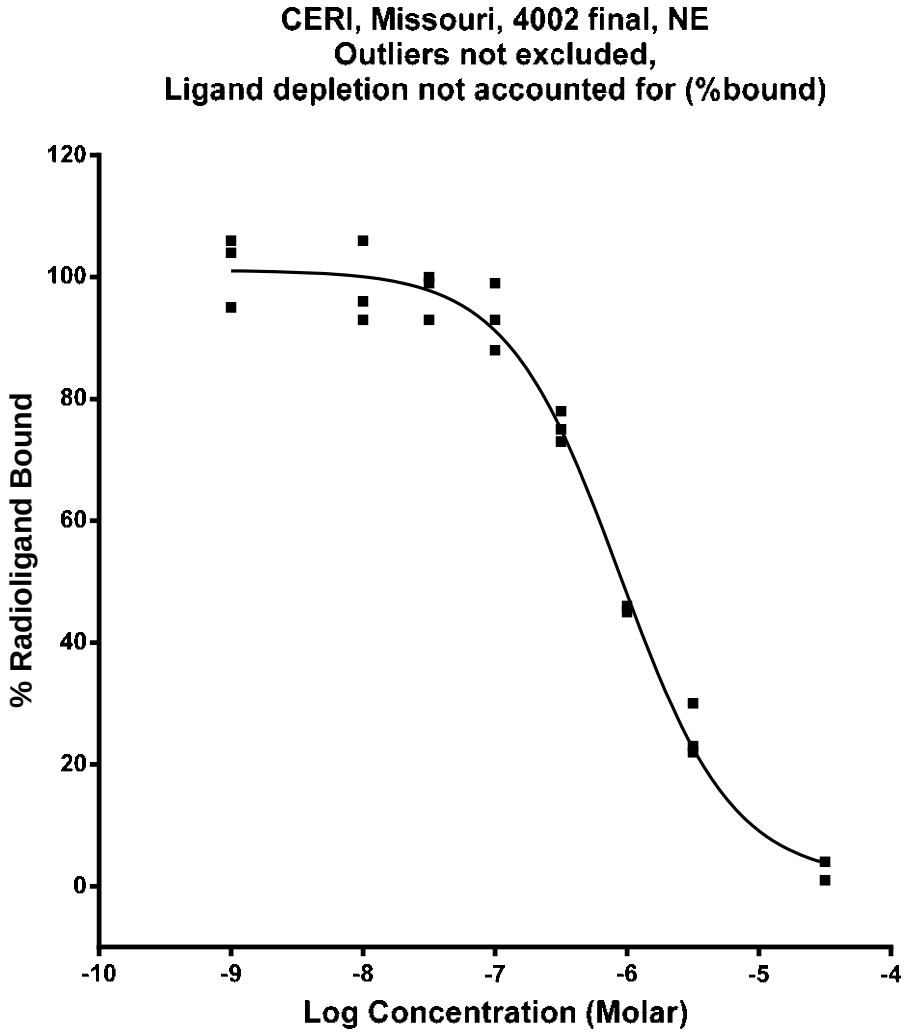
logIC50	
Best-fit values	
Bottom	0.5439
Top	103.7
LogIC50	-6.033
HillSlope	-0.8357
Std. Error	
Bottom	5.112
Top	2.701
LogIC50	0.04655
HillSlope	0.1167
95% Confidence Intervals	
Bottom	-10.12 to 11.21
Top	98.08 to 109.3
LogIC50	-6.130 to -5.936
HillSlope	-1.079 to -0.5922
Goodness of Fit	
Degrees of Freedom	20
R square	0.9770
Sy.x	5.860
Number of points	
Analyzed	24

Subtask 1



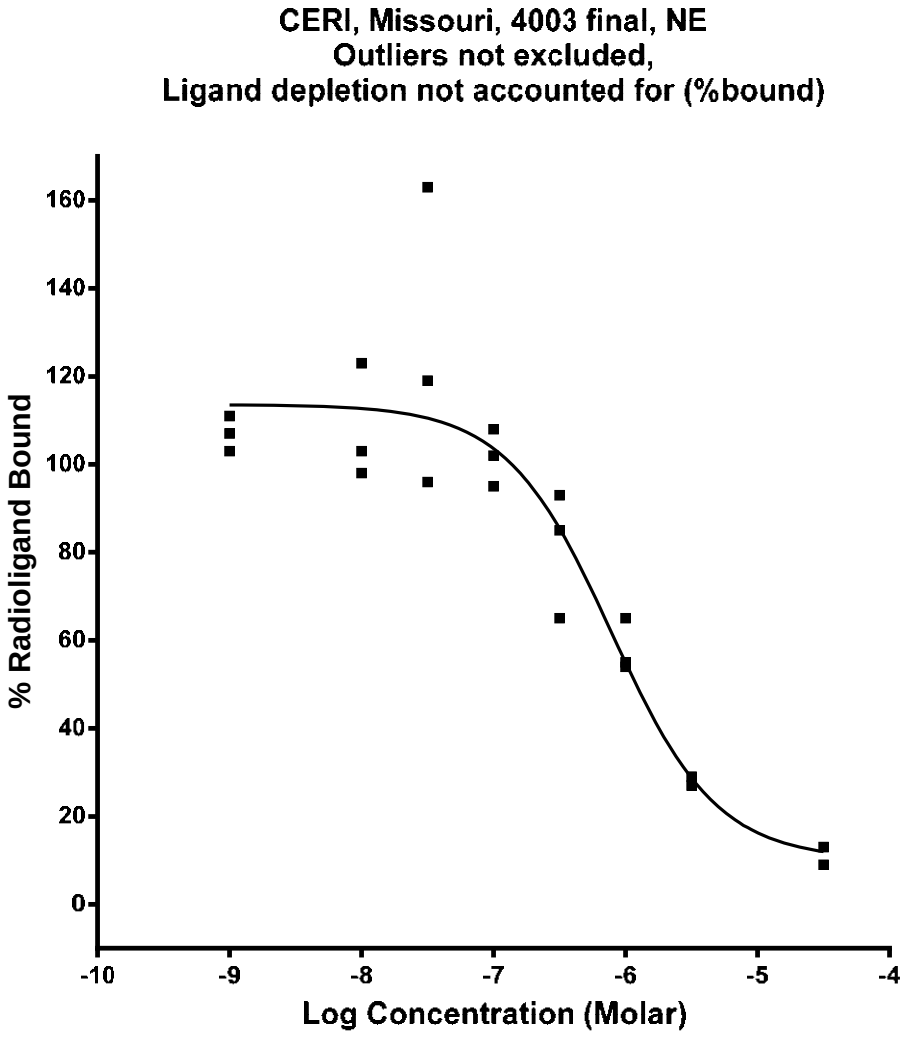
logIC50	
Best-fit values	
Bottom	2.025
Top	107.4
LogIC50	-6.000
HillSlope	-0.8993
Std. Error	
Bottom	4.022
Top	2.183
LogIC50	0.03780
HillSlope	0.1011
95% Confidence Intervals	
Bottom	-6.364 to 10.41
Top	102.9 to 112.0
LogIC50	-6.078 to -5.921
HillSlope	-1.110 to -0.6884
Goodness of Fit	
Degrees of Freedom	20
R square	0.9845
Sy.x	5.025
Number of points	
Analyzed	24

Subtask 14



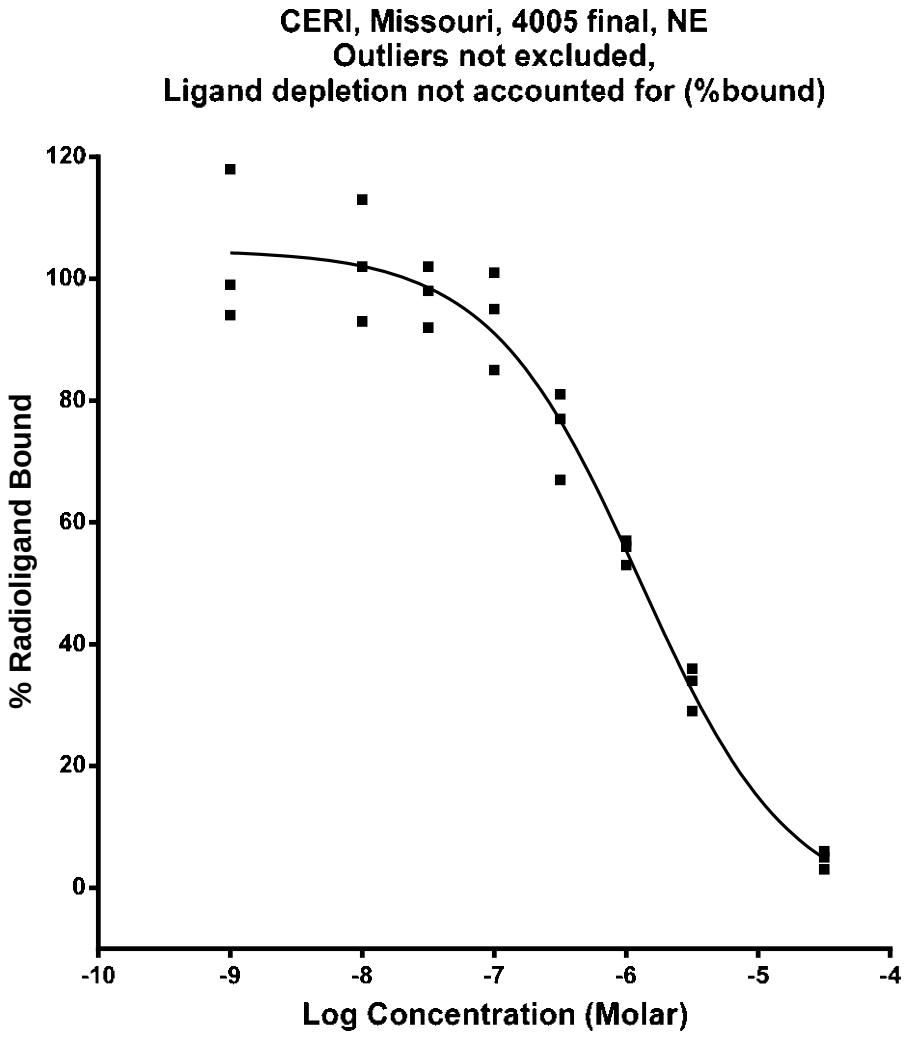
logIC50	
Best-fit values	
Bottom	1.257
Top	101.1
LogIC50	-6.034
HillSlope	-1.014
Std. Error	
Bottom	3.122
Top	1.684
LogIC50	0.03049
HillSlope	0.09853
95% Confidence Intervals	
Bottom	-5.257 to 7.770
Top	97.60 to 104.6
LogIC50	-6.098 to -5.971
HillSlope	-1.220 to -0.8087
Goodness of Fit	
Degrees of Freedom	20
R square	0.9881
Sy.x	4.281
Number of points	
Analyzed	24

Subtask 1



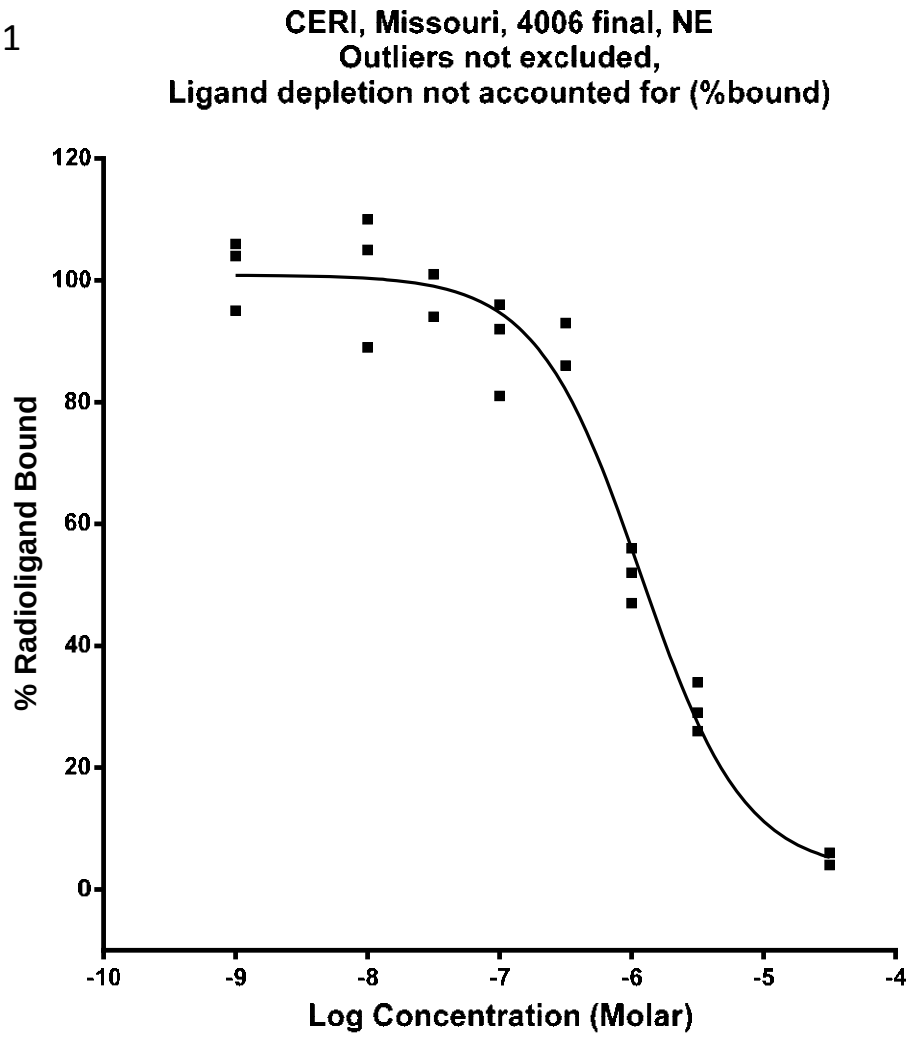
logIC50	
Best-fit values	
Bottom	10.18
Top	113.6
LogIC50	-5.919
HillSlope	-1.087
Std. Error	
Bottom	9.869
Top	5.610
LogIC50	0.1068
HillSlope	0.3381
95% Confidence Intervals	
Bottom	-10.41 to 30.76
Top	101.9 to 125.3
LogIC50	-6.142 to -5.697
HillSlope	-1.792 to -0.3818
Goodness of Fit	
Degrees of Freedom	20
R square	0.8896
Sy.x	14.66
Number of points	
Analyzed	24

Subtask 1



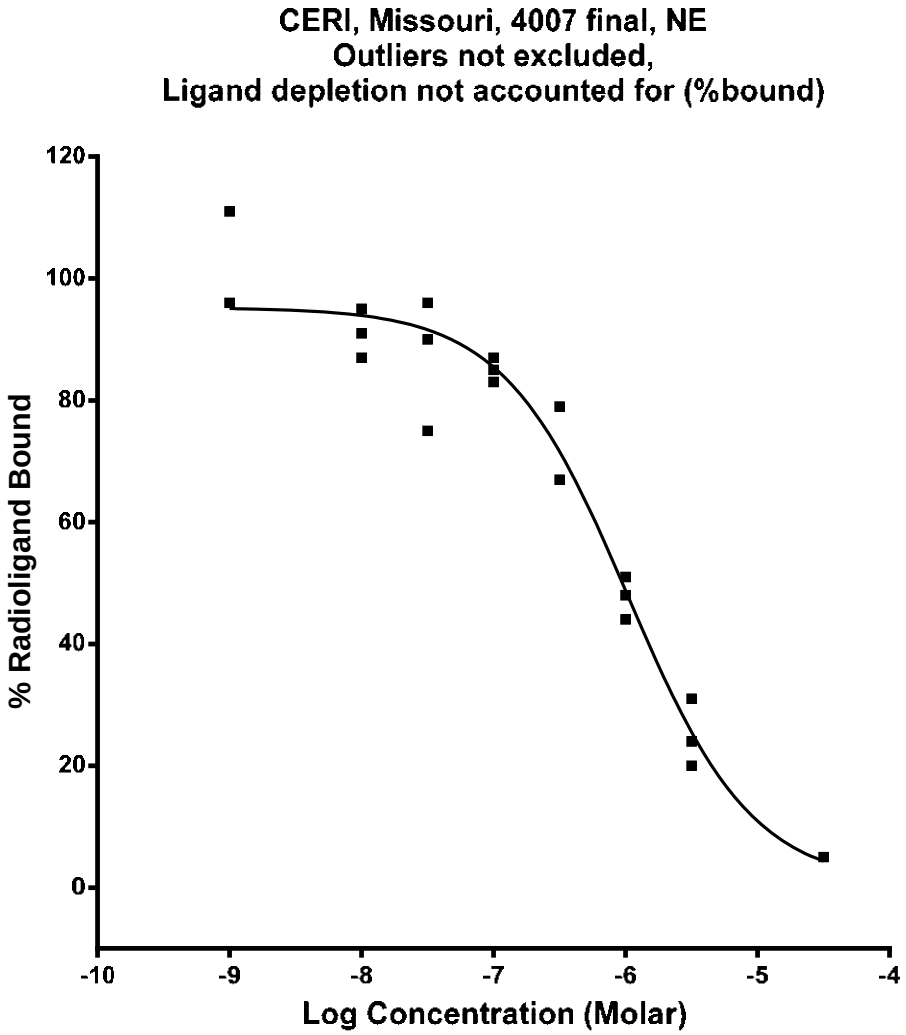
logIC50	
Best-fit values	
Bottom	-3.781
Top	104.7
LogIC50	-5.886
HillSlope	-0.7609
Std. Error	
Bottom	7.638
Top	3.171
LogIC50	0.05612
HillSlope	0.1316
95% Confidence Intervals	
Bottom	-19.72 to 12.15
Top	98.10 to 111.3
LogIC50	-6.003 to -5.769
HillSlope	-1.035 to -0.4864
Goodness of Fit	
Degrees of Freedom	20
R square	0.9695
Sy.x	6.650
Number of points	
Analyzed	24

Subtask 1



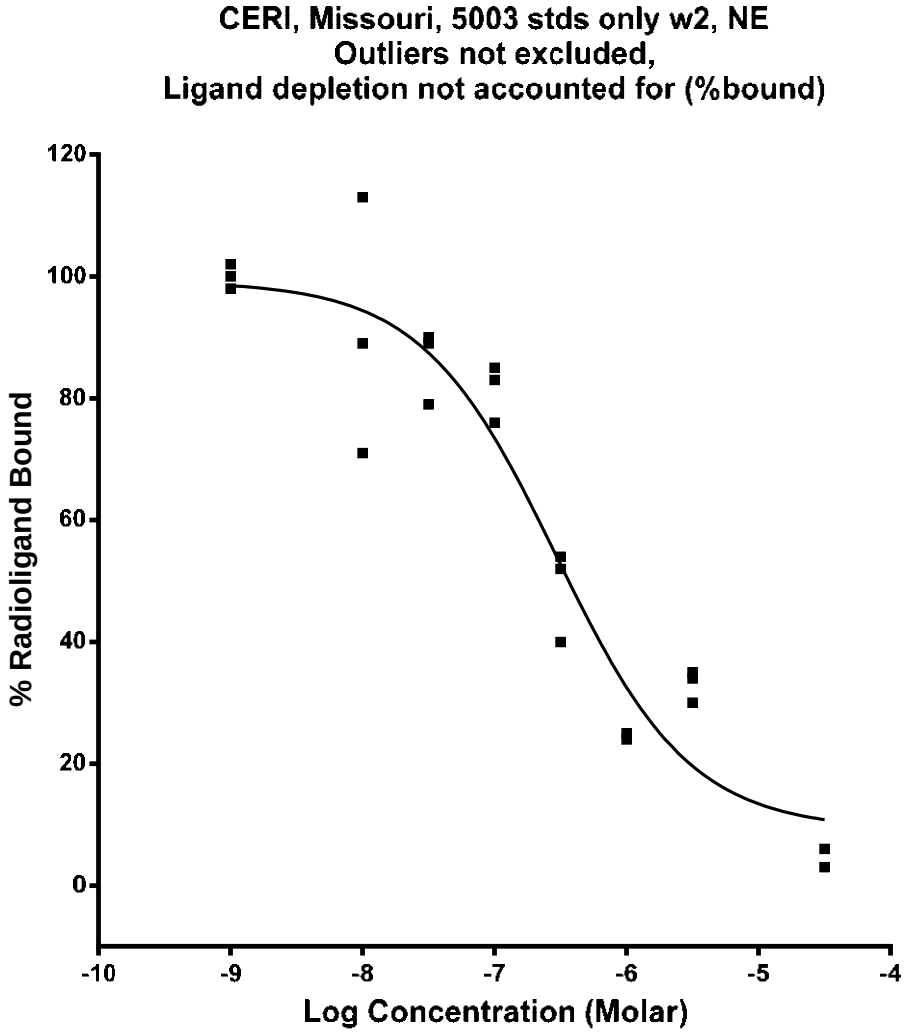
logIC50	
Best-fit values	
Bottom	2.717
Top	100.9
LogIC50	-5.902
HillSlope	-1.099
Std. Error	
Bottom	4.757
Top	2.341
LogIC50	0.04556
HillSlope	0.1674
95% Confidence Intervals	
Bottom	-7.206 to 12.64
Top	95.97 to 105.7
LogIC50	-5.997 to -5.807
HillSlope	-1.448 to -0.7501
Goodness of Fit	
Degrees of Freedom	20
R square	0.9717
Sy.x	6.503
Number of points	
Analyzed	24

Subtask 1



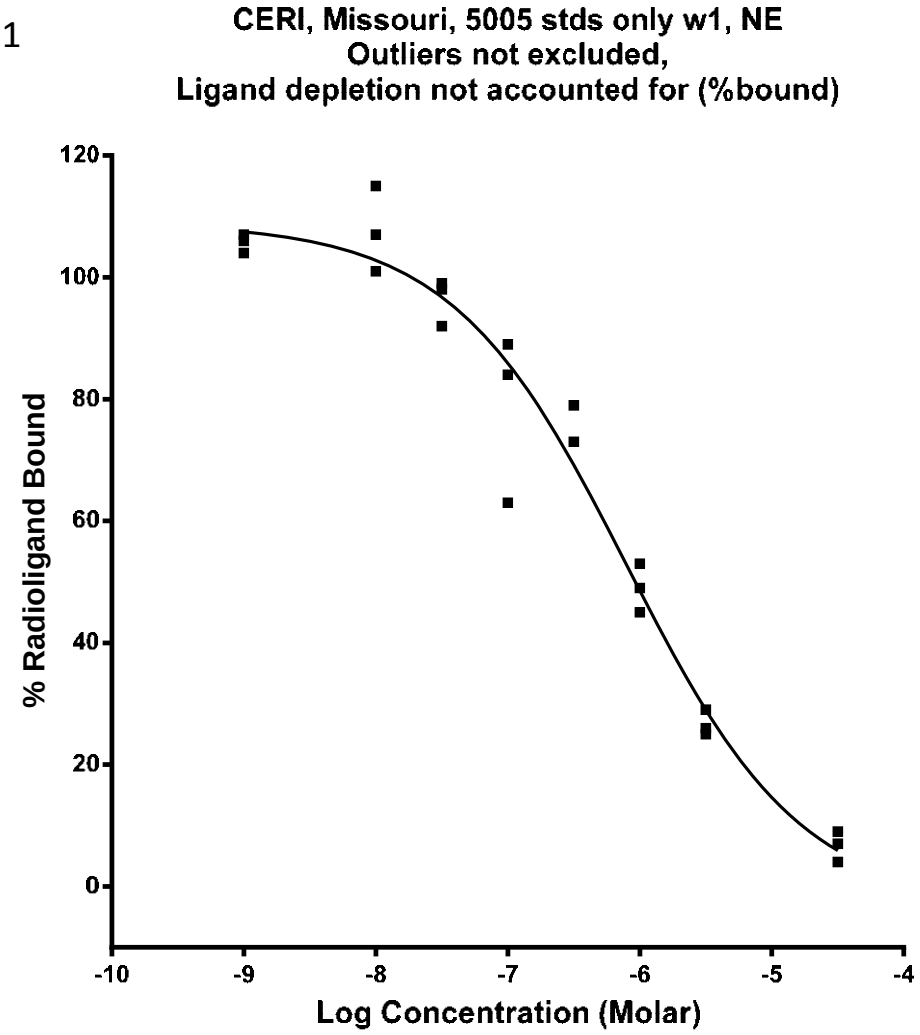
logIC50	
Best-fit values	
Bottom	0.4380
Top	95.21
LogIC50	-6.023
HillSlope	-0.9226
Std. Error	
Bottom	6.587
Top	2.747
LogIC50	0.05329
HillSlope	0.1606
95% Confidence Intervals	
Bottom	-13.35 to 14.23
Top	89.46 to 101.0
LogIC50	-6.135 to -5.911
HillSlope	-1.259 to -0.5863
Goodness of Fit	
Degrees of Freedom	19
R square	0.9622
Sy.x	6.659
Number of points	
Analyzed	23

Subtask 19



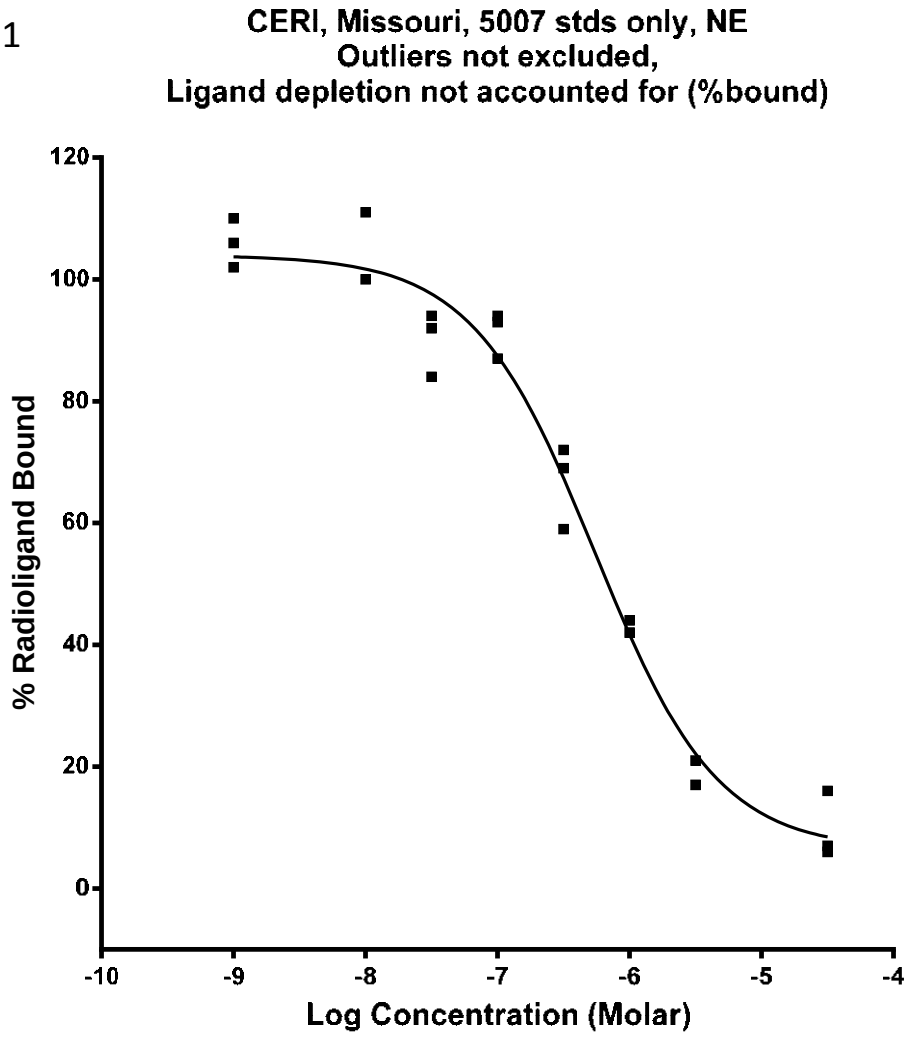
logIC50	
Best-fit values	
Bottom	9.199
Top	99.19
LogIC50	-6.438
HillSlope	-0.8534
Std. Error	
Bottom	7.013
Top	5.735
LogIC50	0.09327
HillSlope	0.2262
95% Confidence Intervals	
Bottom	-5.429 to 23.83
Top	87.22 to 111.1
LogIC50	-6.632 to -6.243
HillSlope	-1.325 to -0.3815
Goodness of Fit	
Degrees of Freedom	20
R square	0.9182
Sy.x	10.62
Number of points	
Analyzed	24

Subtask 1



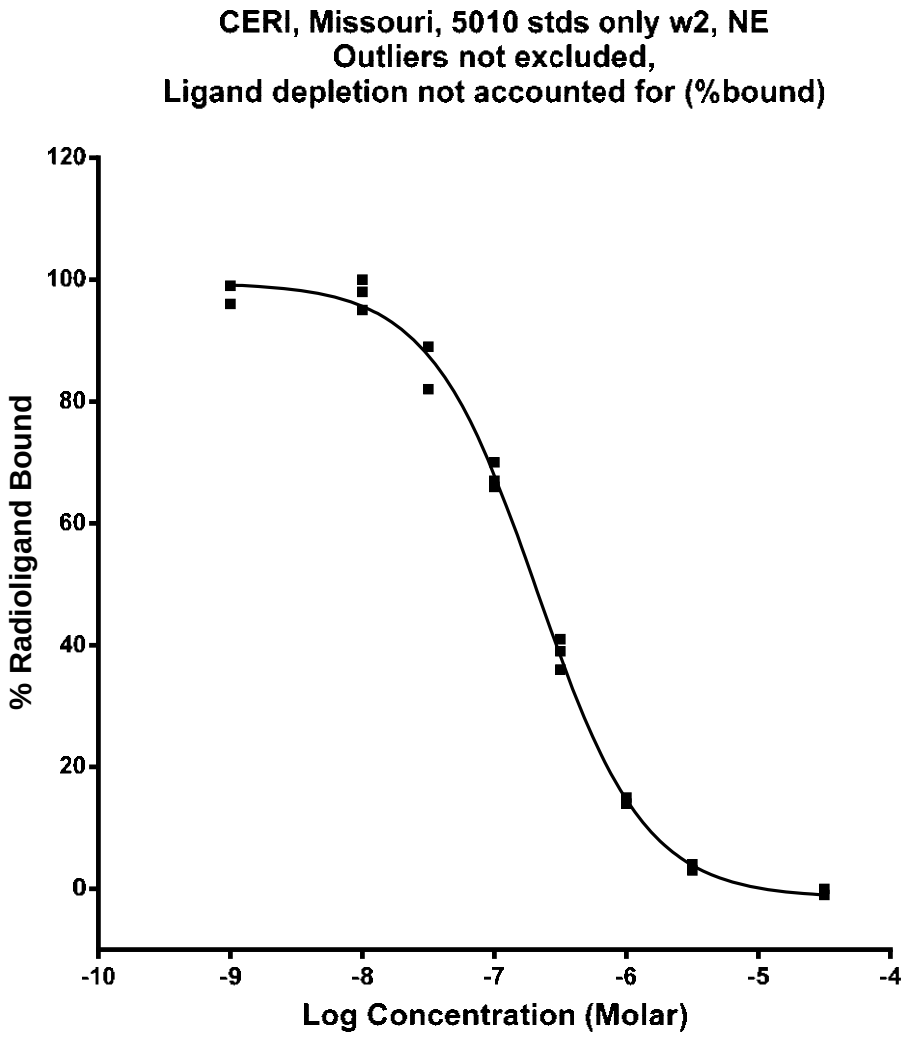
logIC50	
Best-fit values	
Bottom	-3.313
Top	108.8
LogIC50	-6.034
HillSlope	-0.6556
Std. Error	
Bottom	8.179
Top	4.130
LogIC50	0.06118
HillSlope	0.1171
95% Confidence Intervals	
Bottom	-20.38 to 13.75
Top	100.2 to 117.4
LogIC50	-6.161 to -5.906
HillSlope	-0.8999 to -0.4112
Goodness of Fit	
Degrees of Freedom	20
R square	0.9684
Sy.x	6.883
Number of points	
Analyzed	24

Subtask 1



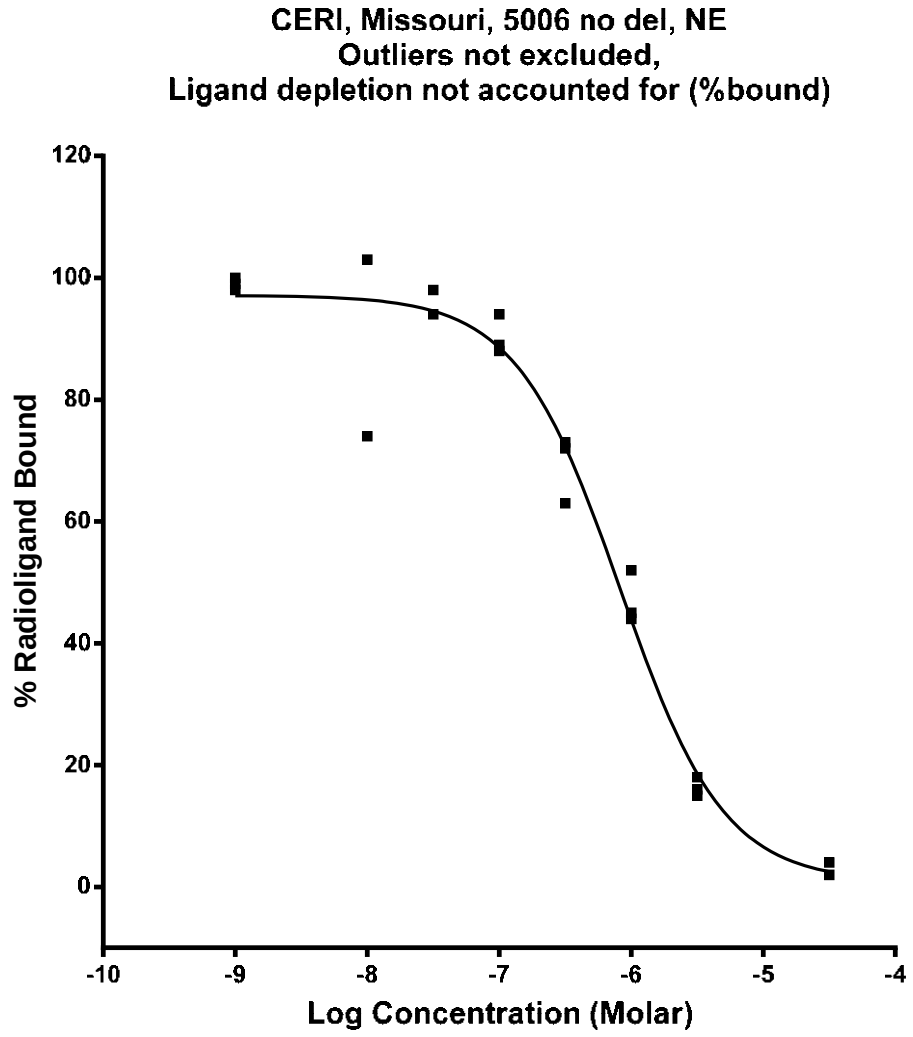
logIC50	
Best-fit values	
Bottom	6.313
Top	104.0
LogIC50	-6.163
HillSlope	-0.9358
Std. Error	
Bottom	3.969
Top	2.544
LogIC50	0.04412
HillSlope	0.1214
95% Confidence Intervals	
Bottom	-1.966 to 14.59
Top	98.66 to 109.3
LogIC50	-6.255 to -6.071
HillSlope	-1.189 to -0.6826
Goodness of Fit	
Degrees of Freedom	20
R square	0.9789
Sy.x	5.675
Number of points	
Analyzed	24

Subtask



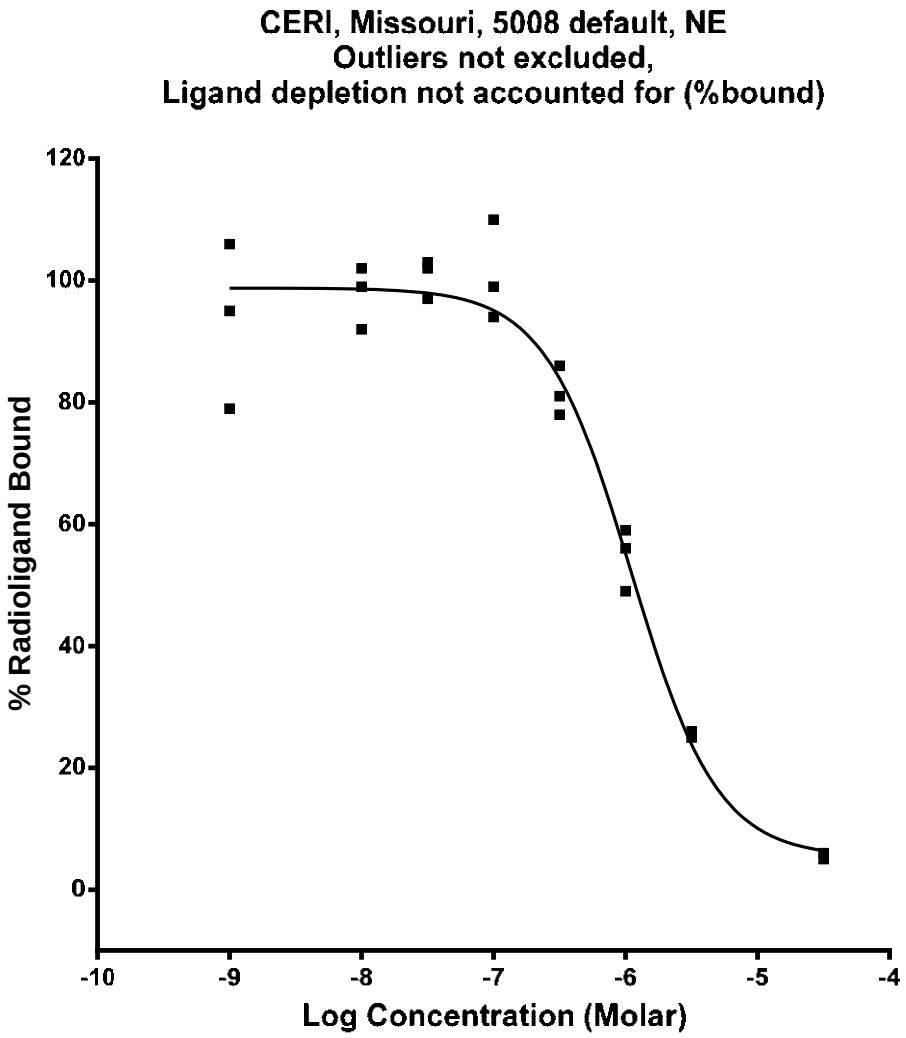
logIC50	
Best-fit values	
Bottom	-1.499
Top	99.46
LogIC50	-6.694
HillSlope	-1.063
Std. Error	
Bottom	1.139
Top	1.065
LogIC50	0.01469
HillSlope	0.04894
95% Confidence Intervals	
Bottom	-3.876 to 0.8773
Top	97.23 to 101.7
LogIC50	-6.724 to -6.663
HillSlope	-1.165 to -0.9609
Goodness of Fit	
Degrees of Freedom	20
R square	0.9974
Sy.x	2.182
Number of points	
Analyzed	24

Subtask3



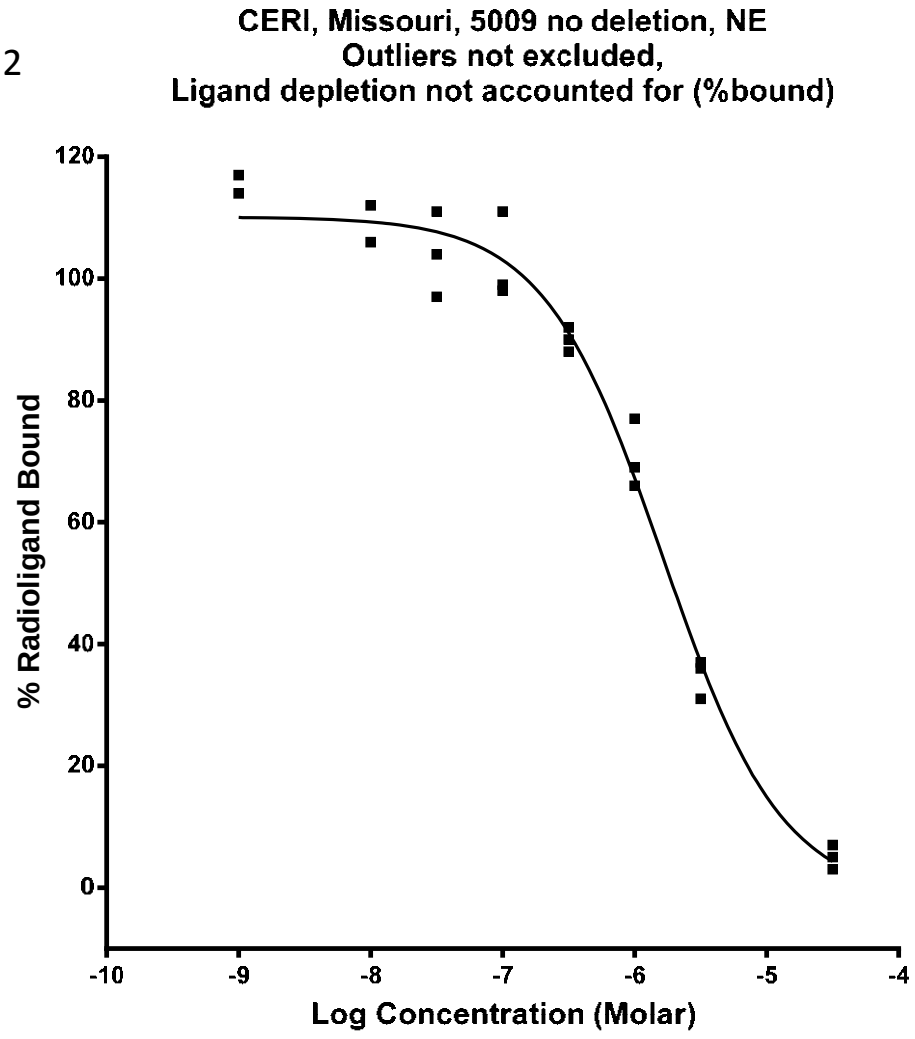
logIC50	
Best-fit values	
Bottom	0.8222
Top	97.13
LogIC50	-6.100
HillSlope	-1.103
Std. Error	
Bottom	4.327
Top	2.417
LogIC50	0.04449
HillSlope	0.1615
95% Confidence Intervals	
Bottom	-8.203 to 9.847
Top	92.09 to 102.2
LogIC50	-6.193 to -6.007
HillSlope	-1.439 to -0.7657
Goodness of Fit	
Degrees of Freedom	20
R square	0.9732
Sy.x	6.419
Number of points	
Analyzed	24

Subtask



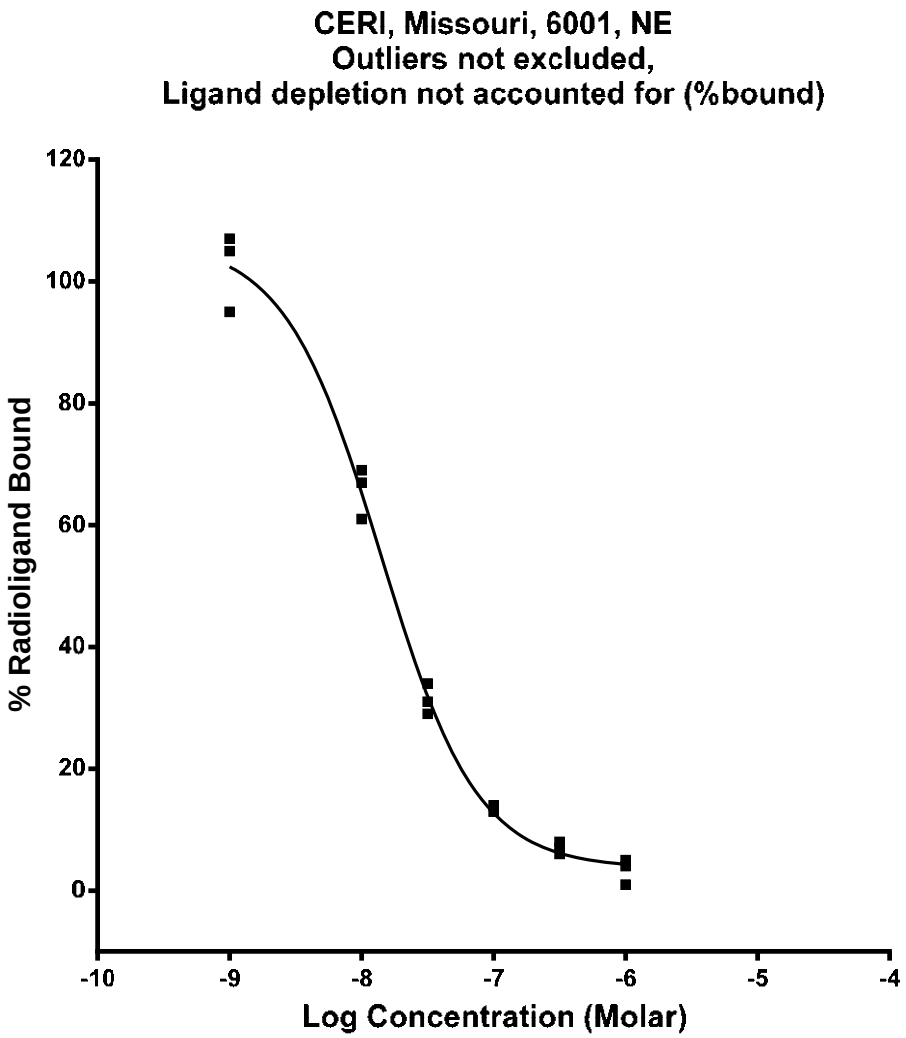
logIC50	
Best-fit values	
Bottom	5.326
Top	98.77
LogIC50	-5.926
HillSlope	-1.329
Std. Error	
Bottom	4.301
Top	2.215
LogIC50	0.04363
HillSlope	0.2113
95% Confidence Intervals	
Bottom	-3.645 to 14.30
Top	94.15 to 103.4
LogIC50	-6.017 to -5.835
HillSlope	-1.770 to -0.8886
Goodness of Fit	
Degrees of Freedom	20
R square	0.9697
Sy.x	6.724
Number of points	
Analyzed	24

Subtask 2



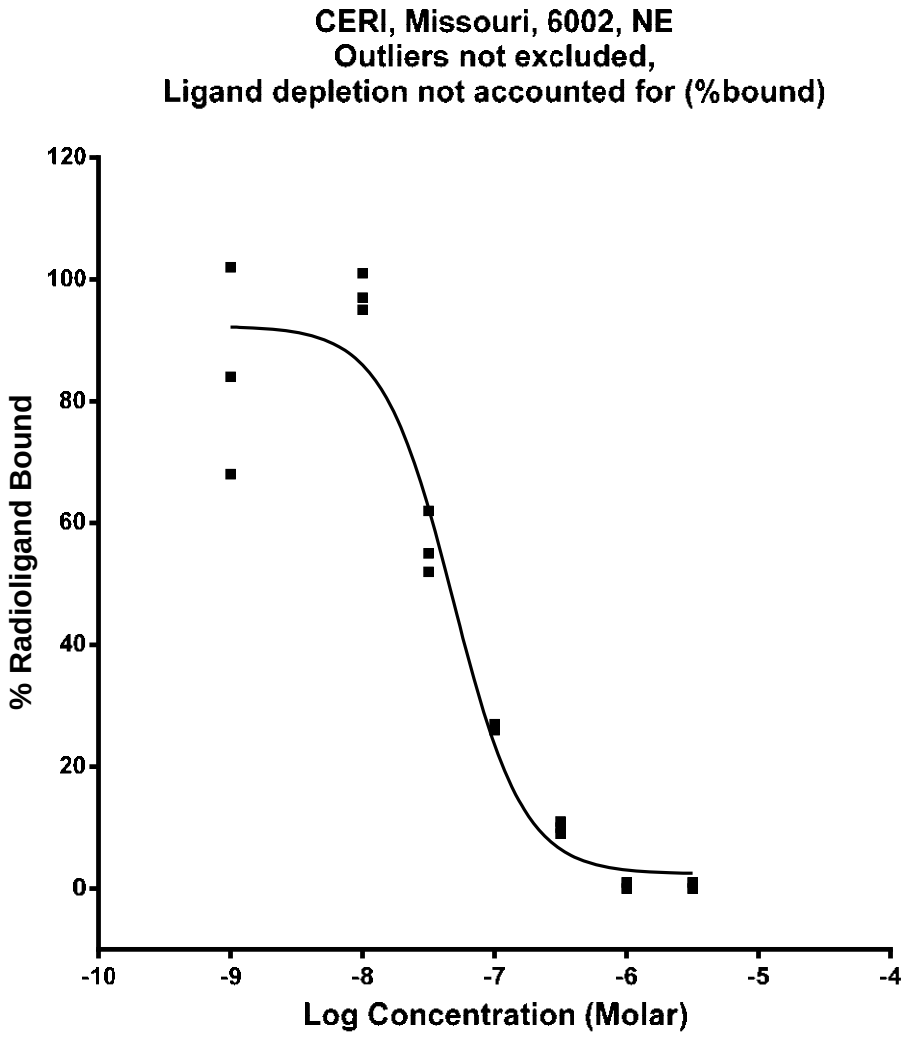
logIC50	
Best-fit values	
Bottom	-1.819
Top	110.1
LogIC50	-5.716
HillSlope	-0.9623
Std. Error	
Bottom	4.729
Top	1.910
LogIC50	0.03724
HillSlope	0.1138
95% Confidence Intervals	
Bottom	-11.68 to 8.045
Top	106.1 to 114.1
LogIC50	-5.794 to -5.639
HillSlope	-1.200 to -0.7249
Goodness of Fit	
Degrees of Freedom	20
R square	0.9846
Sy.x	5.055
Number of points	
Analyzed	24

Subtask 3



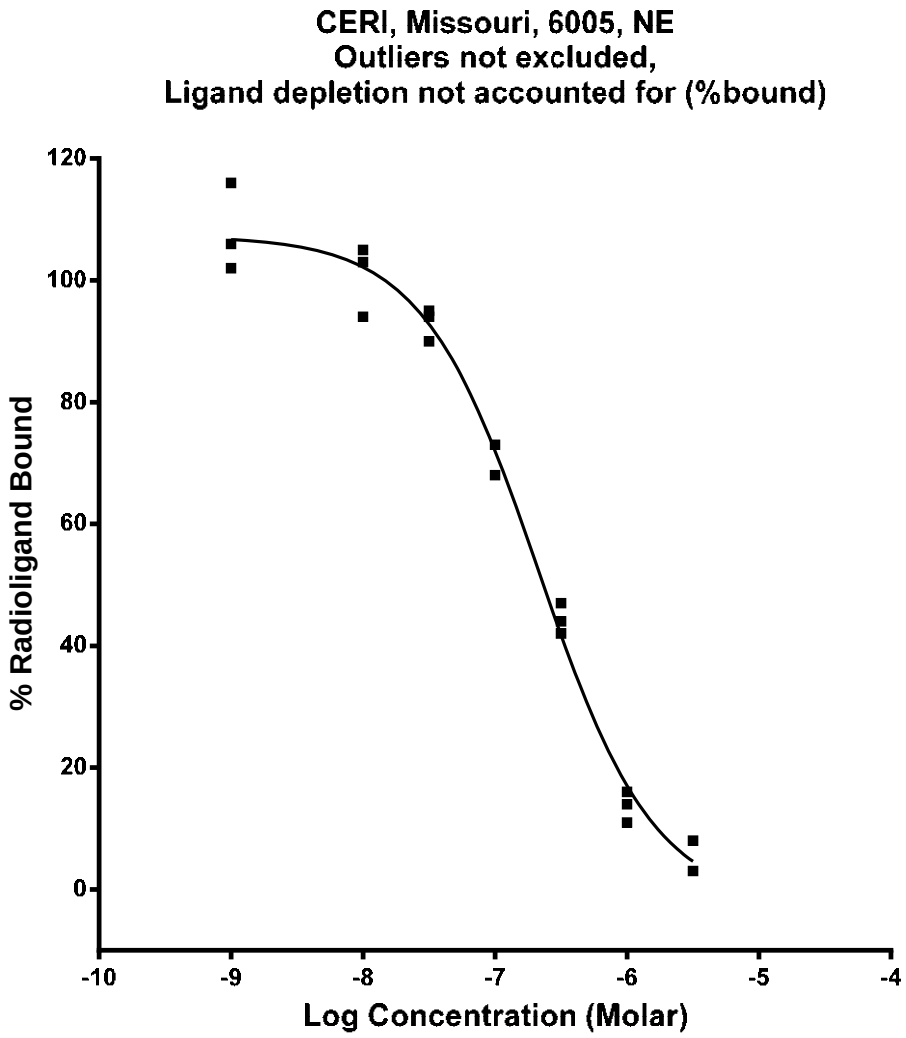
logIC50	
Best-fit values	
Bottom	3.695
Top	106.7
LogIC50	-7.780
HillSlope	-1.190
Std. Error	
Bottom	1.655
Top	2.819
LogIC50	0.02149
HillSlope	0.1155
95% Confidence Intervals	
Bottom	0.1444 to 7.246
Top	100.6 to 112.7
LogIC50	-7.826 to -7.734
HillSlope	-1.437 to -0.9419
Goodness of Fit	
Degrees of Freedom	14
R square	0.9936
Sy.x	3.264
Number of points	
Analyzed	18

Subtask 3[2]



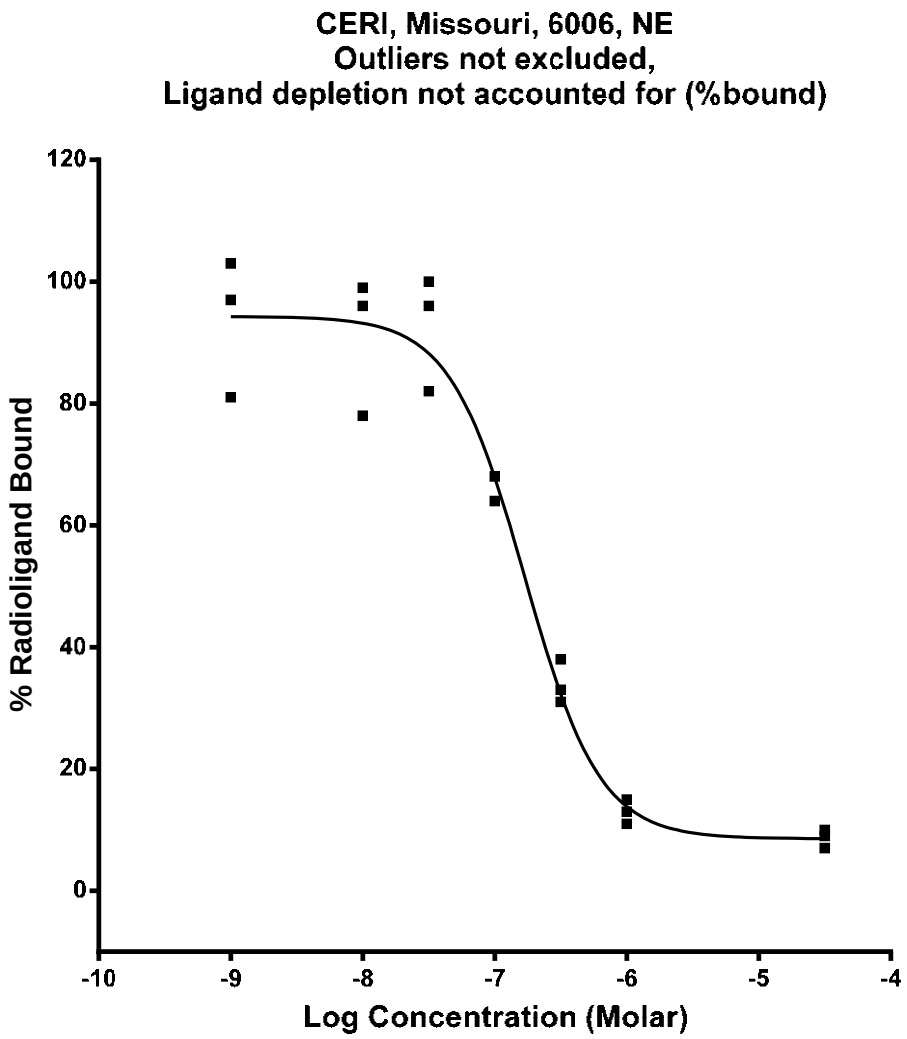
logIC50	
Best-fit values	
Bottom	2.384
Top	92.34
LogIC50	-7.344
HillSlope	-1.627
Std. Error	
Bottom	3.649
Top	4.826
LogIC50	0.05551
HillSlope	0.3676
95% Confidence Intervals	
Bottom	-5.316 to 10.08
Top	82.16 to 102.5
LogIC50	-7.461 to -7.227
HillSlope	-2.403 to -0.8515
Goodness of Fit	
Degrees of Freedom	17
R square	0.9521
Sy.x	9.184
Number of points	
Analyzed	21

Subtask 3



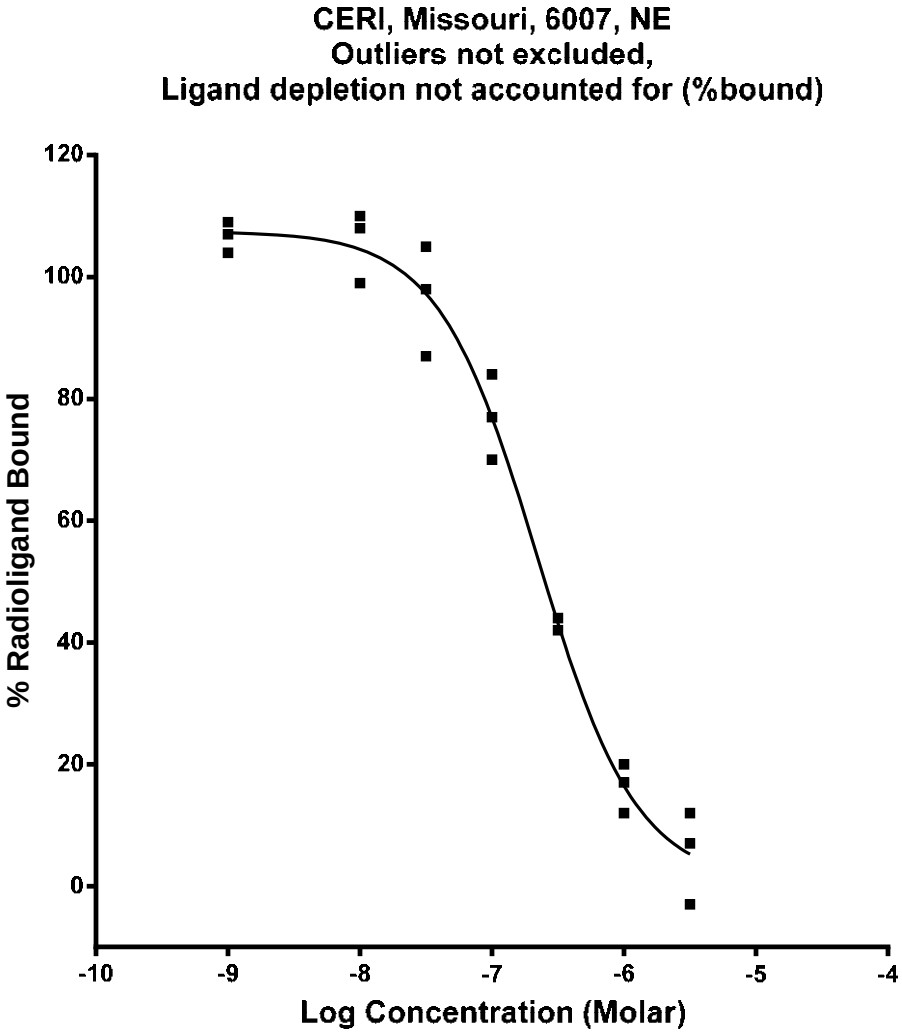
logIC50	
Best-fit values	
Bottom	-2.504
Top	107.2
LogIC50	-6.632
HillSlope	-0.9897
Std. Error	
Bottom	4.605
Top	2.341
LogIC50	0.03024
HillSlope	0.1114
95% Confidence Intervals	
Bottom	-12.22 to 7.212
Top	102.3 to 112.2
LogIC50	-6.696 to -6.569
HillSlope	-1.225 to -0.7546
Goodness of Fit	
Degrees of Freedom	17
R square	0.9898
Sy.x	4.345
Number of points	
Analyzed	21

Subtask 4



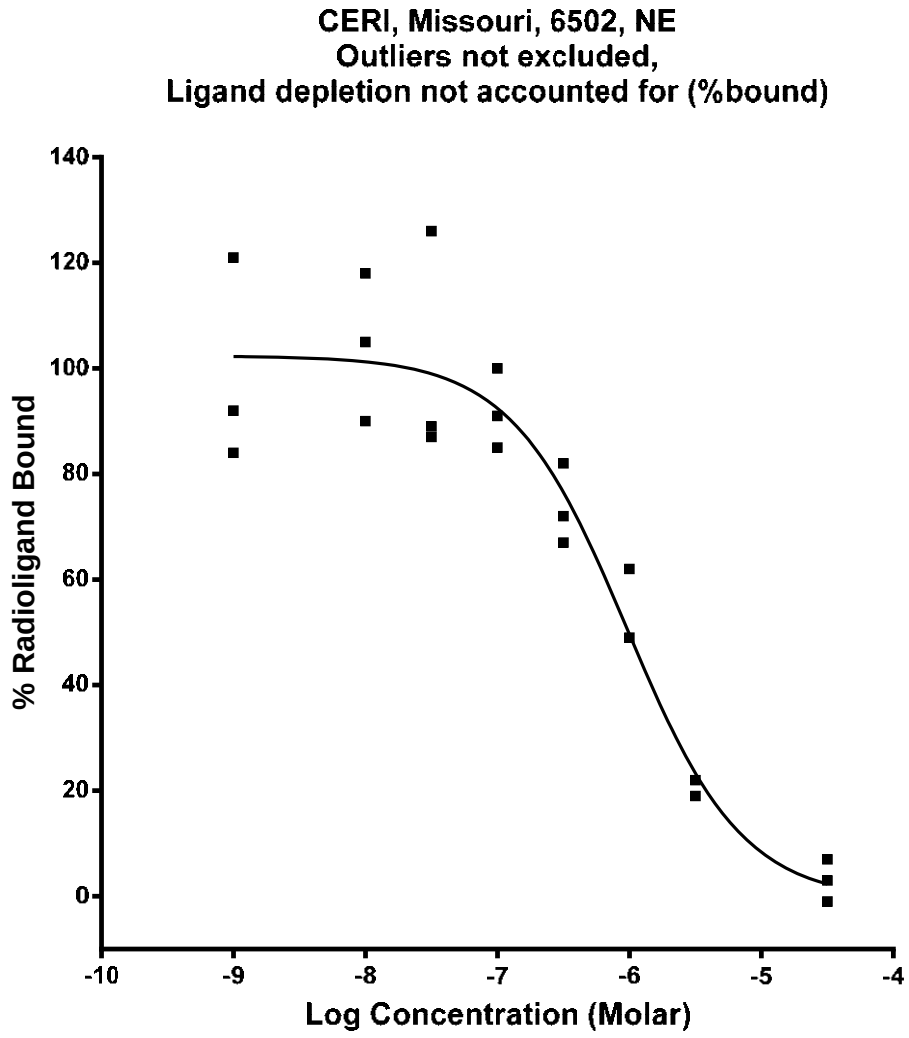
logIC50	
Best-fit values	
Bottom	8.546
Top	94.29
LogIC50	-6.752
HillSlope	-1.534
Std. Error	
Bottom	3.607
Top	2.937
LogIC50	0.04613
HillSlope	0.2728
95% Confidence Intervals	
Bottom	0.9345 to 16.16
Top	88.09 to 100.5
LogIC50	-6.849 to -6.655
HillSlope	-2.110 to -0.9587
Goodness of Fit	
Degrees of Freedom	17
R square	0.9682
Sy.x	7.067
Number of points	
Analyzed	21

Subtask 3



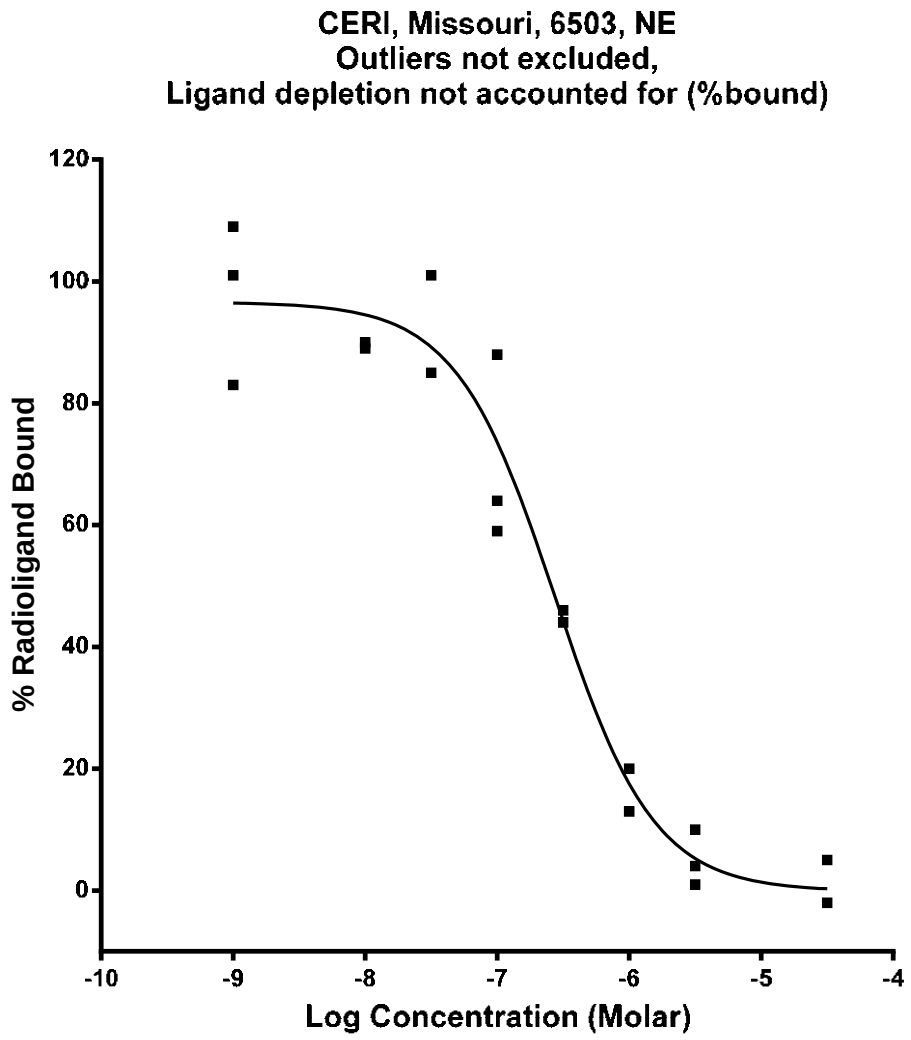
logIC50	
Best-fit values	
Bottom	0.4982
Top	107.5
LogIC50	-6.598
HillSlope	-1.154
Std. Error	
Bottom	4.759
Top	2.586
LogIC50	0.03523
HillSlope	0.1553
95% Confidence Intervals	
Bottom	-9.544 to 10.54
Top	102.0 to 112.9
LogIC50	-6.672 to -6.524
HillSlope	-1.482 to -0.8265
Goodness of Fit	
Degrees of Freedom	17
R square	0.9849
Sy.x	5.434
Number of points	
Analyzed	21

Subtask 3



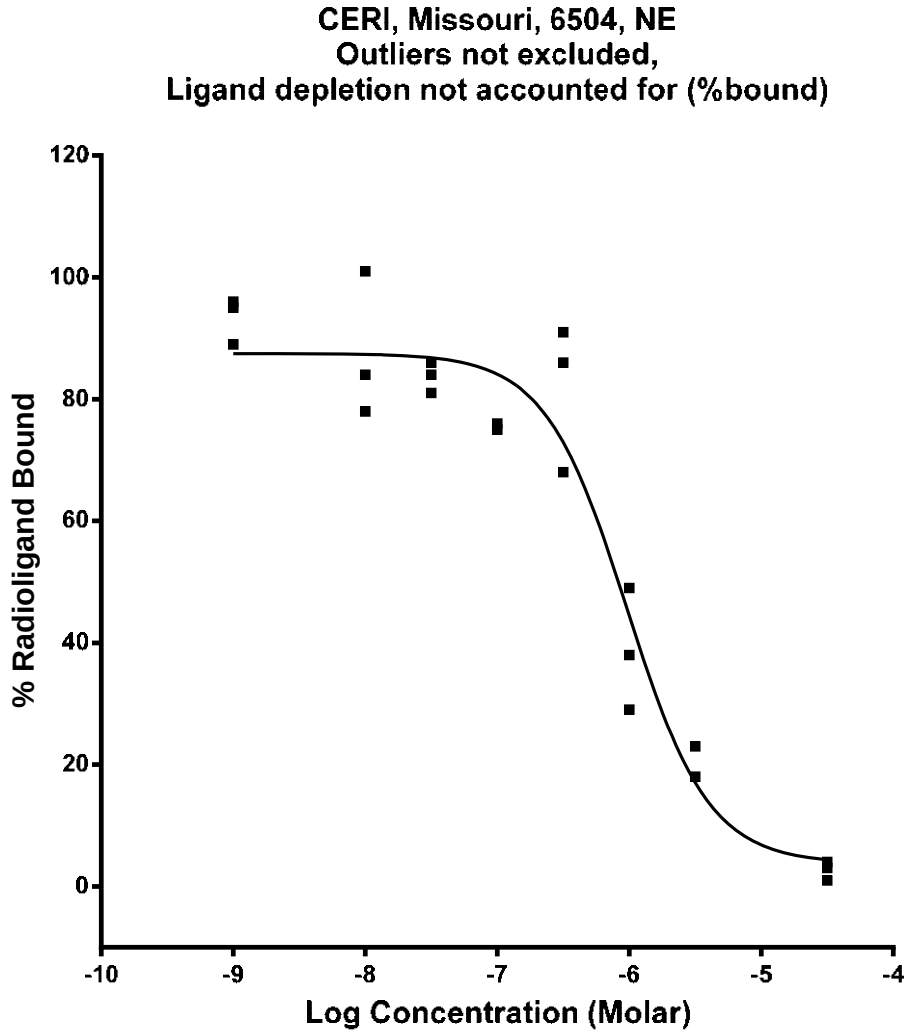
logIC50	
Best-fit values	
Bottom	-0.7623
Top	102.4
LogIC50	-6.005
HillSlope	-0.9926
Std. Error	
Bottom	8.730
Top	4.543
LogIC50	0.08068
HillSlope	0.2557
95% Confidence Intervals	
Bottom	-18.97 to 17.45
Top	92.90 to 111.9
LogIC50	-6.174 to -5.837
HillSlope	-1.526 to -0.4592
Goodness of Fit	
Degrees of Freedom	20
R square	0.9228
Sy.x	11.51
Number of points	
Analyzed	24

Subtask 3



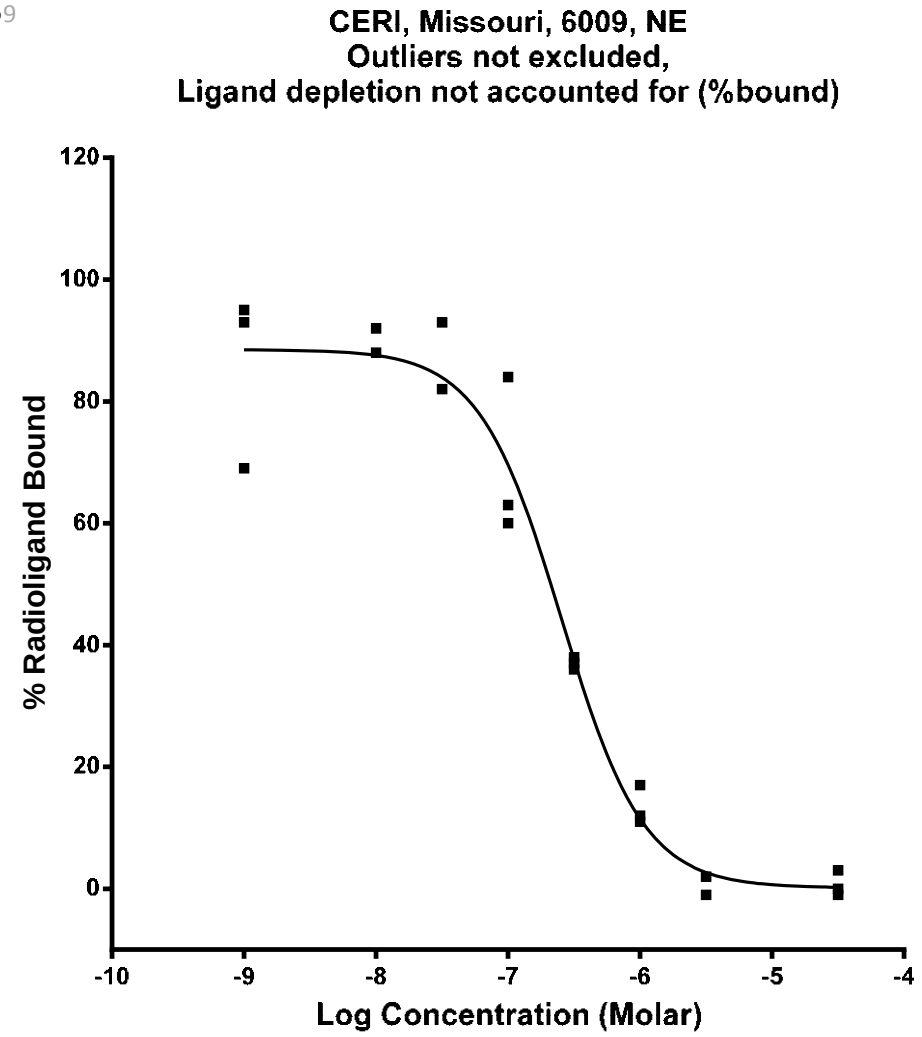
logIC50	
Best-fit values	
Bottom	-0.1095
Top	96.59
LogIC50	-6.589
HillSlope	-1.157
Std. Error	
Bottom	4.316
Top	3.634
LogIC50	0.05529
HillSlope	0.2101
95% Confidence Intervals	
Bottom	-9.113 to 8.894
Top	89.01 to 104.2
LogIC50	-6.704 to -6.474
HillSlope	-1.595 to -0.7189
Goodness of Fit	
Degrees of Freedom	20
R square	0.9628
Sy.x	8.253
Number of points	
Analyzed	24

Subtask 3



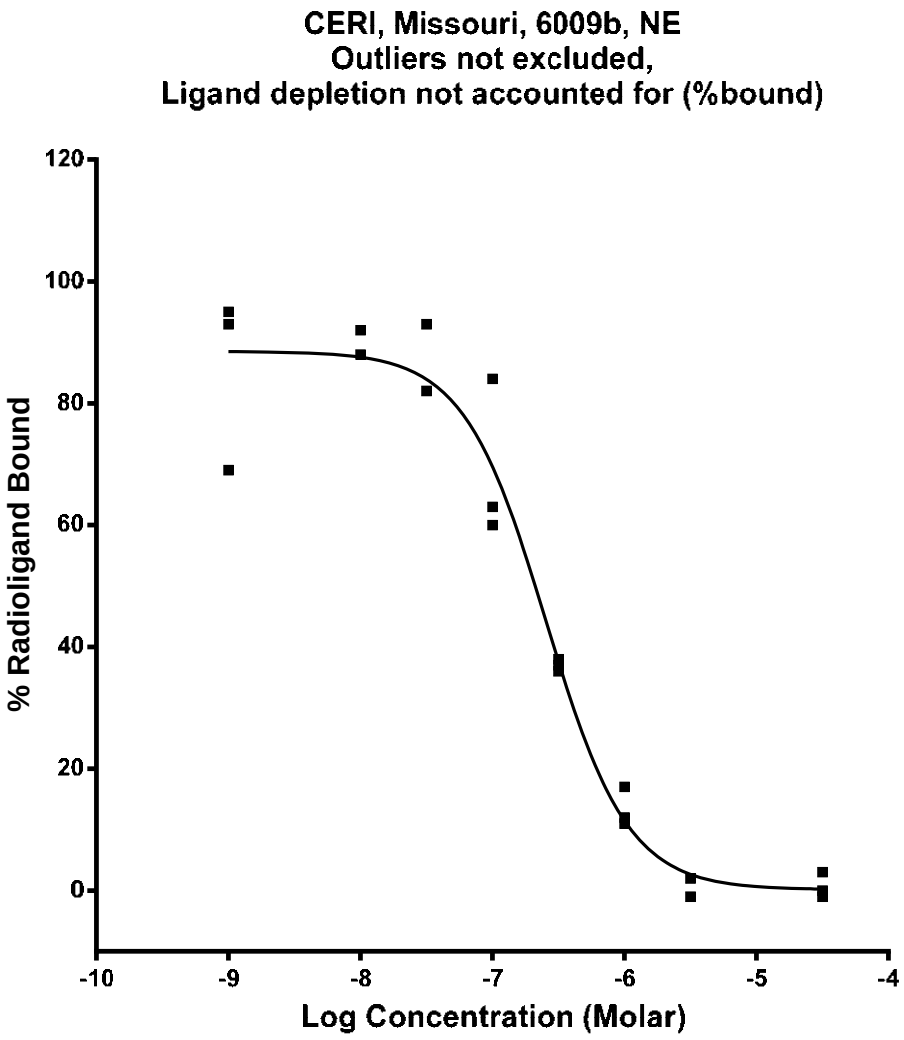
logIC50	
Best-fit values	
Bottom	3.782
Top	87.49
LogIC50	-6.082
HillSlope	-1.400
Std. Error	
Bottom	5.399
Top	2.923
LogIC50	0.06232
HillSlope	0.3261
95% Confidence Intervals	
Bottom	-7.481 to 15.04
Top	81.39 to 93.59
LogIC50	-6.212 to -5.952
HillSlope	-2.080 to -0.7200
Goodness of Fit	
Degrees of Freedom	20
R square	0.9391
Sy.x	8.894
Number of points	
Analyzed	24

Subtask 3[9]



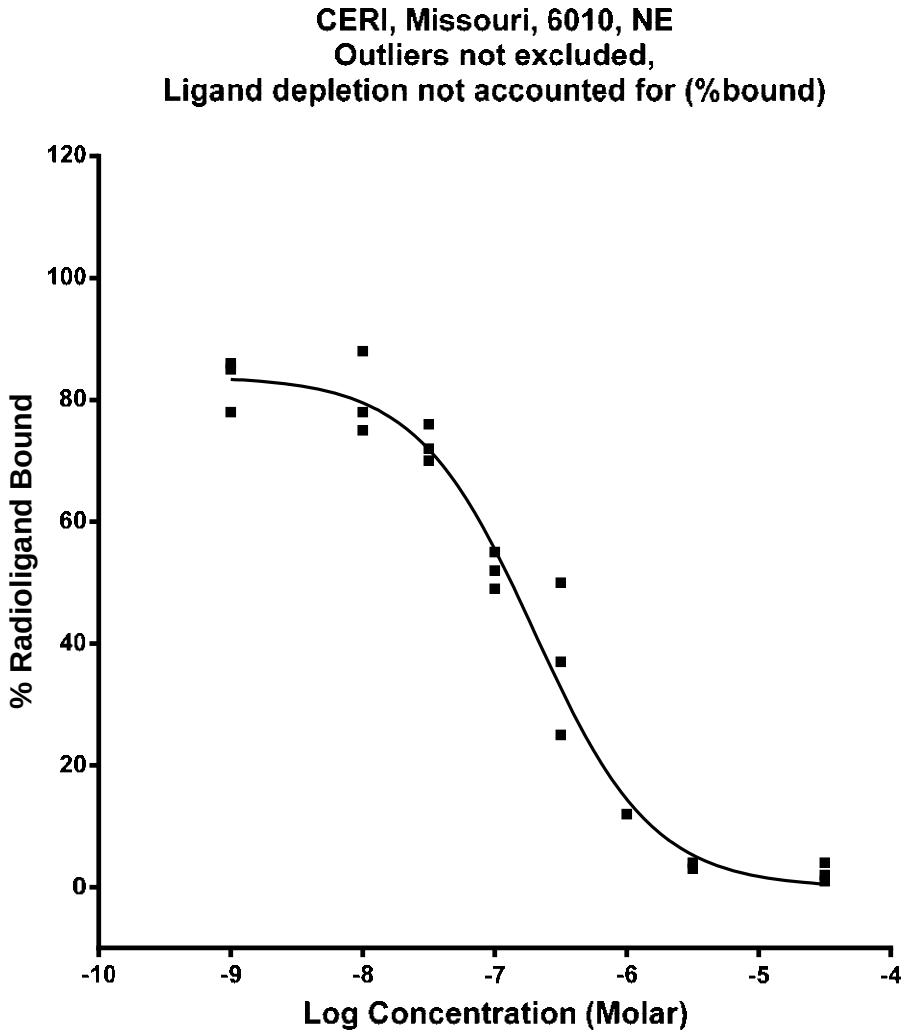
logIC50	
Best-fit values	
Bottom	0.1349
Top	88.51
LogIC50	-6.675
HillSlope	-1.397
Std. Error	
Bottom	3.200
Top	2.796
LogIC50	0.04662
HillSlope	0.2349
95% Confidence Intervals	
Bottom	-6.540 to 6.810
Top	82.68 to 94.35
LogIC50	-6.772 to -6.578
HillSlope	-1.886 to -0.9066
Goodness of Fit	
Degrees of Freedom	20
R square	0.9713
Sy.x	6.902
Number of points	
Analyzed	24

Subtask 3



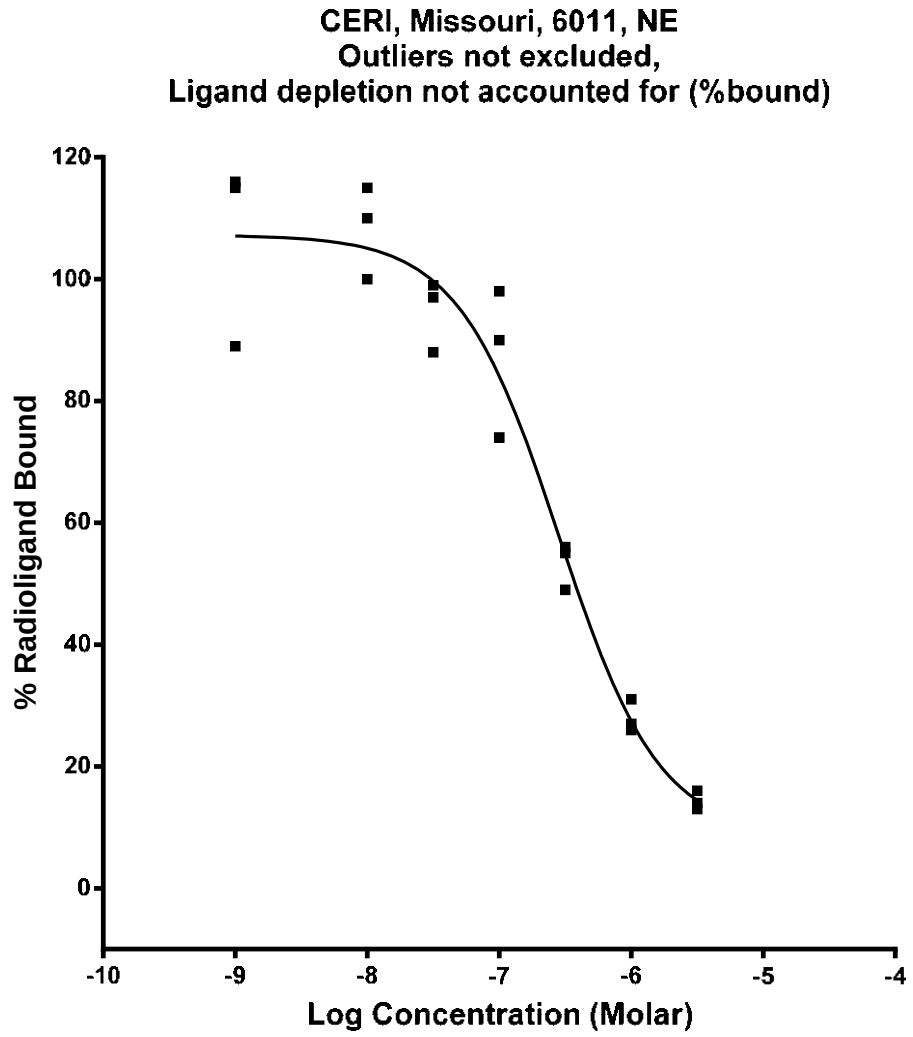
logIC50	
Best-fit values	
Bottom	0.1349
Top	88.51
LogIC50	-6.675
HillSlope	-1.397
Std. Error	
Bottom	3.200
Top	2.796
LogIC50	0.04662
HillSlope	0.2349
95% Confidence Intervals	
Bottom	-6.540 to 6.810
Top	82.68 to 94.35
LogIC50	-6.772 to -6.578
HillSlope	-1.886 to -0.9066
Goodness of Fit	
Degrees of Freedom	20
R square	0.9713
Sy.x	6.902
Number of points	
Analyzed	24

Subtask 1



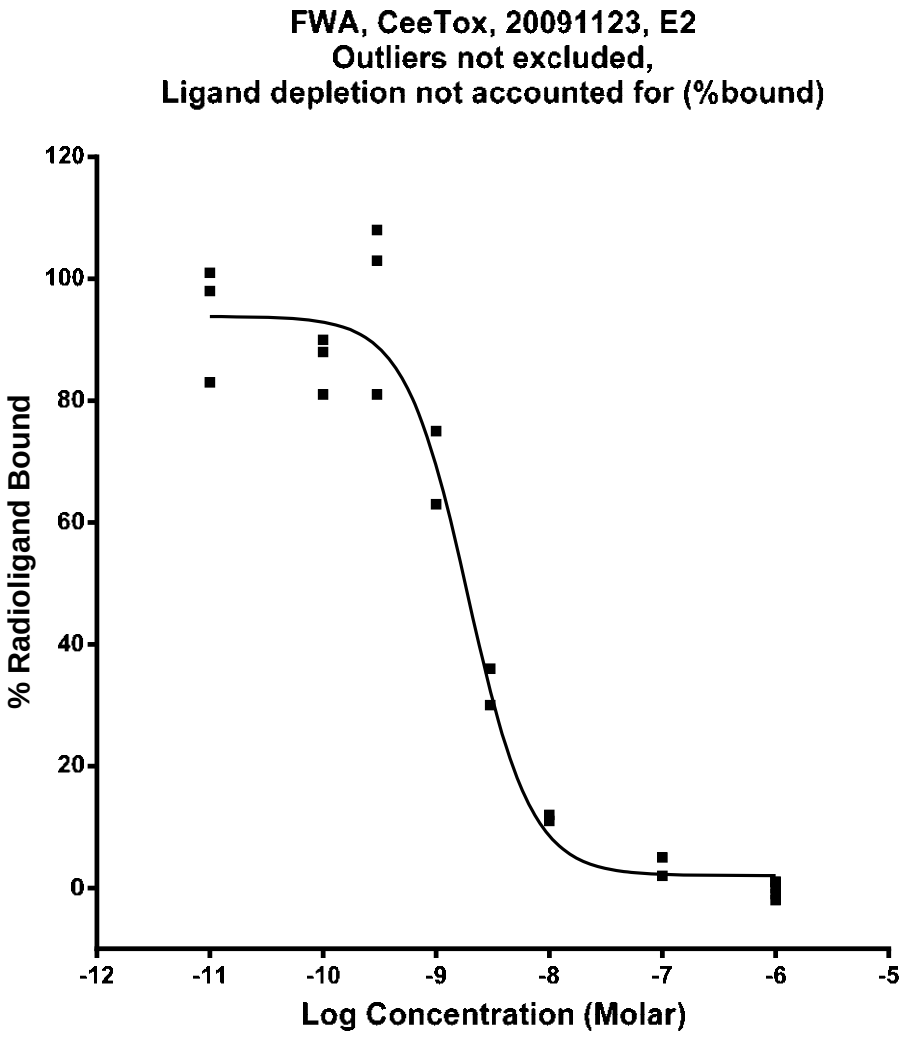
logIC50	
Best-fit values	
Bottom	-0.08222
Top	83.85
LogIC50	-6.873
HillSlope	-0.9721
Std. Error	
Bottom	3.079
Top	2.936
LogIC50	0.05063
HillSlope	0.1398
95% Confidence Intervals	
Bottom	-6.505 to 6.341
Top	77.72 to 89.97
LogIC50	-6.979 to -6.768
HillSlope	-1.264 to -0.6806
Goodness of Fit	
Degrees of Freedom	20
R square	0.9749
Sy.x	5.598
Number of points	
Analyzed	24

Subtask

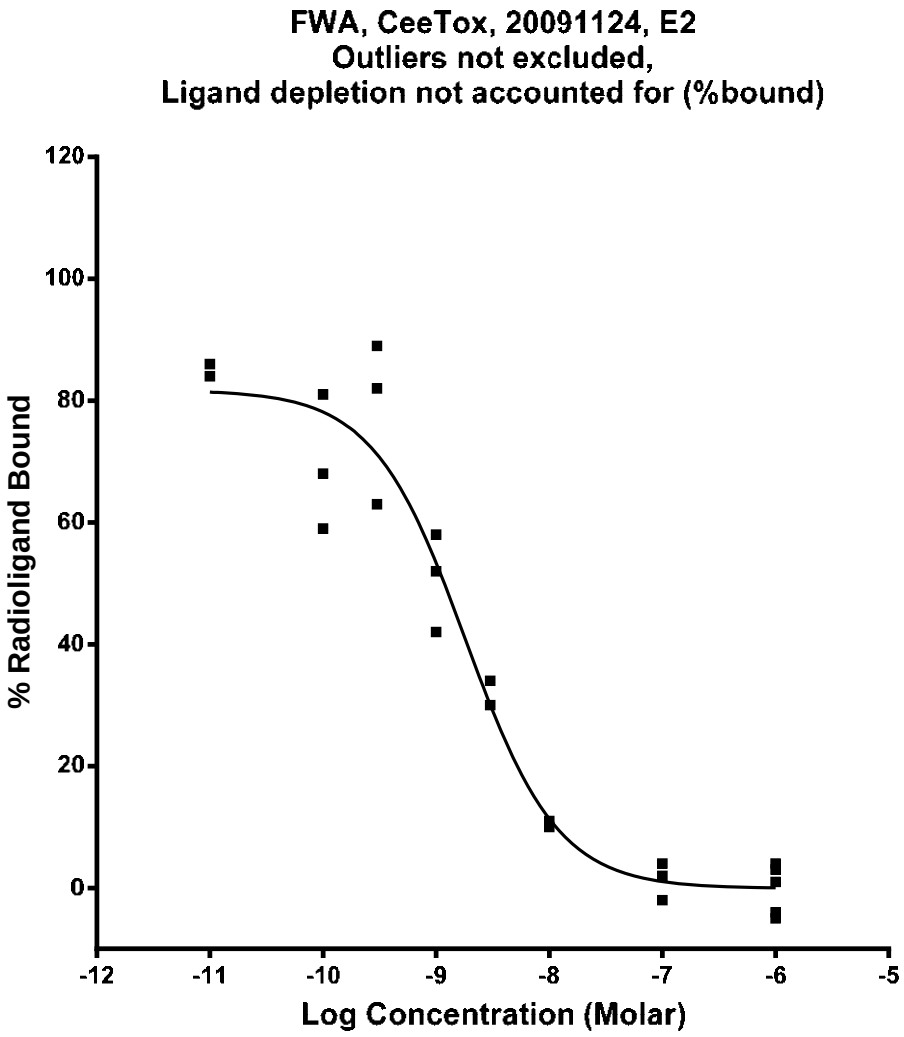


logIC50	
Best-fit values	
Bottom	8.431
Top	107.2
LogIC50	-6.425
HillSlope	-1.137
Std. Error	
Bottom	8.436
Top	3.792
LogIC50	0.06233
HillSlope	0.2641
95% Confidence Intervals	
Bottom	-9.369 to 26.23
Top	99.22 to 115.2
LogIC50	-6.556 to -6.293
HillSlope	-1.694 to -0.5800
Goodness of Fit	
Degrees of Freedom	17
R square	0.9584
Sy.x	8.213
Number of points	
Analyzed	21

Subtask 1


FWA, CeeTox, 20091123, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 1


FWA, CeeTox, 20091124, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
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20
0
-12
-11
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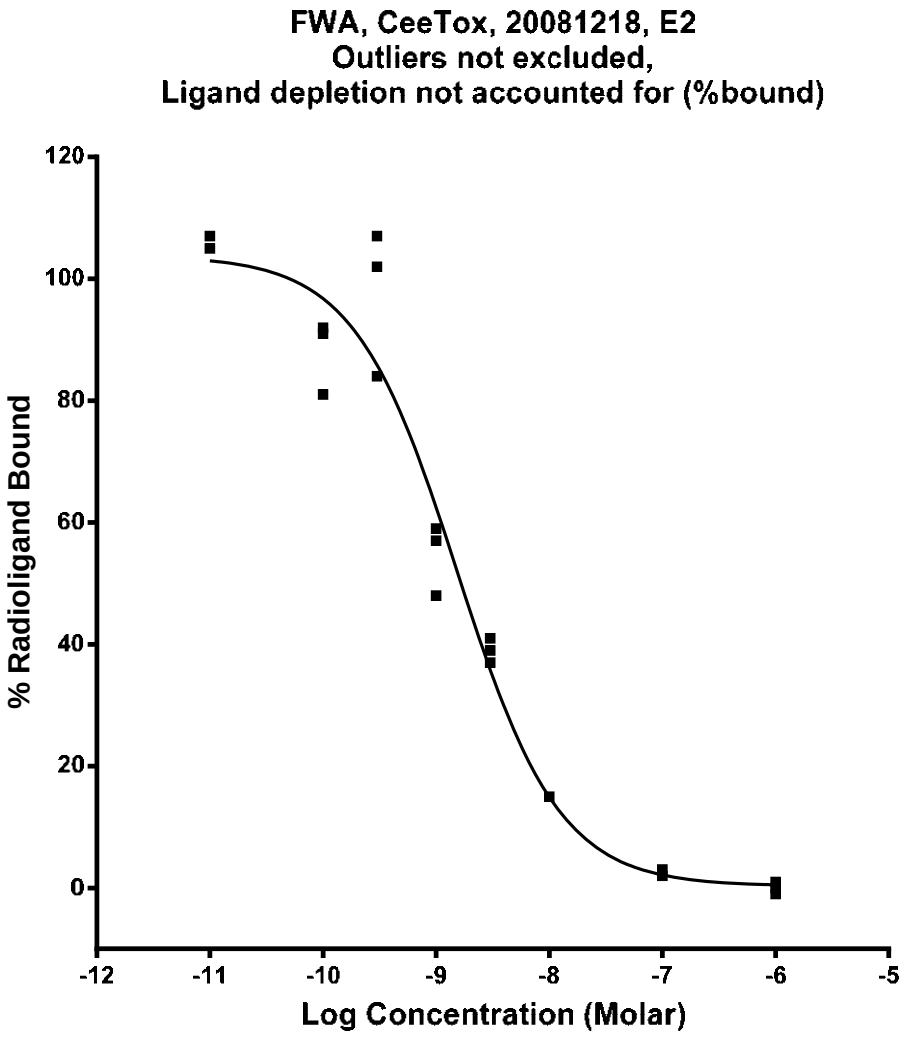
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 1


FWA, CeeTox, 20081218, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
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0
-12
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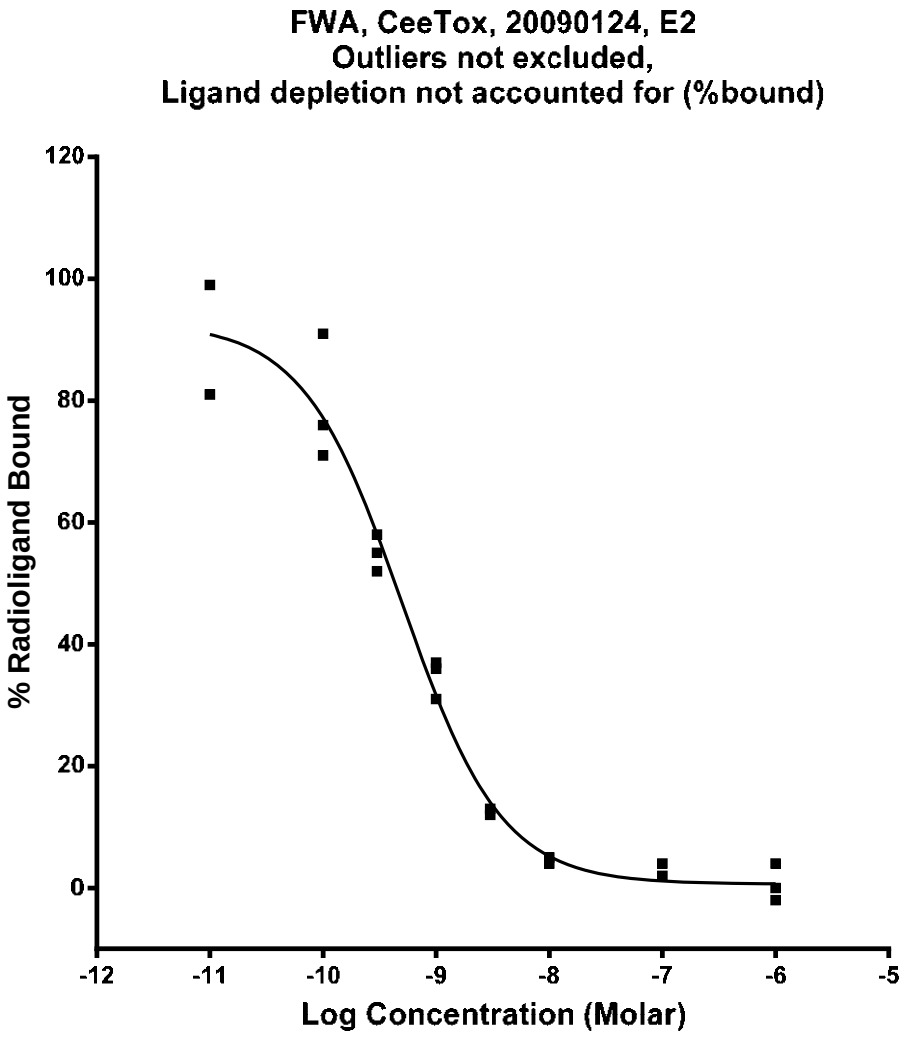
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090124, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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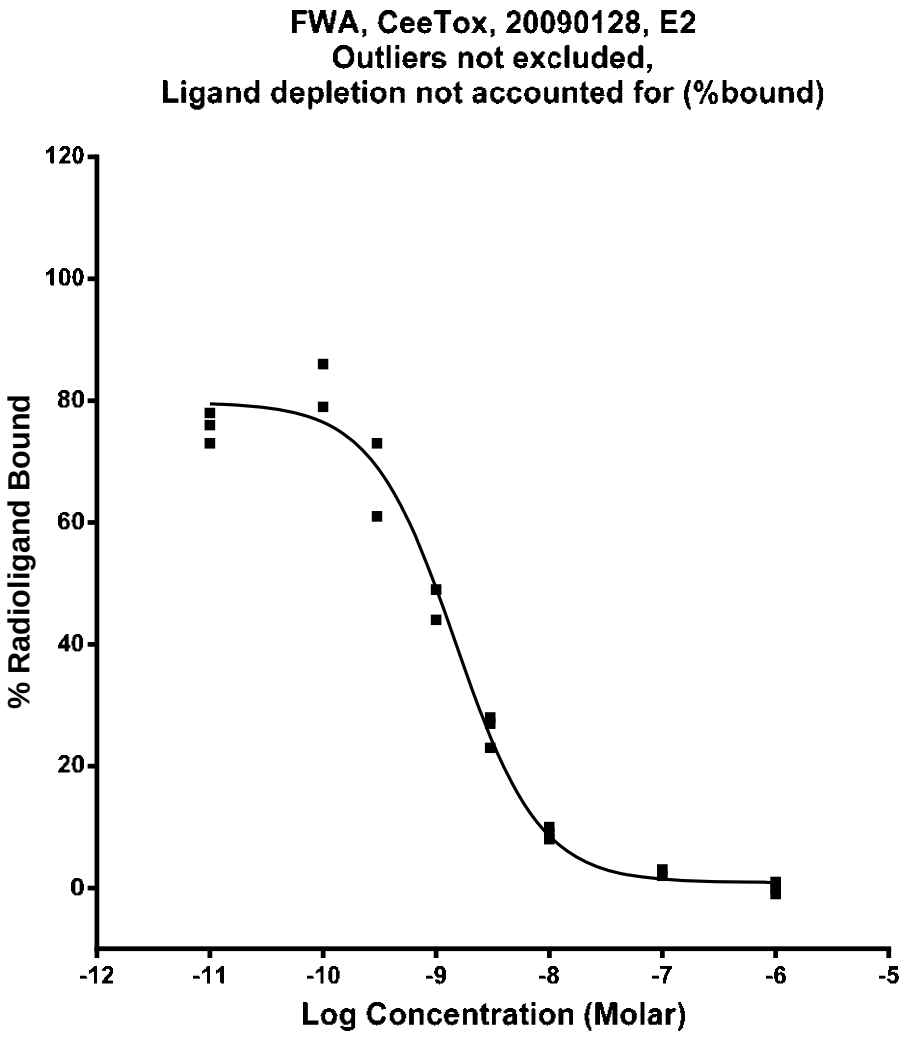
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090128, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
80
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-12
-11
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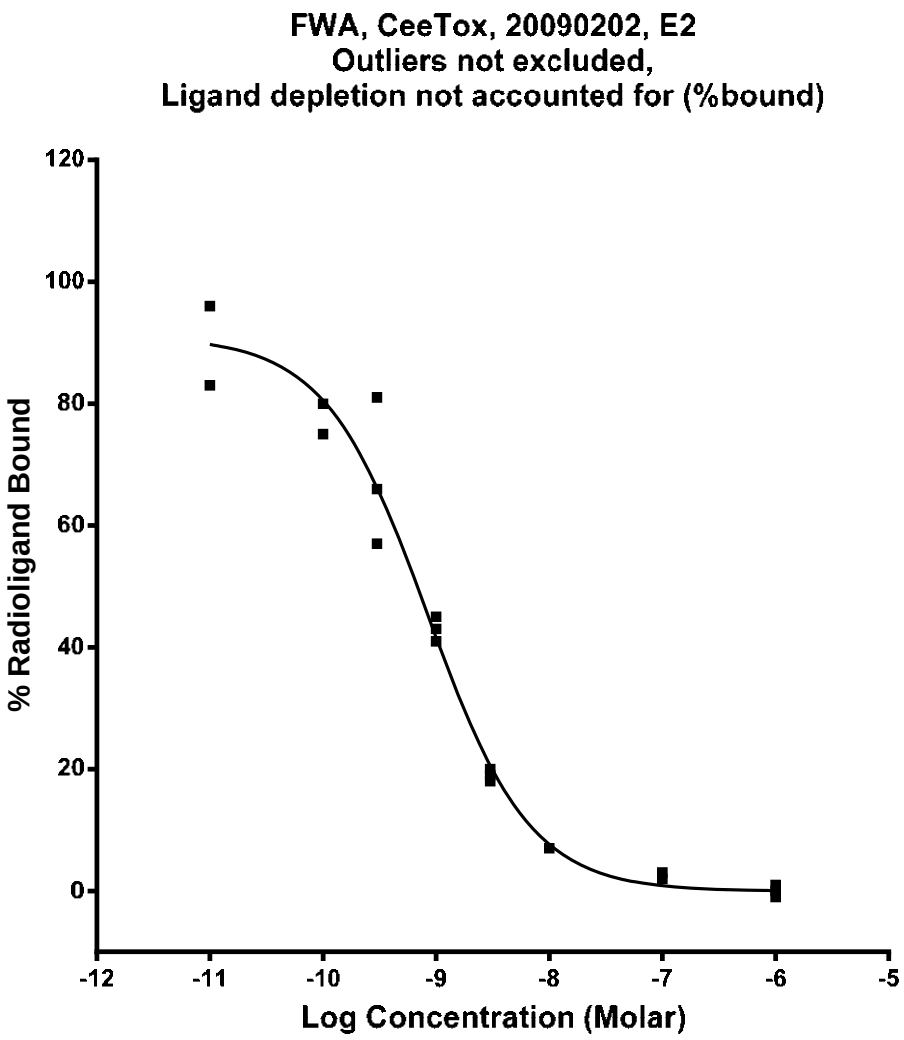
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090202, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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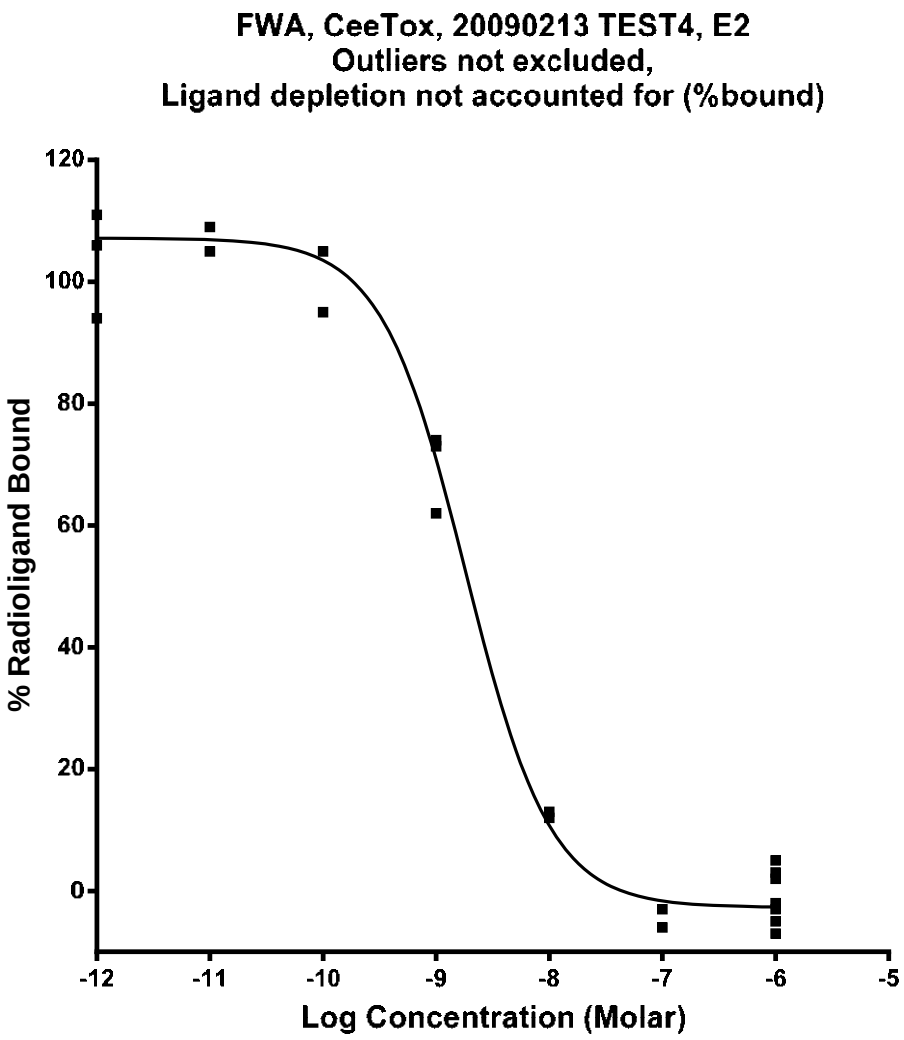
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090213 TEST4, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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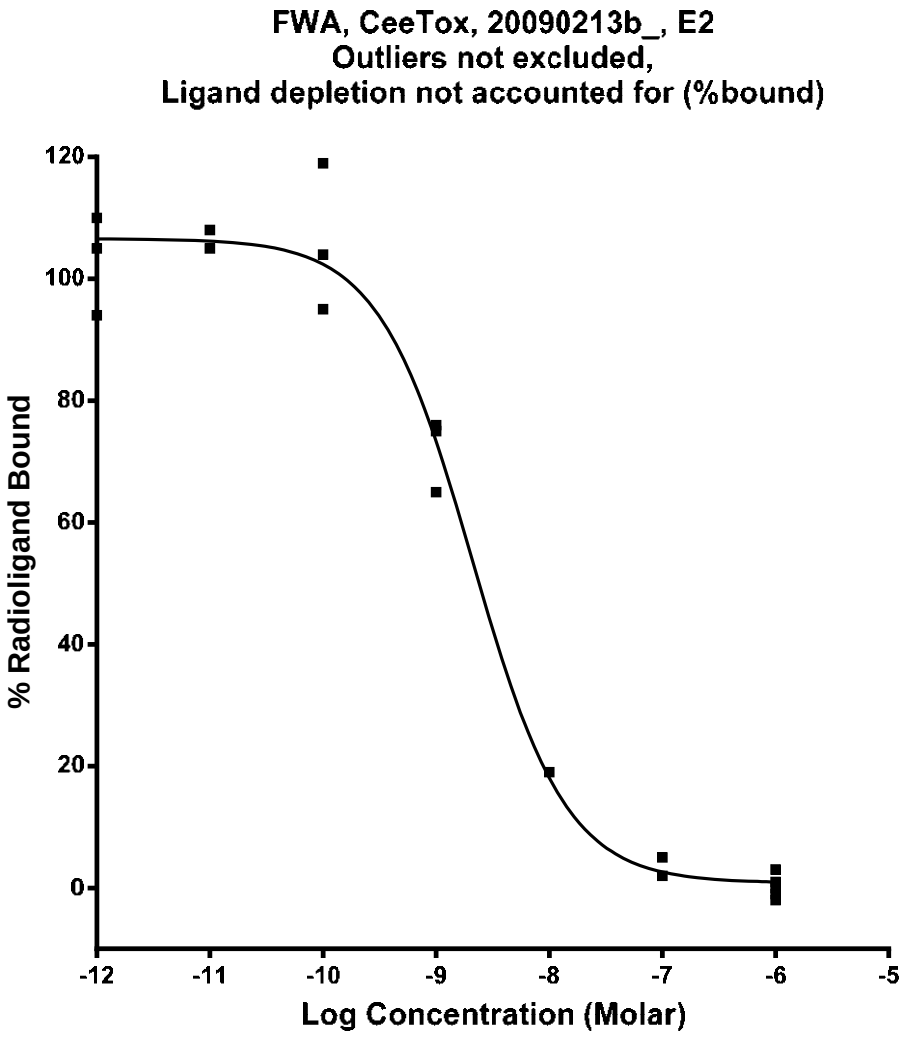
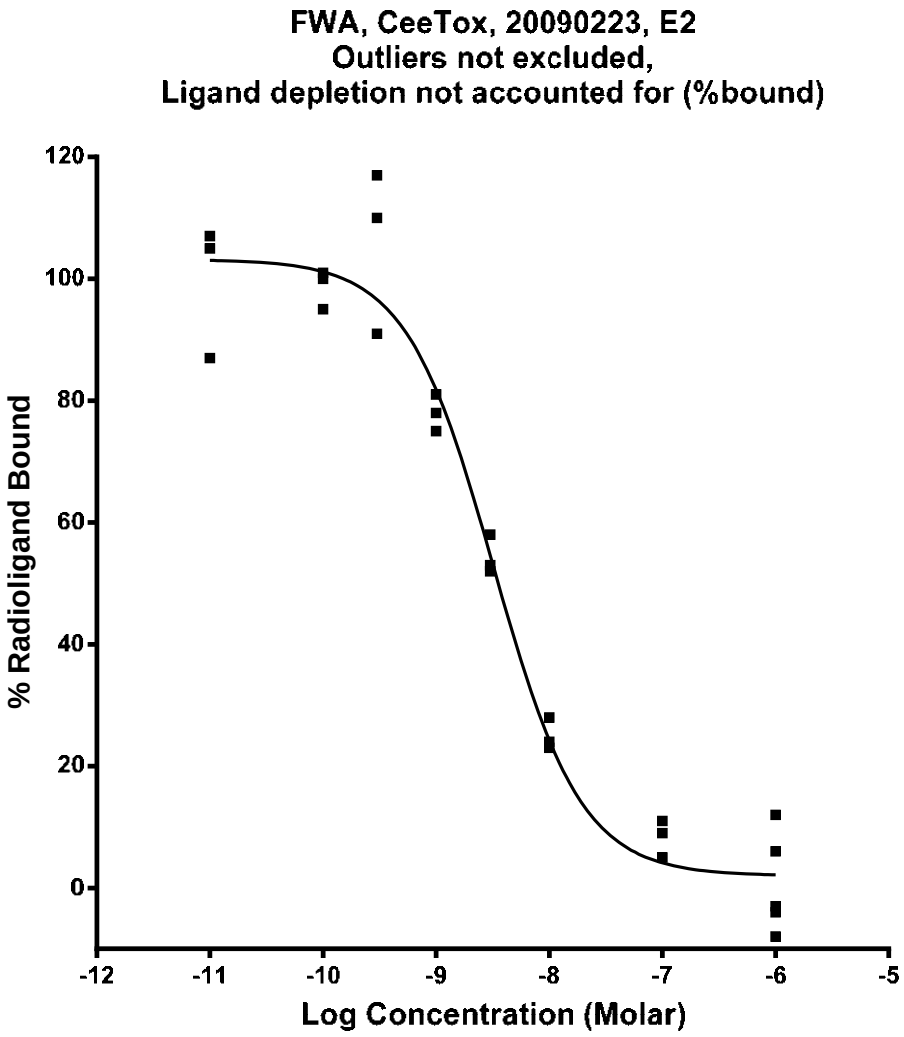
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

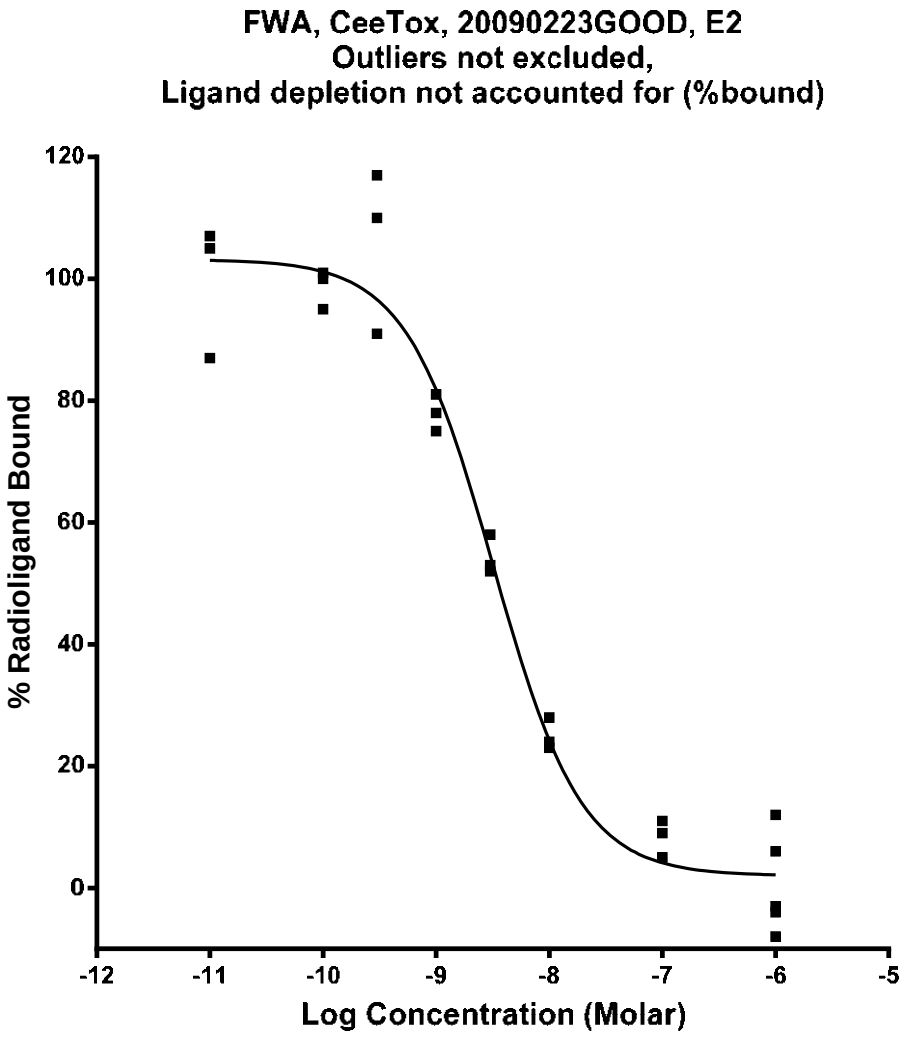
Subtask 2


FWA, CeeTox, 20090213b_, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

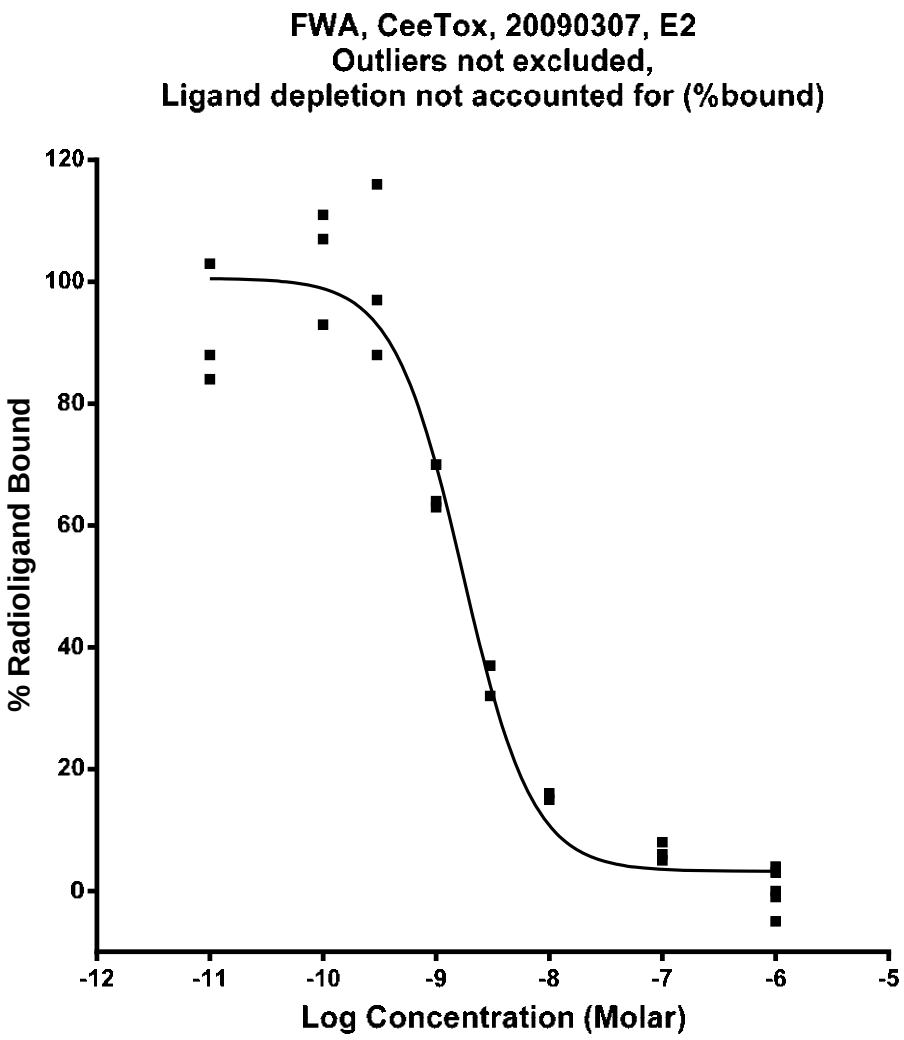
Subtask 2


FWA, CeeTox, 20090223, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

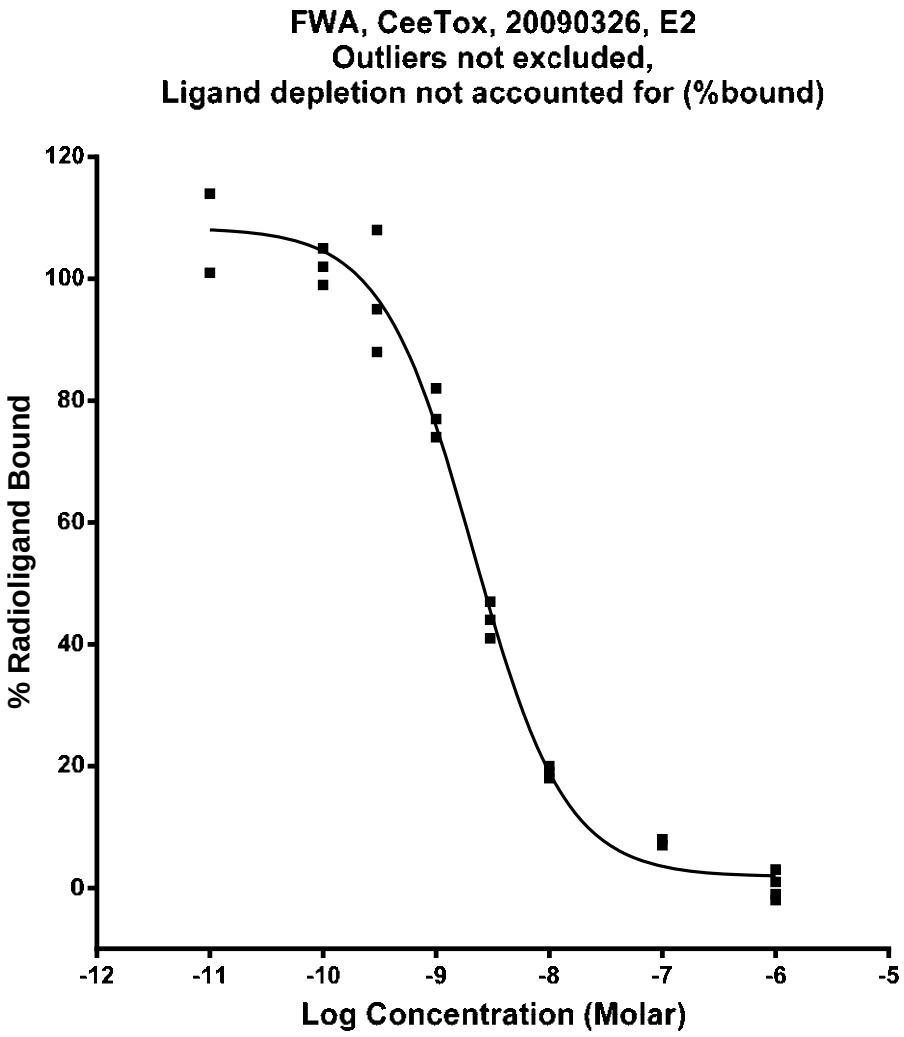
Subtask 2


FWA, CeeTox, 20090223GOOD, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

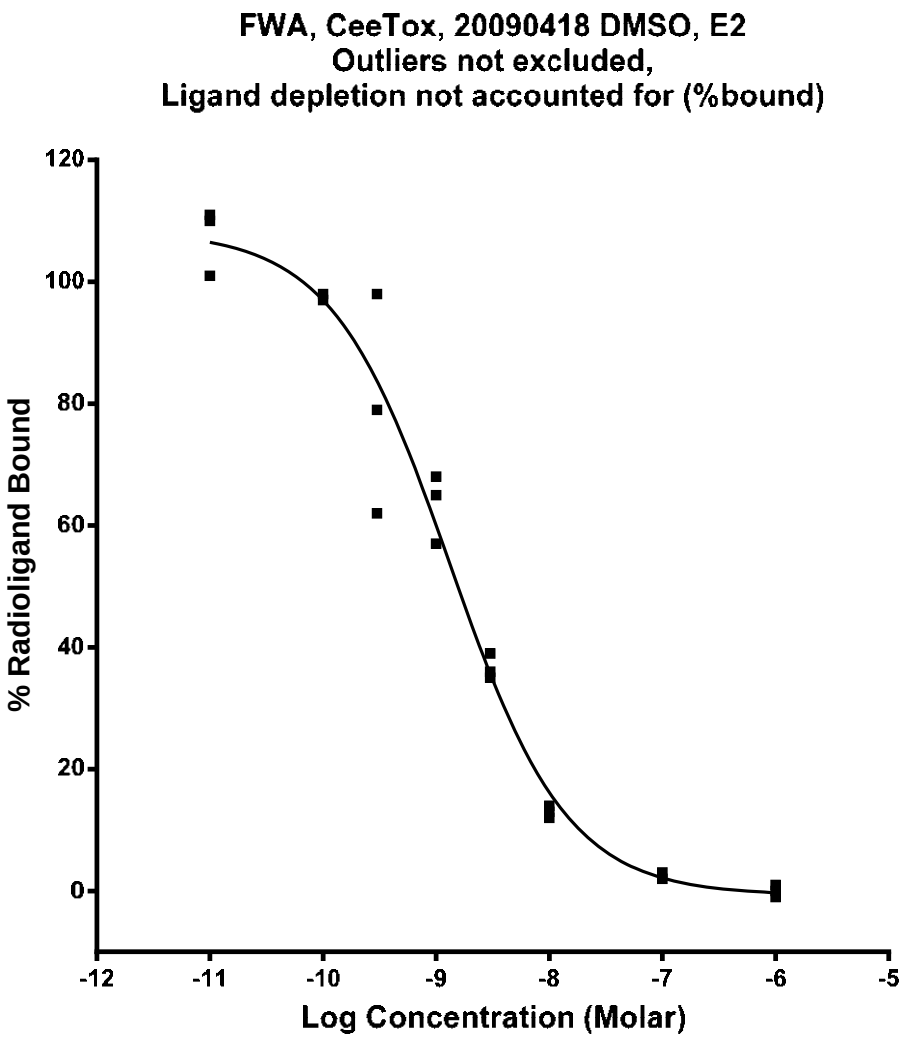
Subtask 2


FWA, CeeTox, 20090307, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

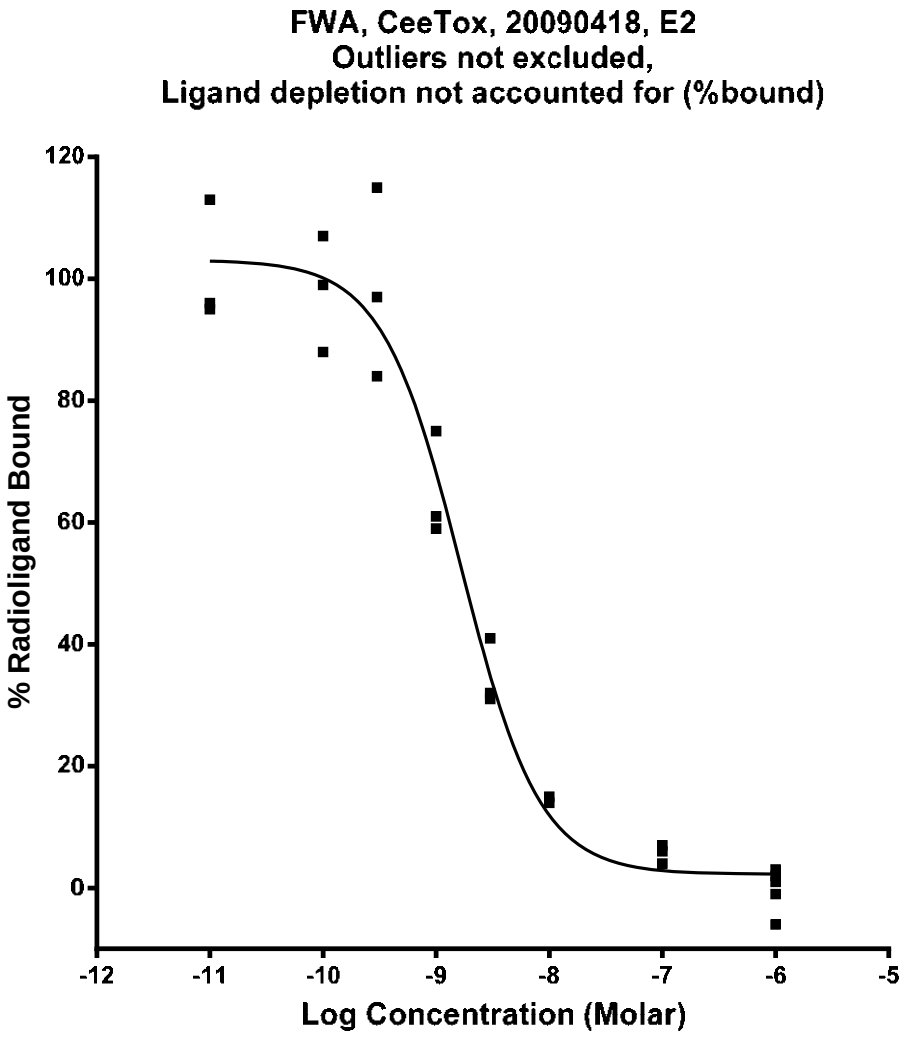
Subtask 2


FWA, CeeTox, 20090326, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418 DMSO, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
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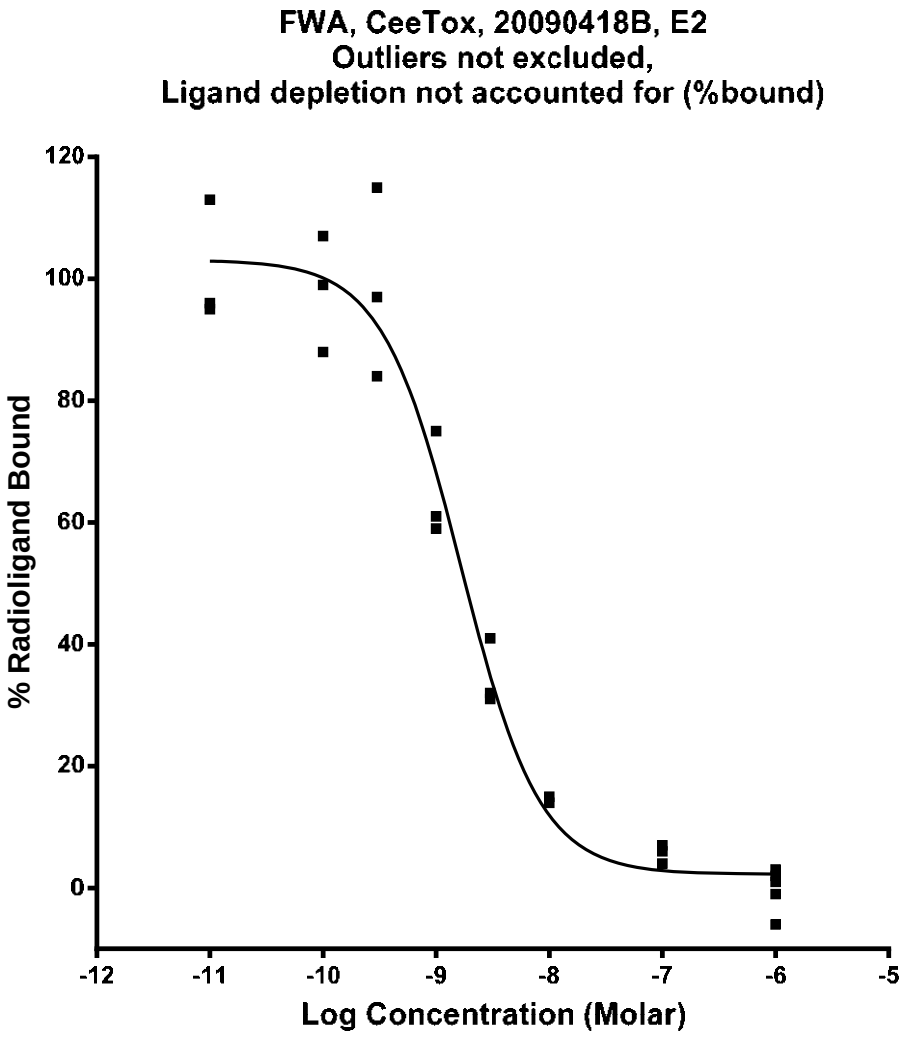
-6
-5
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418B, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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-12
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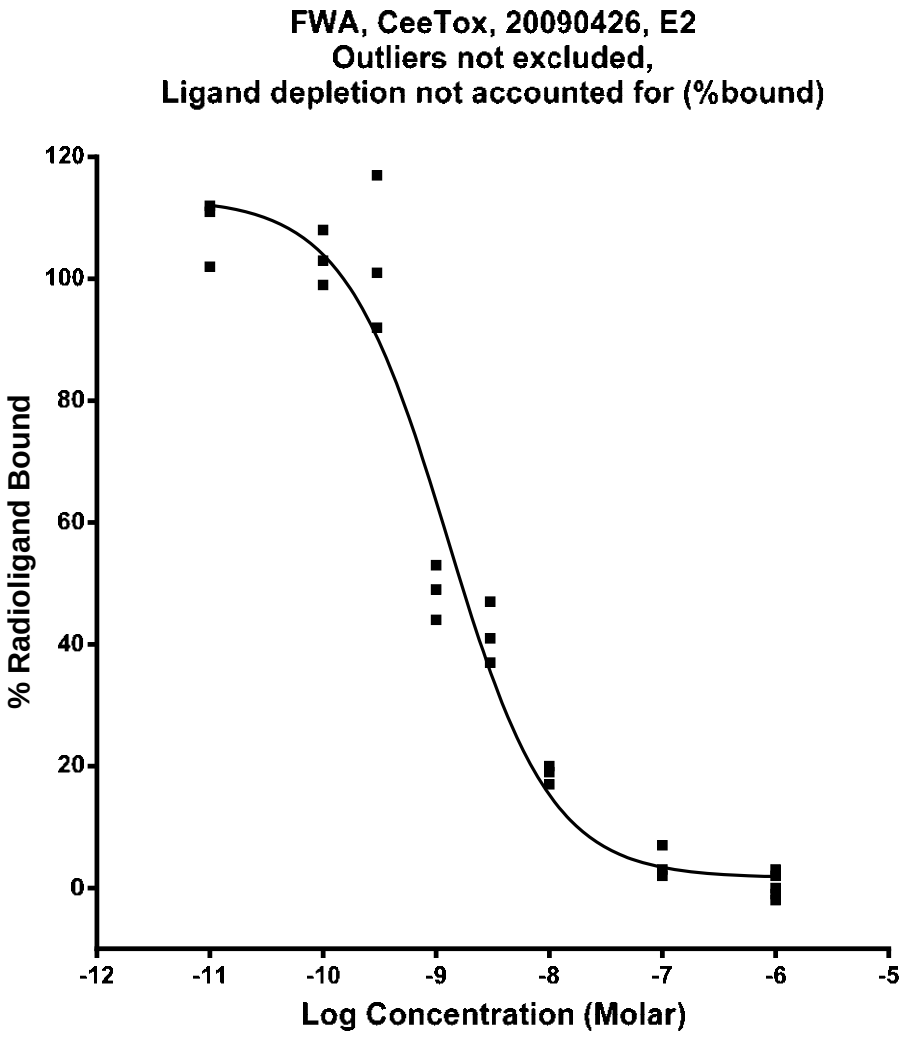
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090426, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
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0
-12
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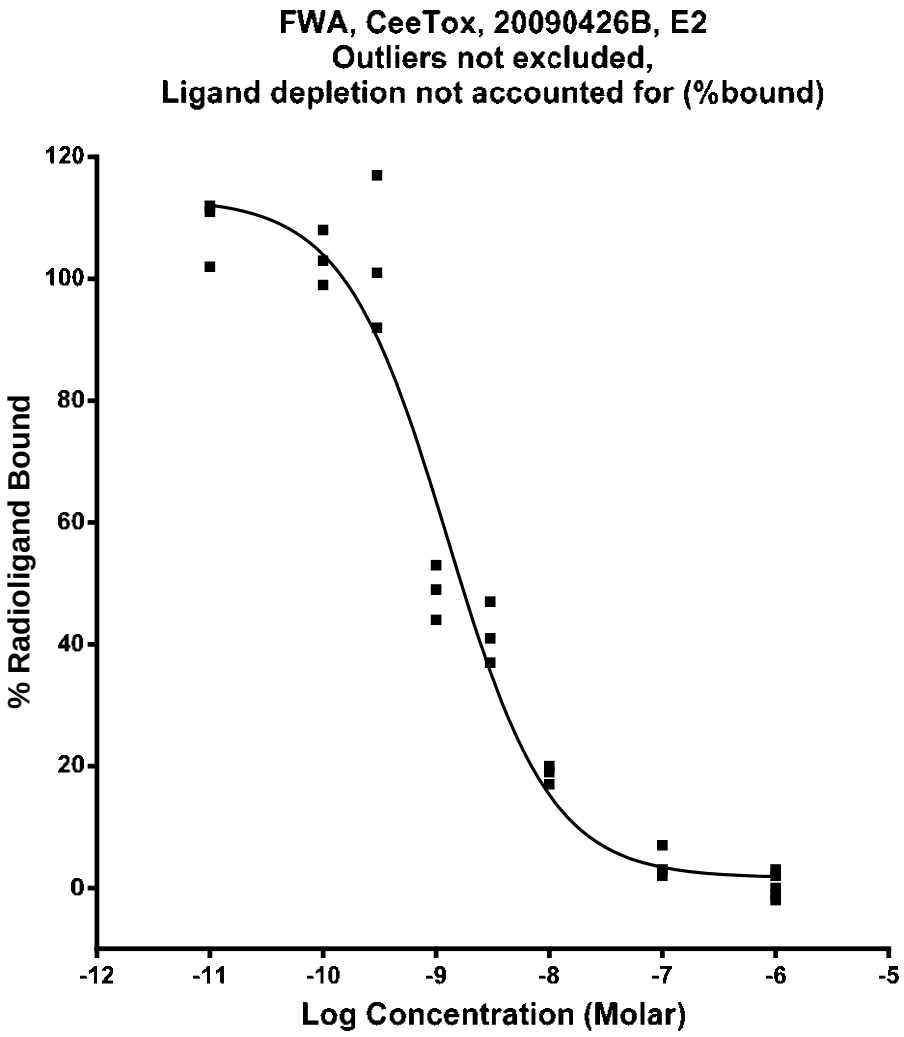
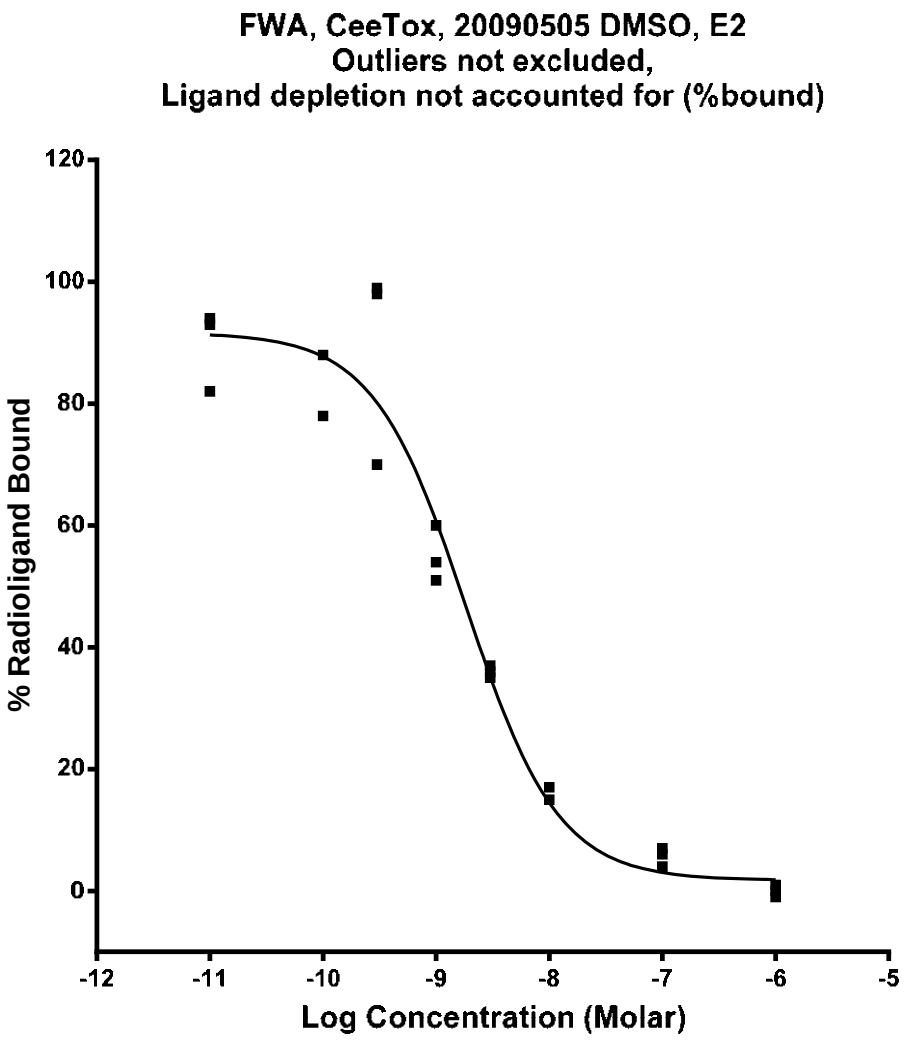
-8
-7
-6
-5
Log Concentration (Molar)

Subtask 3



Same data as for 20090426

Subtask 3


FWA, CeeTox, 20090505 DMSO, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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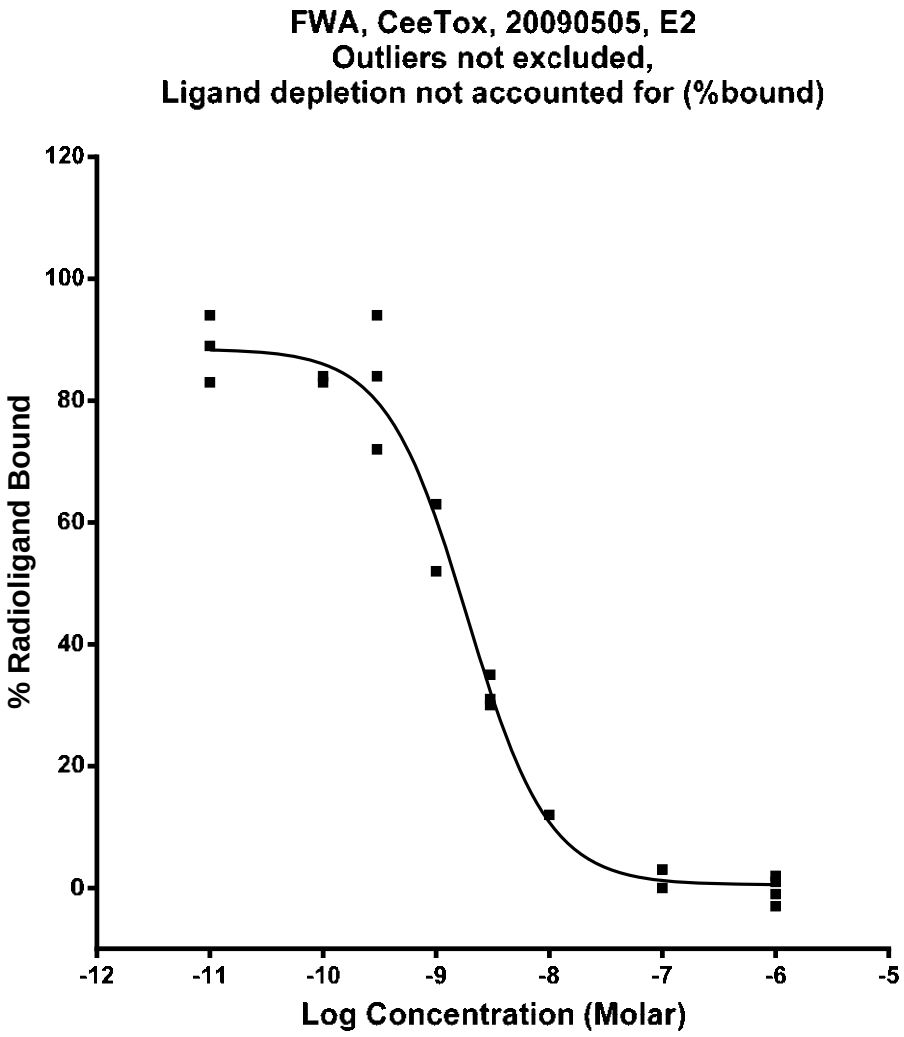
-5
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090505, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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40
20
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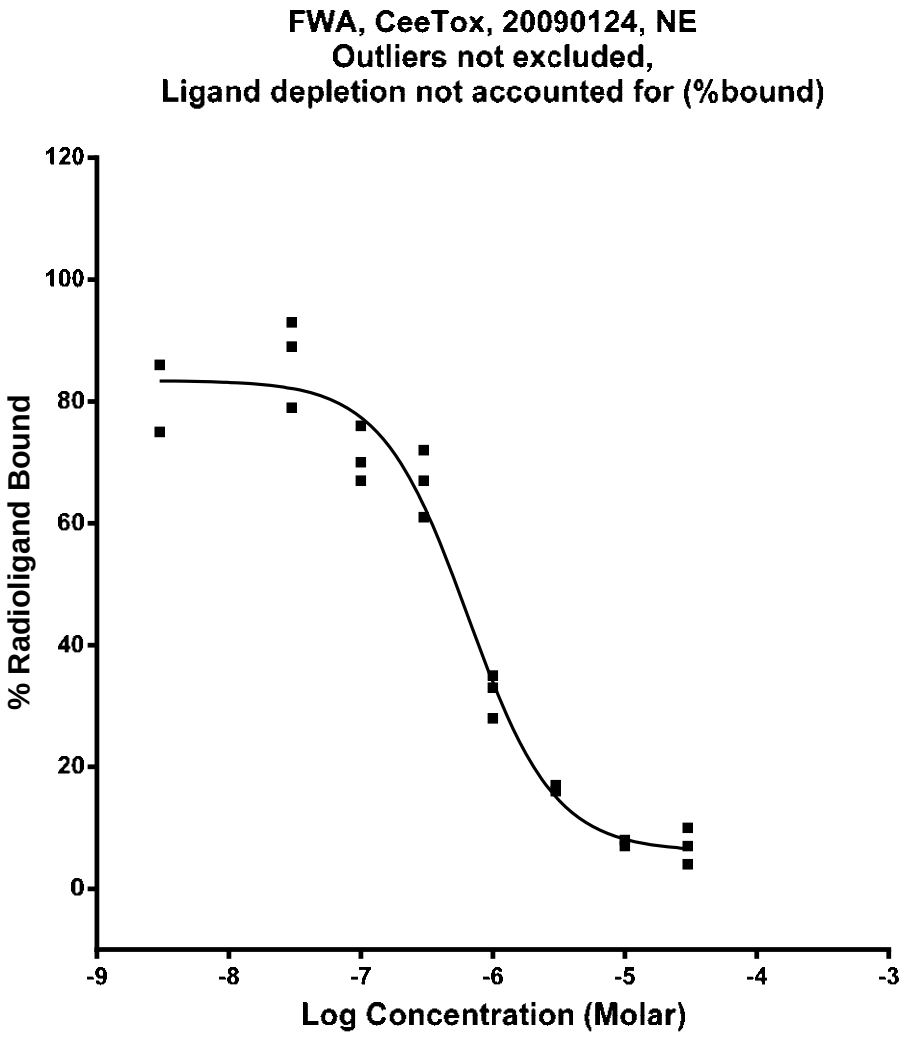
-5
Log Concentration (Molar)

FW ASSAY, (Ceetox Laboratory) Control Norethynodrel (NE) Curves 30 Nov 2014

Begin Here

Subtask 2


FWA, CeeTox, 20090124, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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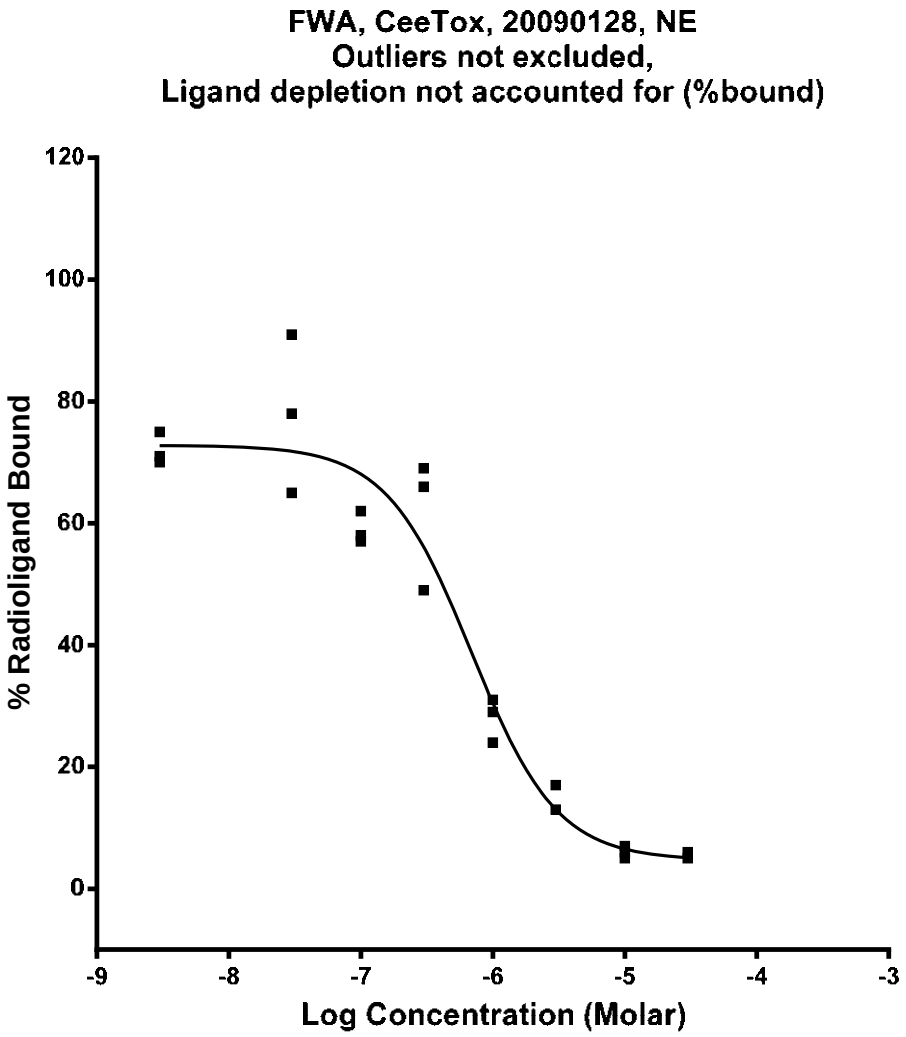
-3
Log Concentration (Molar)

Subtask 2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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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-5
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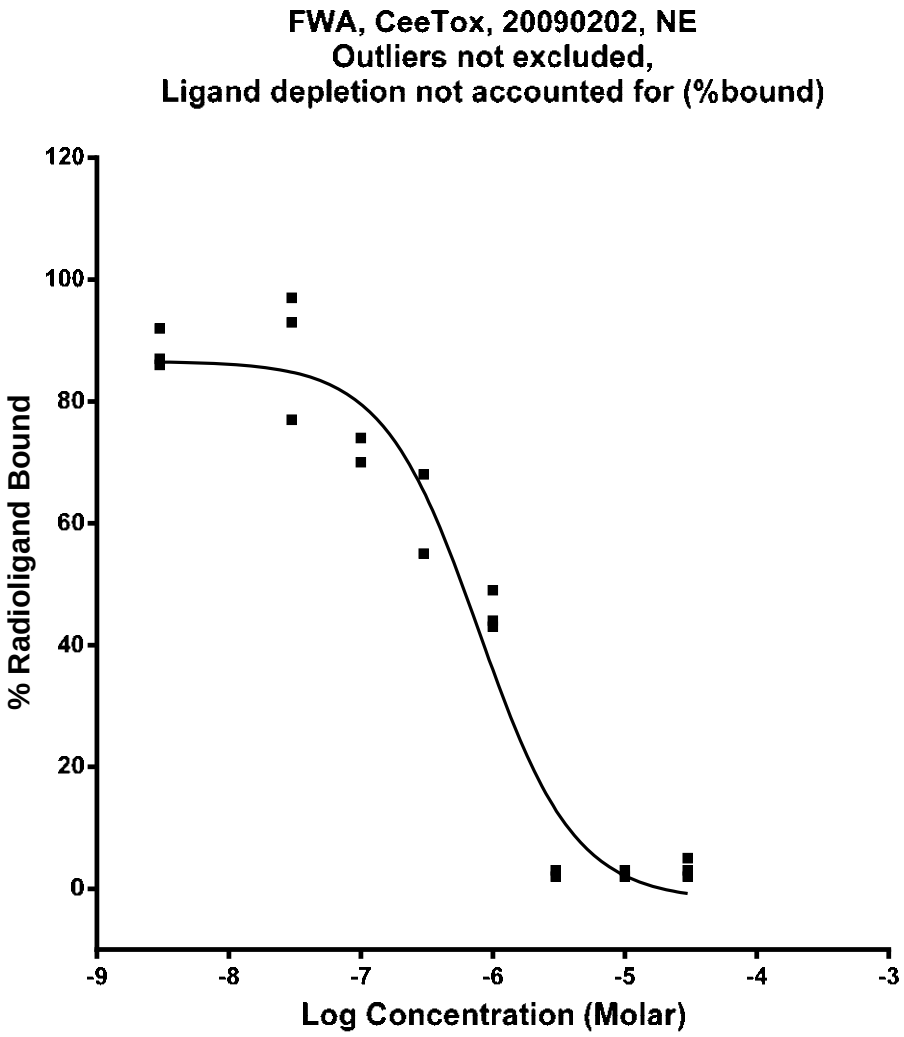
-3
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090202, NE
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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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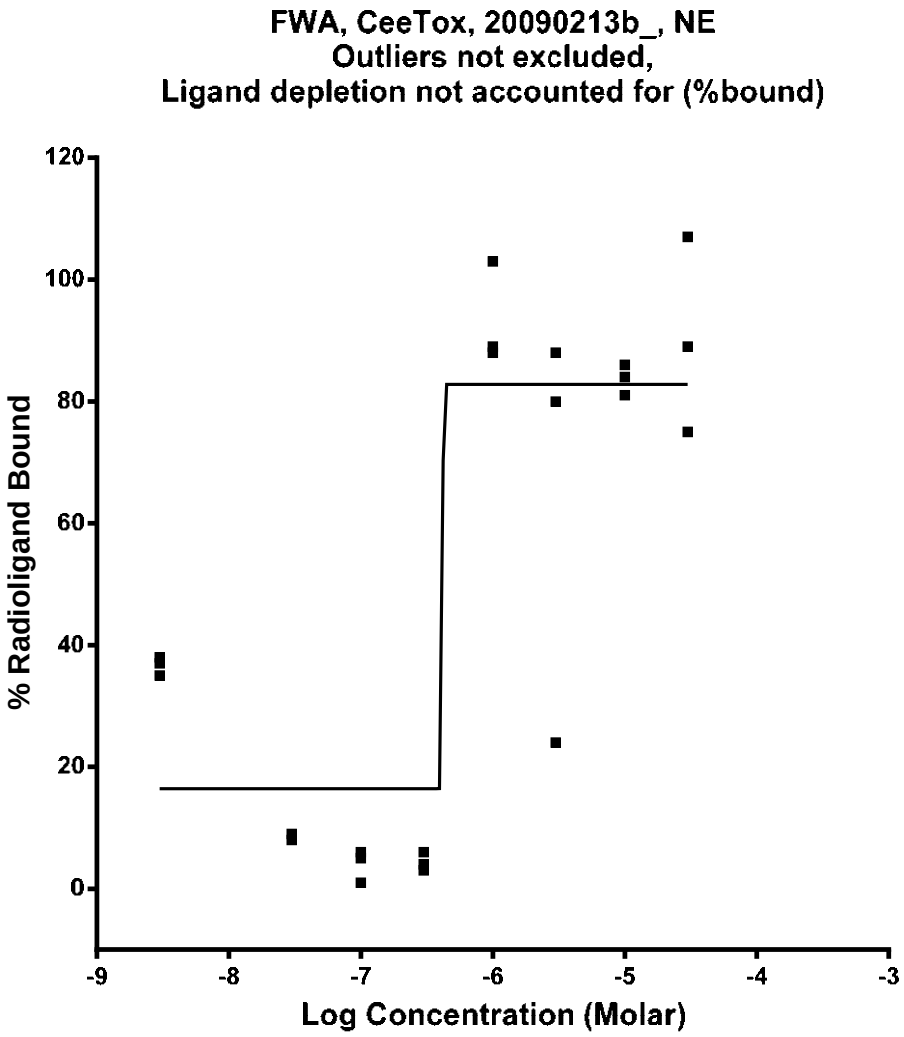
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090213b_, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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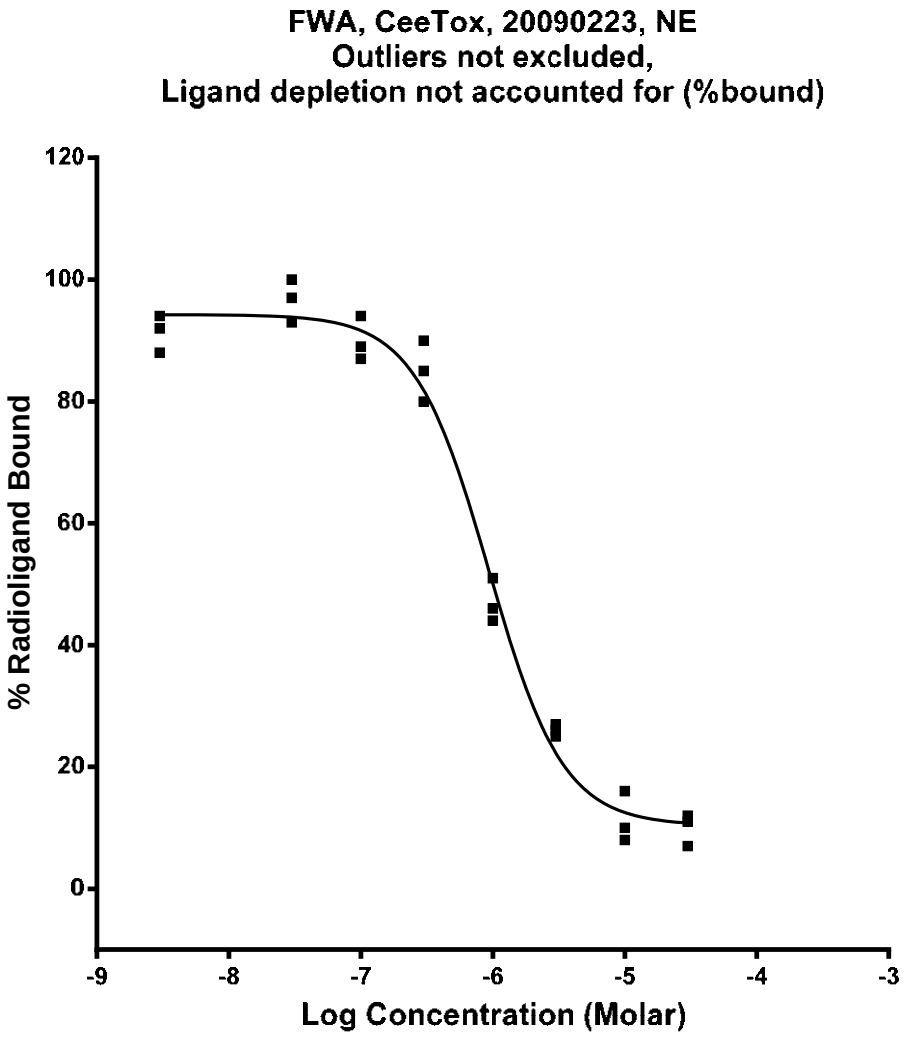
-3
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090223, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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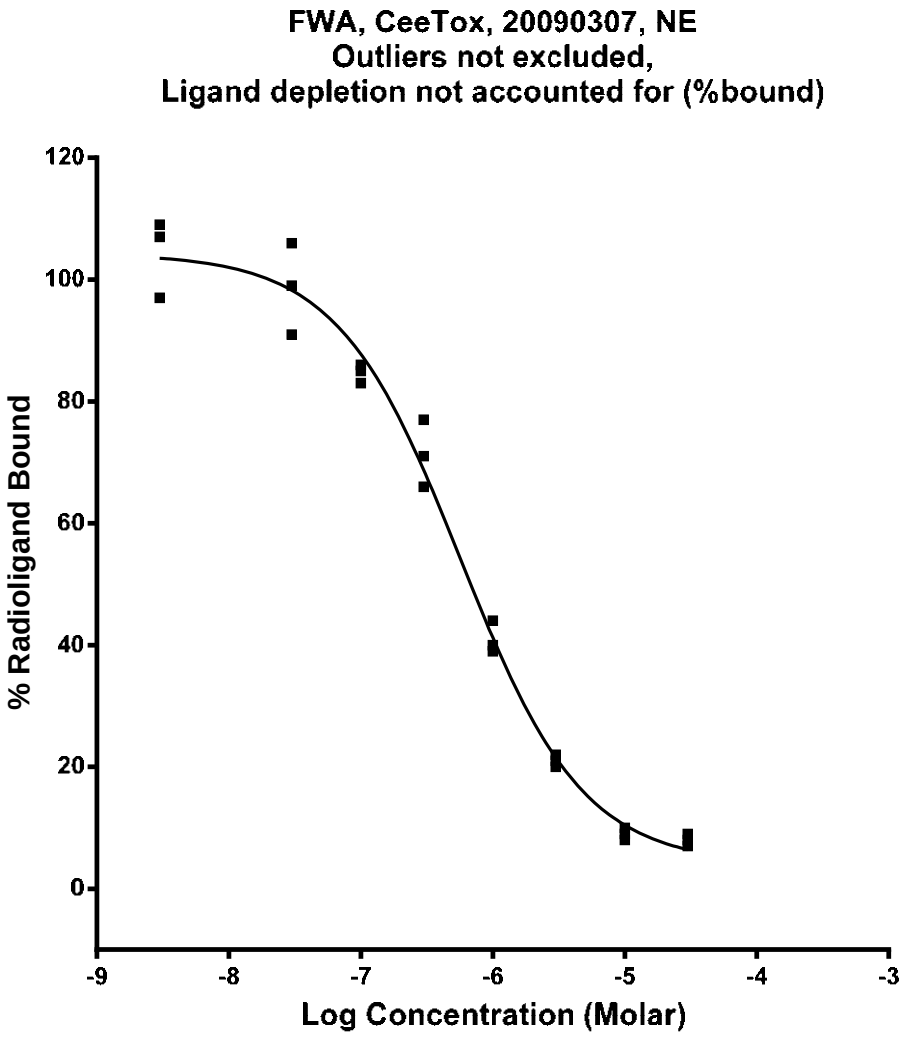
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090307, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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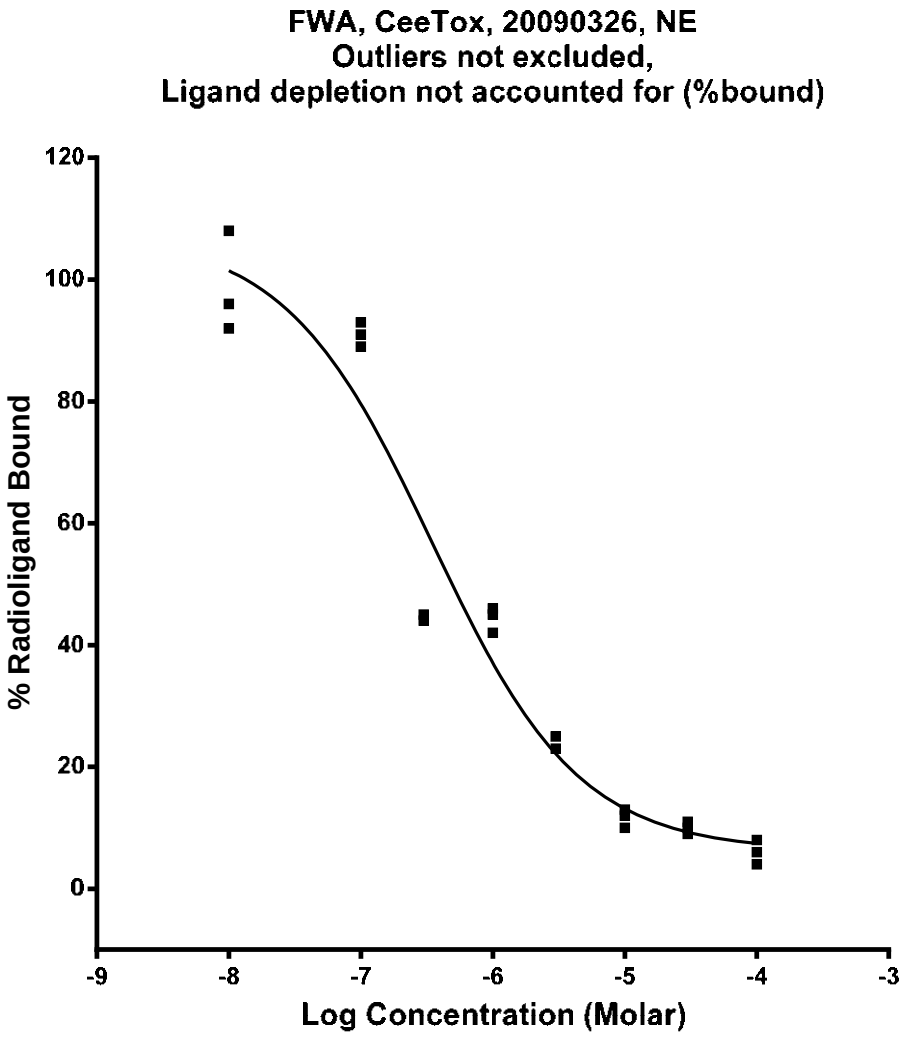
Log Concentration (Molar)

Subtask 2


FWA, CeeTox, 20090326, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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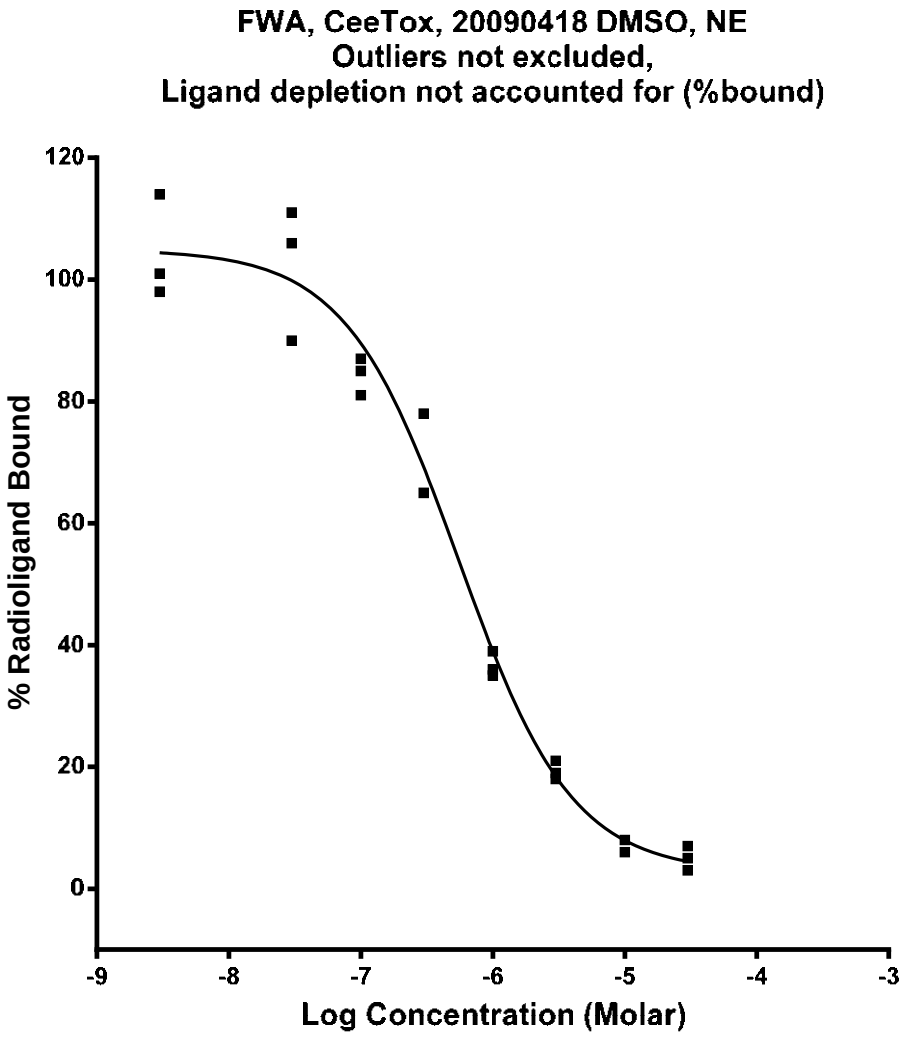
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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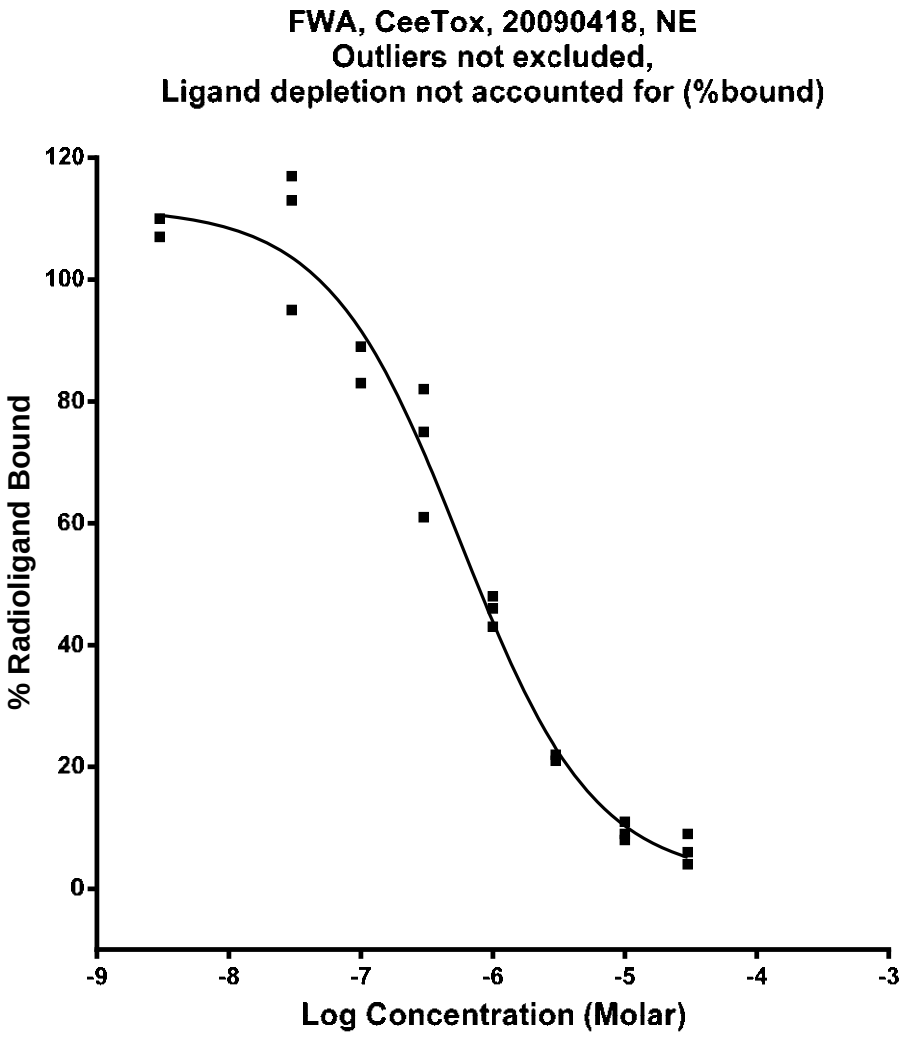
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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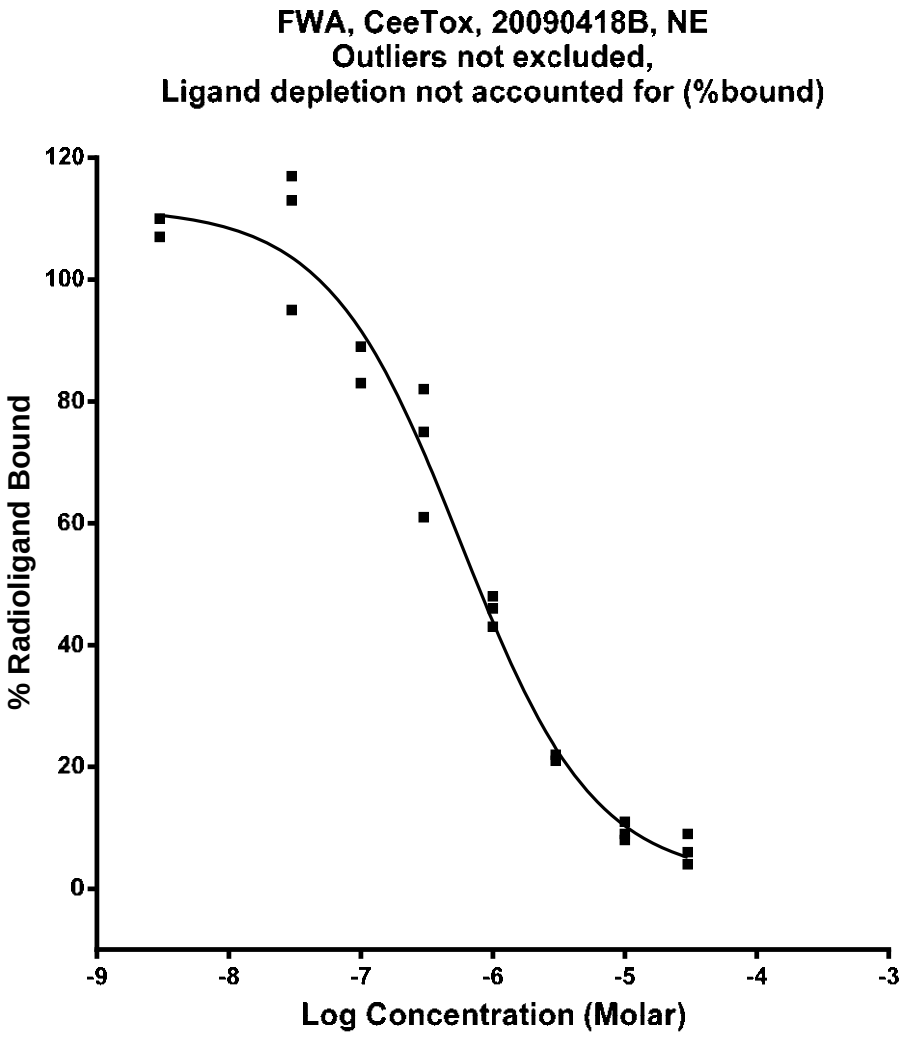
-3
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090418B, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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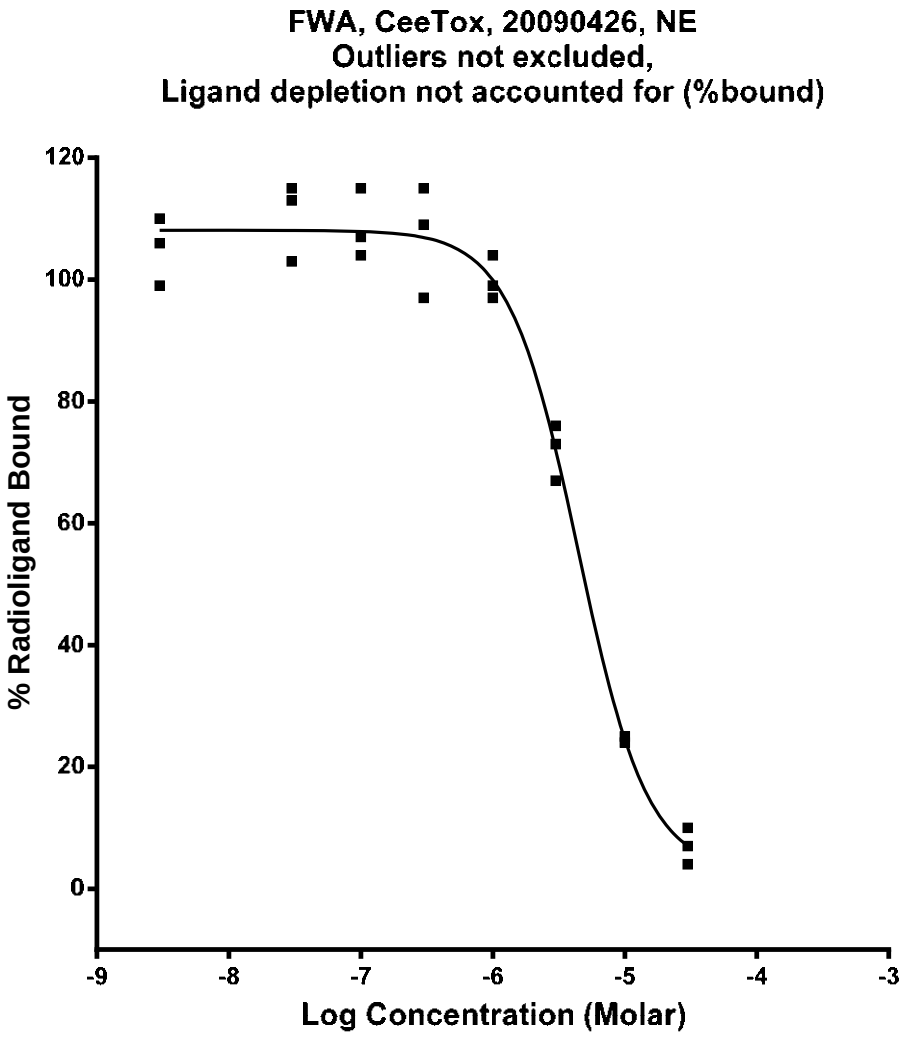
-3
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090426, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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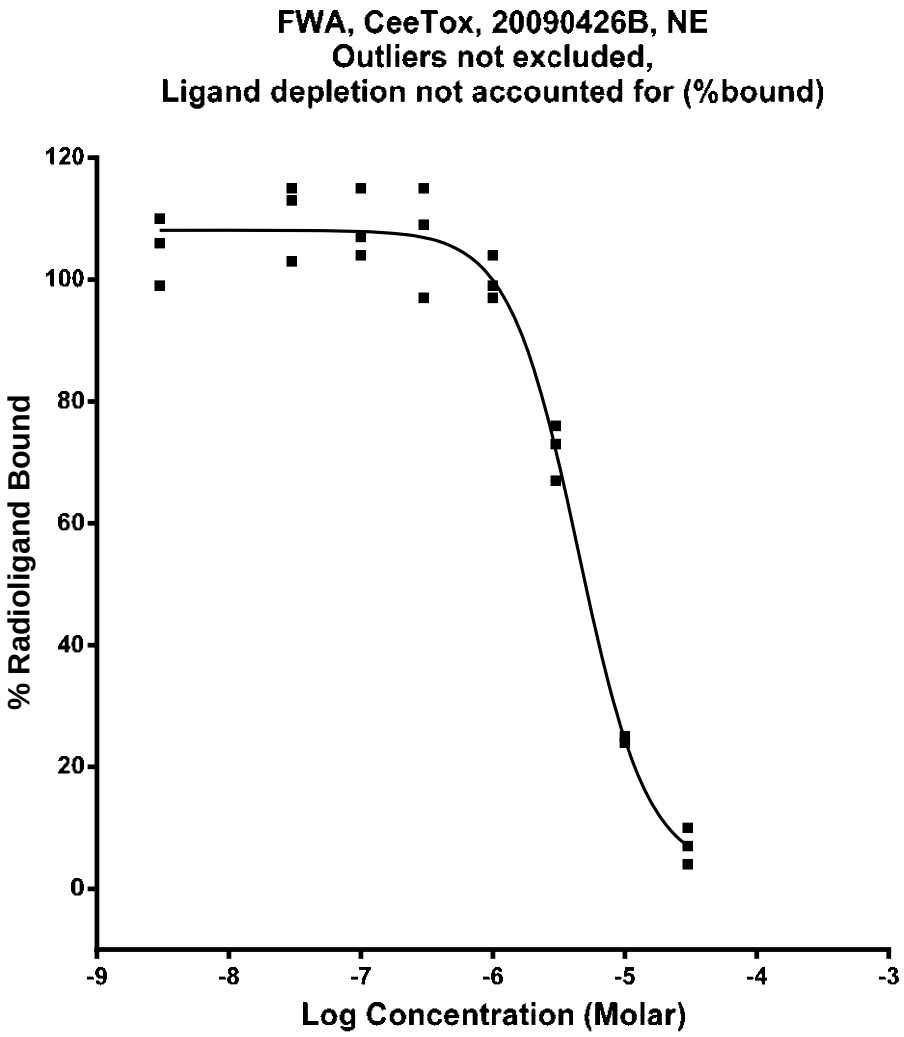
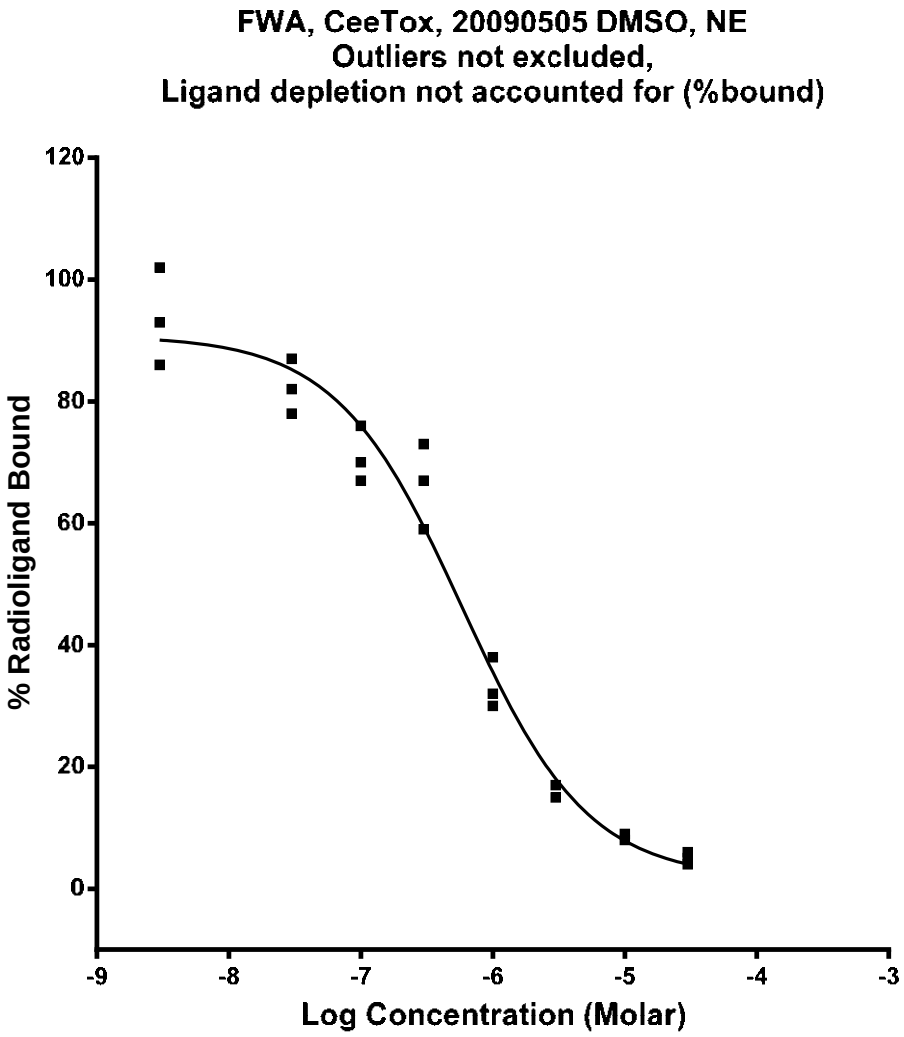
-5
-4
-3
Log Concentration (Molar)

Subtask 3



Same data as for 20090426

Subtask 3


FWA, CeeTox, 20090505 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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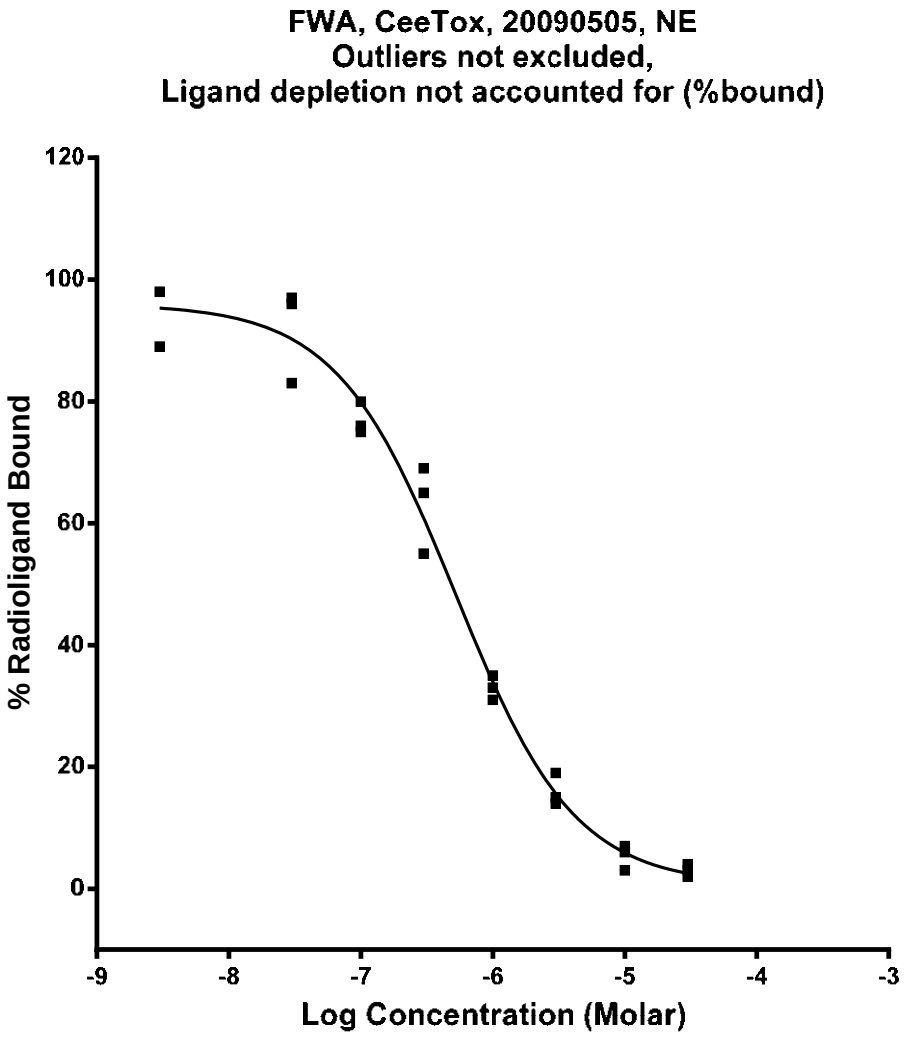
-3
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090505, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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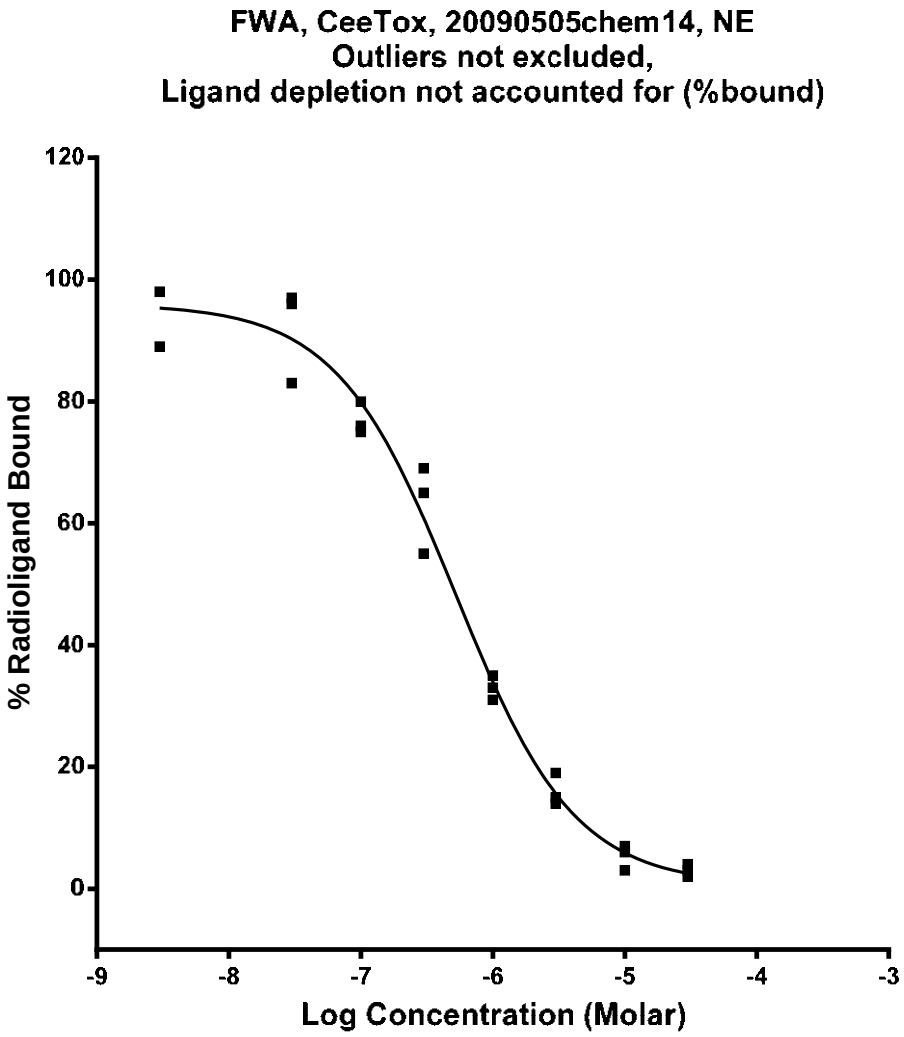
-3
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090505chem14, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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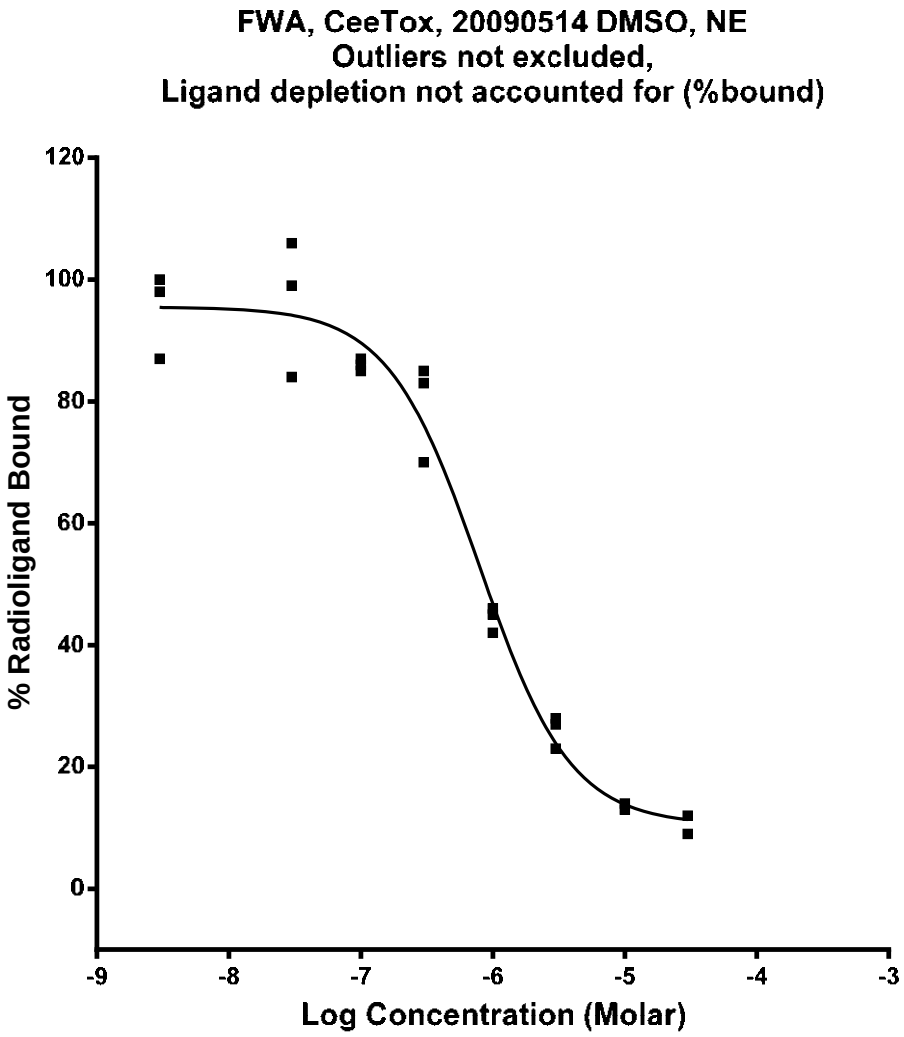
Log Concentration (Molar)

Subtask 3


FWA, CeeTox, 20090514 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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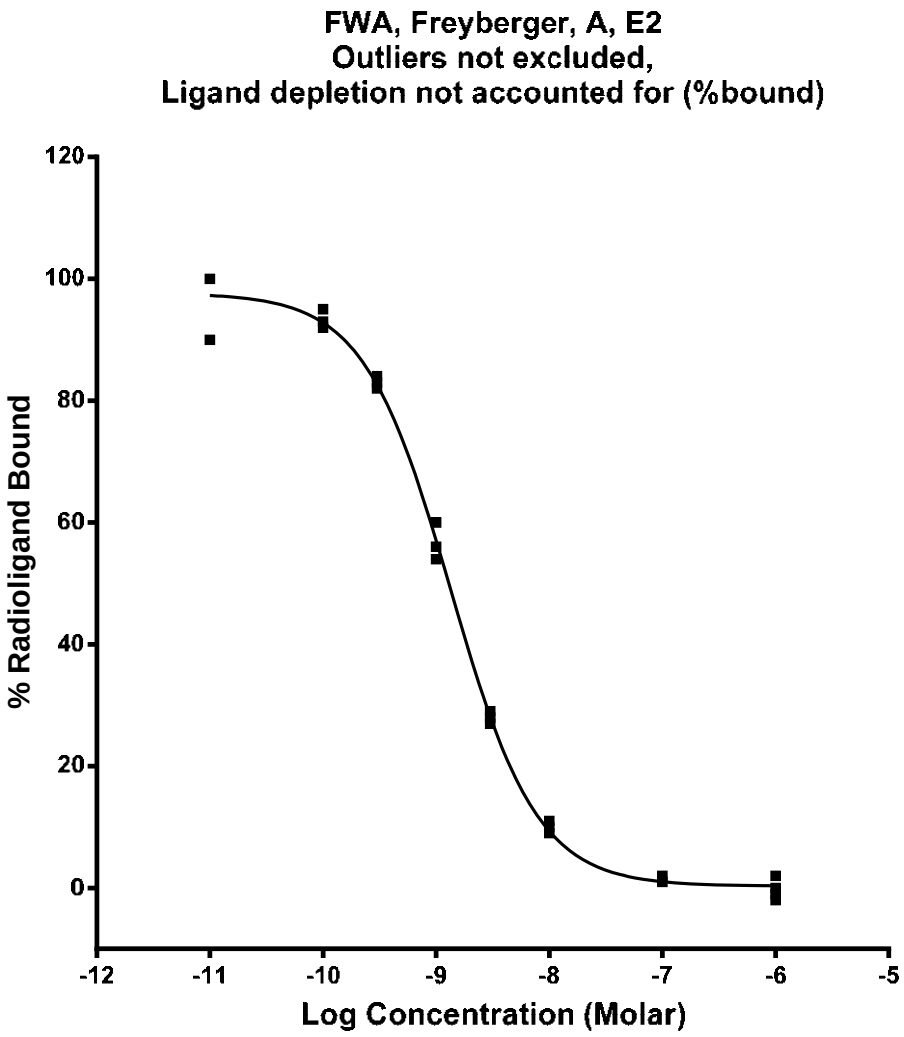
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, A, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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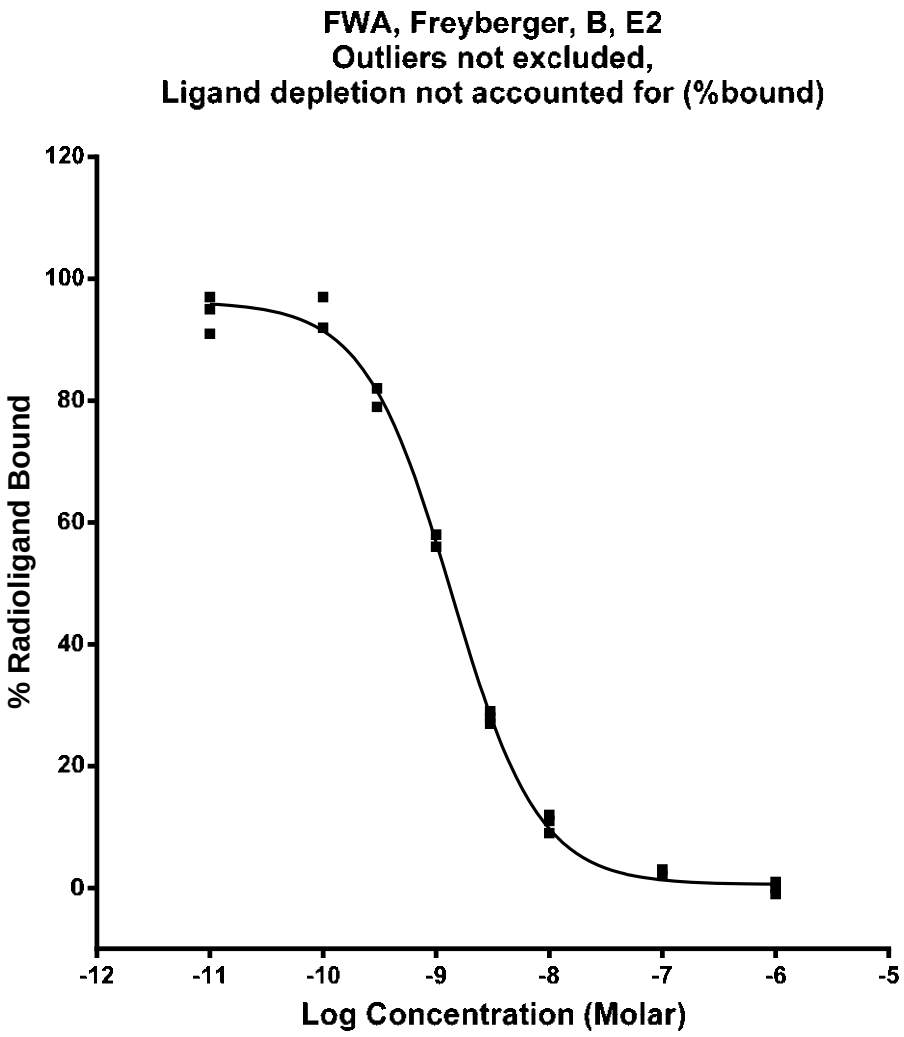
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, B, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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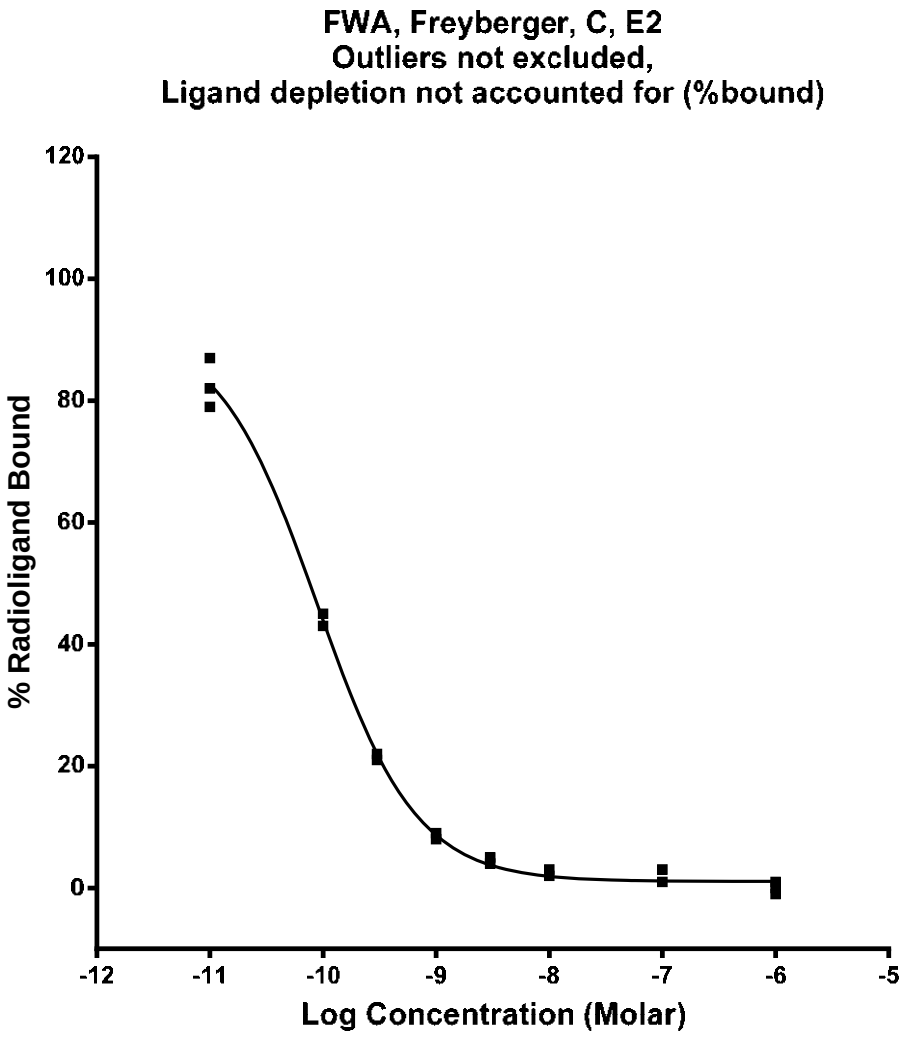
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, C, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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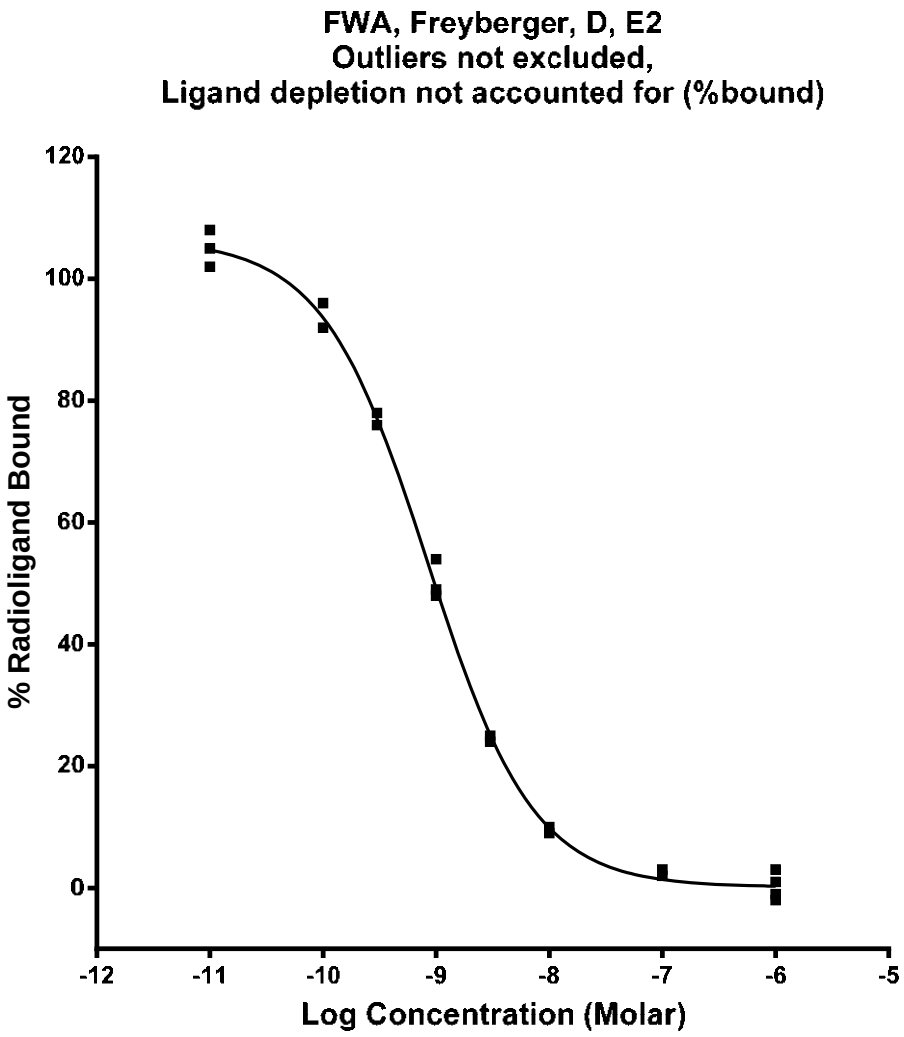
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, D, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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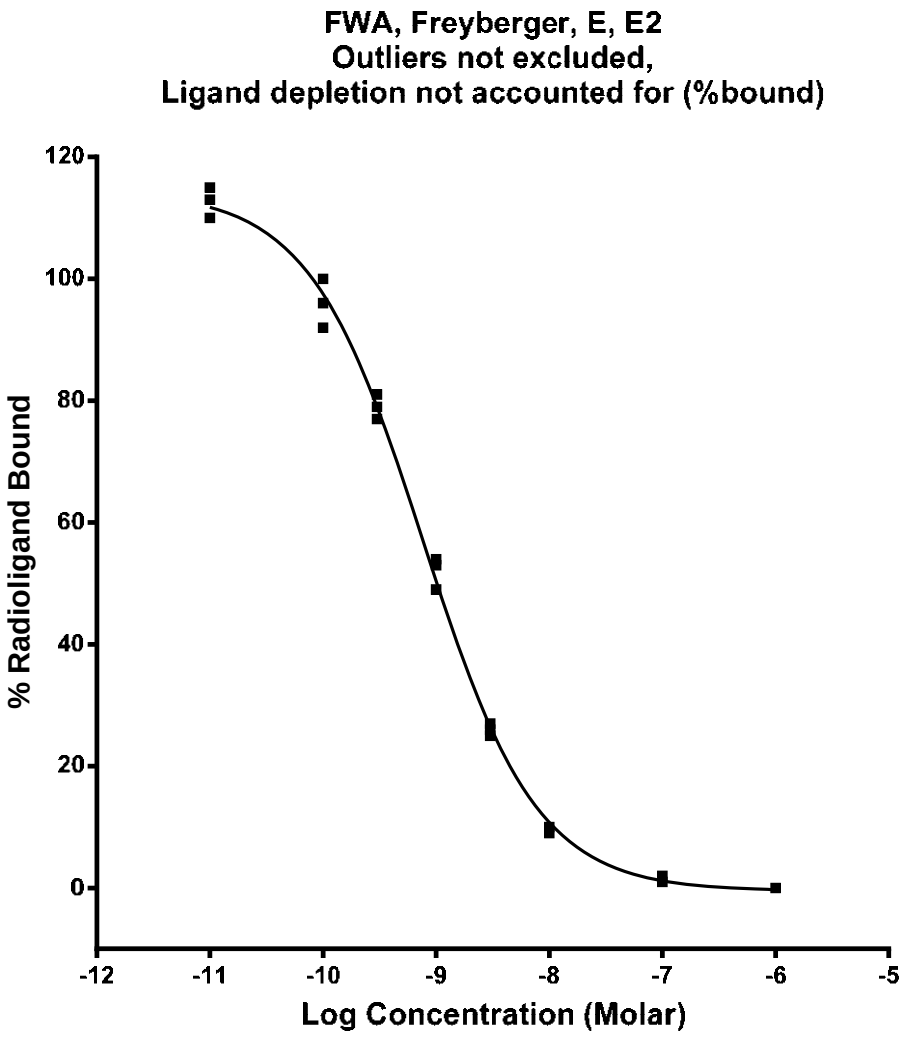
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, E, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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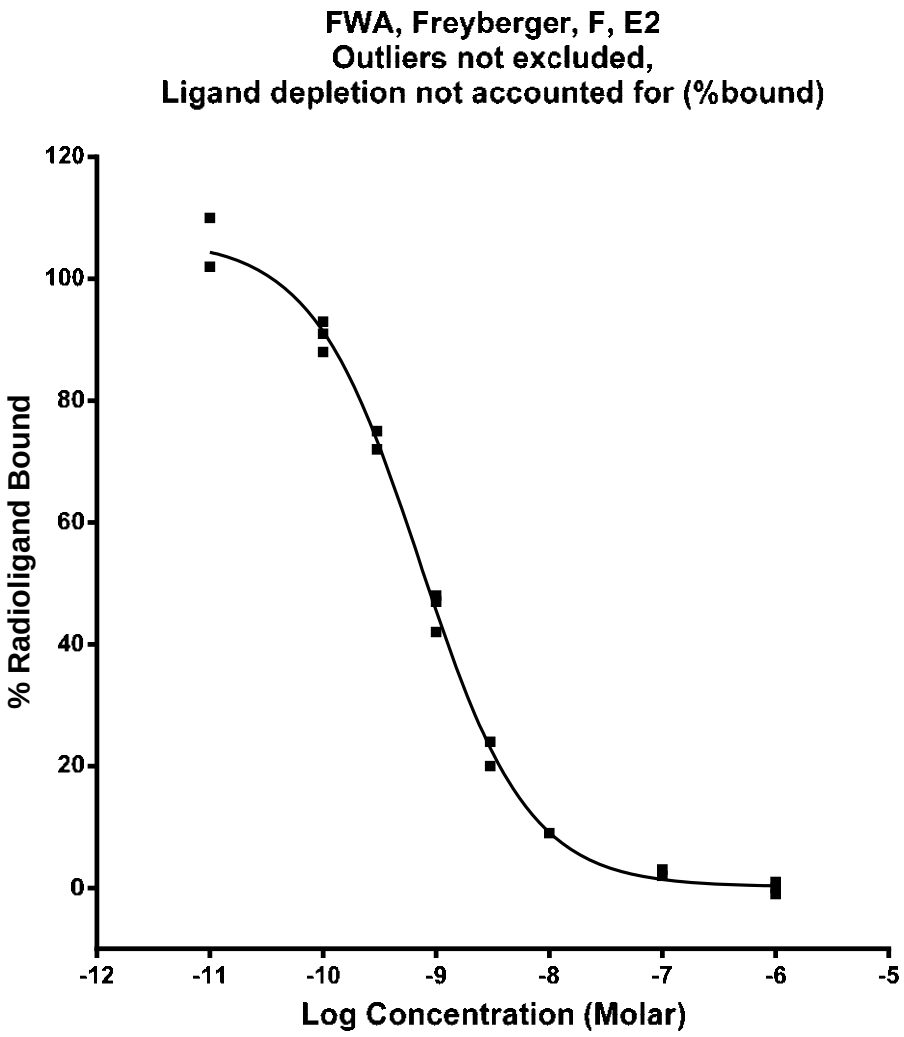
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, F, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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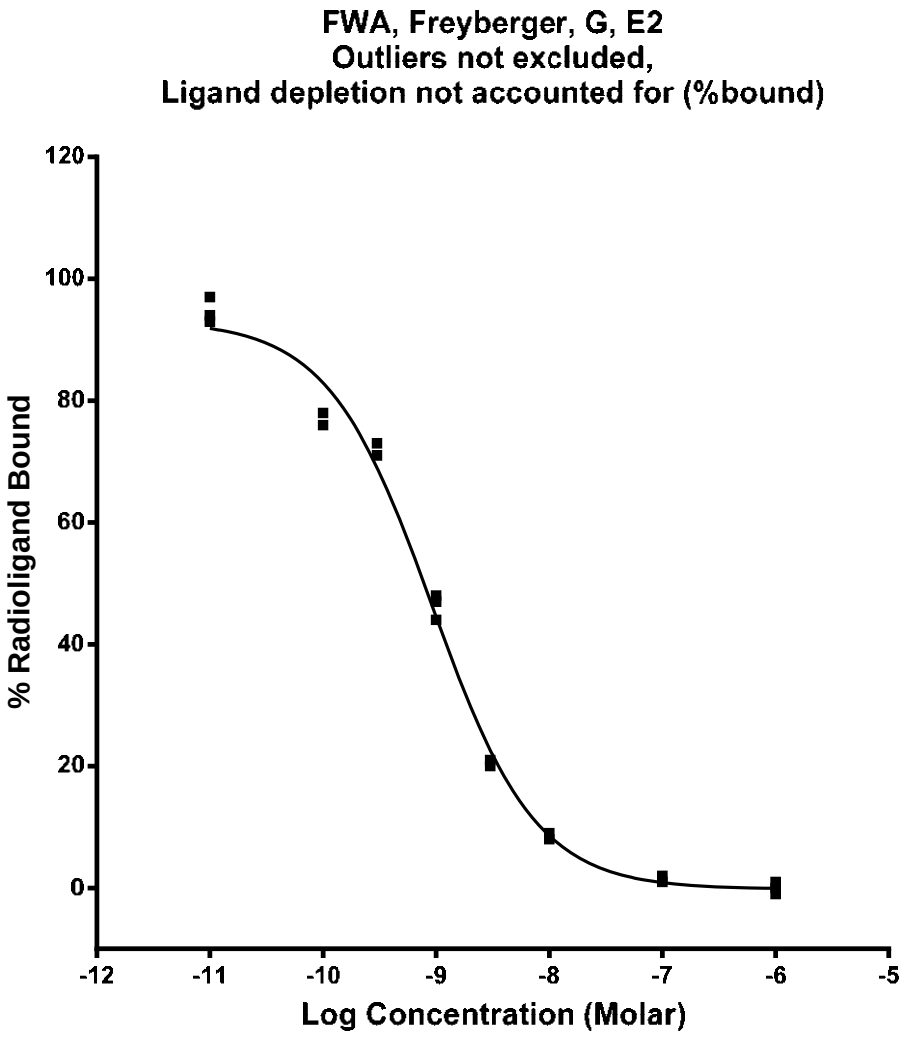
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, G, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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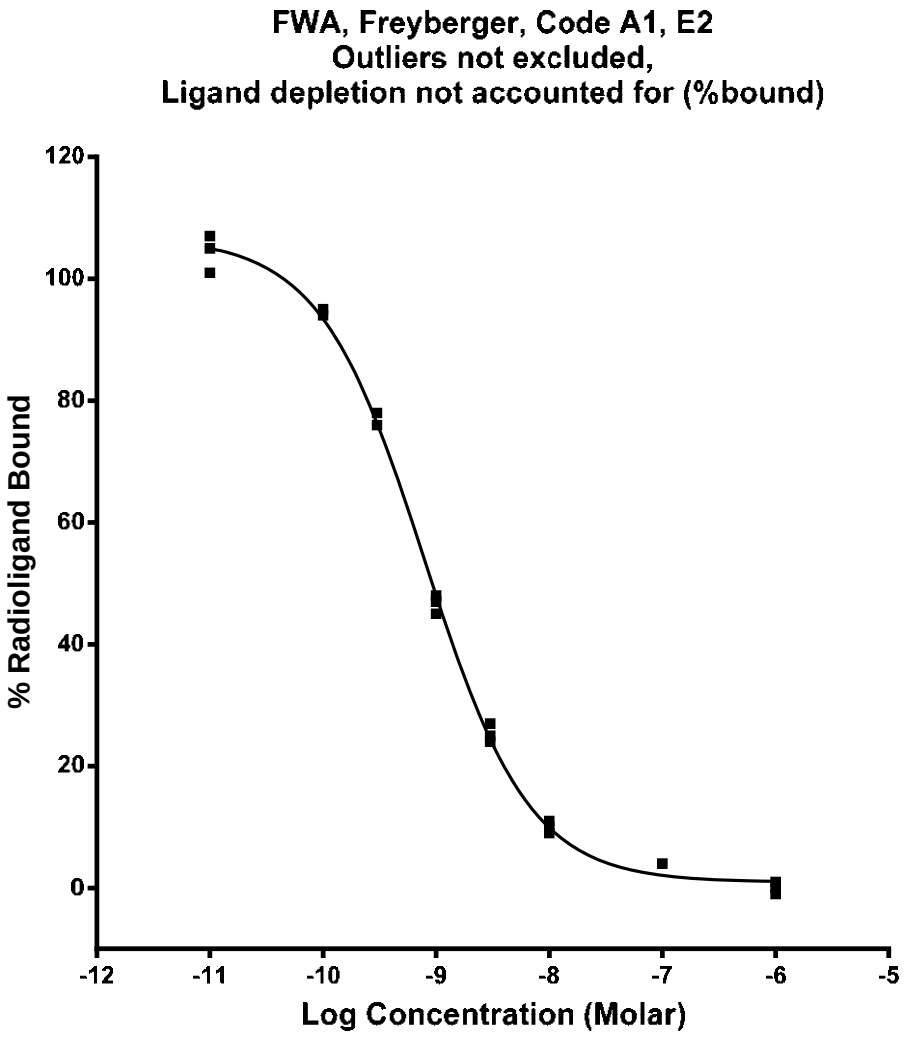
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code A1, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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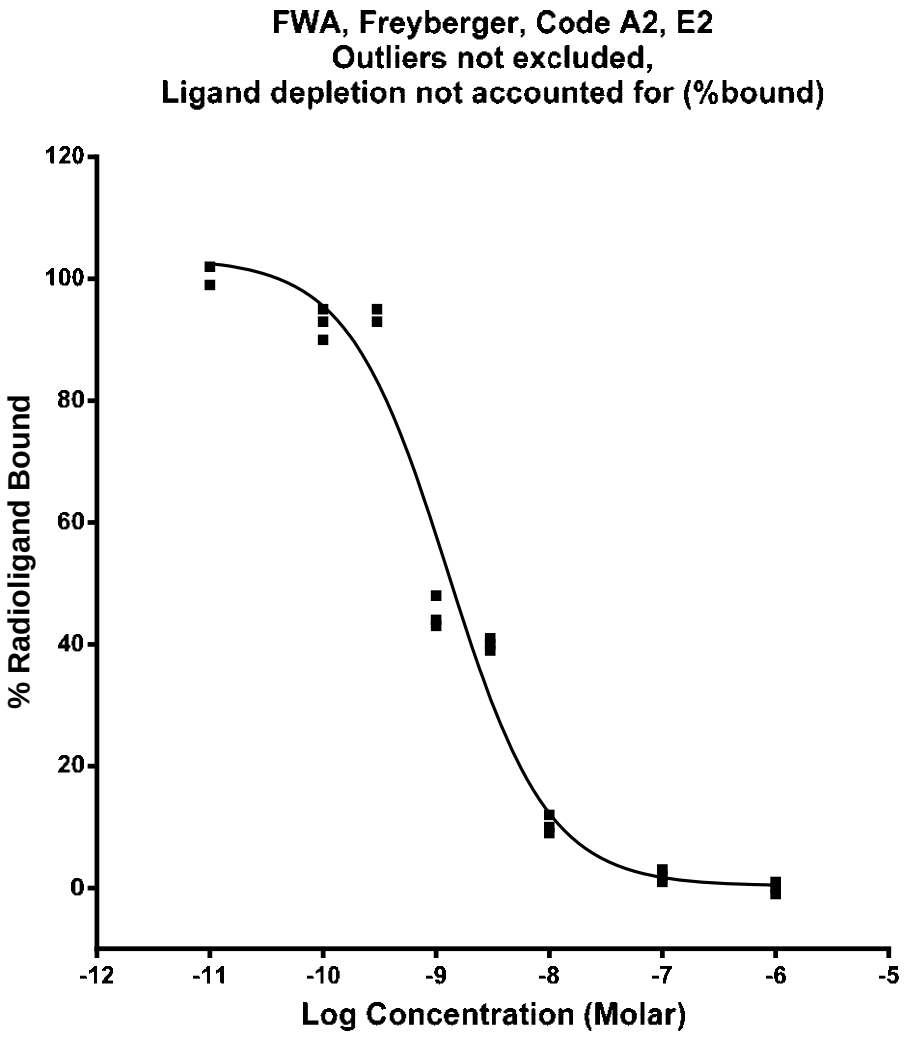
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code A2, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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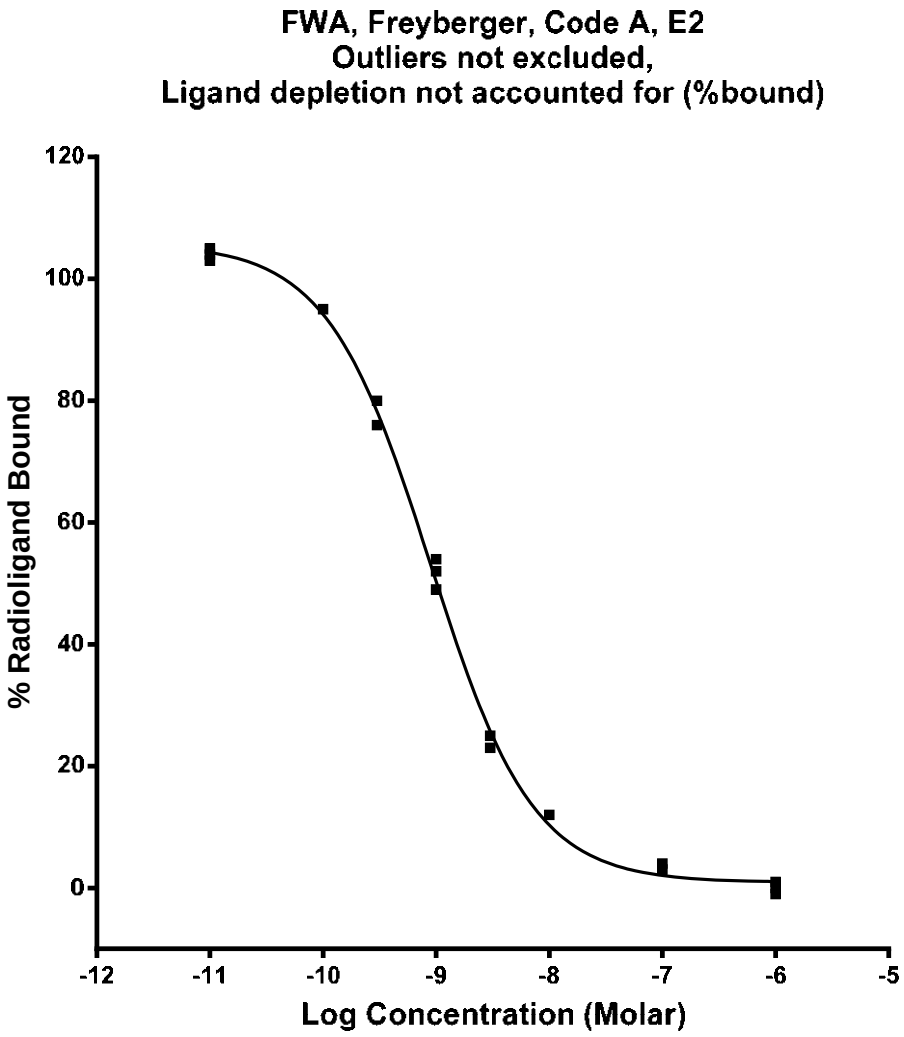
-5
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code A, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
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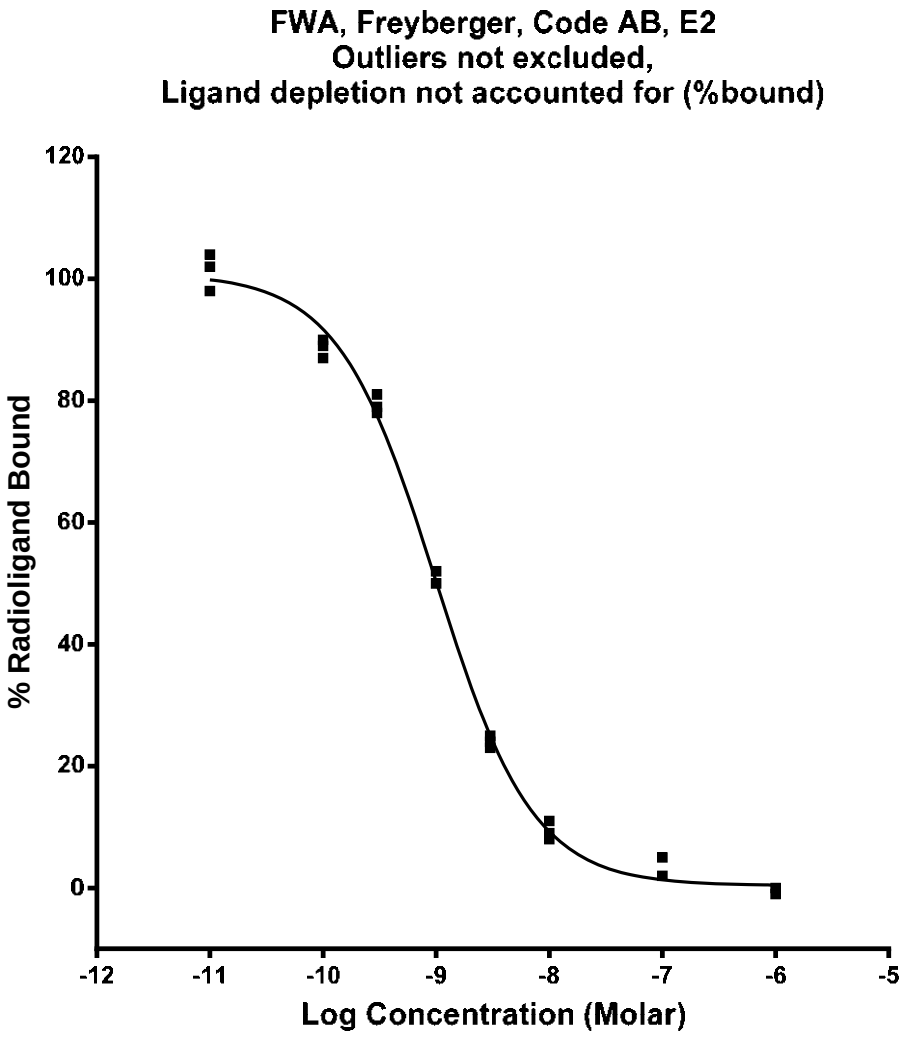
-5
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code AB, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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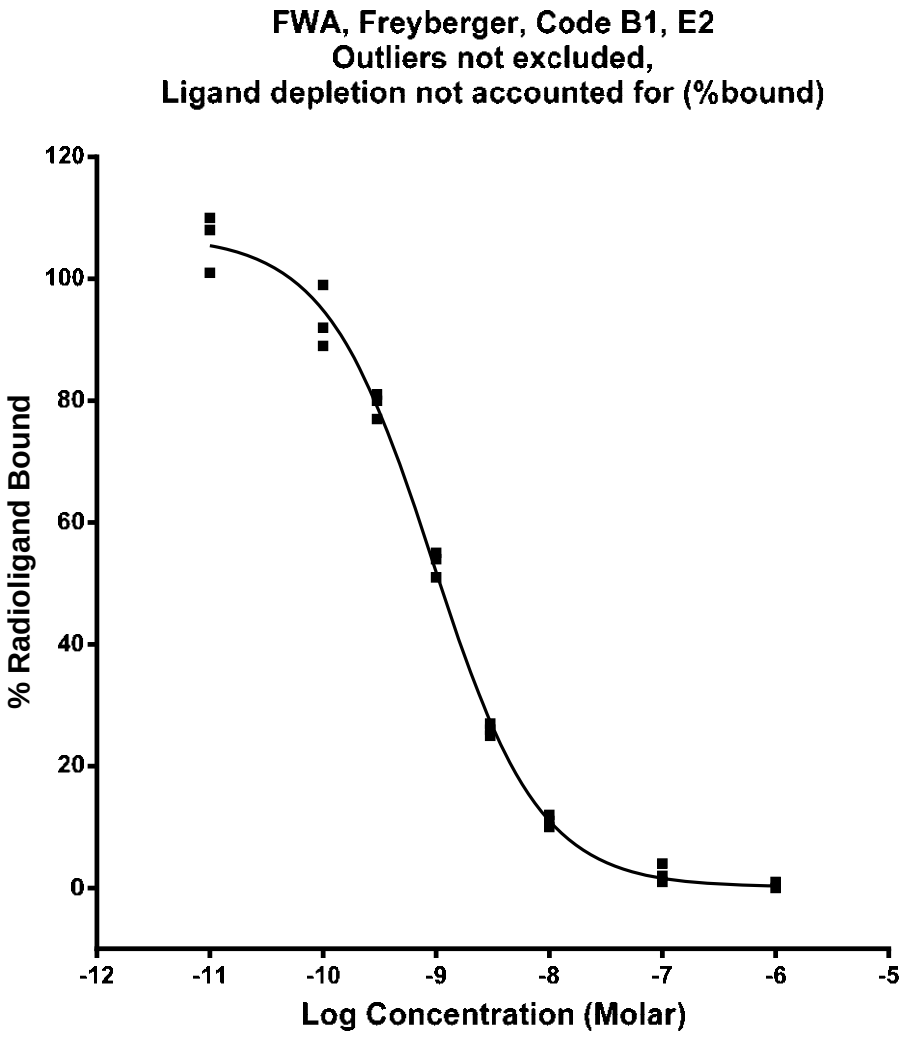
-5
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code B1, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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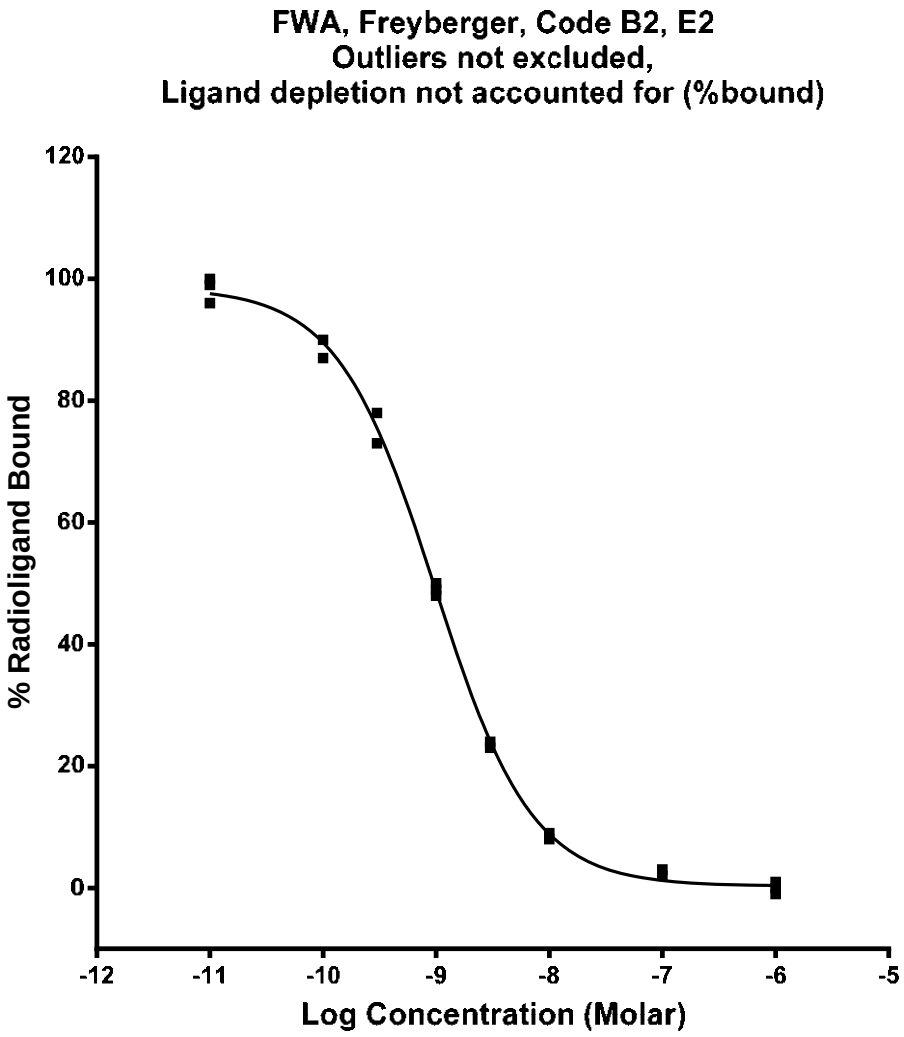
Log Concentration (Molar)

Subtask 3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% Radioligand Bound
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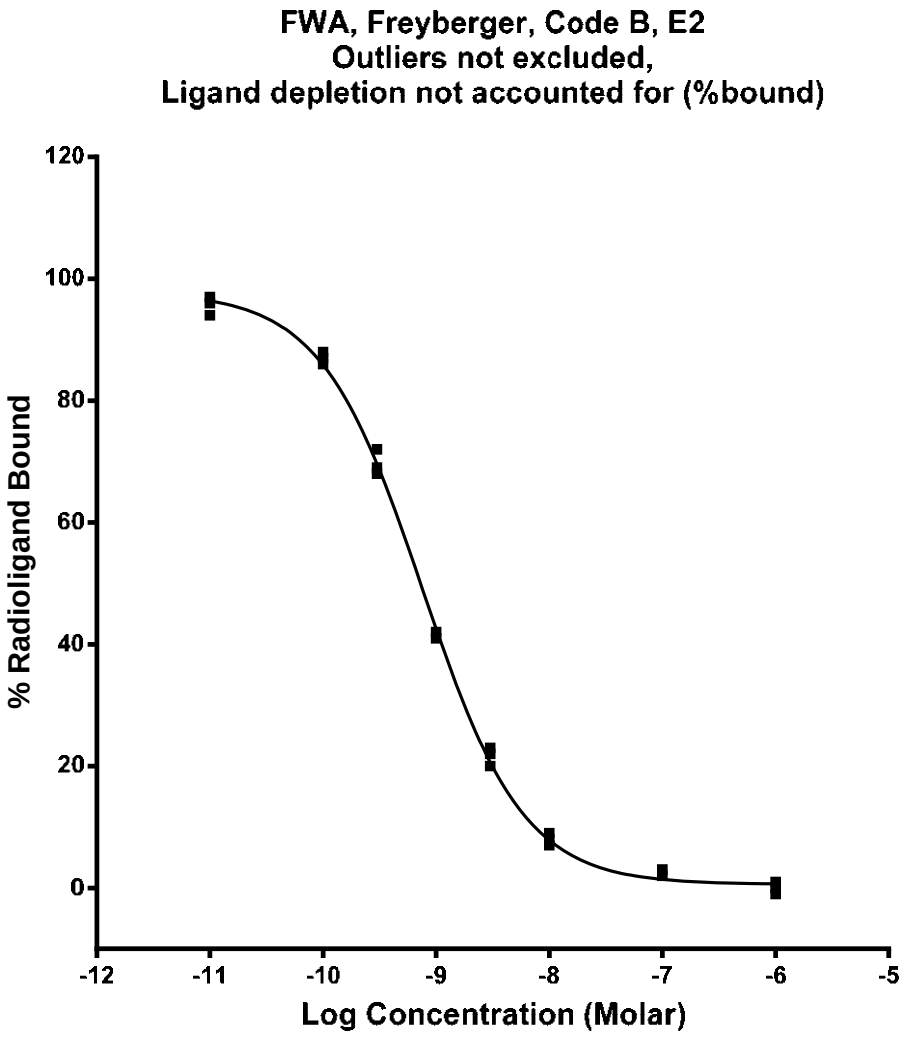
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code B, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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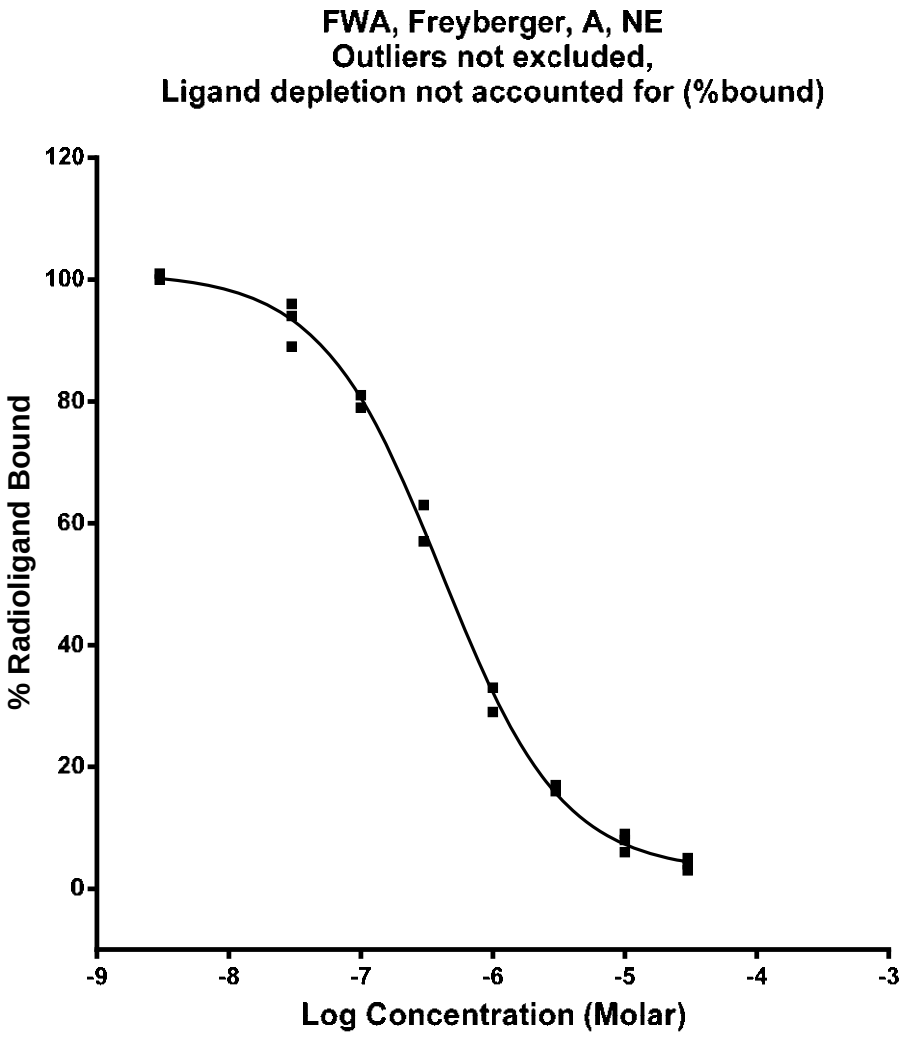
-5
Log Concentration (Molar)

FW Assay, (Freyberger Lab) Control Norethynodrel (NE) Curves 30 Nov 2014

Begin Here

Subtask 2


FWA, Freyberger, A, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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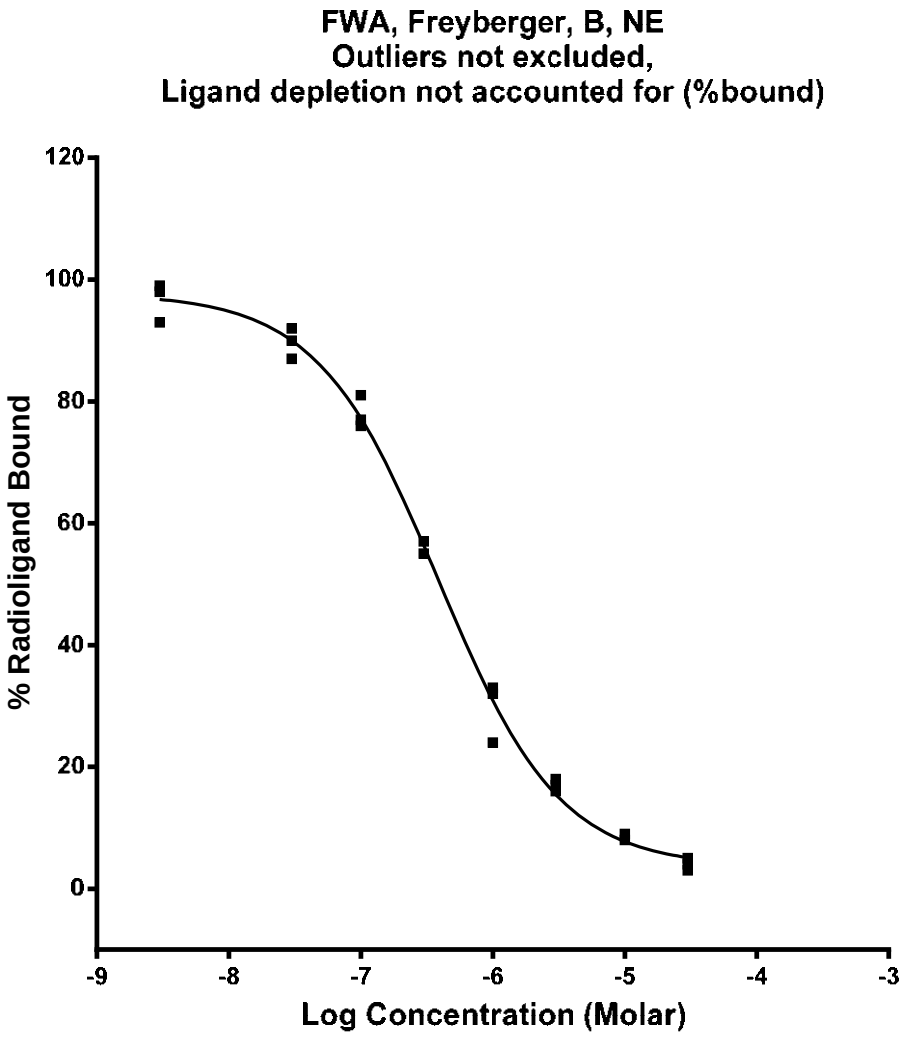
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, B, NE
Outliers not excluded,
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% Radioligand Bound
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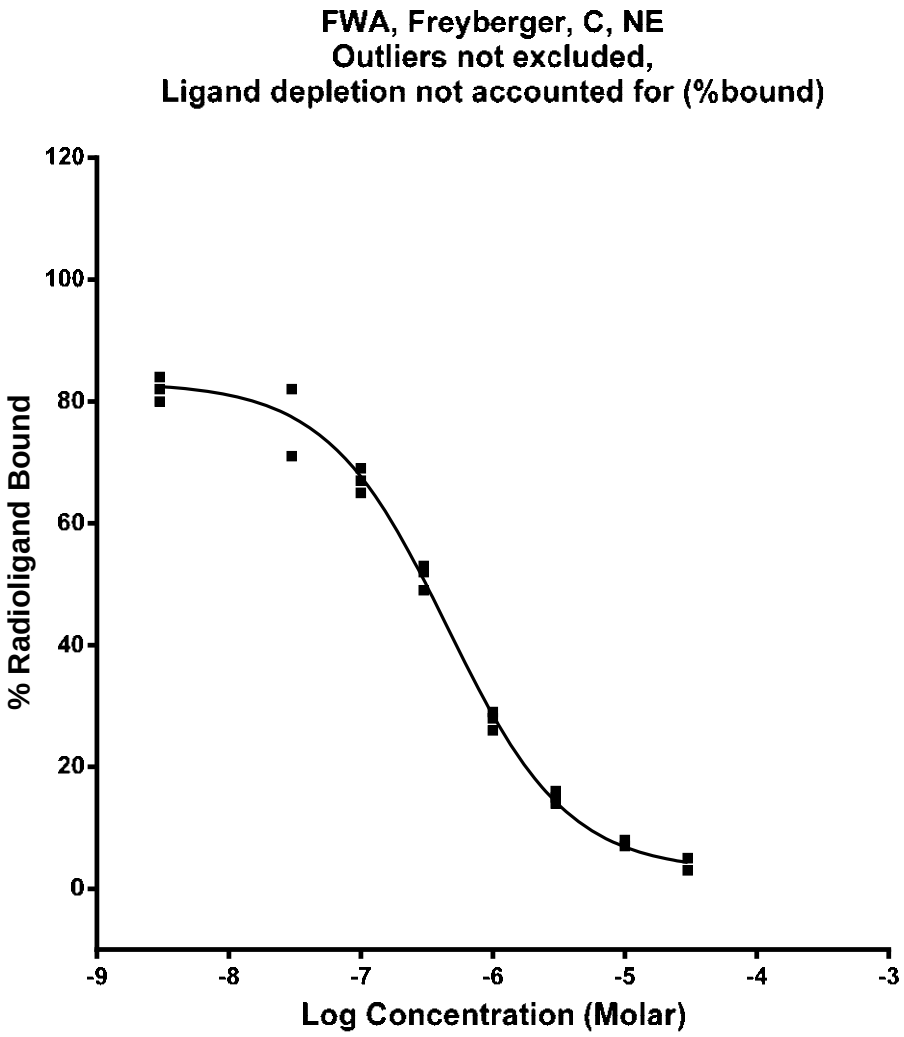
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, C, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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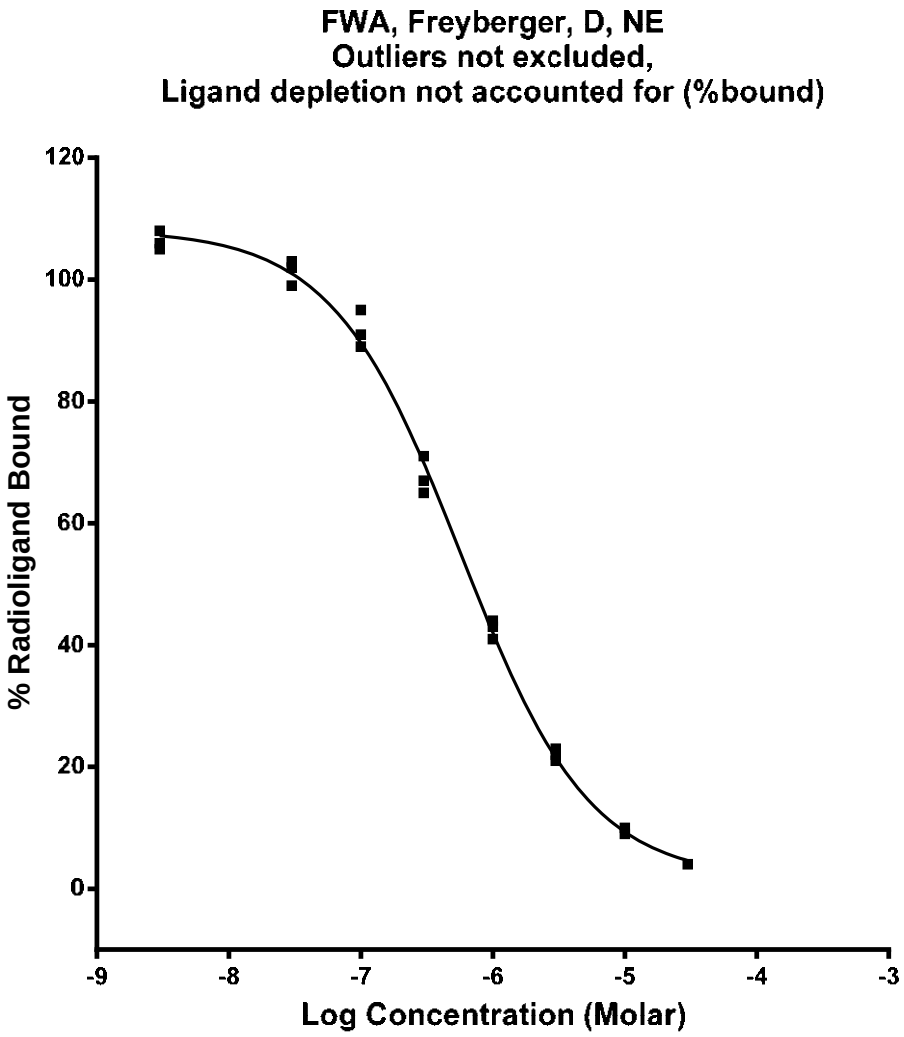
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, D, NE
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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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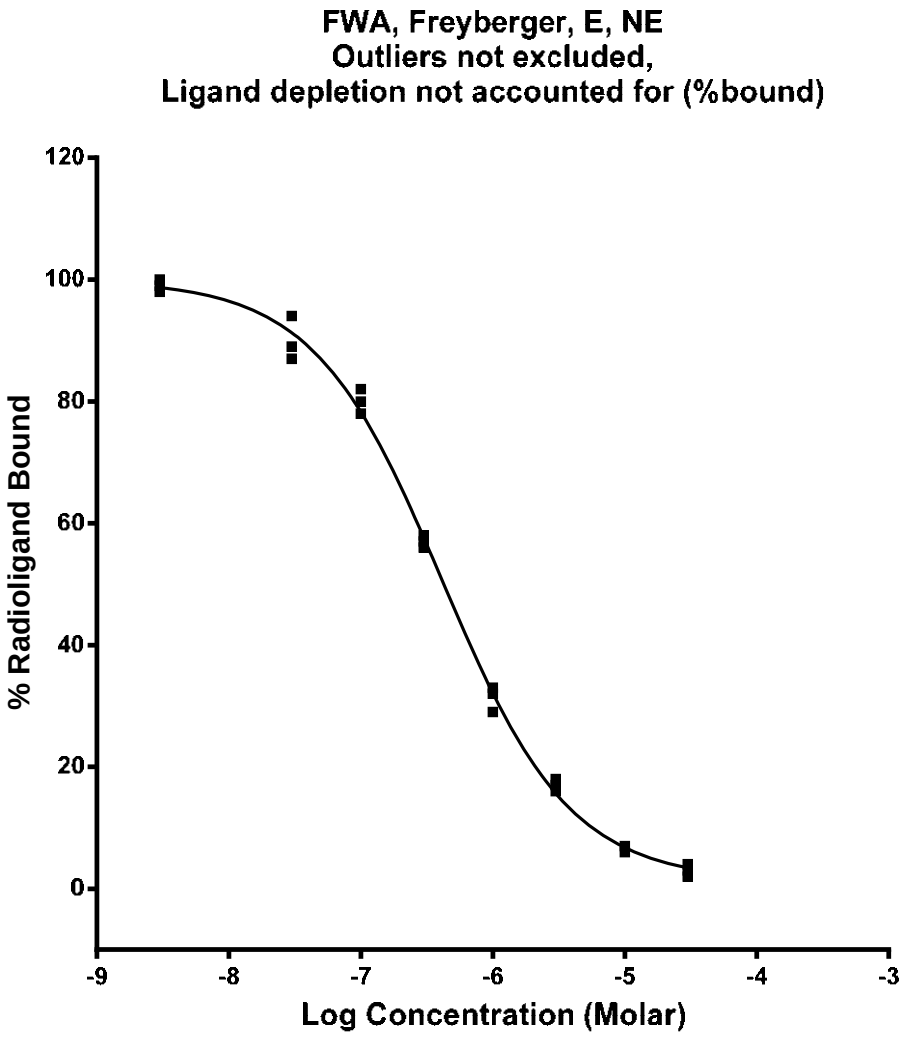
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, E, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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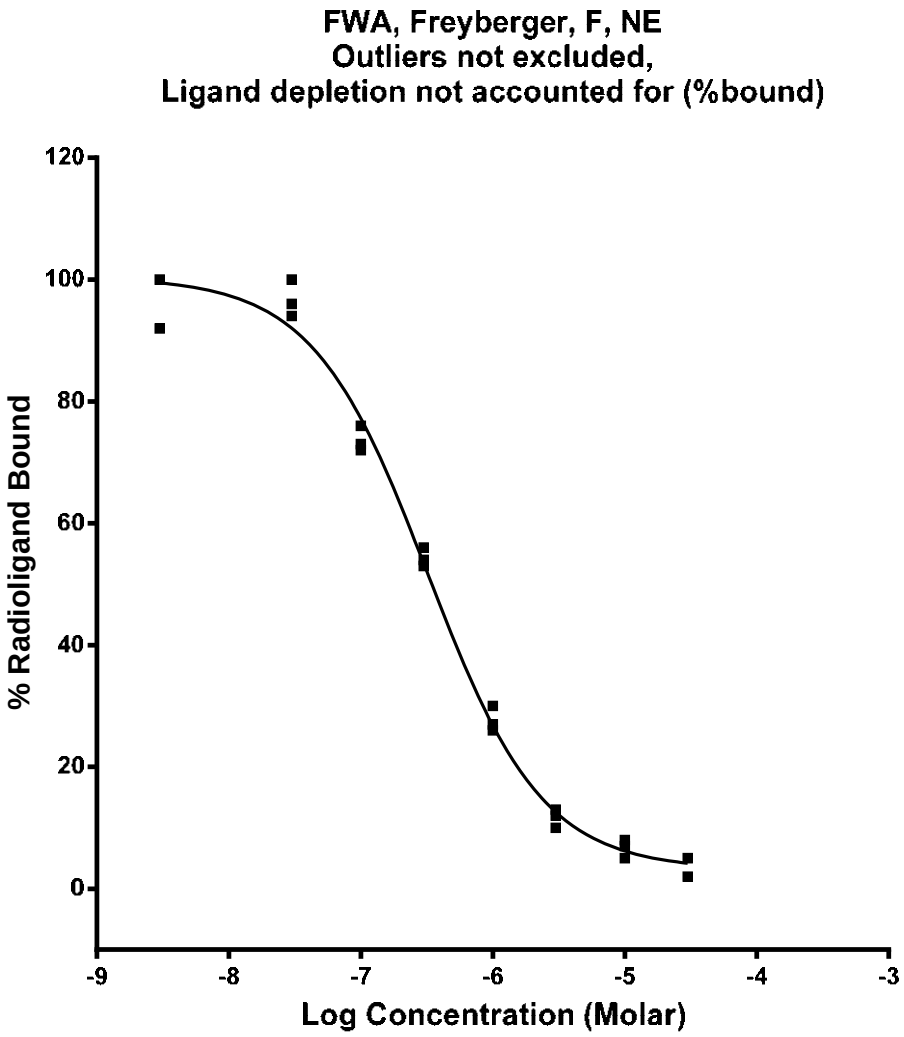
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, F, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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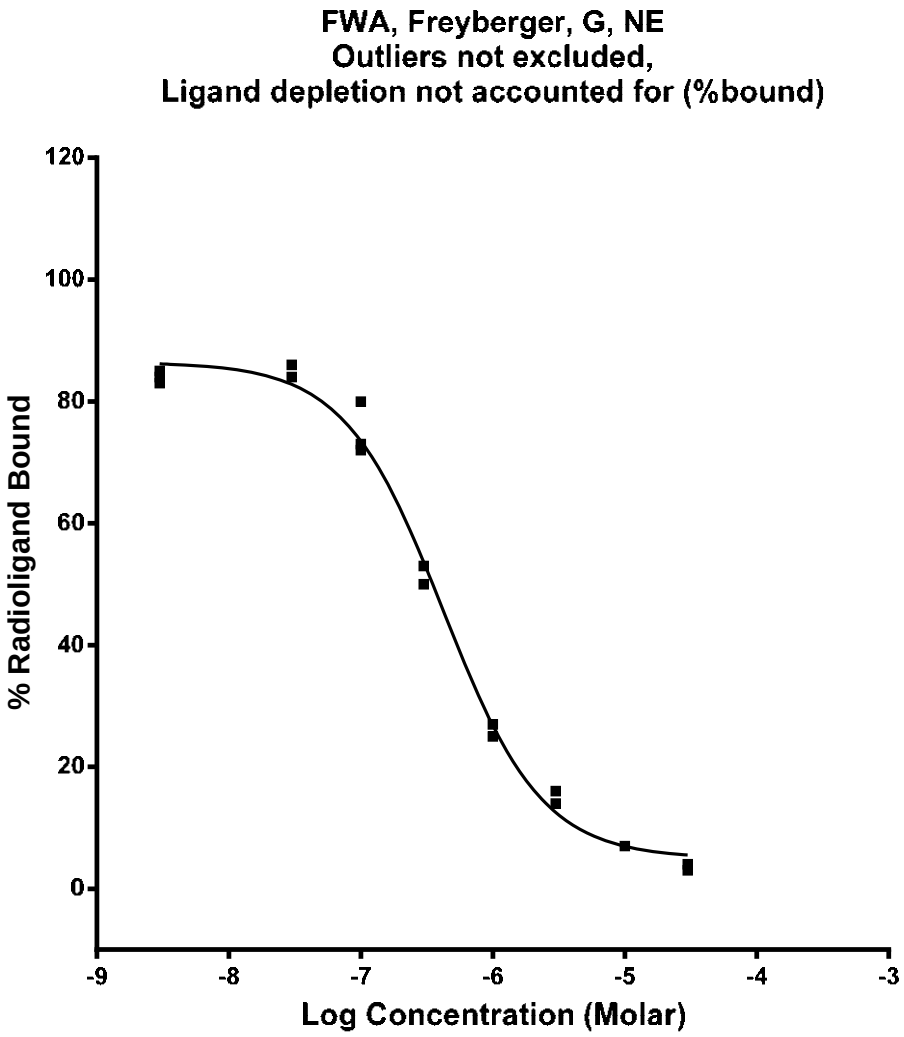
Log Concentration (Molar)

Subtask 2


FWA, Freyberger, G, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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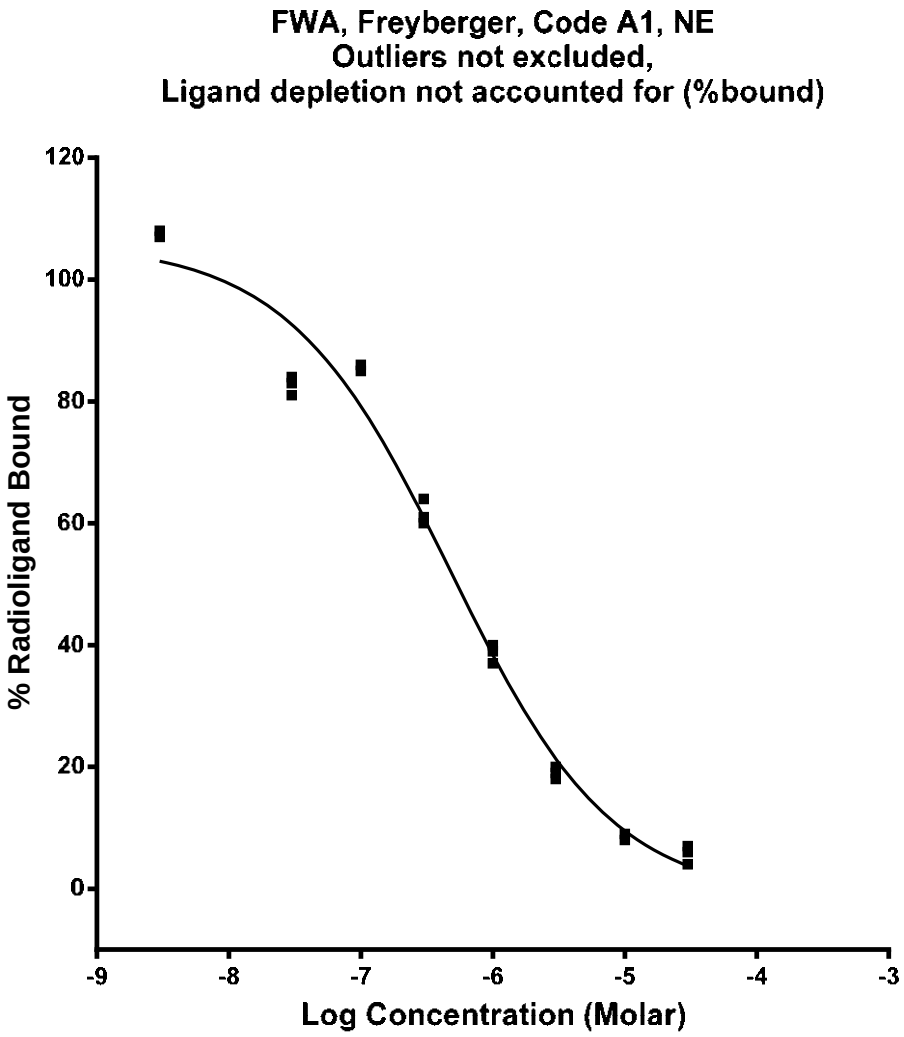
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code A1, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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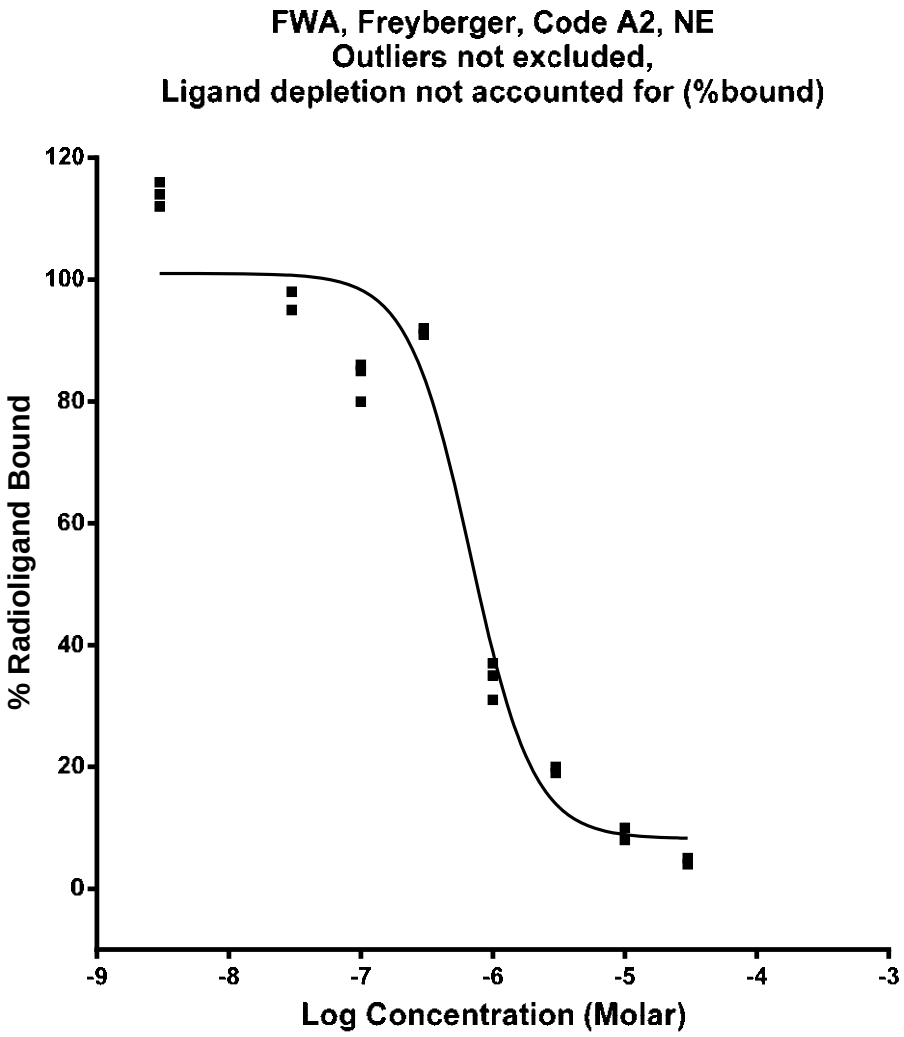
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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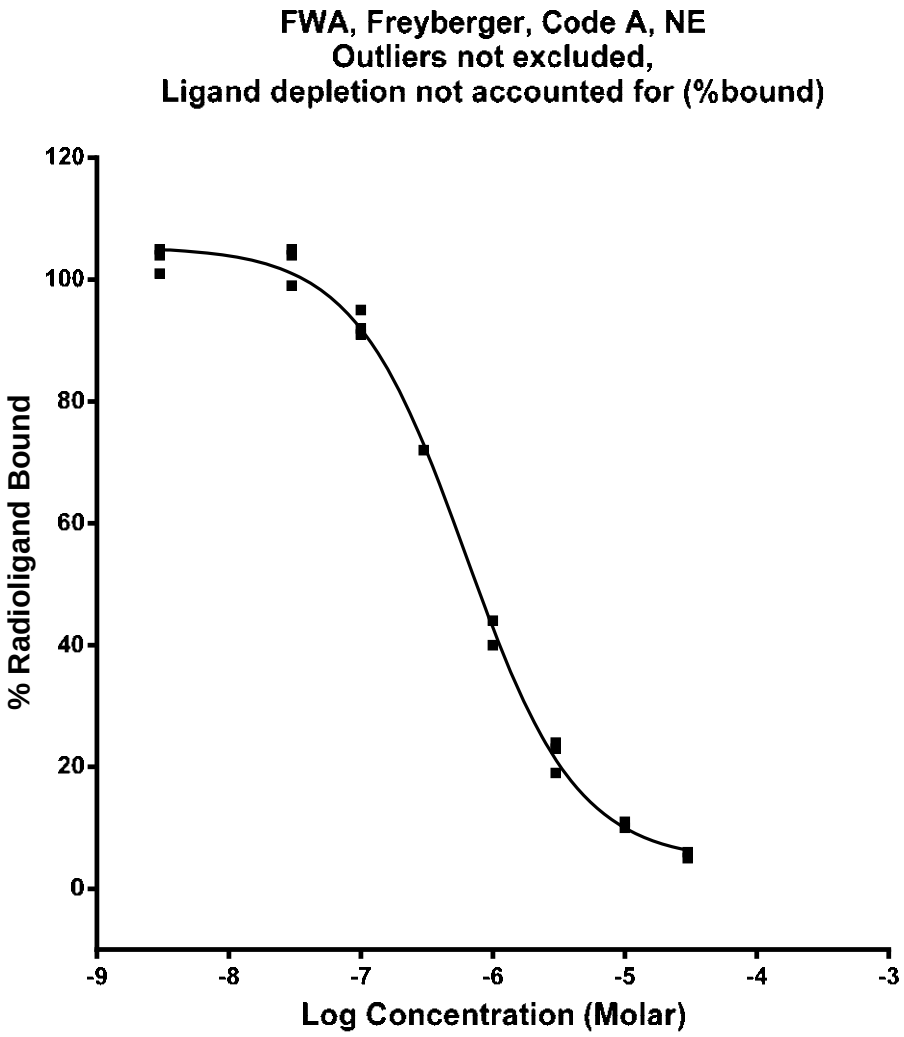
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code A, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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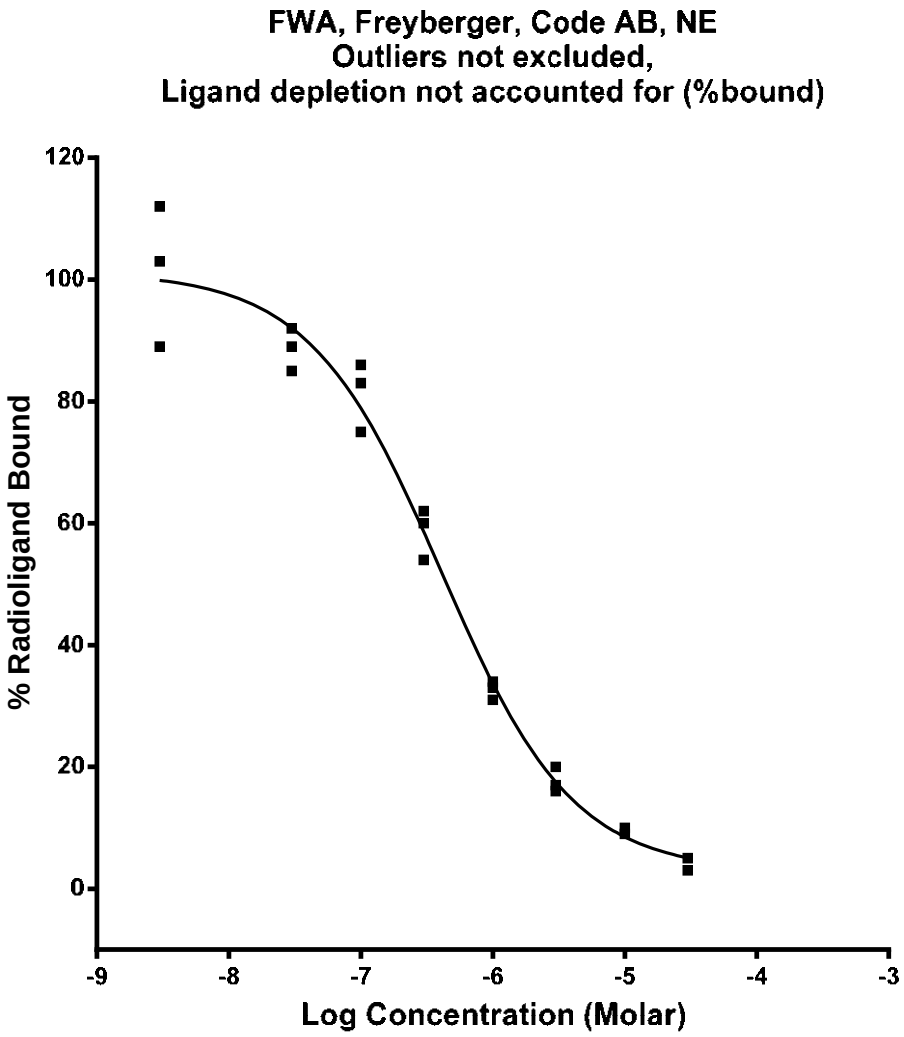
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code AB, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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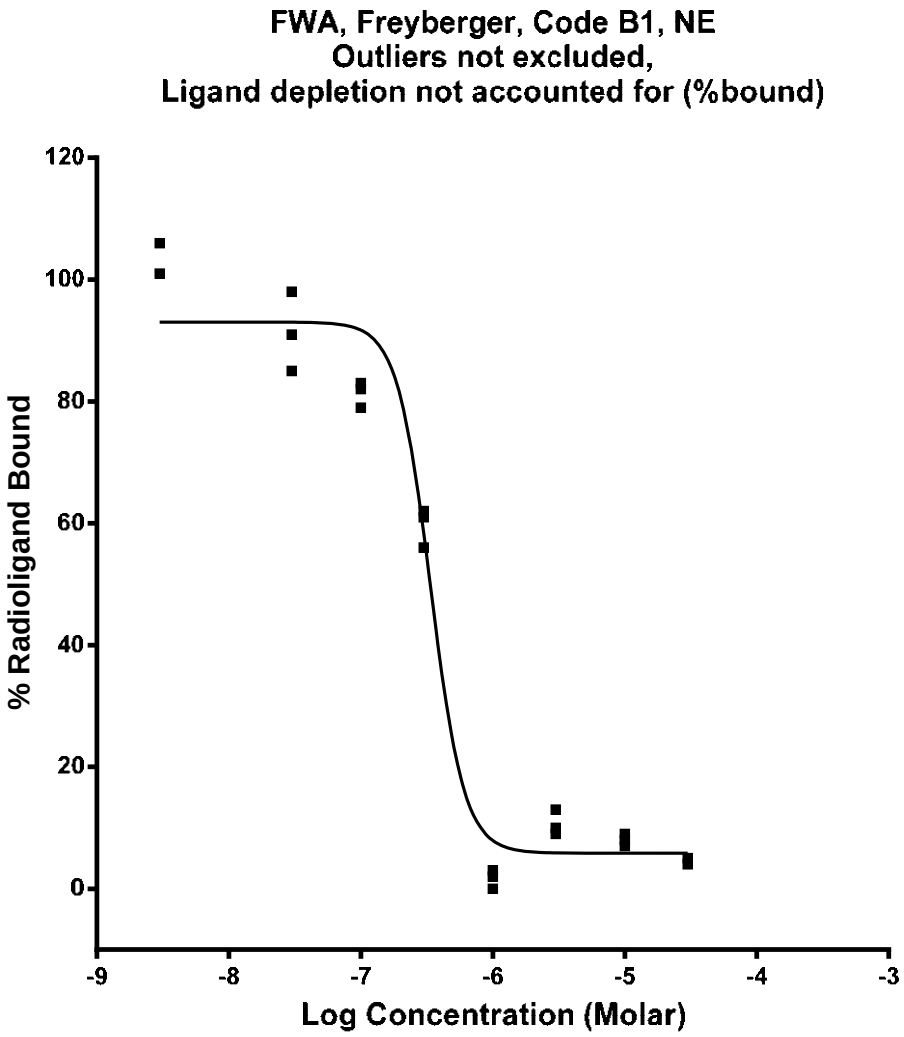
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code B1, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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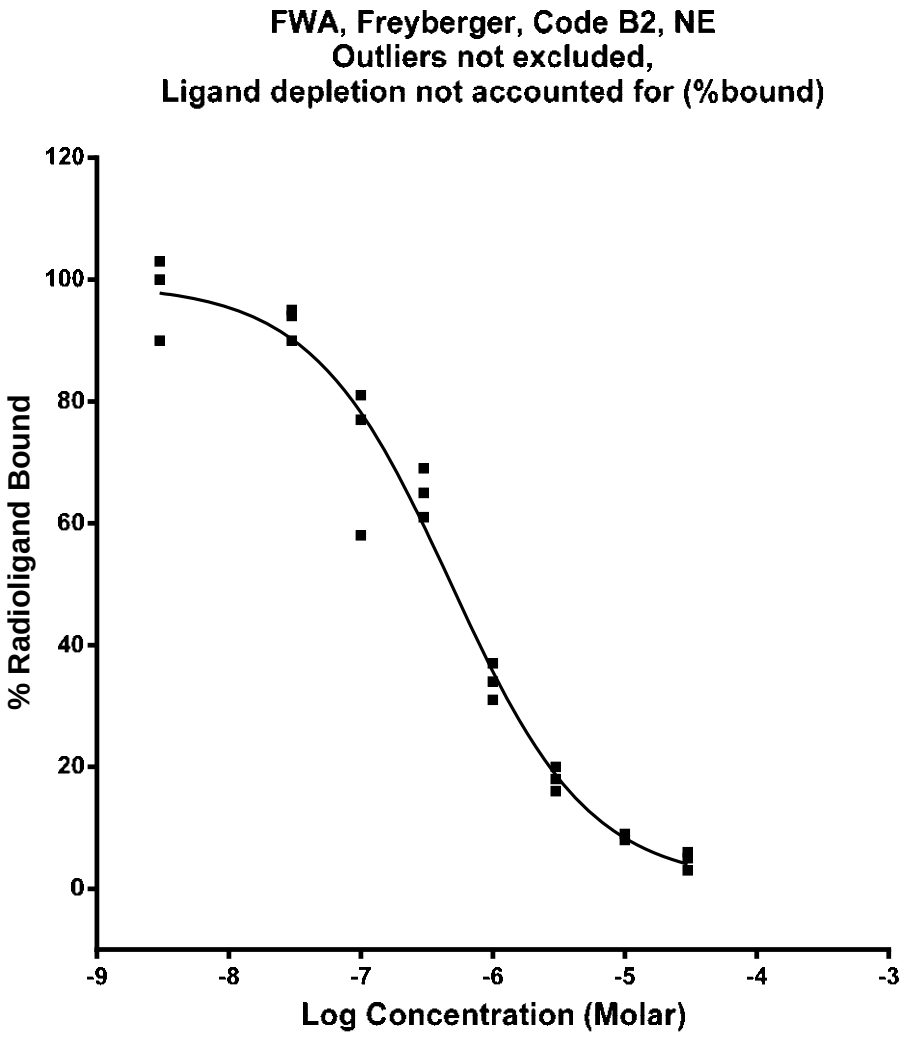
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code B2, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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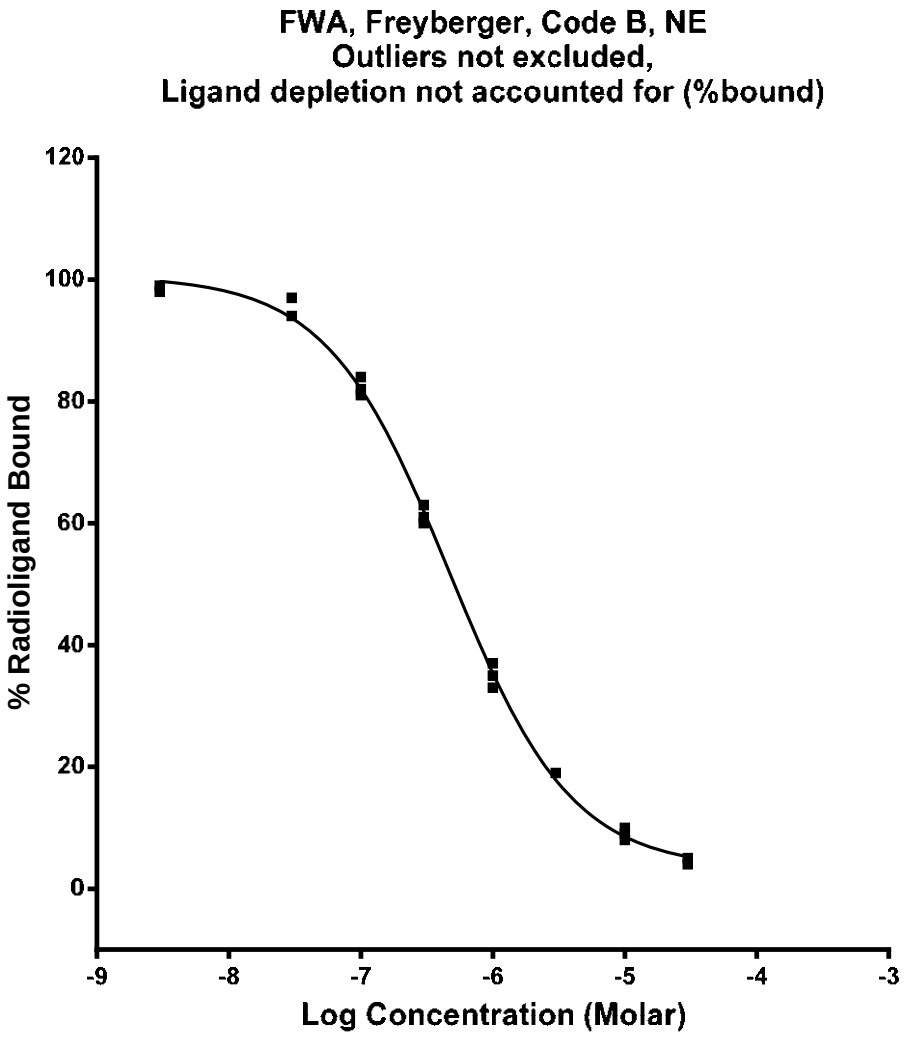
Log Concentration (Molar)

Subtask 3


FWA, Freyberger, Code B, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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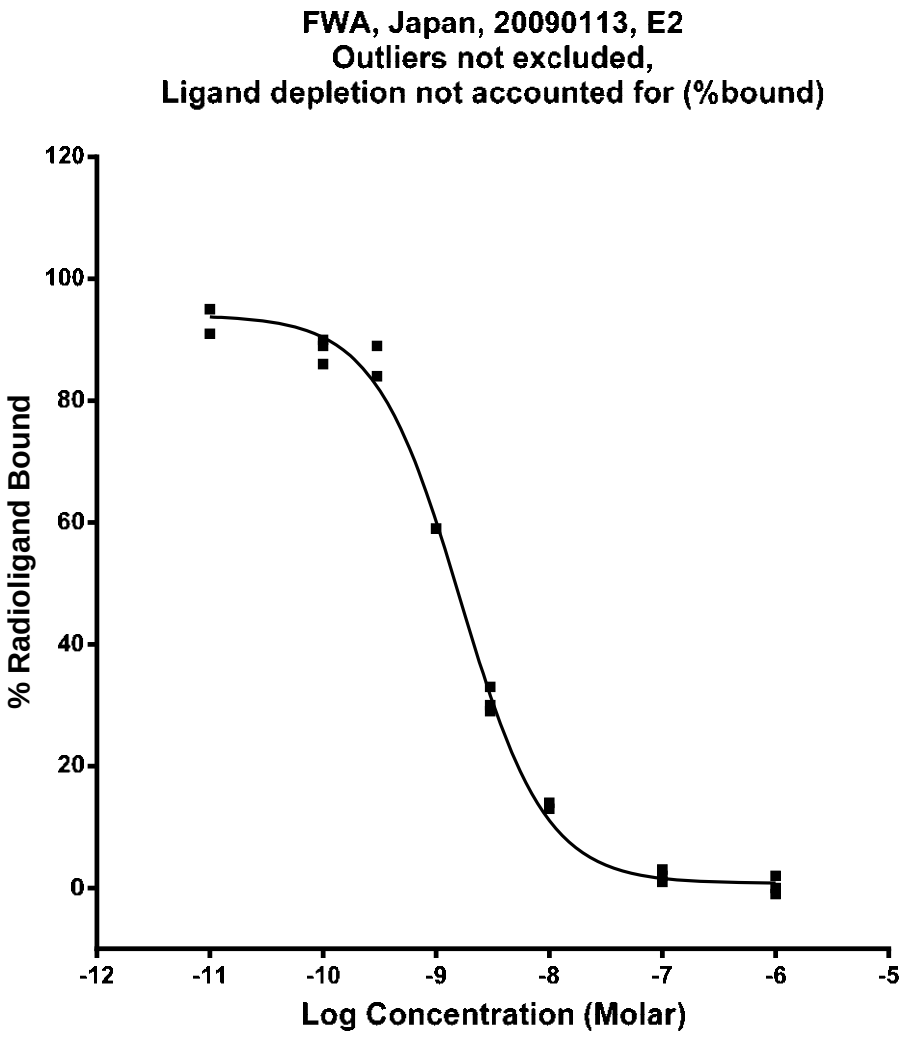
Log Concentration (Molar)

Subtask 1


FWA, Japan, 20090113, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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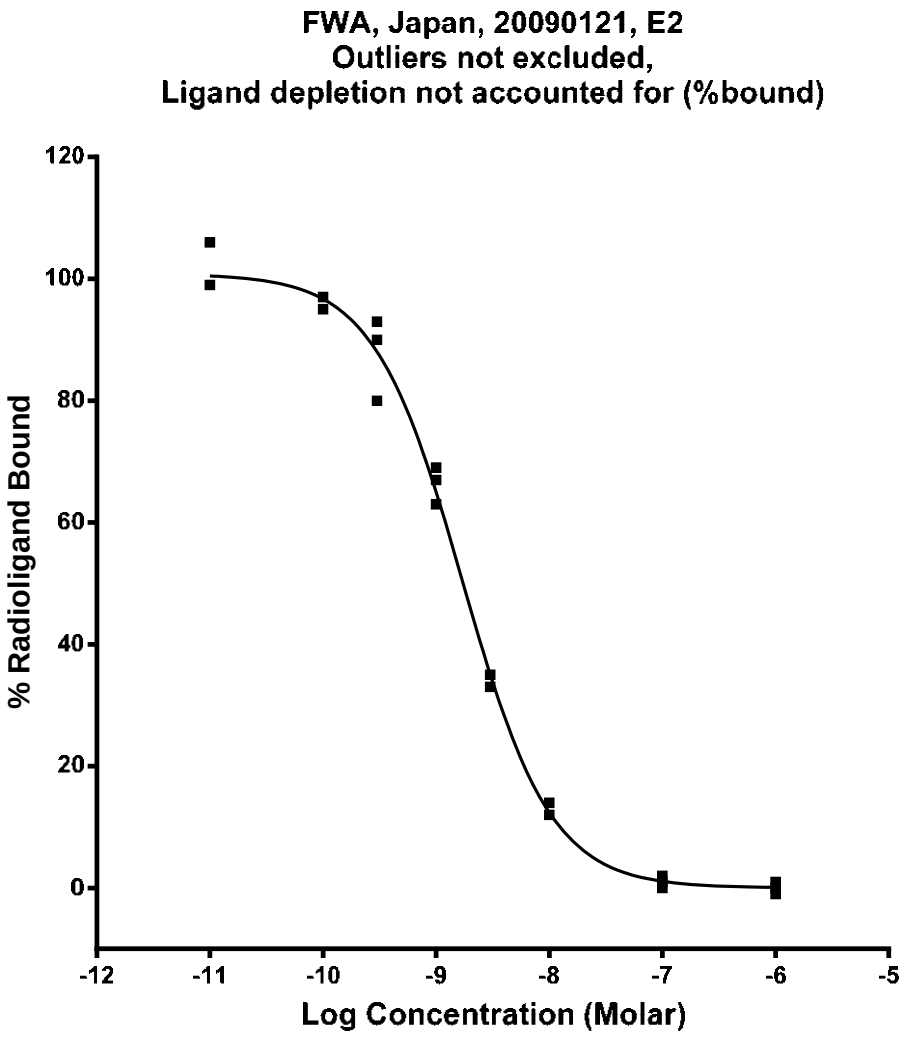
Log Concentration (Molar)

Subtask 1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Ligand depletion not accounted for (%bound)
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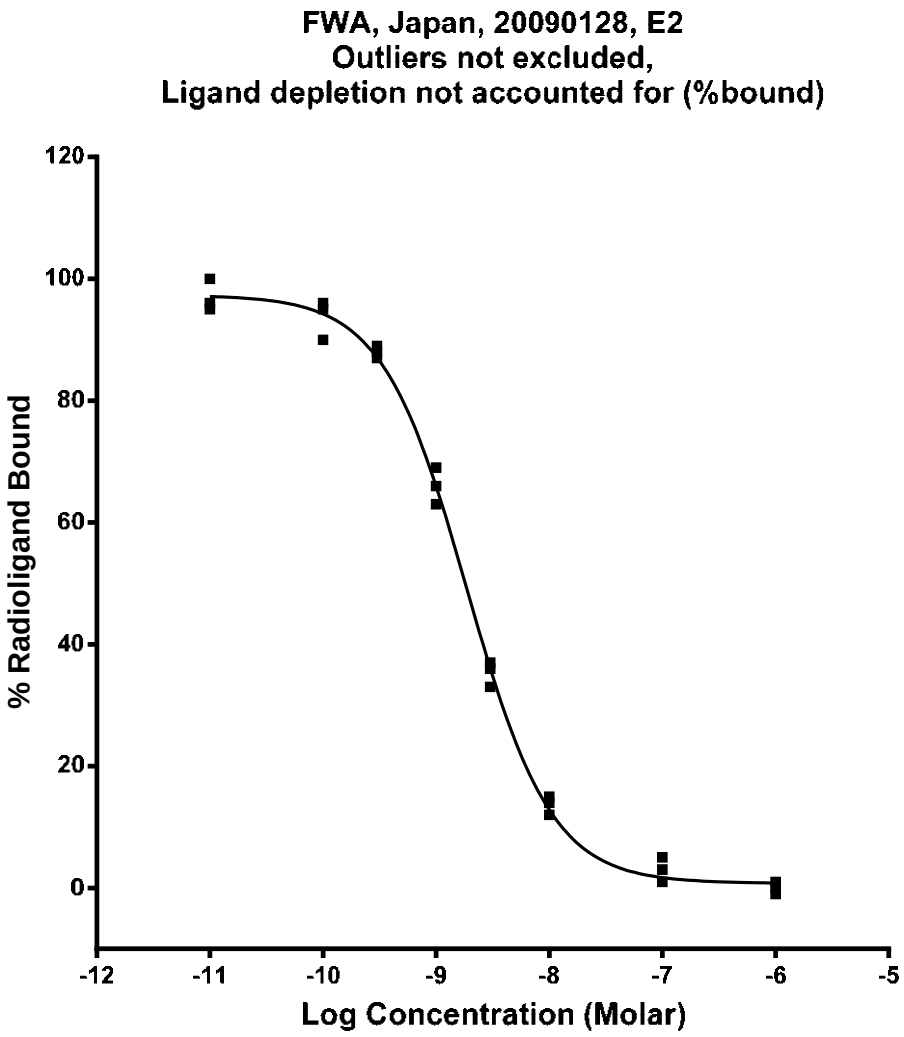
Log Concentration (Molar)

Subtask 1


FWA, Japan, 20090128, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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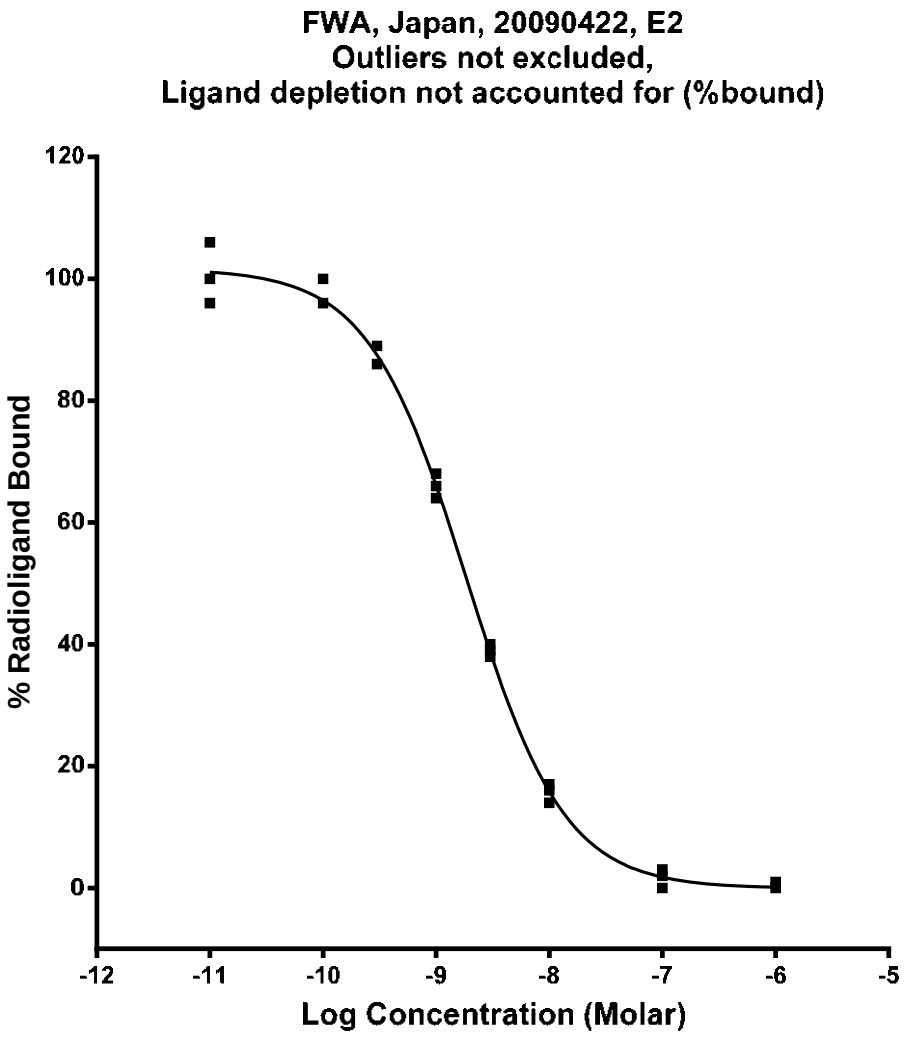
Log Concentration (Molar)

Subtask 2


FWA, Japan, 20090422, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
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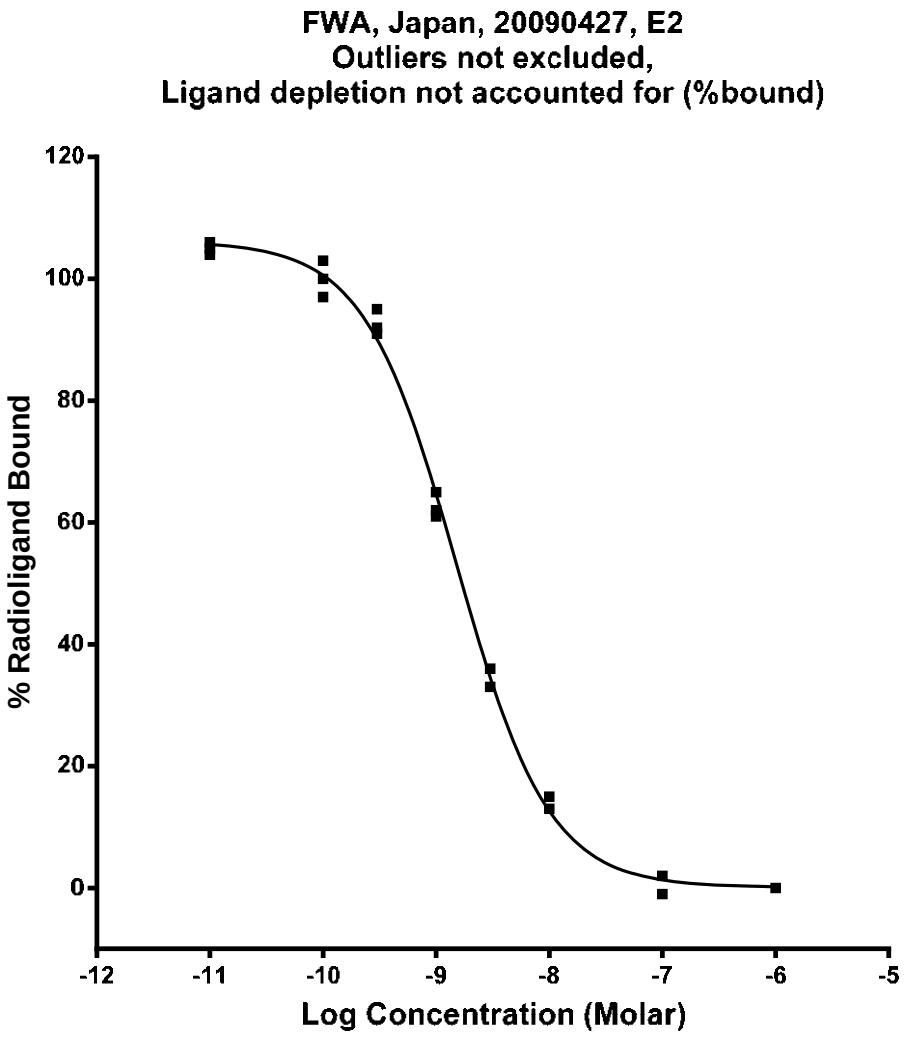
Log Concentration (Molar)

Subtask 2


FWA, Japan, 20090427, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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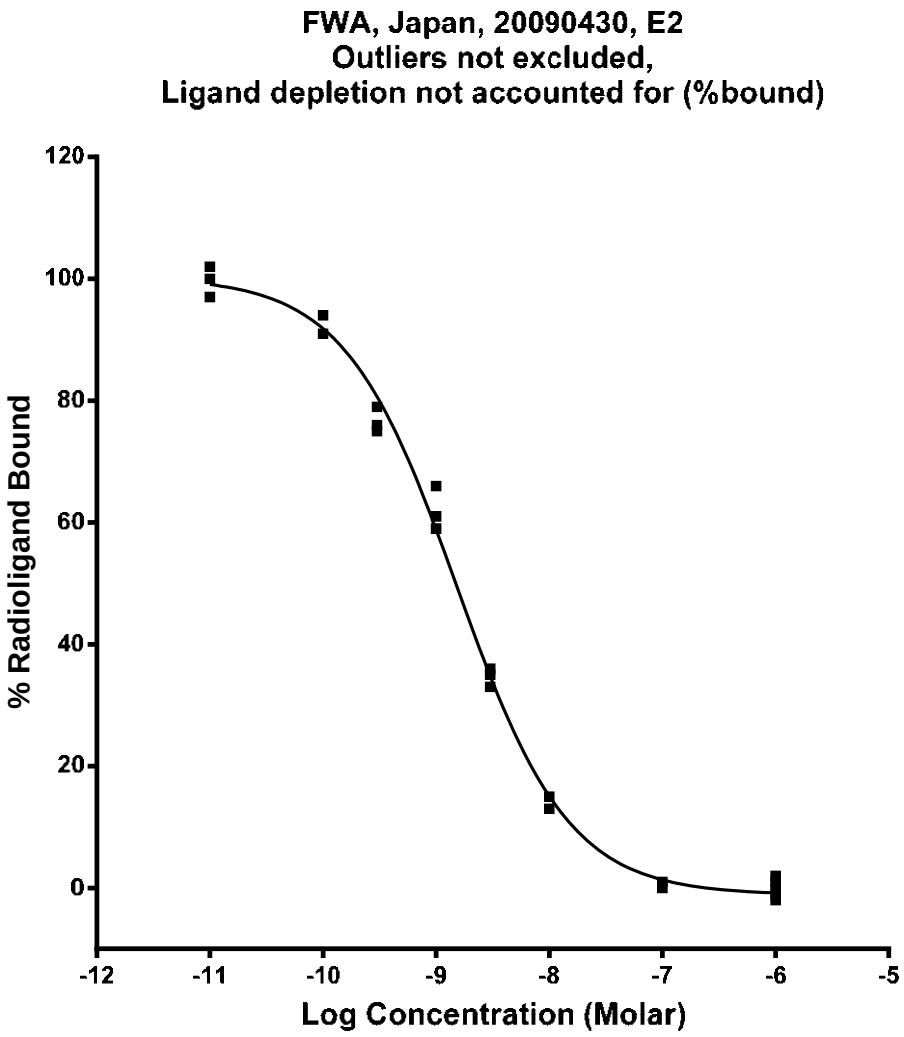
Log Concentration (Molar)

Subtask 2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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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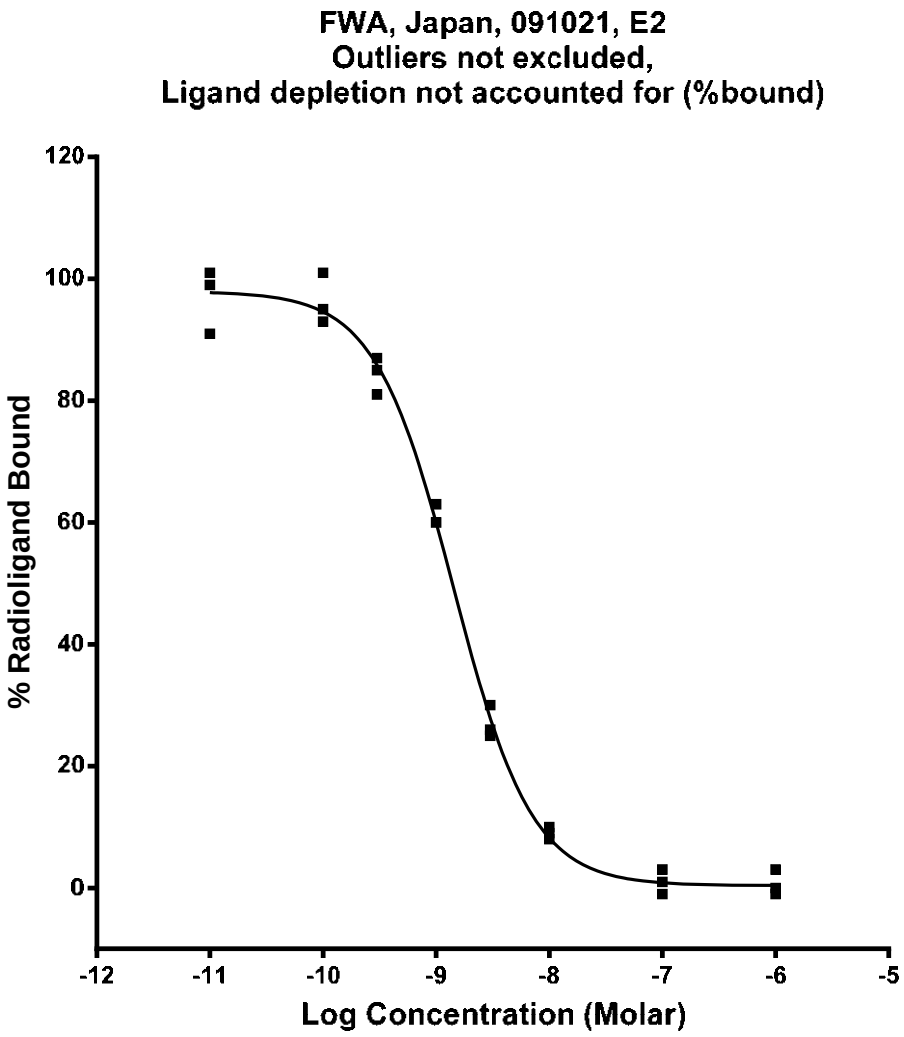
Log Concentration (Molar)

Subtask 3


FWA, Japan, 091021, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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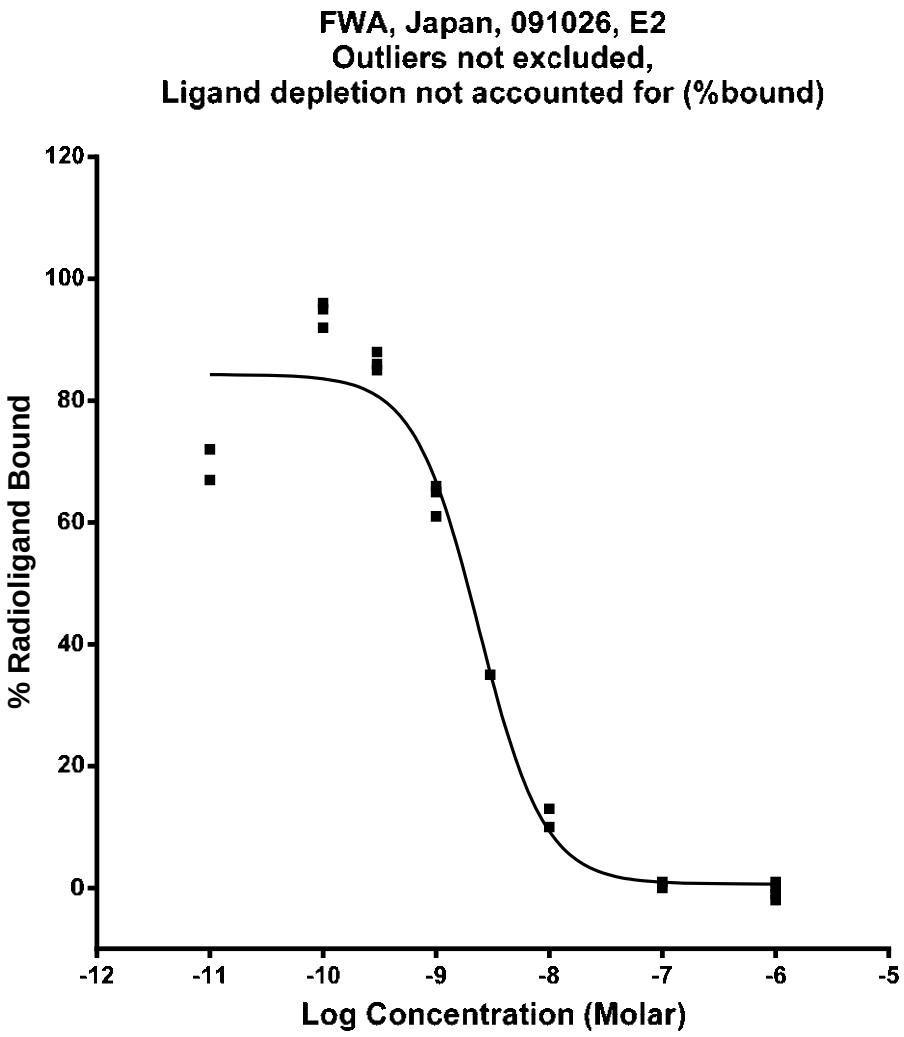
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
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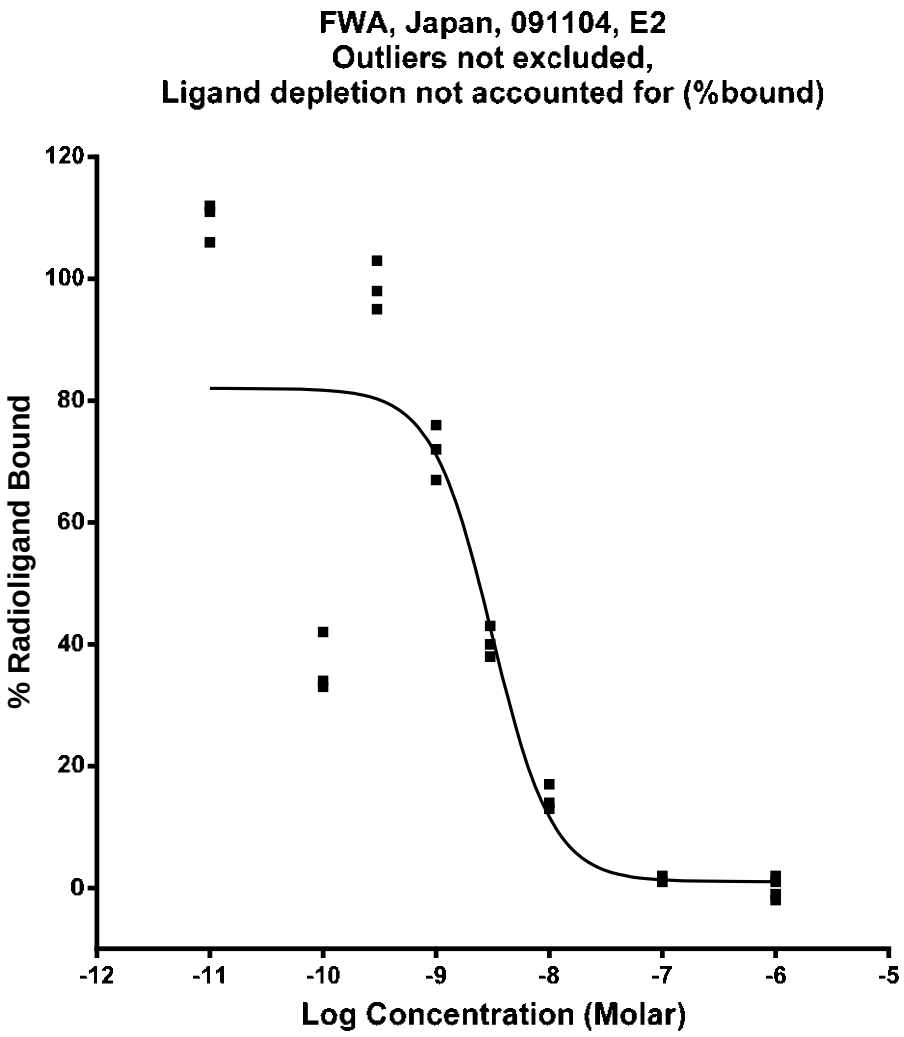
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
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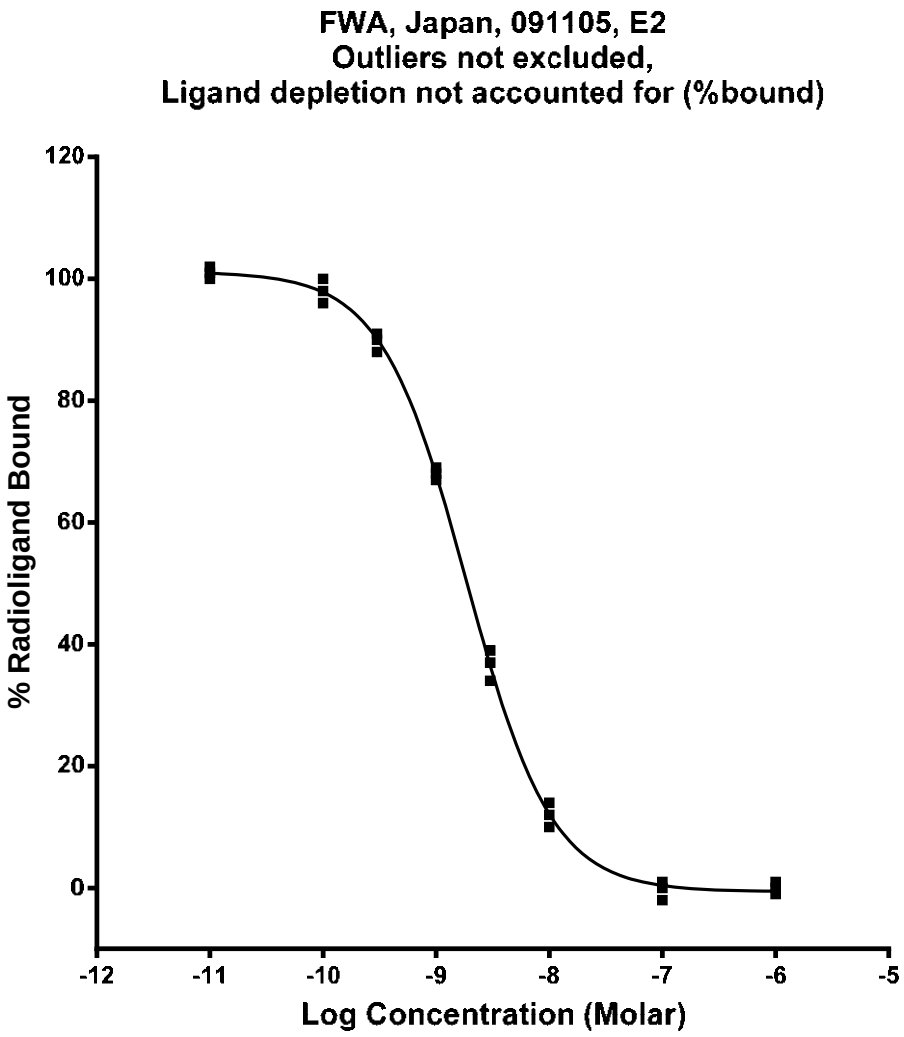
Log Concentration (Molar)

Subtask 3


FWA, Japan, 091105, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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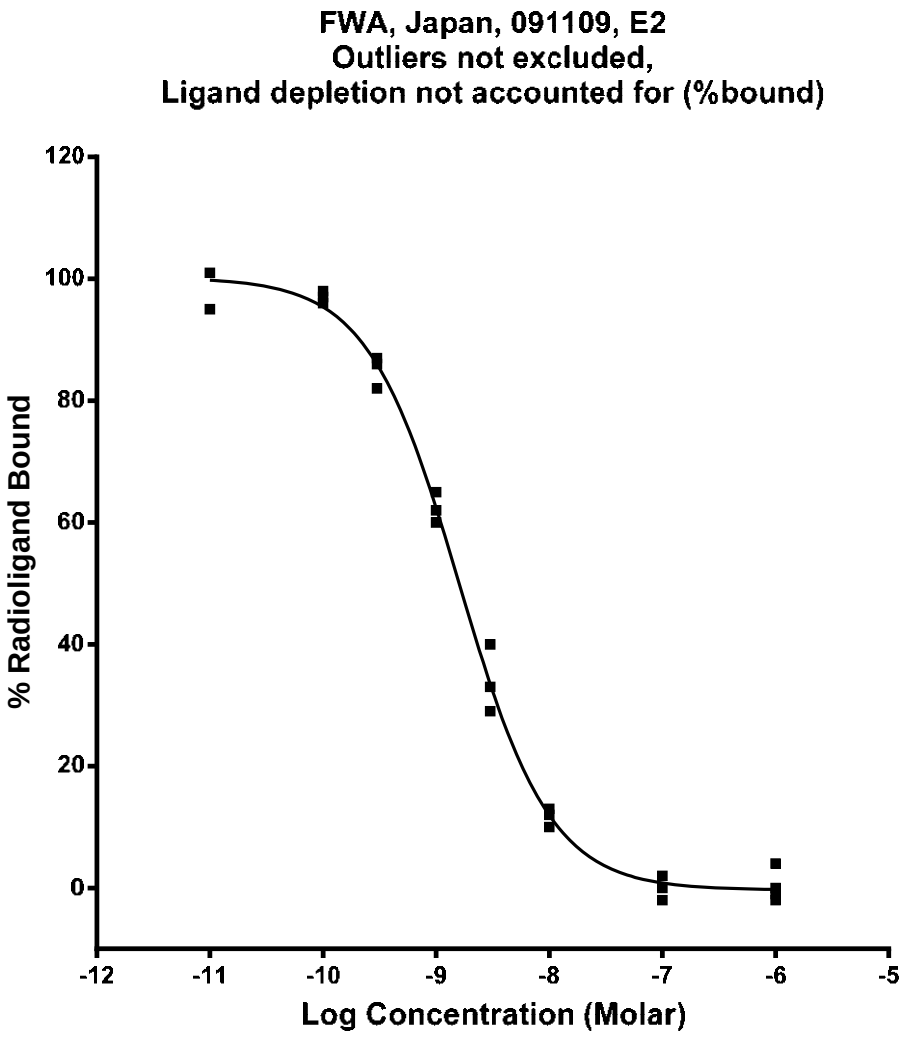
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
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% Radioligand Bound
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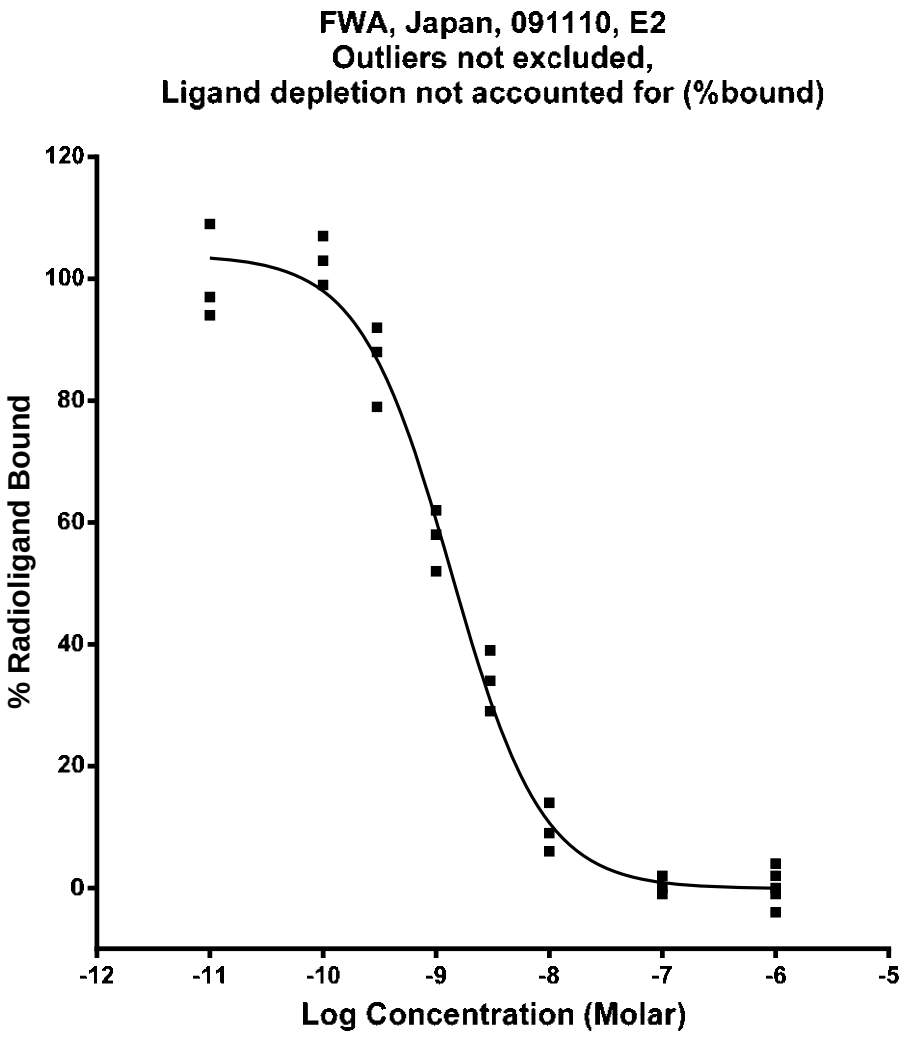
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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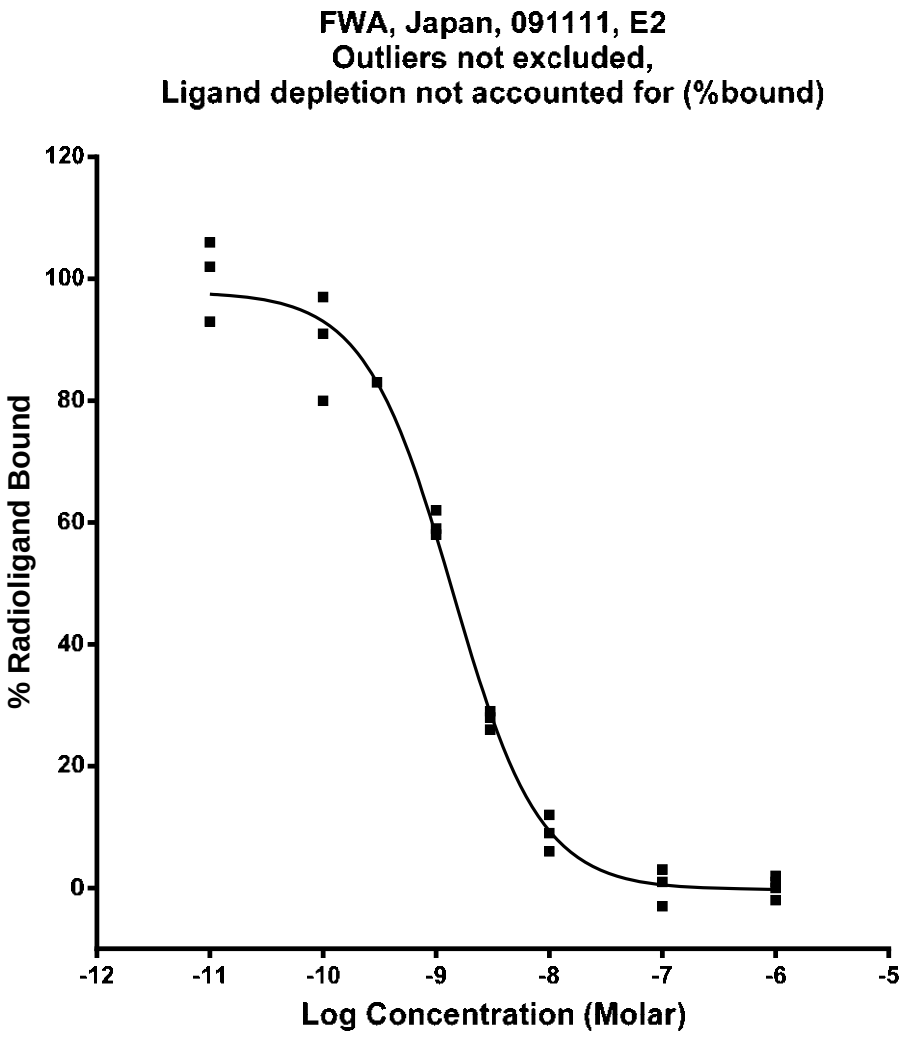
Log Concentration (Molar)

Subtask 3


FWA, Japan, 091111, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
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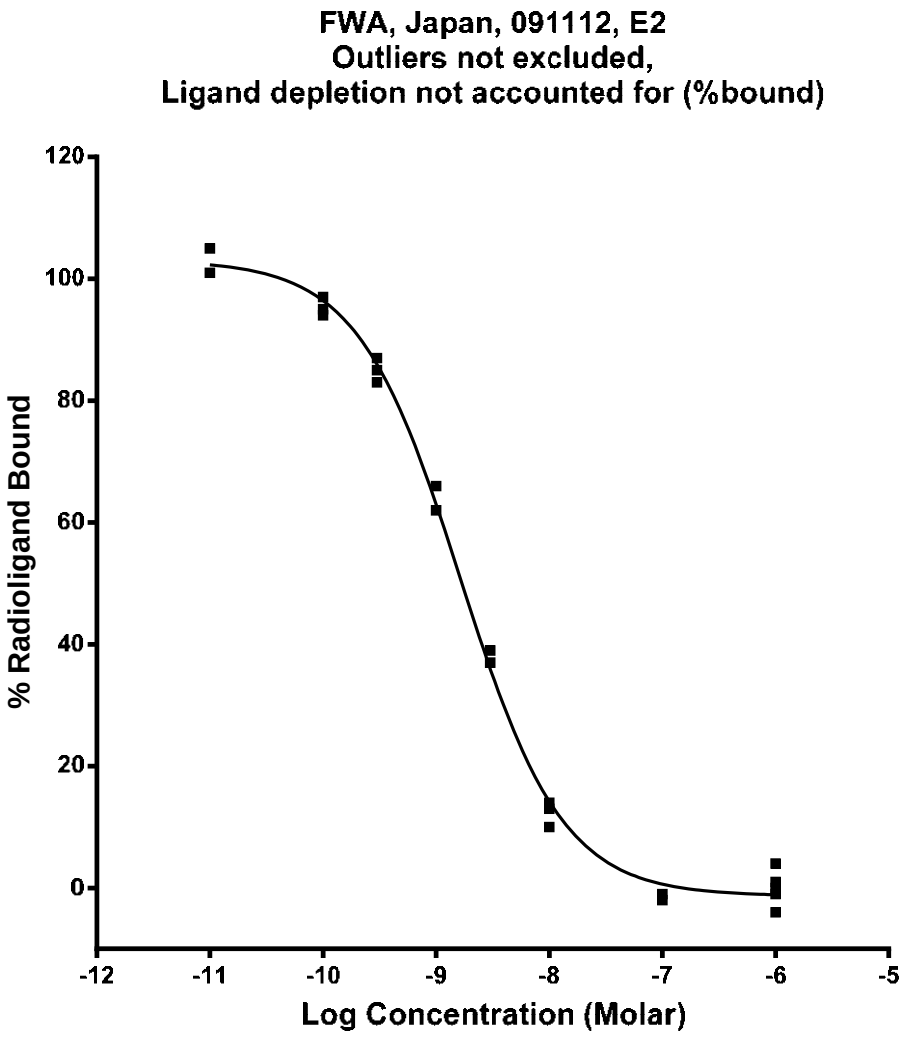
Log Concentration (Molar)

Subtask 3


FWA, Japan, 091112, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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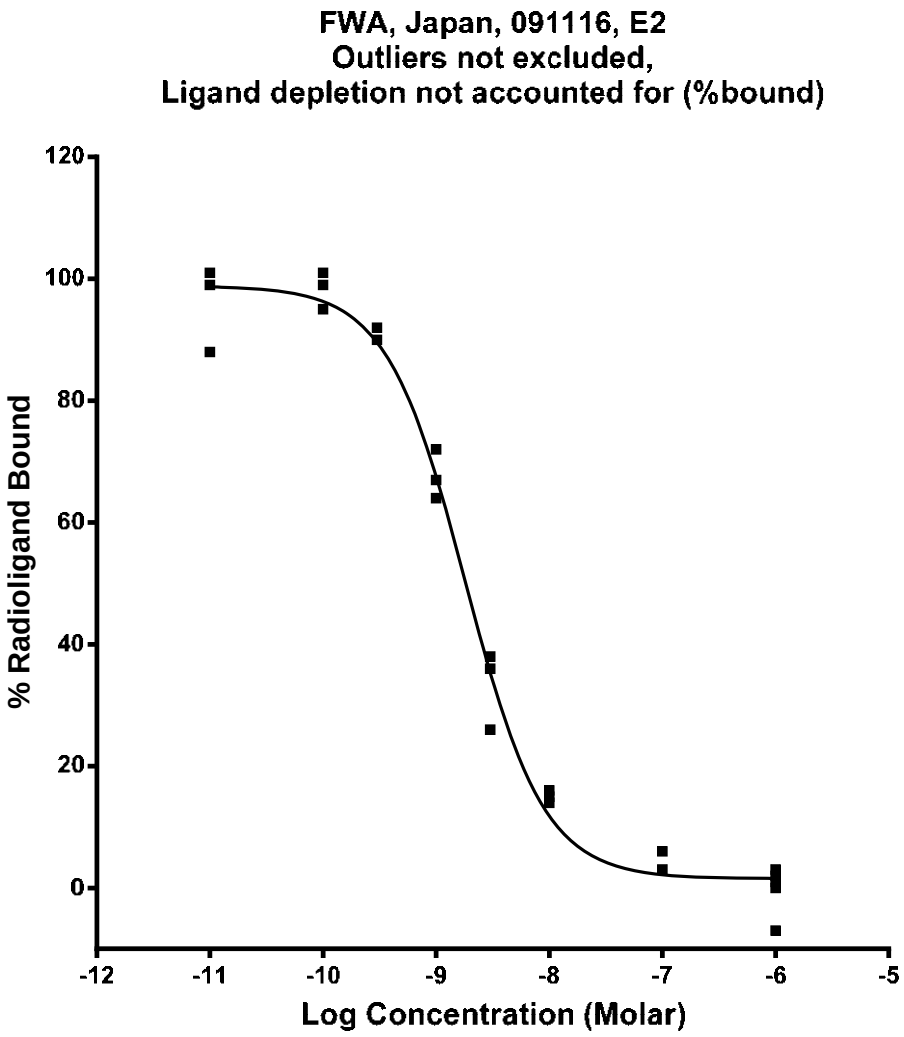
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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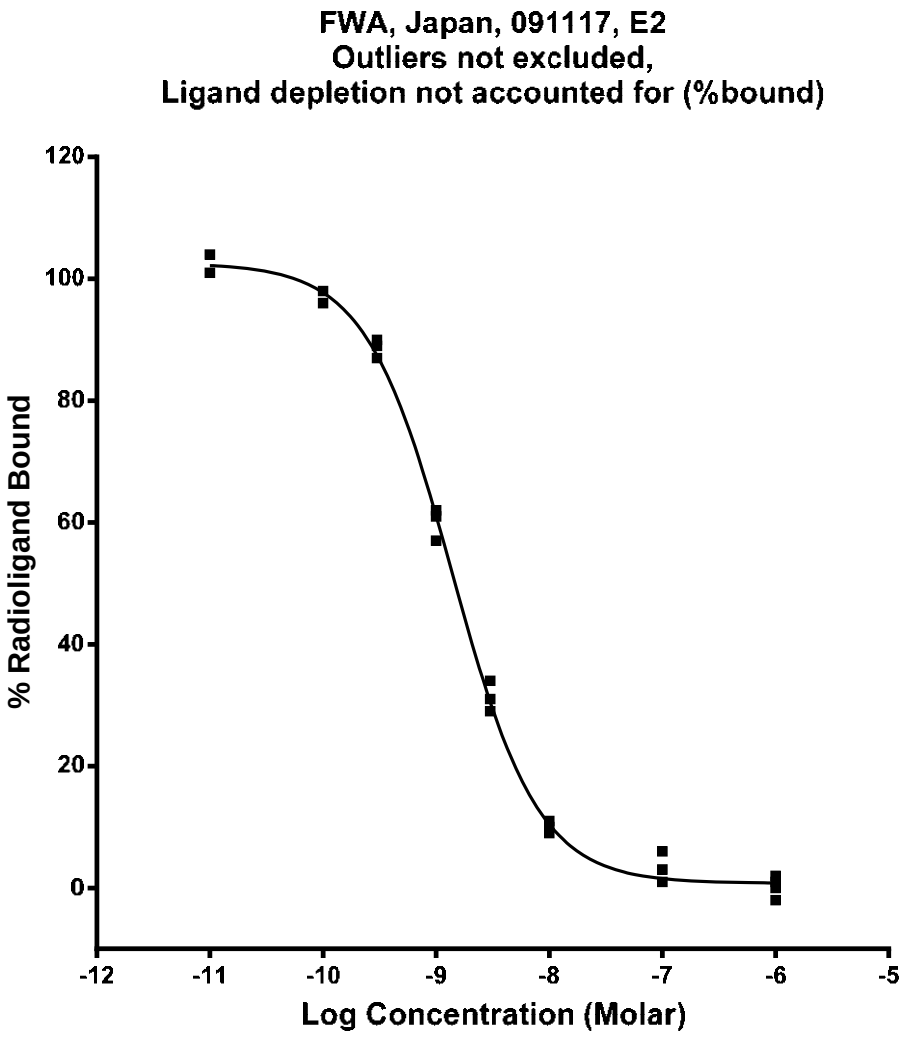
Log Concentration (Molar)

Subtask 3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% Radioligand Bound
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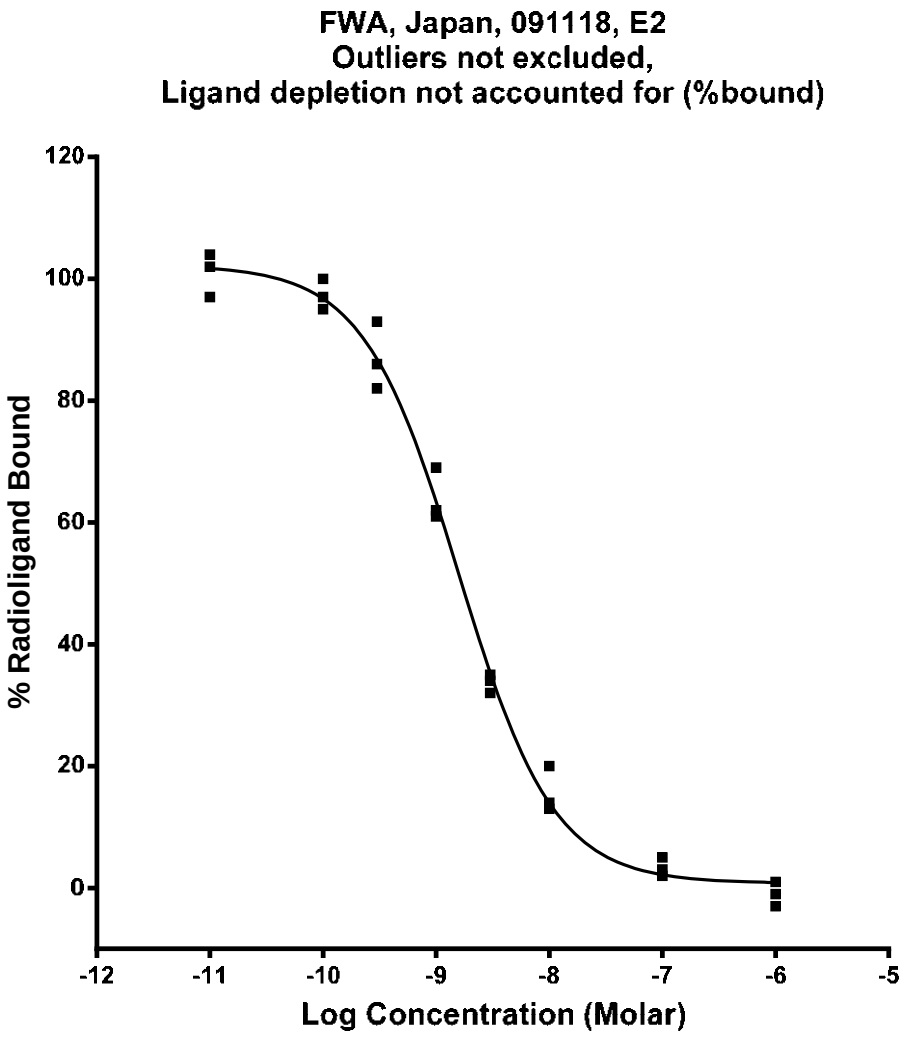
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
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% Radioligand Bound
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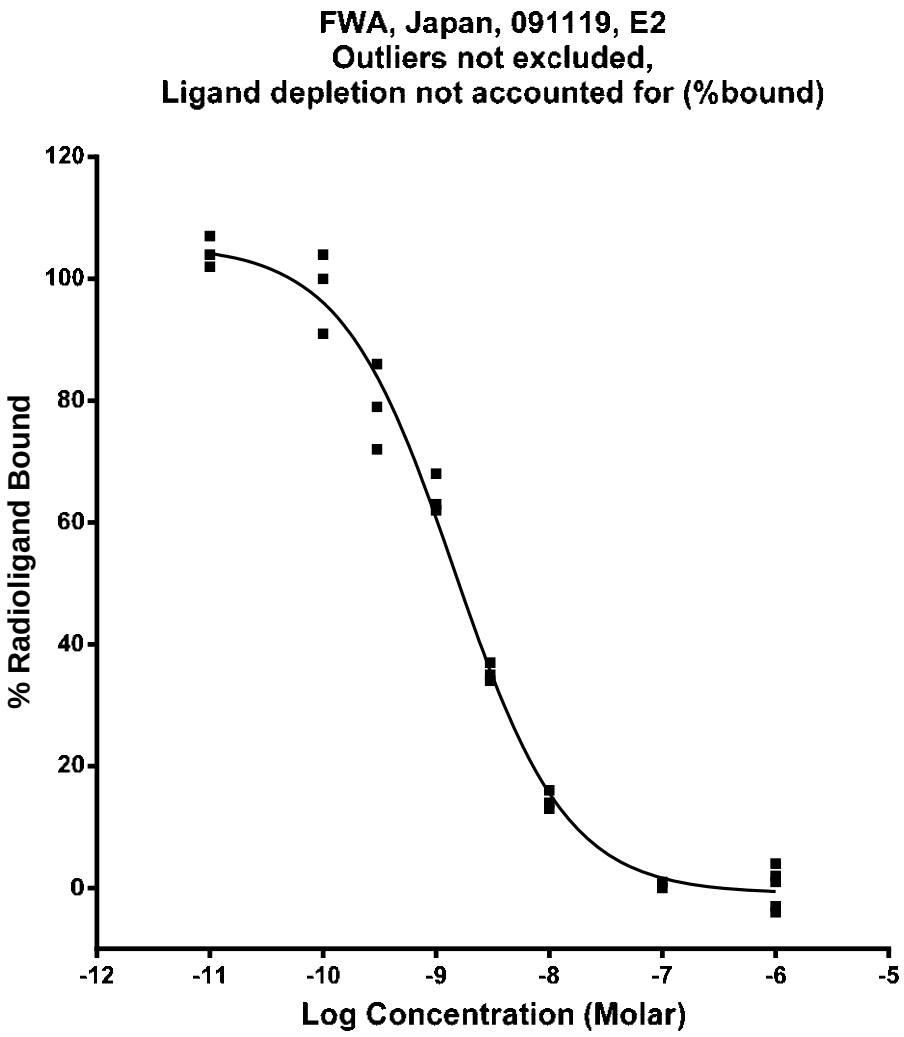
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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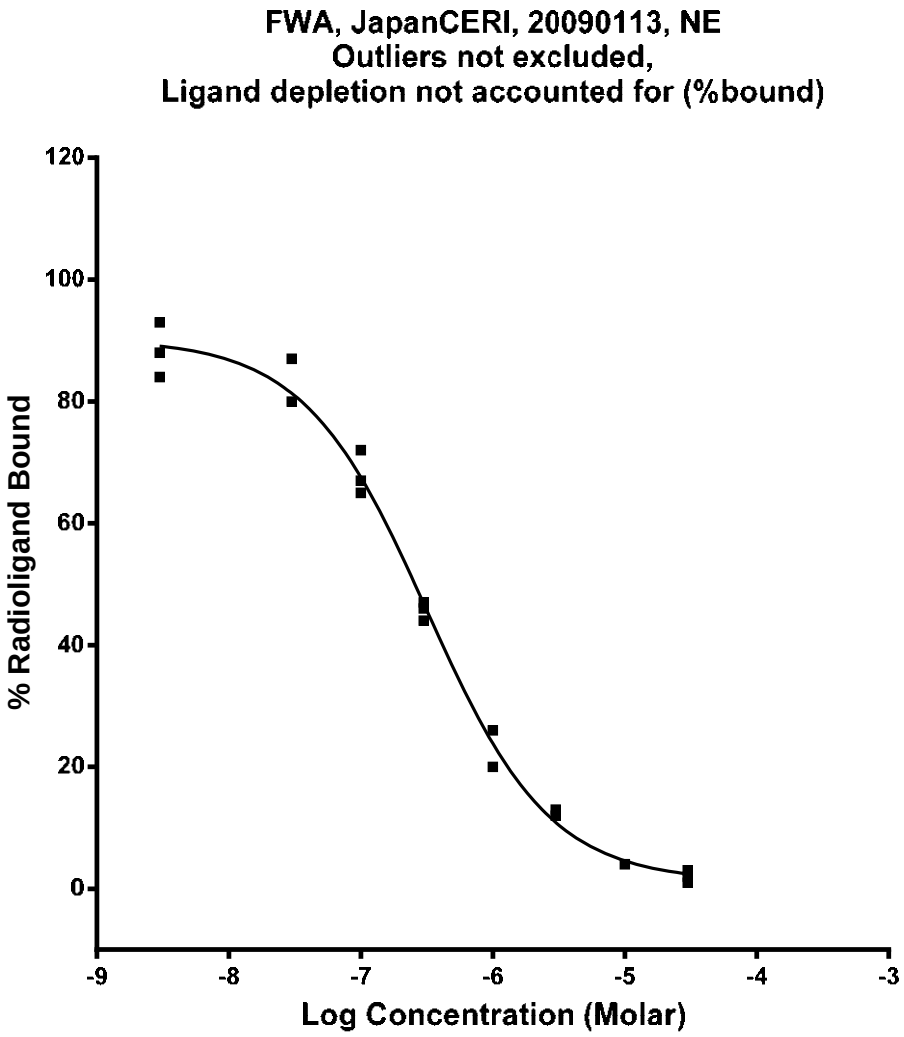
Log Concentration (Molar)

FW ASSAY, (JapanCERI Lab) Control Norethynodrel (NE) 30 Nov 2014

Begin Here

Subtask 1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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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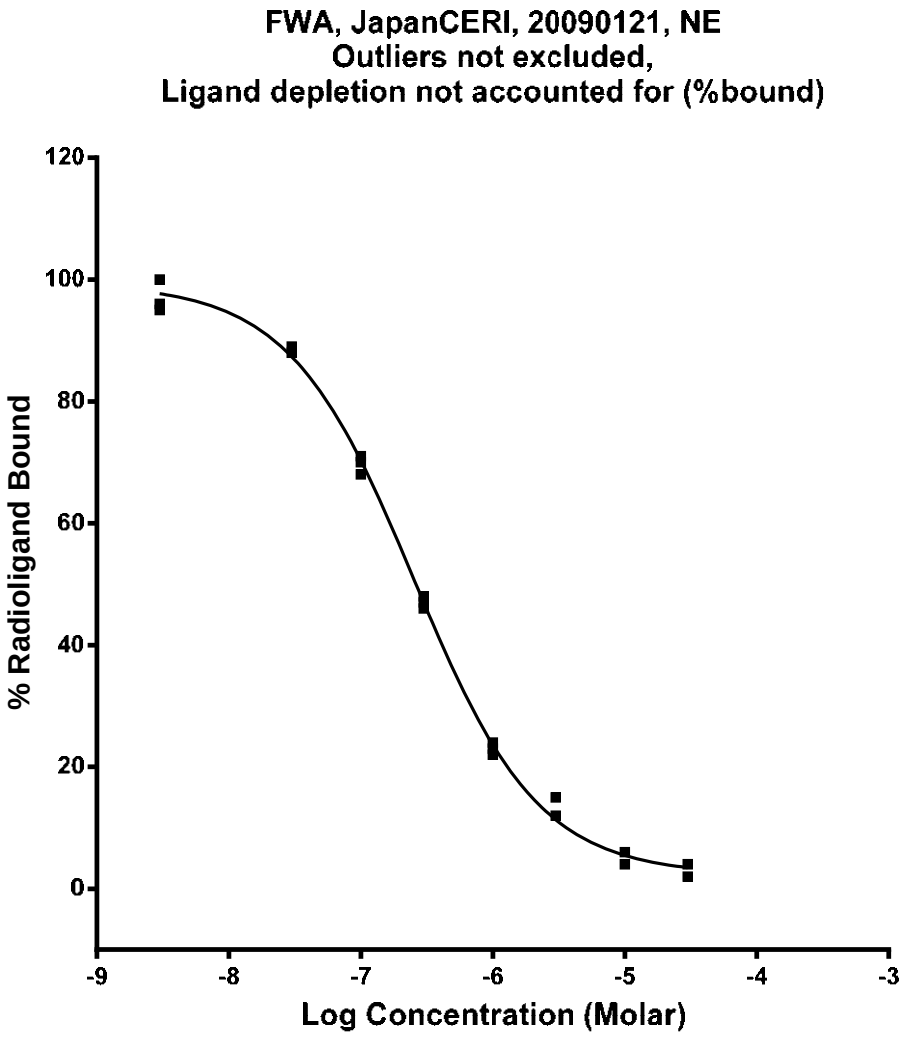
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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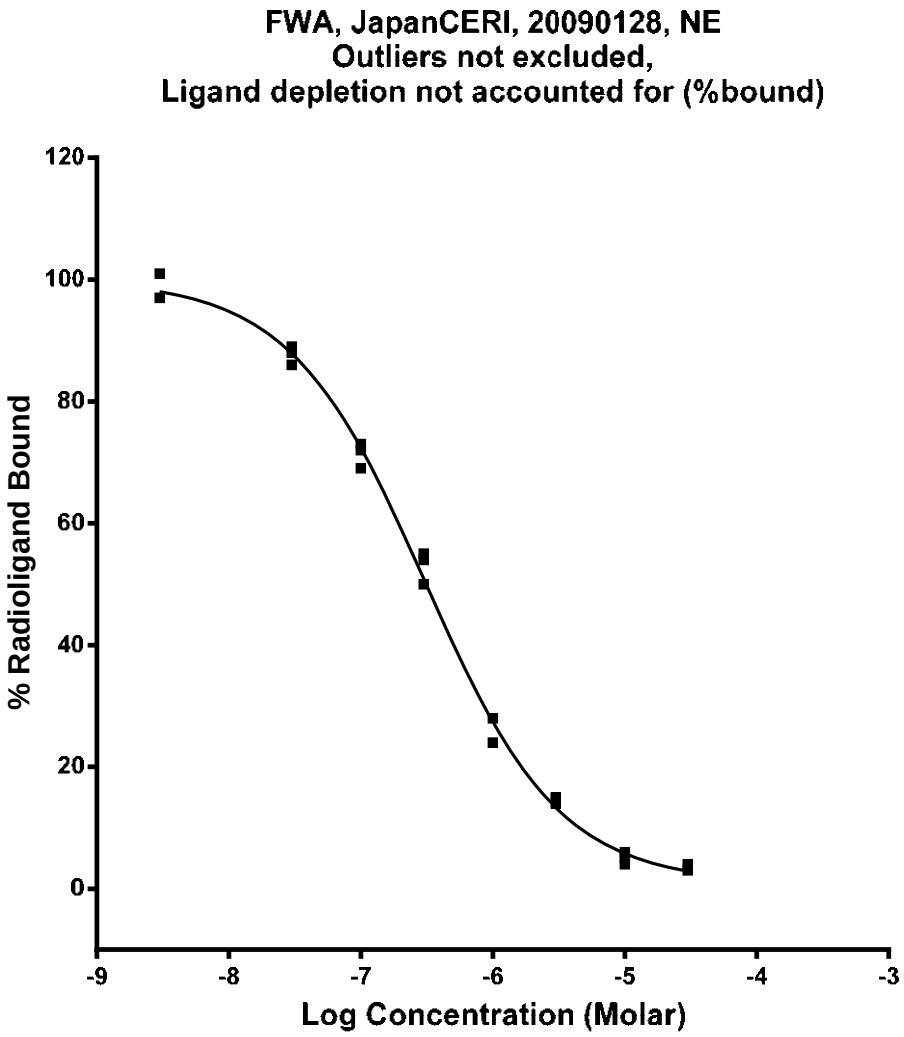
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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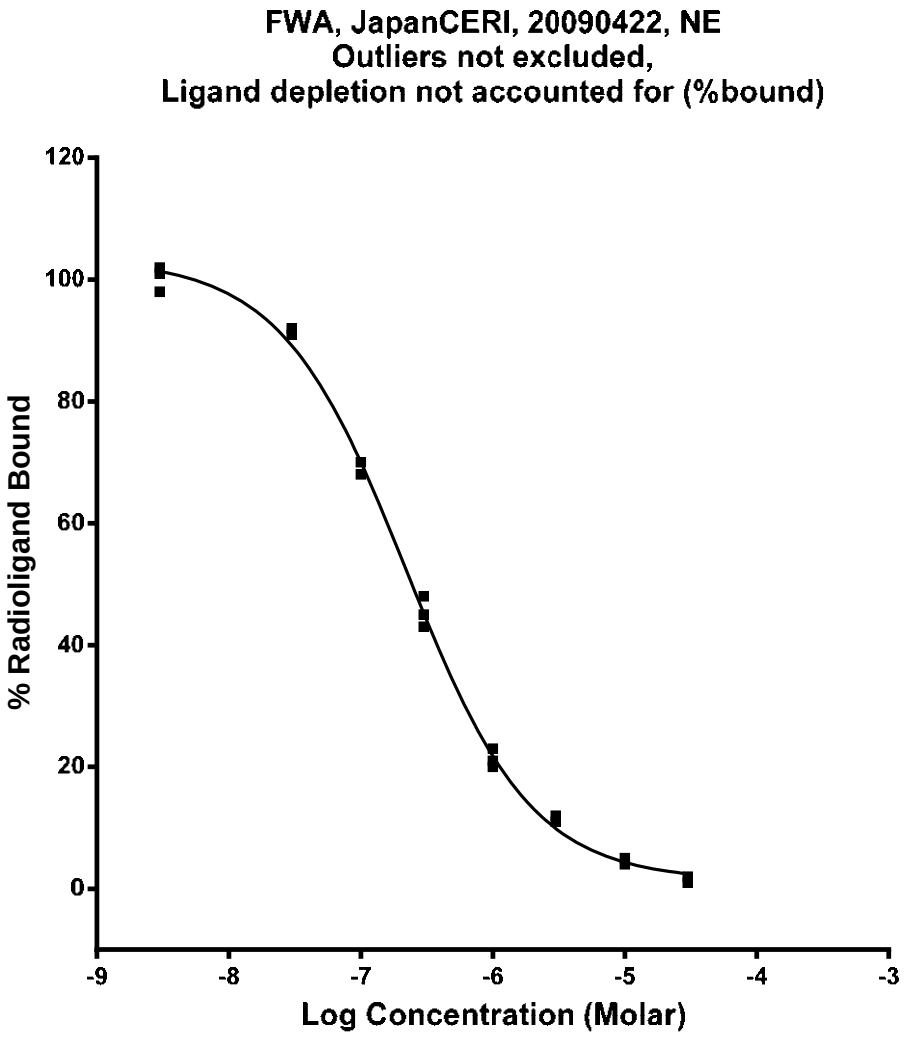
Log Concentration (Molar)

Subtask 2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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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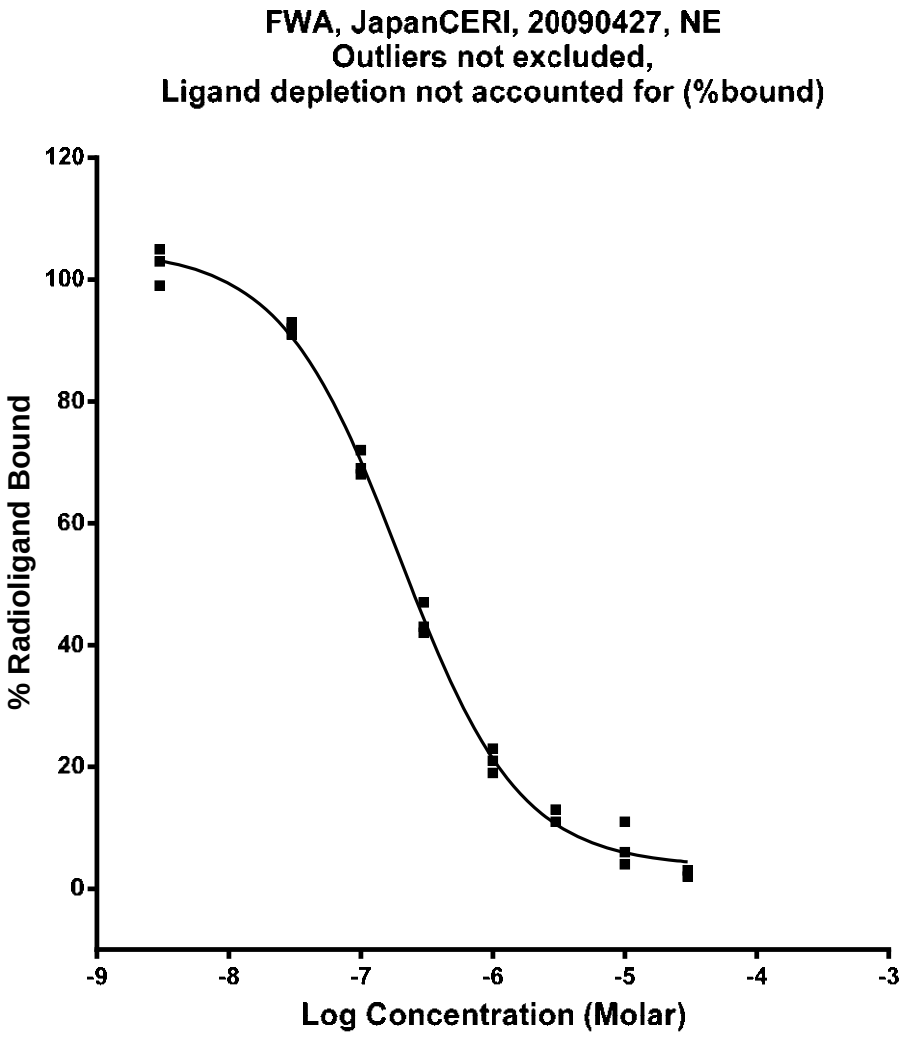
Log Concentration (Molar)

Subtask 2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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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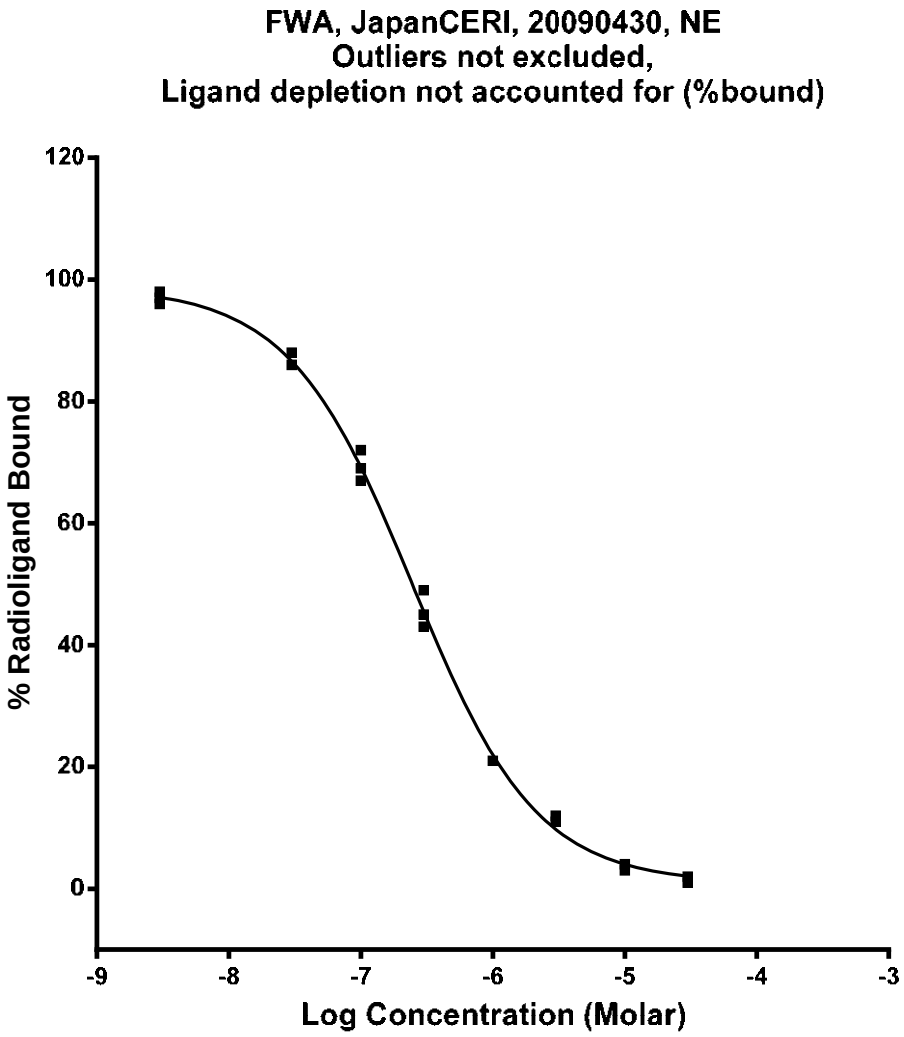
Log Concentration (Molar)

Subtask 2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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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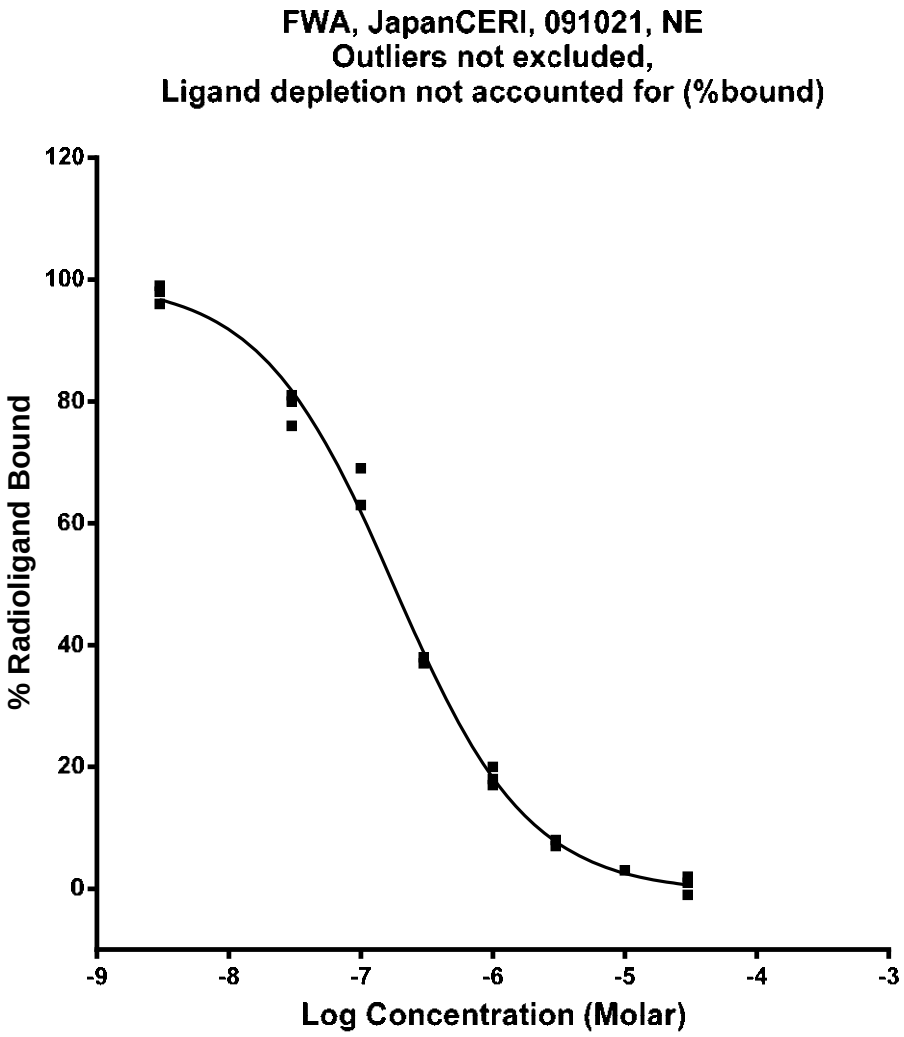
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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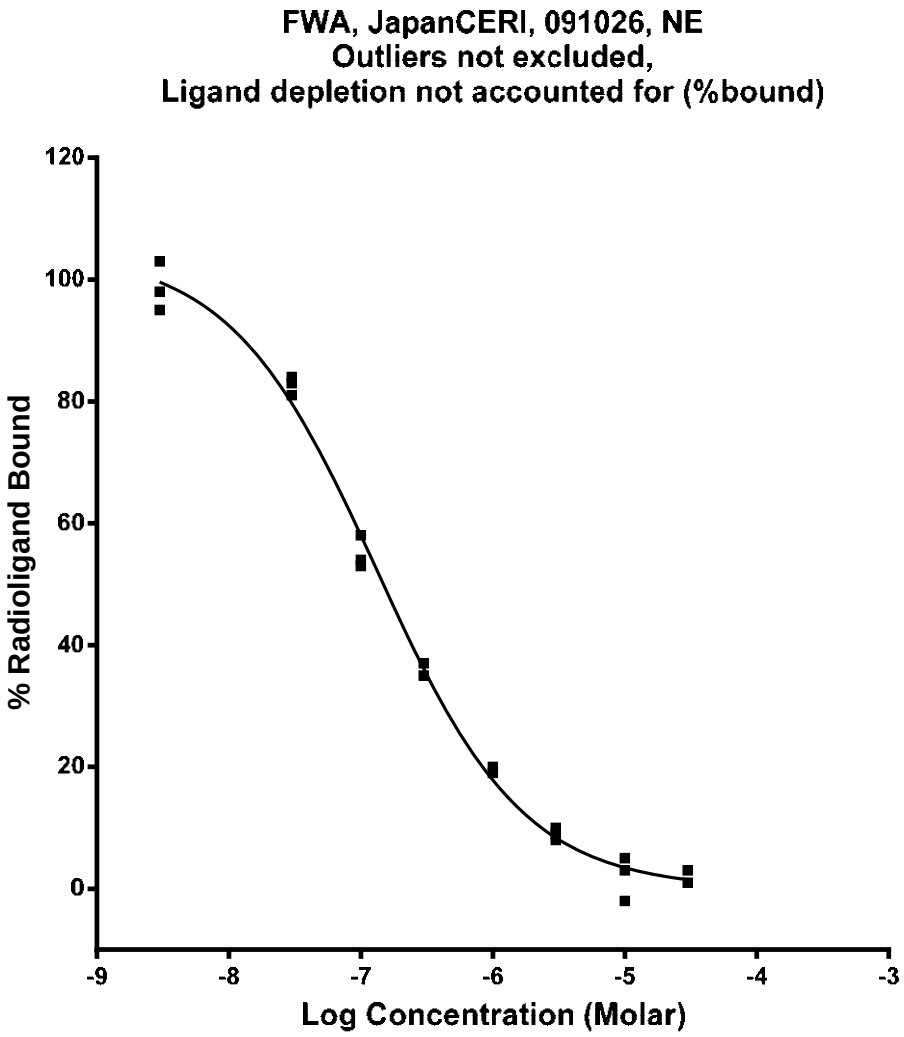
Log Concentration (Molar)

Subtask 3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% Radioligand Bound
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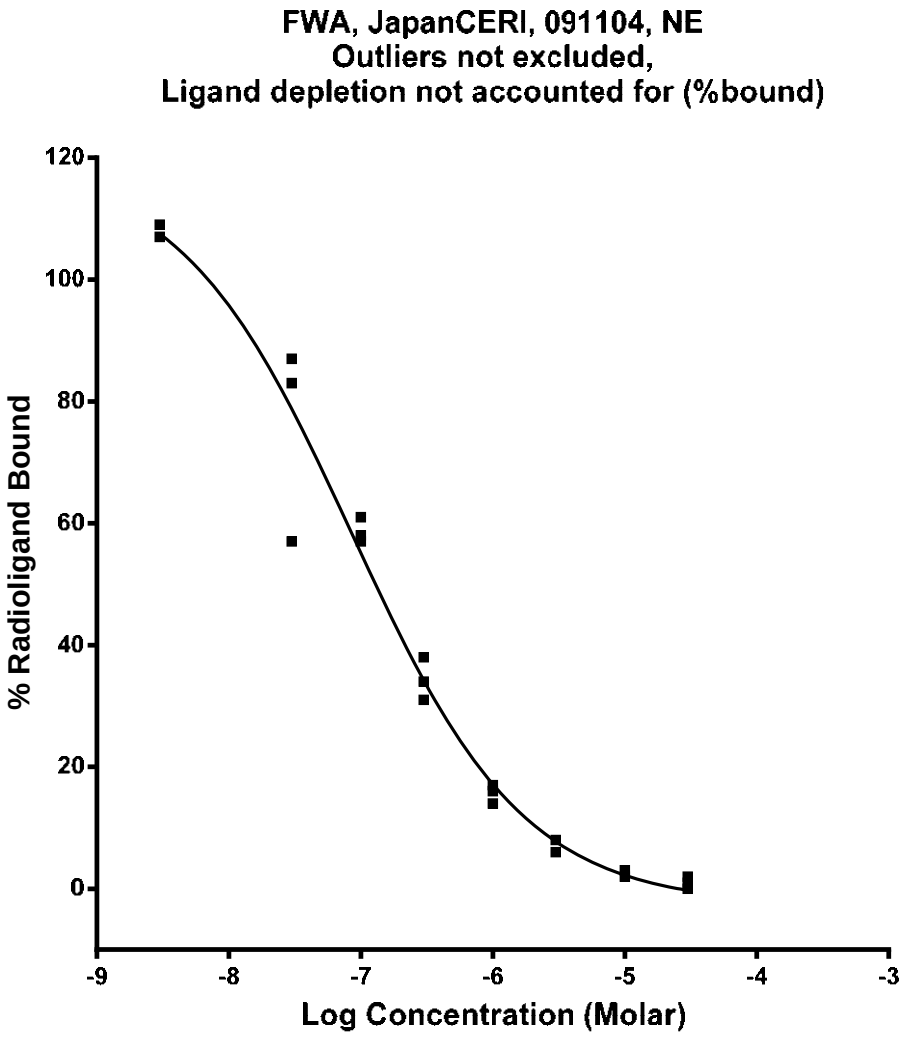
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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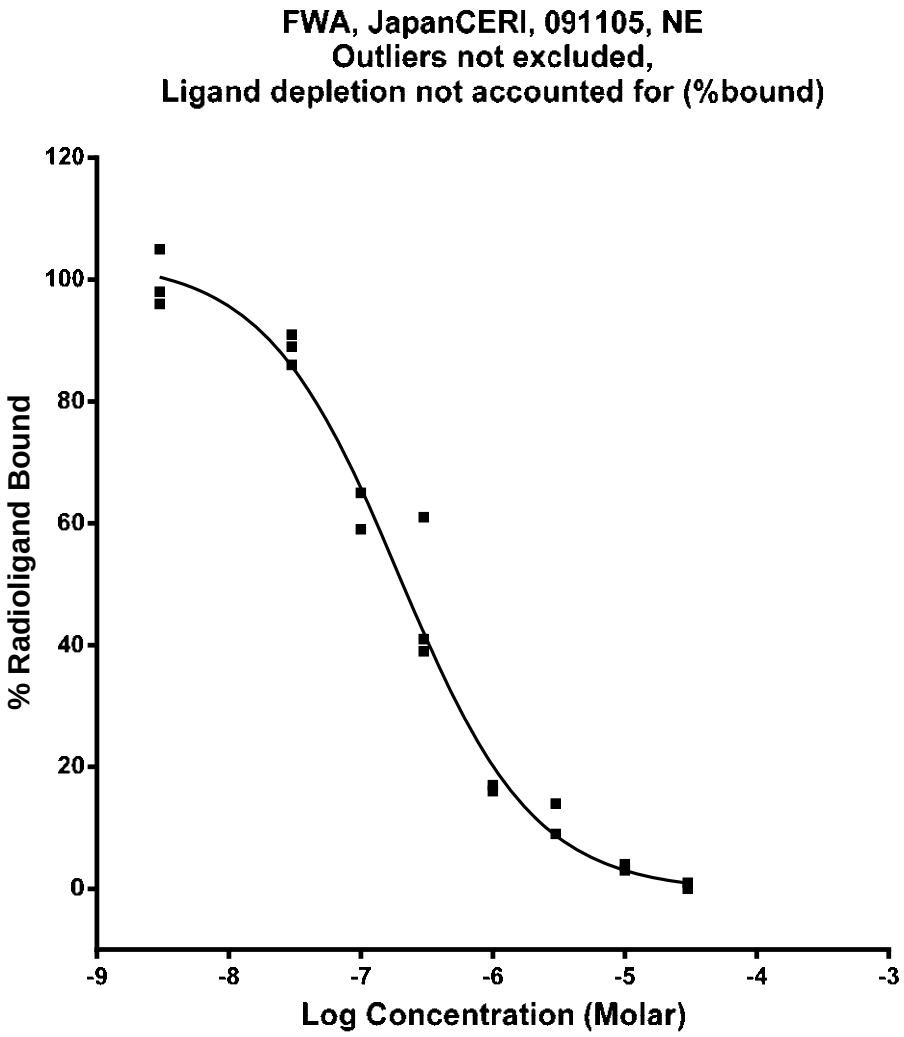
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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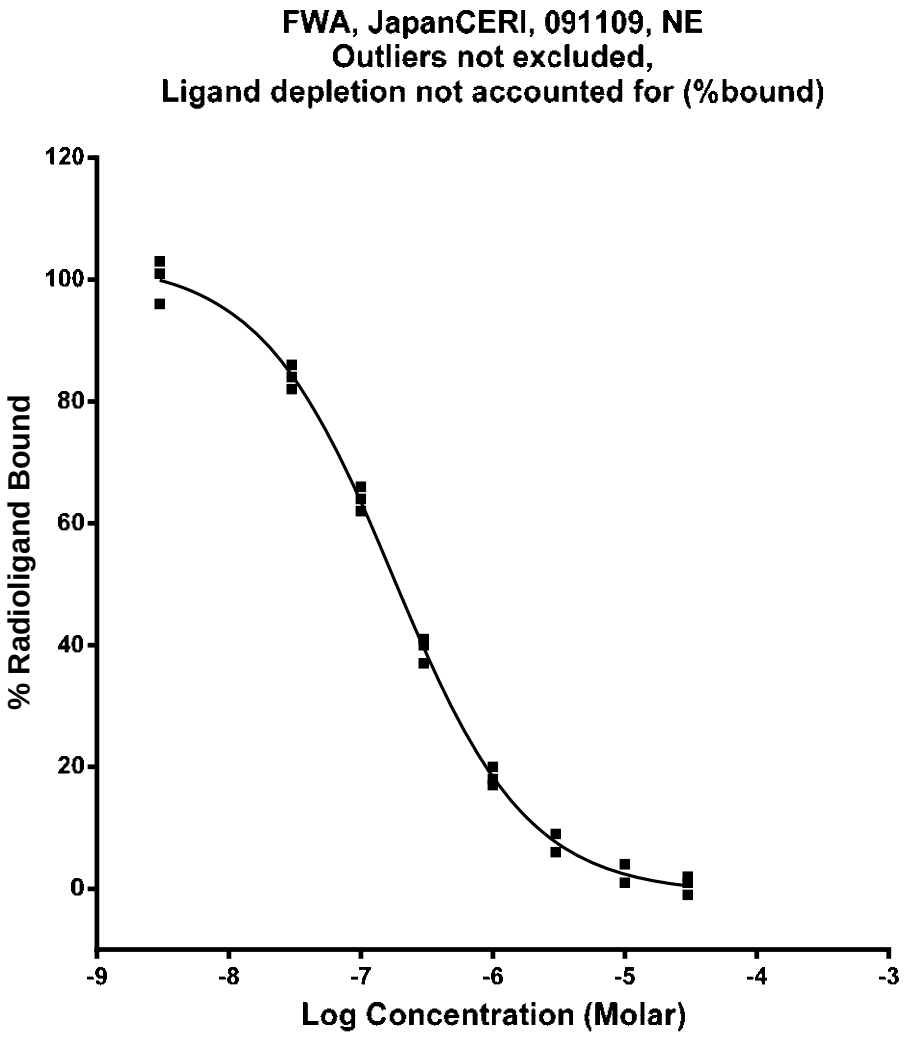
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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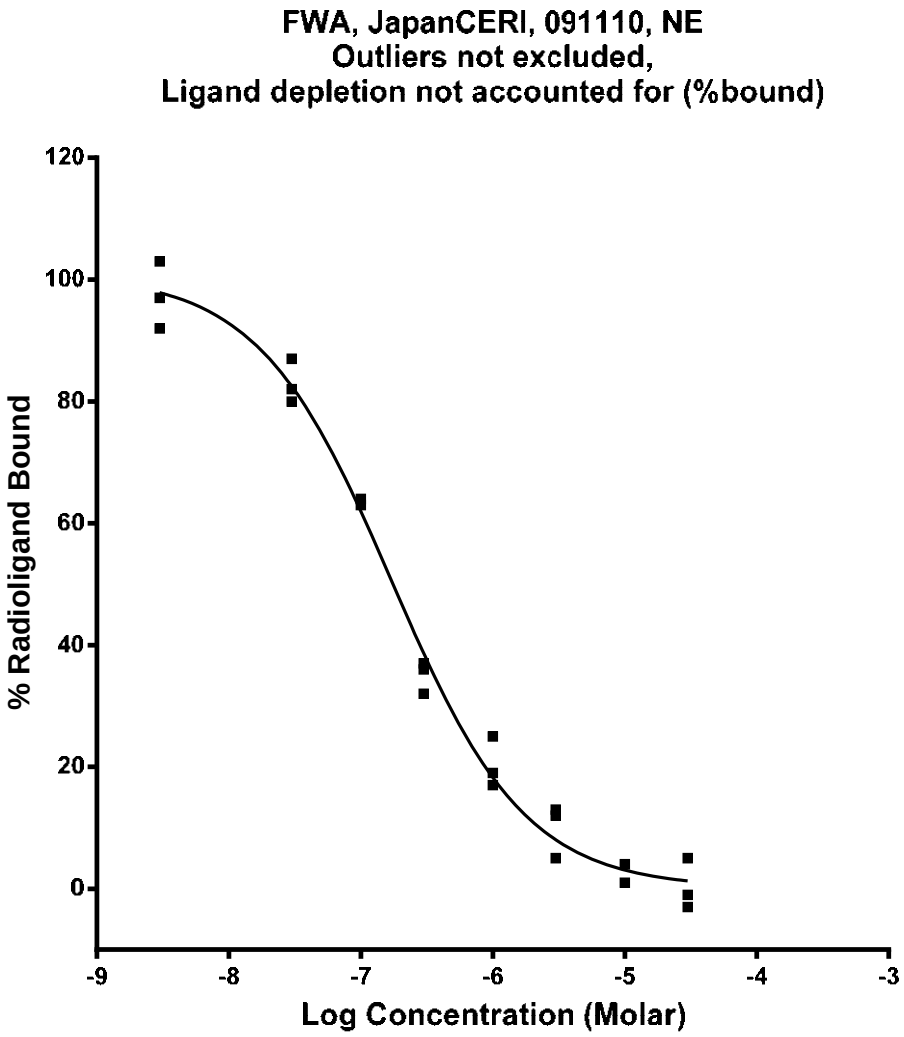
Log Concentration (Molar)

Subtask 3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Ligand depletion not accounted for (%bound)
% Radioligand Bound
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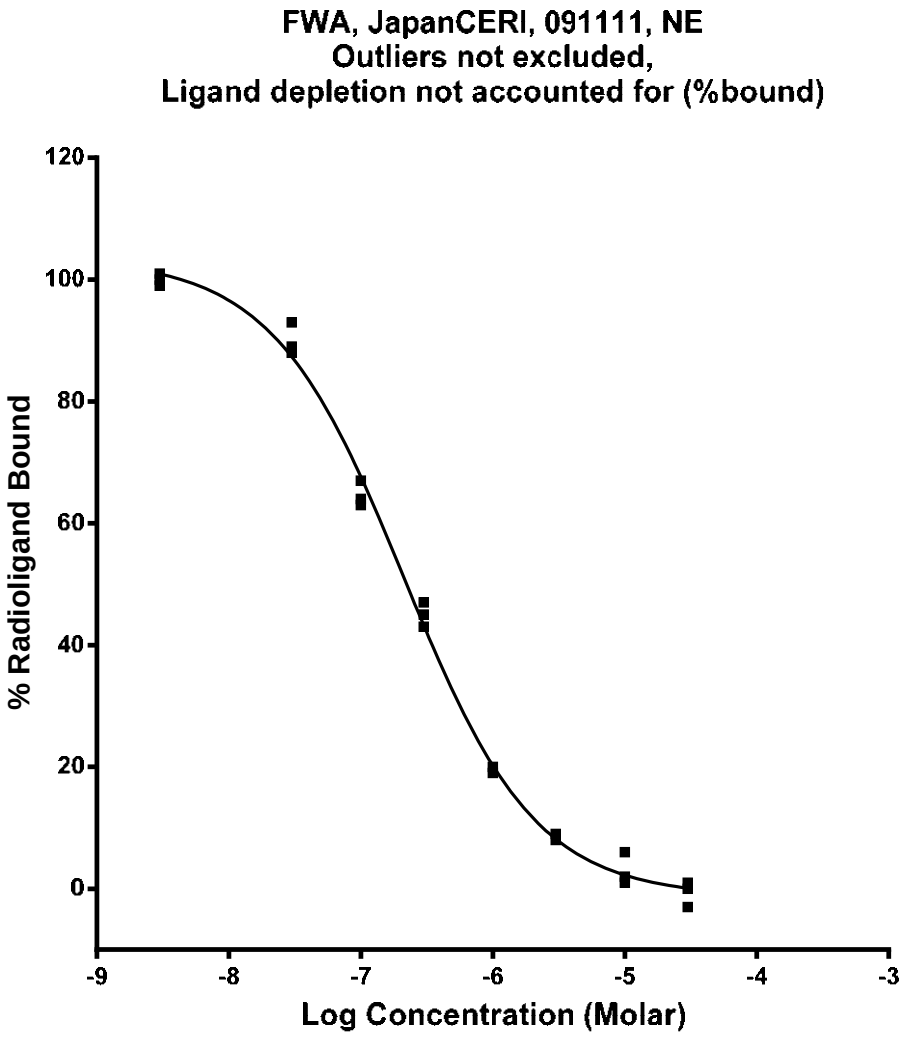
Log Concentration (Molar)

Subtask 3


FWA, JapanCERI, 091111, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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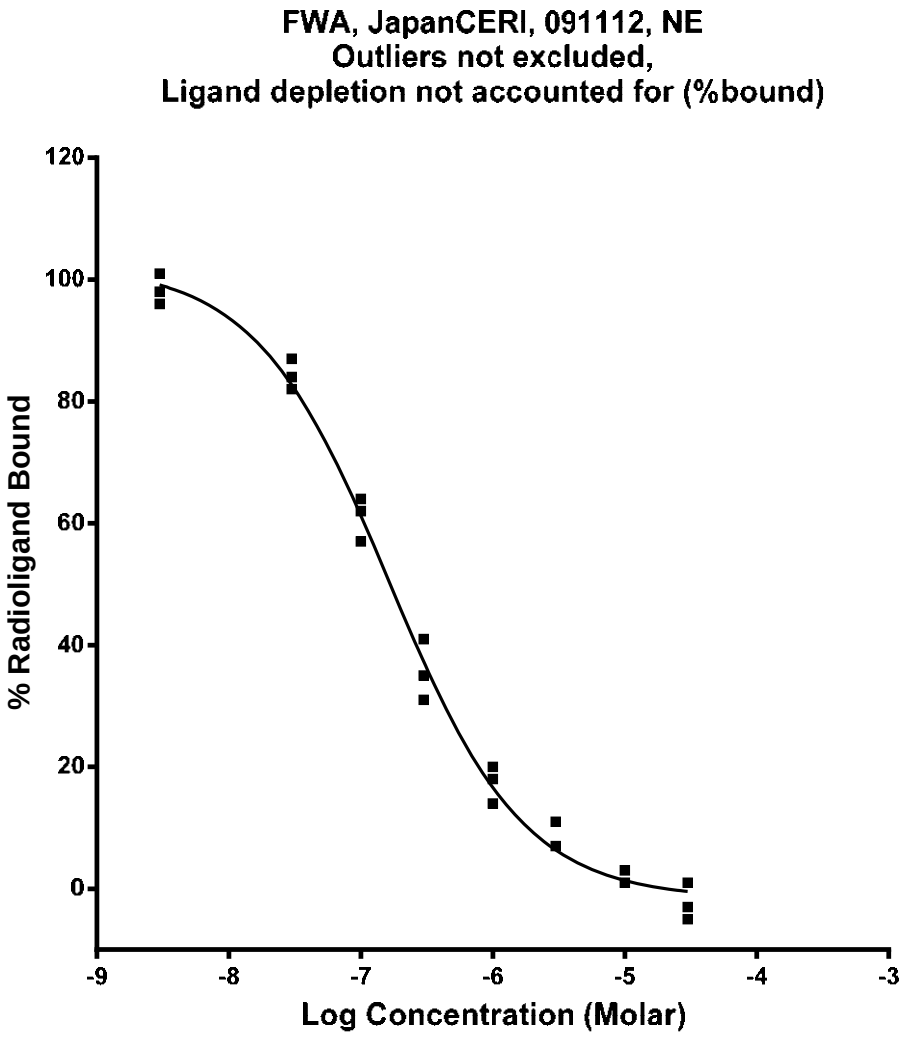
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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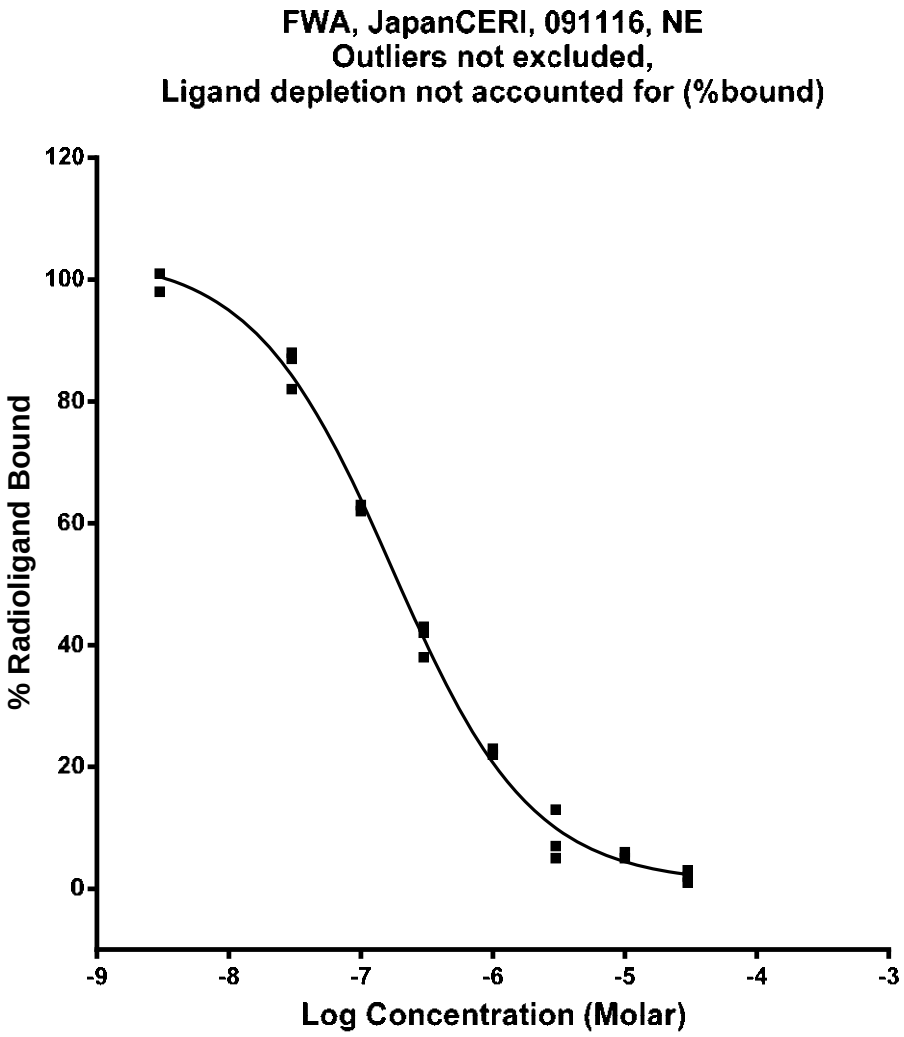
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
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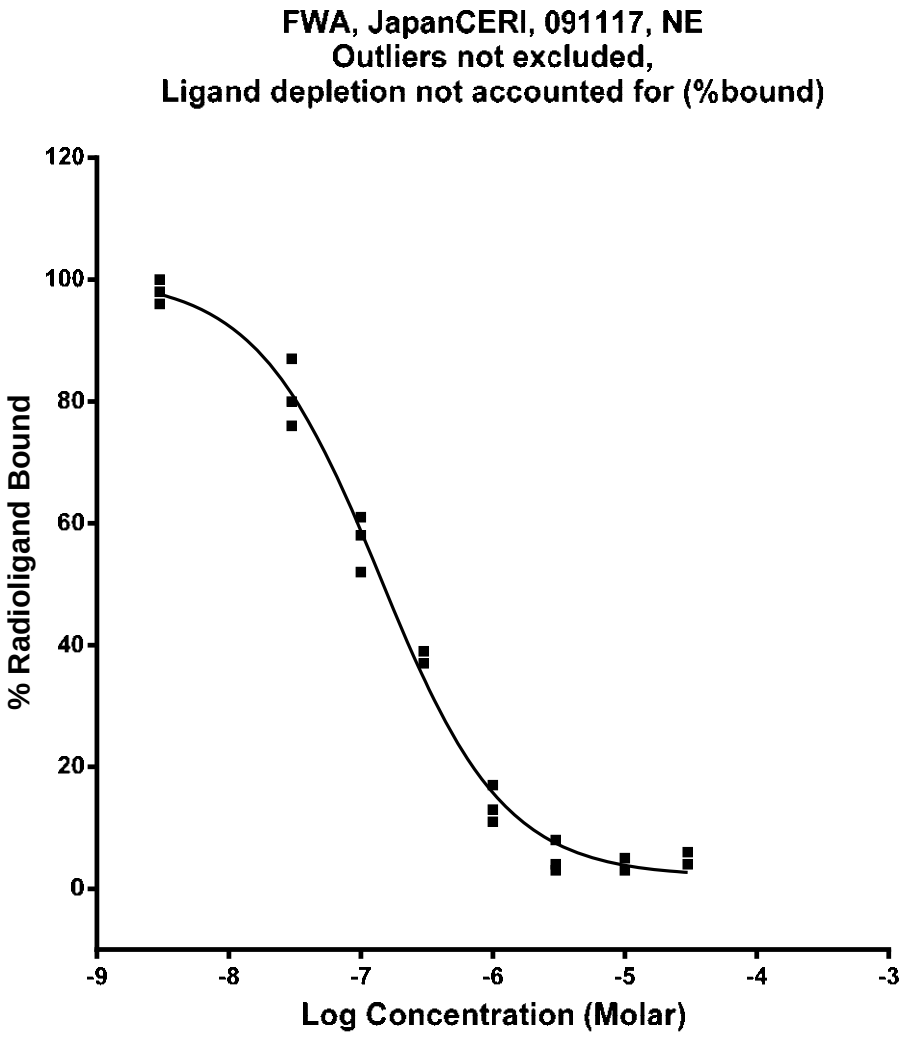
Log Concentration (Molar)

Subtask 3


FWA, JapanCERI, 091117, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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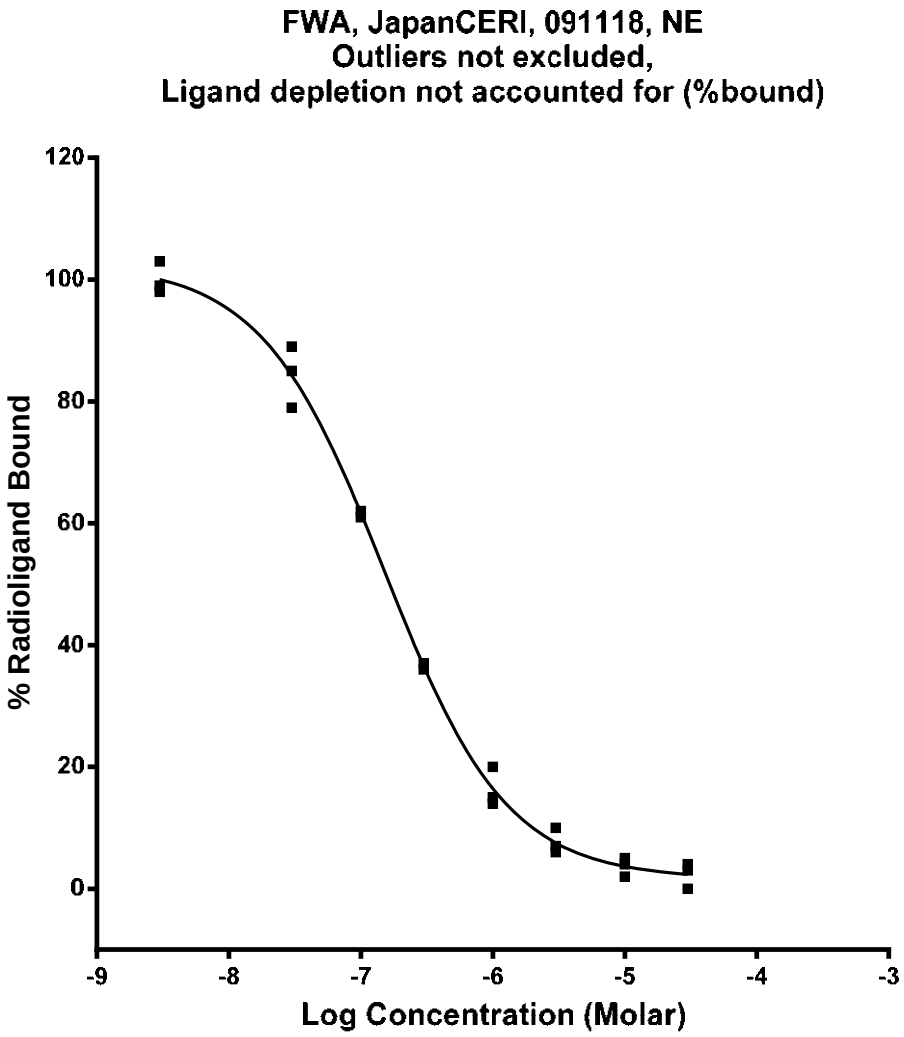
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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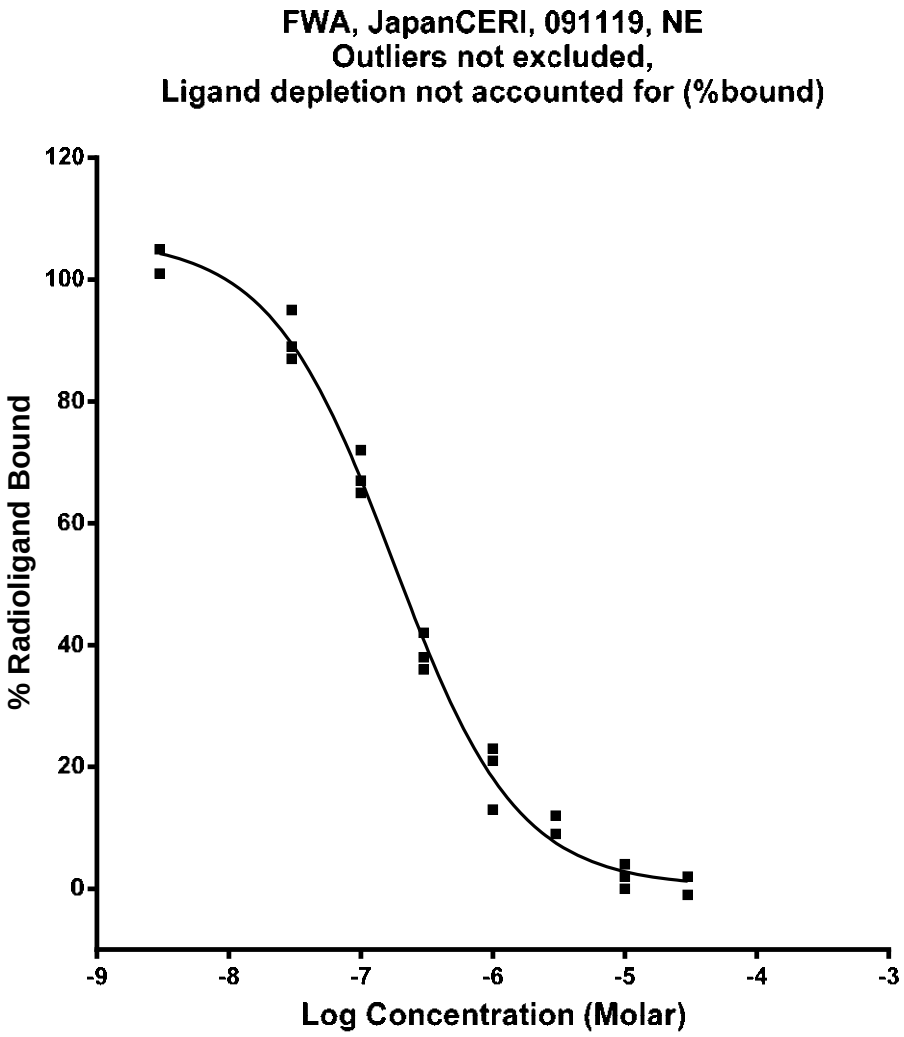
Log Concentration (Molar)

Subtask 3


FWA, JapanCERI, 091119, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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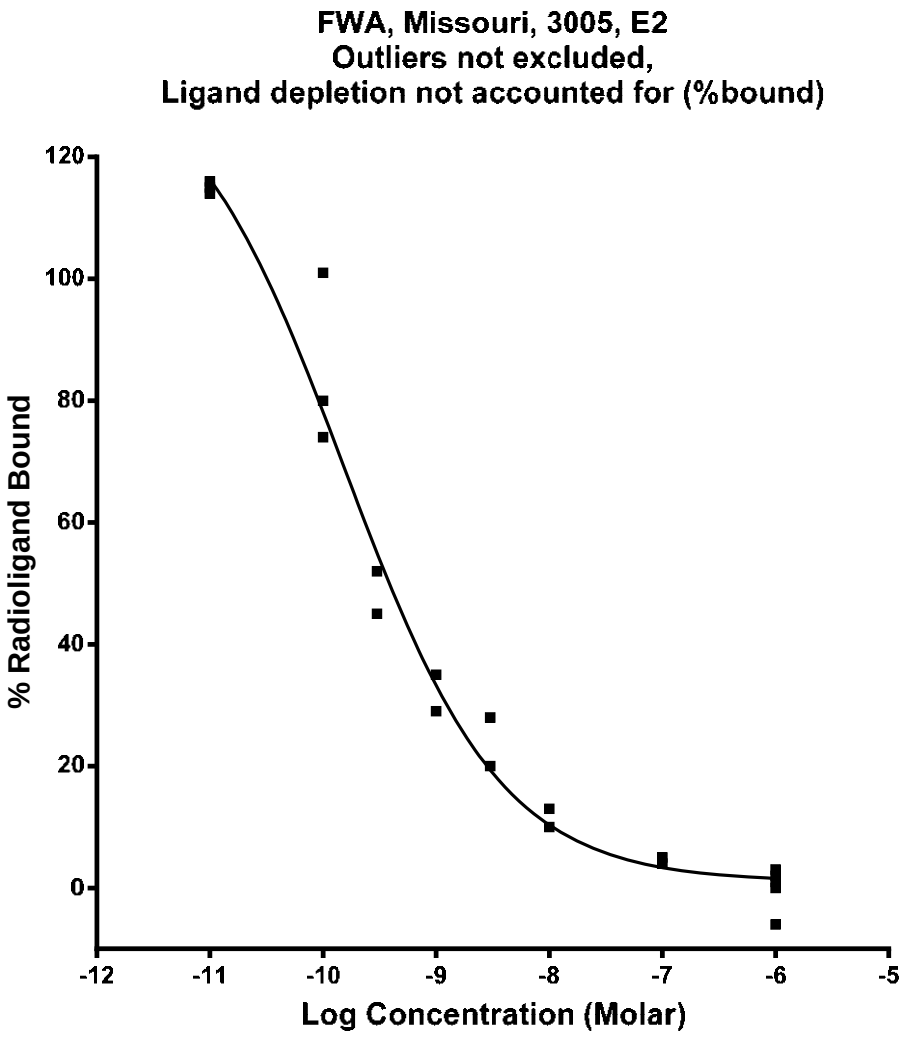
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 3005, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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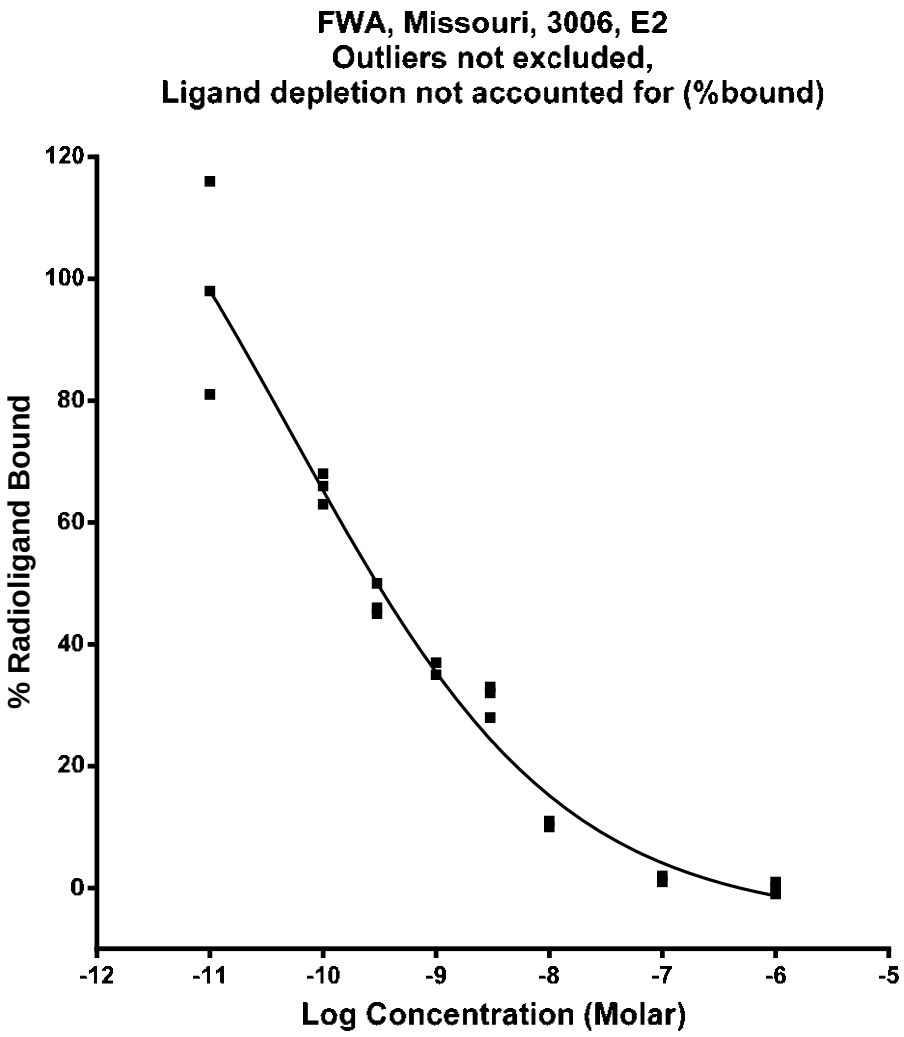
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 3006, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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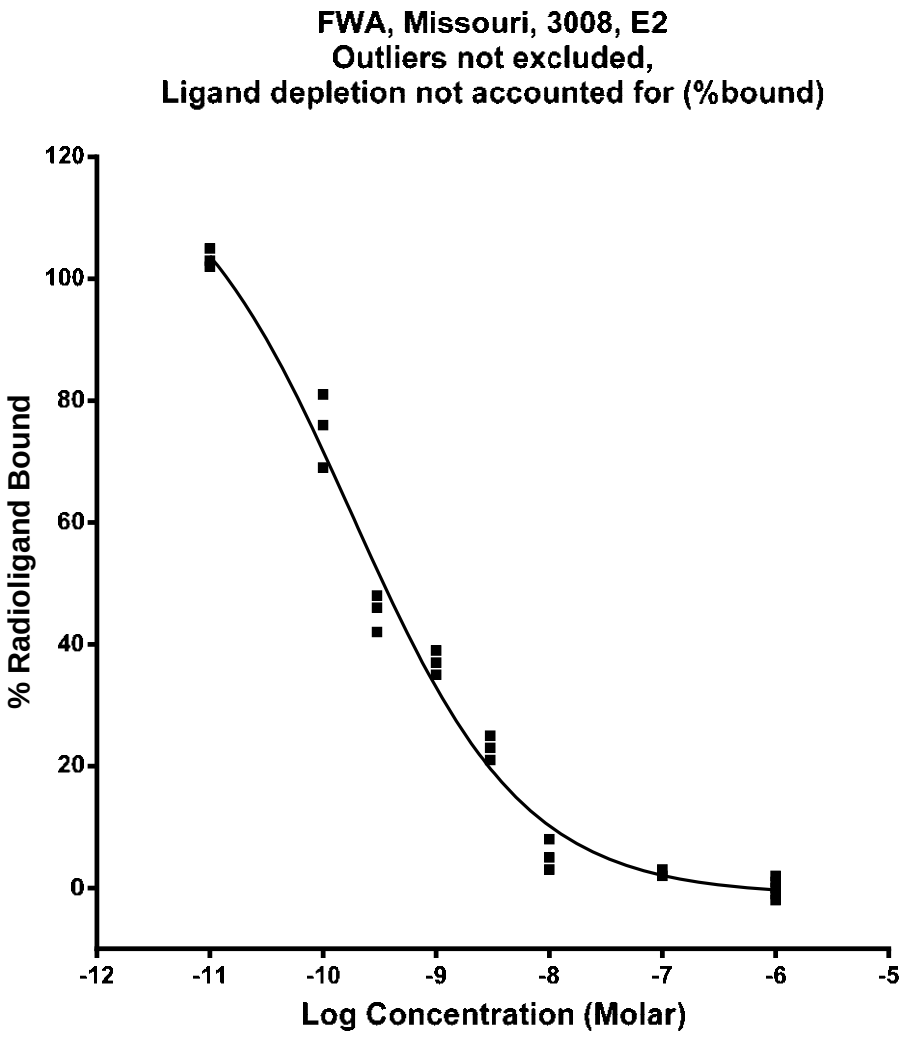
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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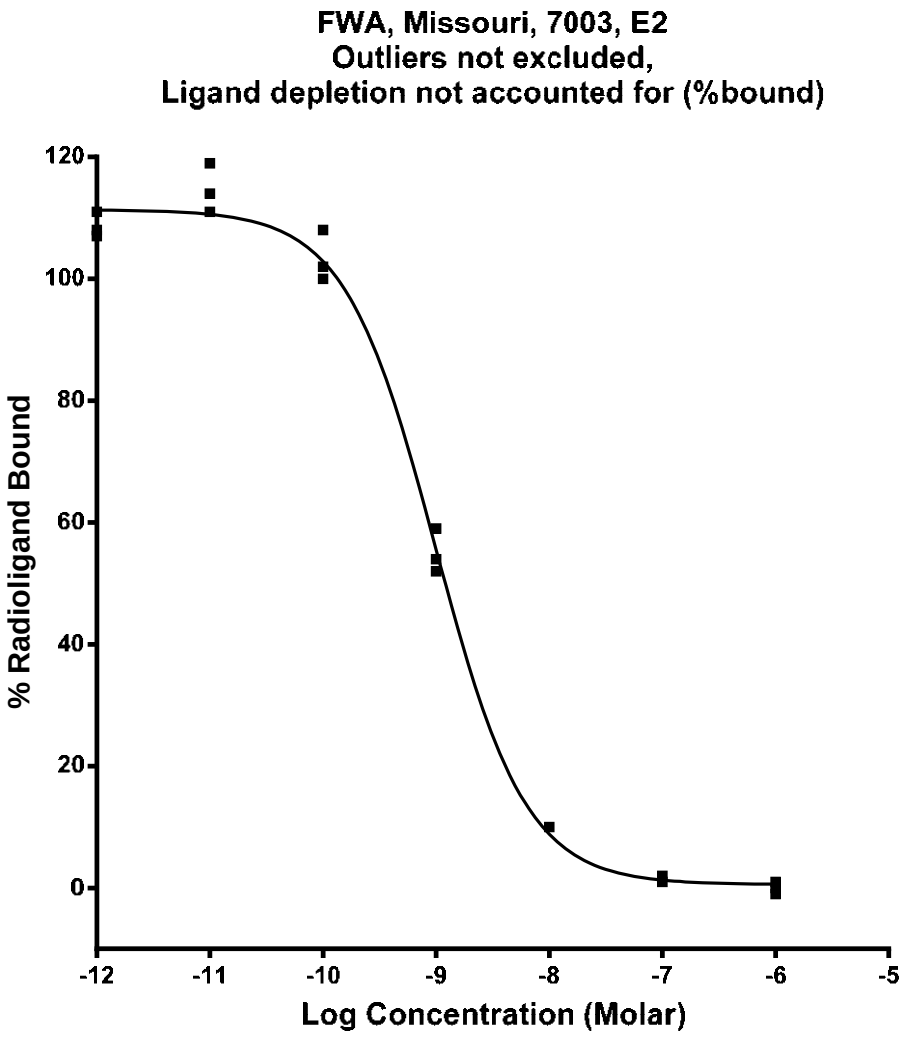
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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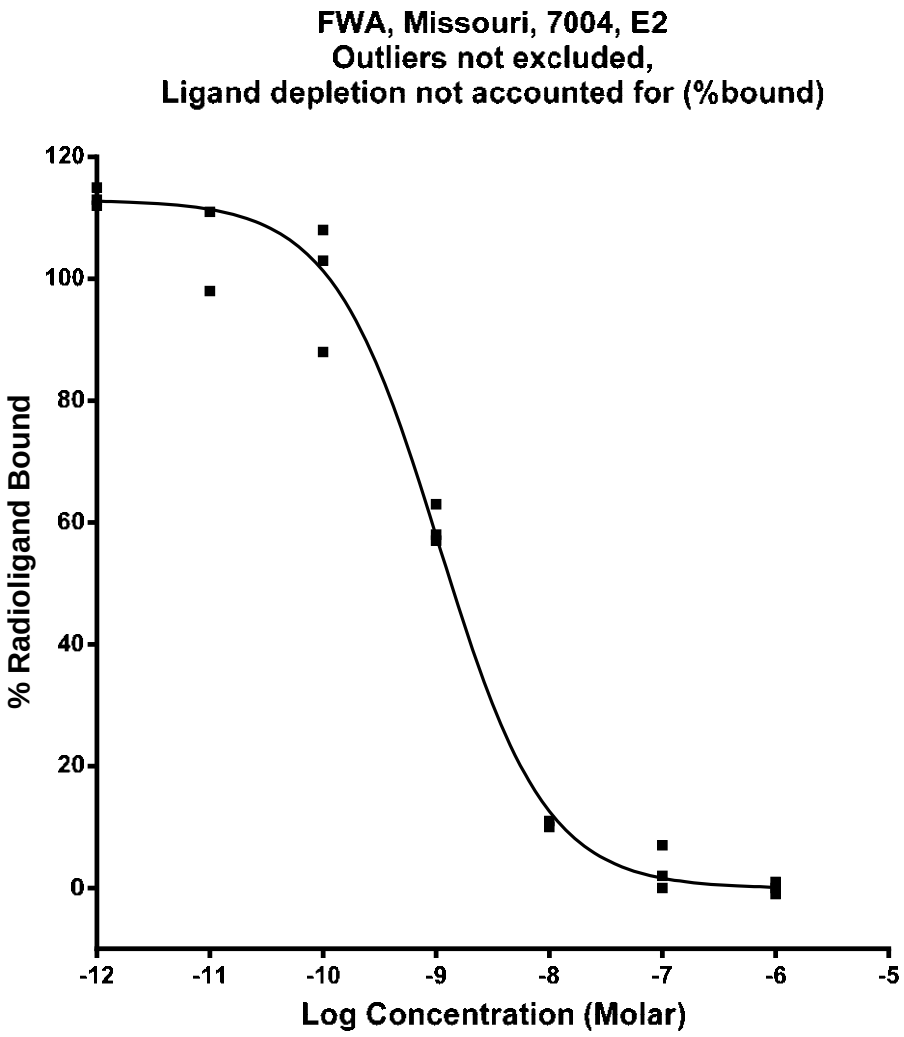
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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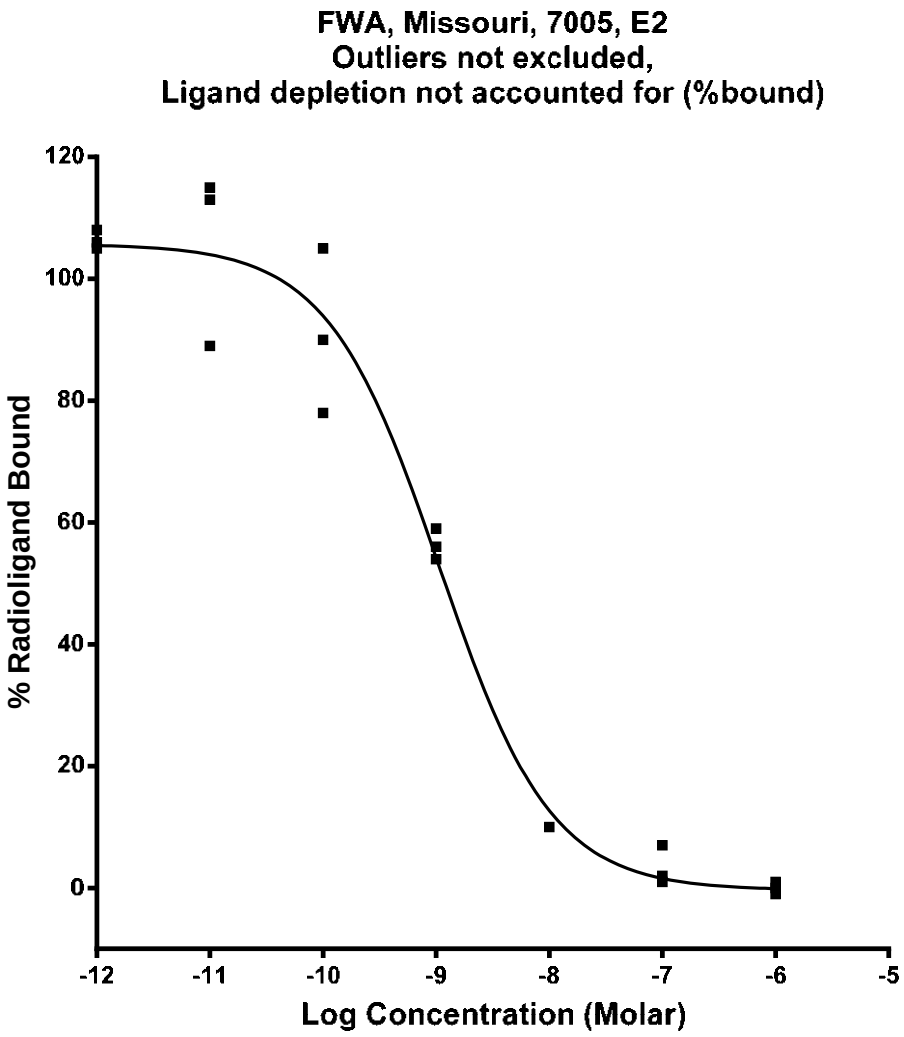
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7005, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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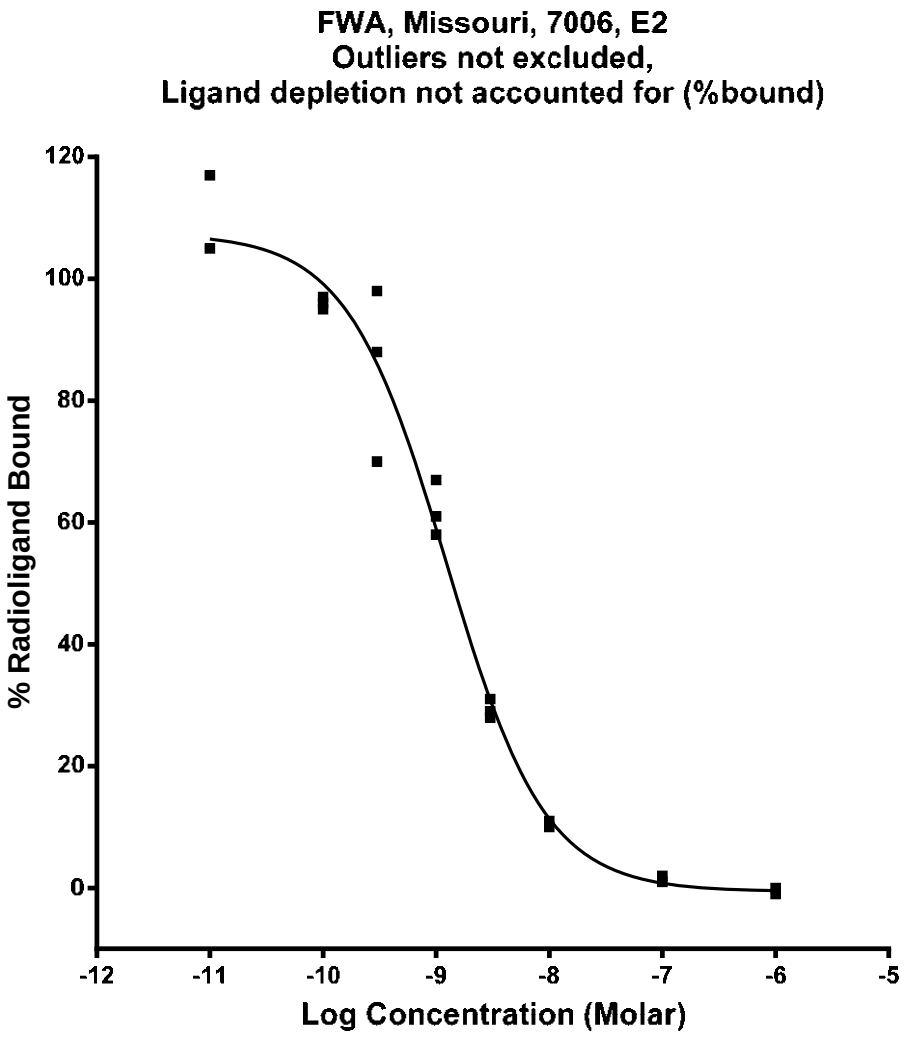
Log Concentration (Molar)

Subtask 1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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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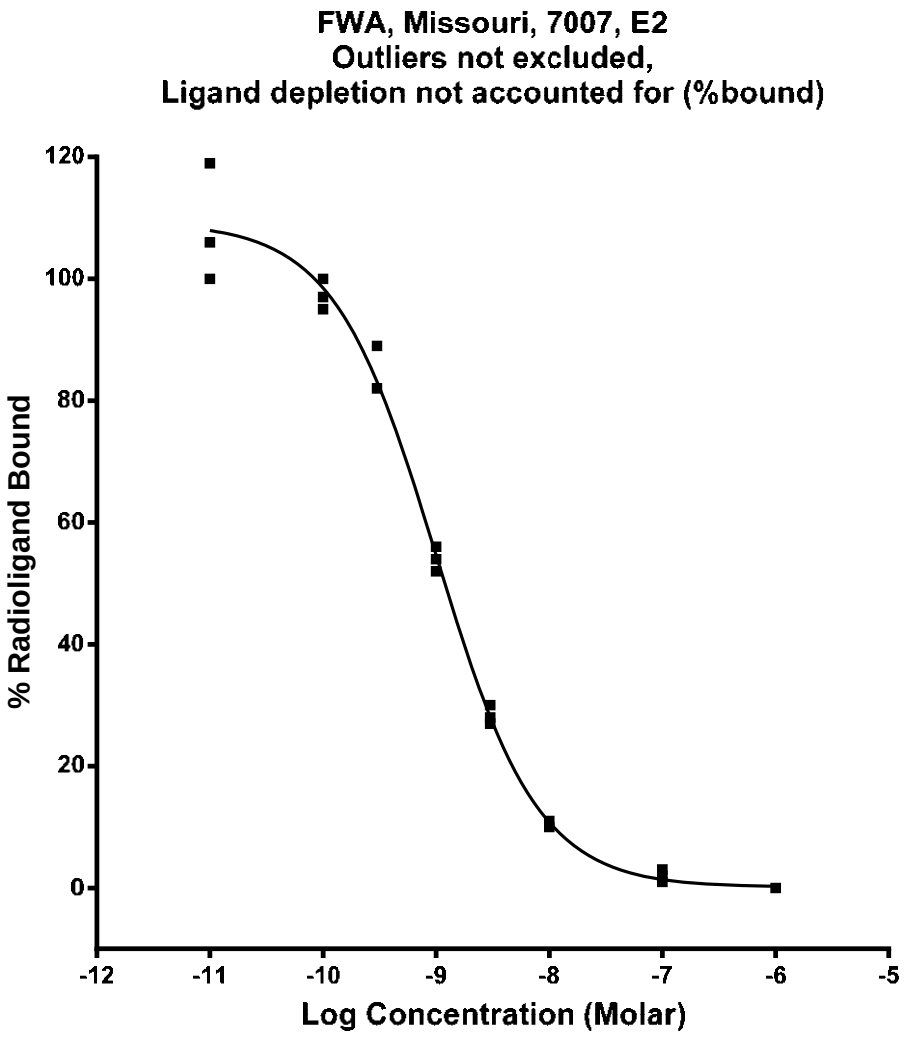
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7007, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
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-11
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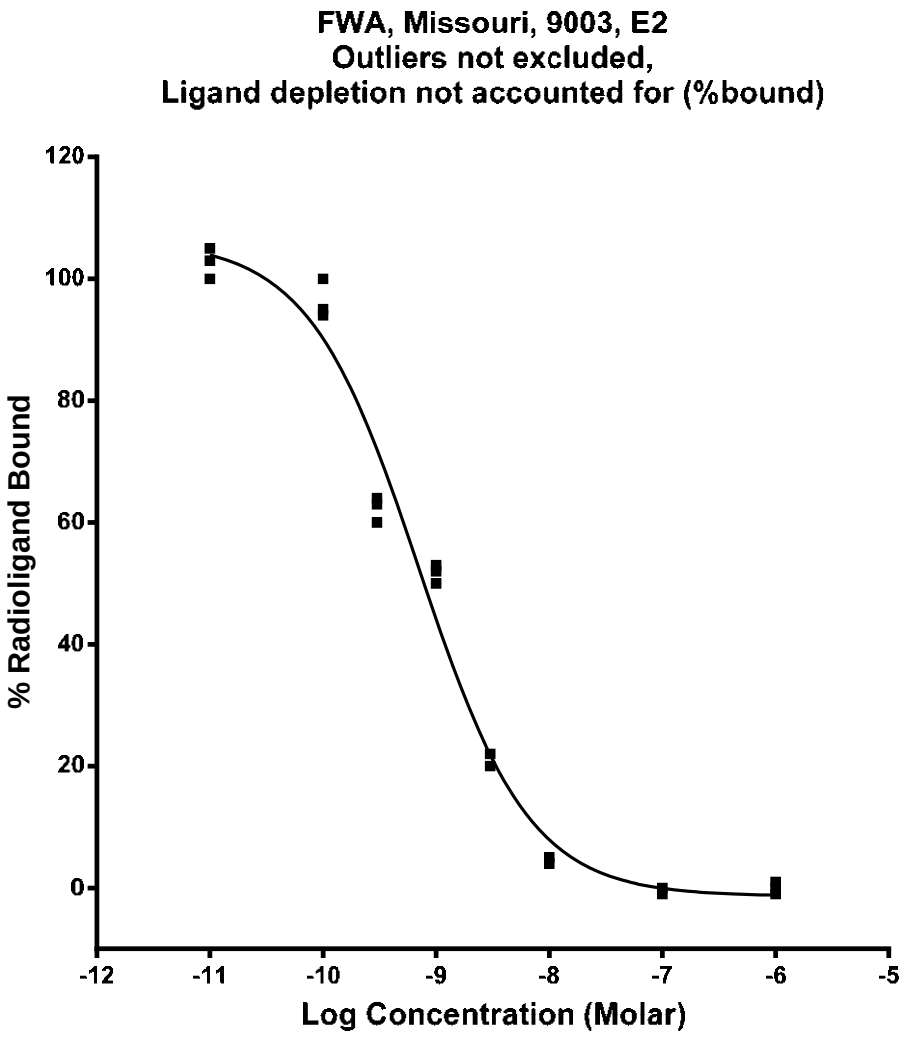
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9003, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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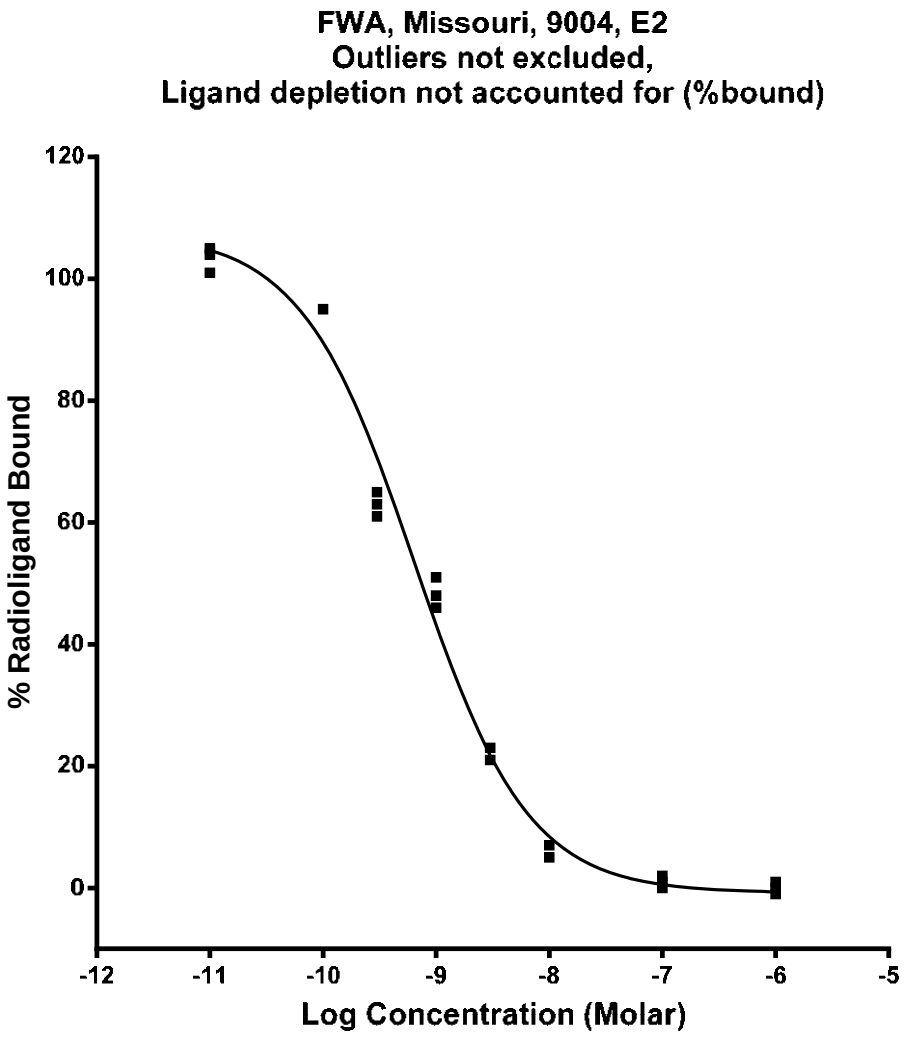
-6
-5
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9004, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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100
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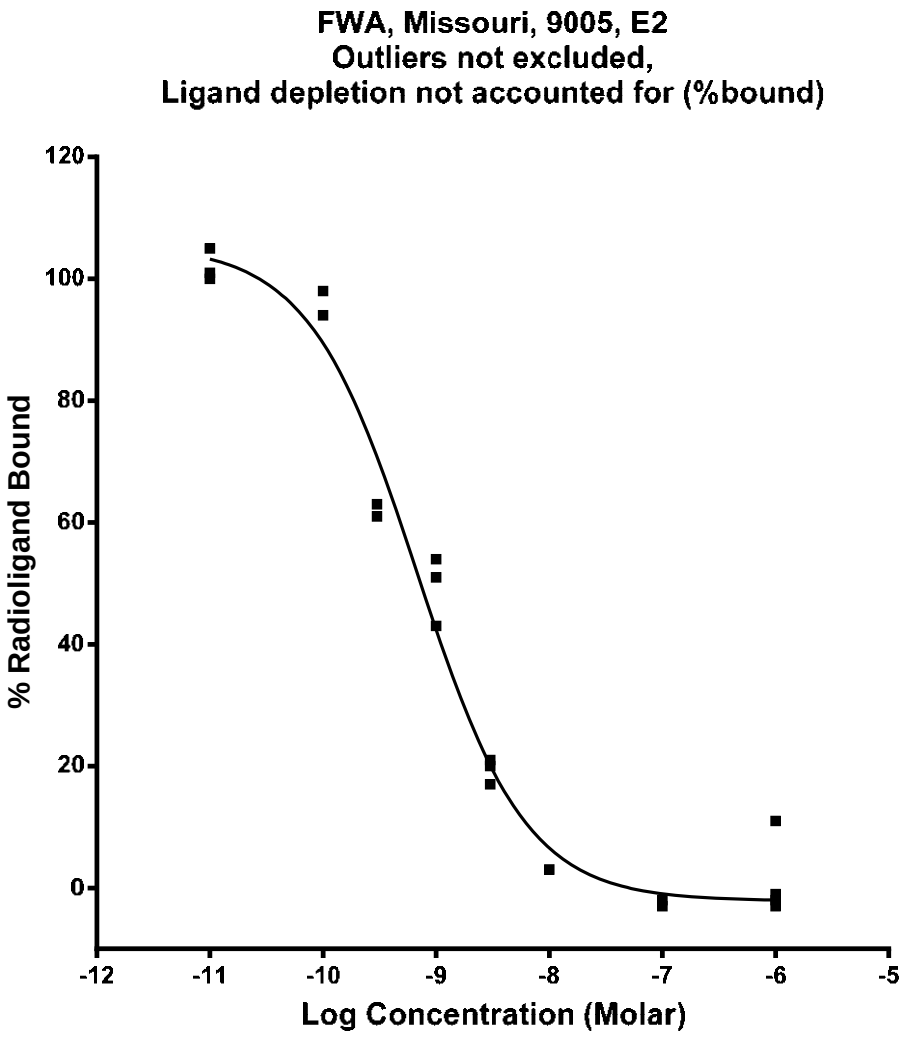
-5
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9005, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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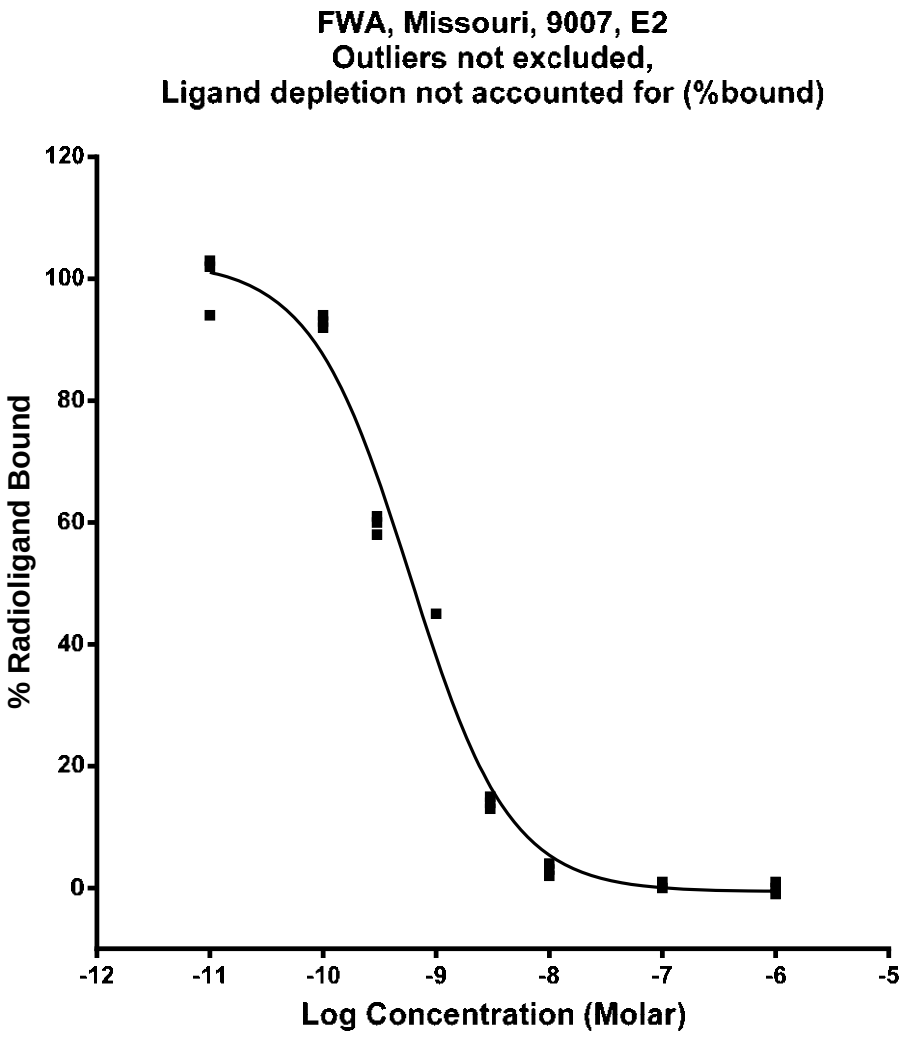
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9007, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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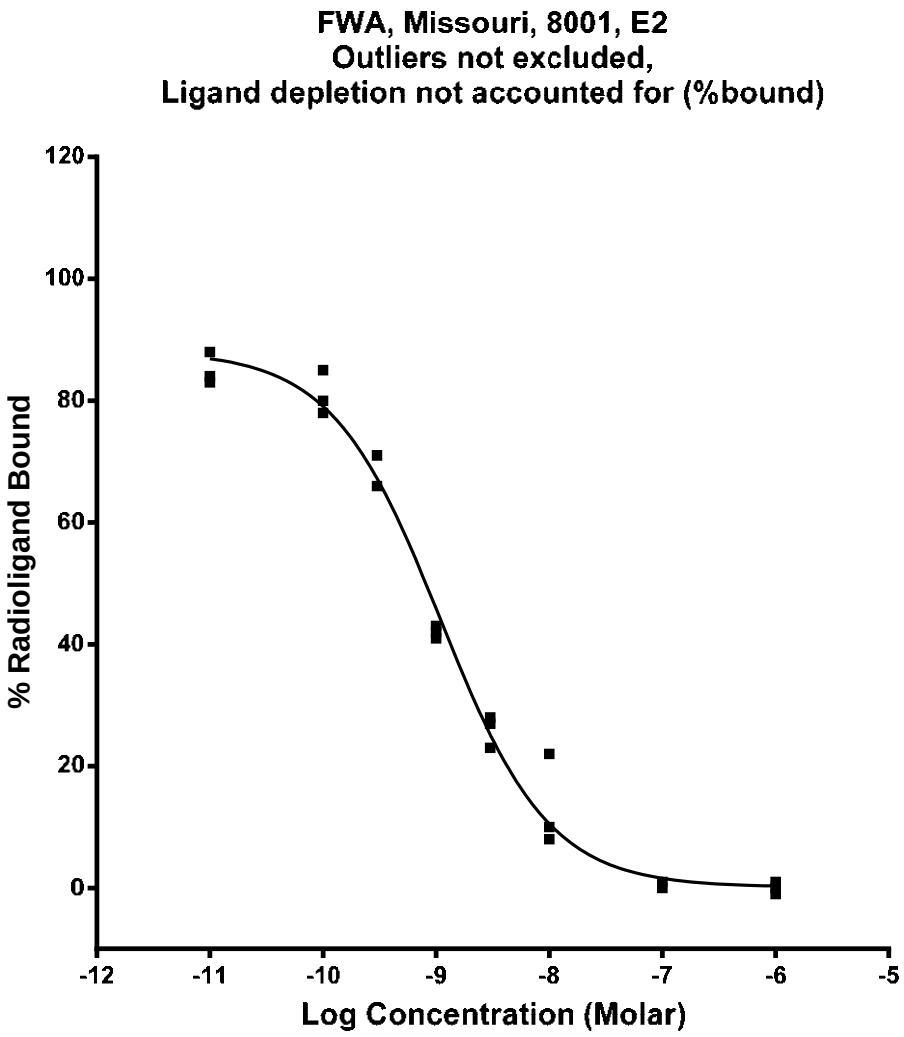
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8001, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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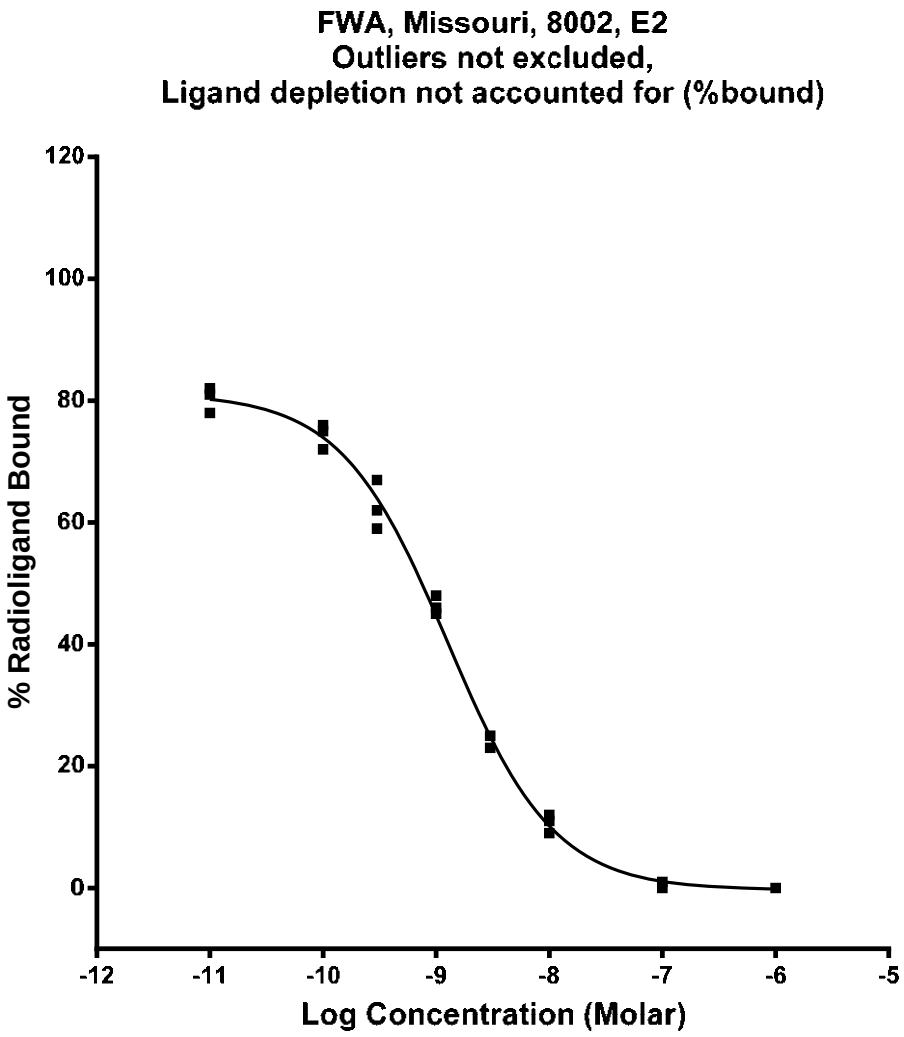
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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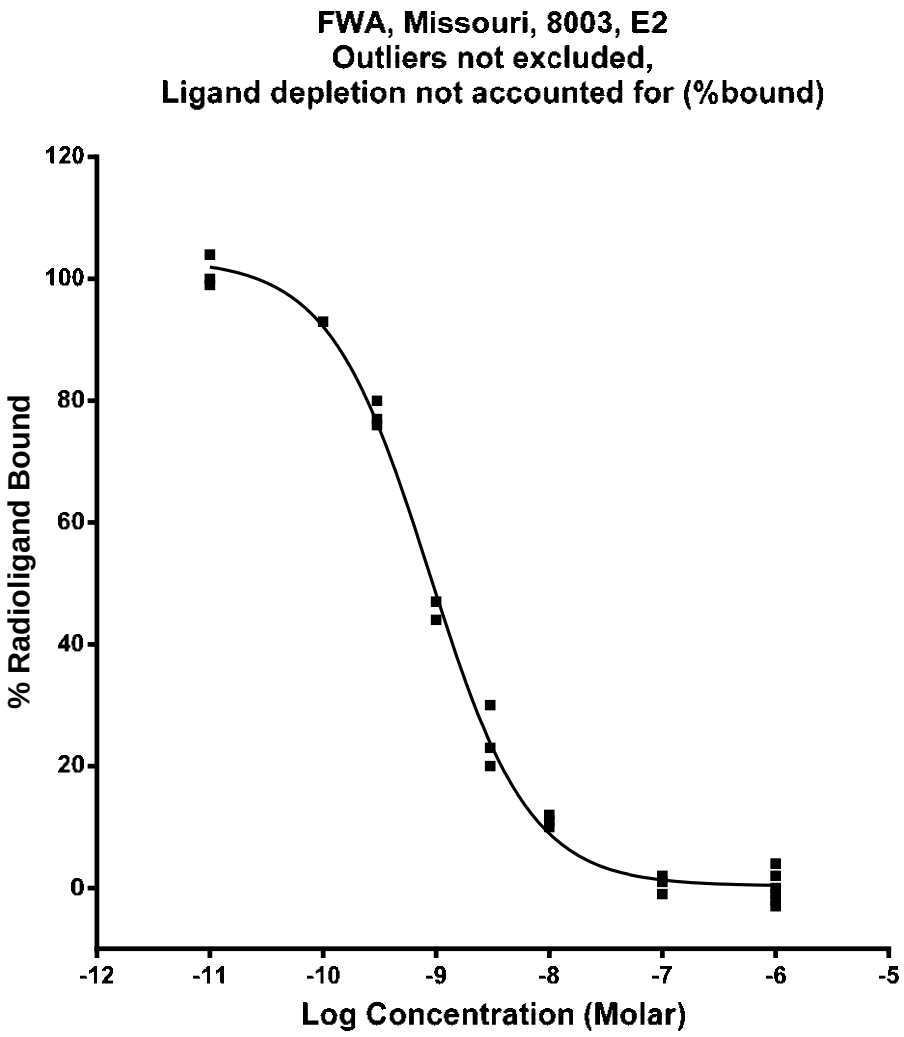
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8003, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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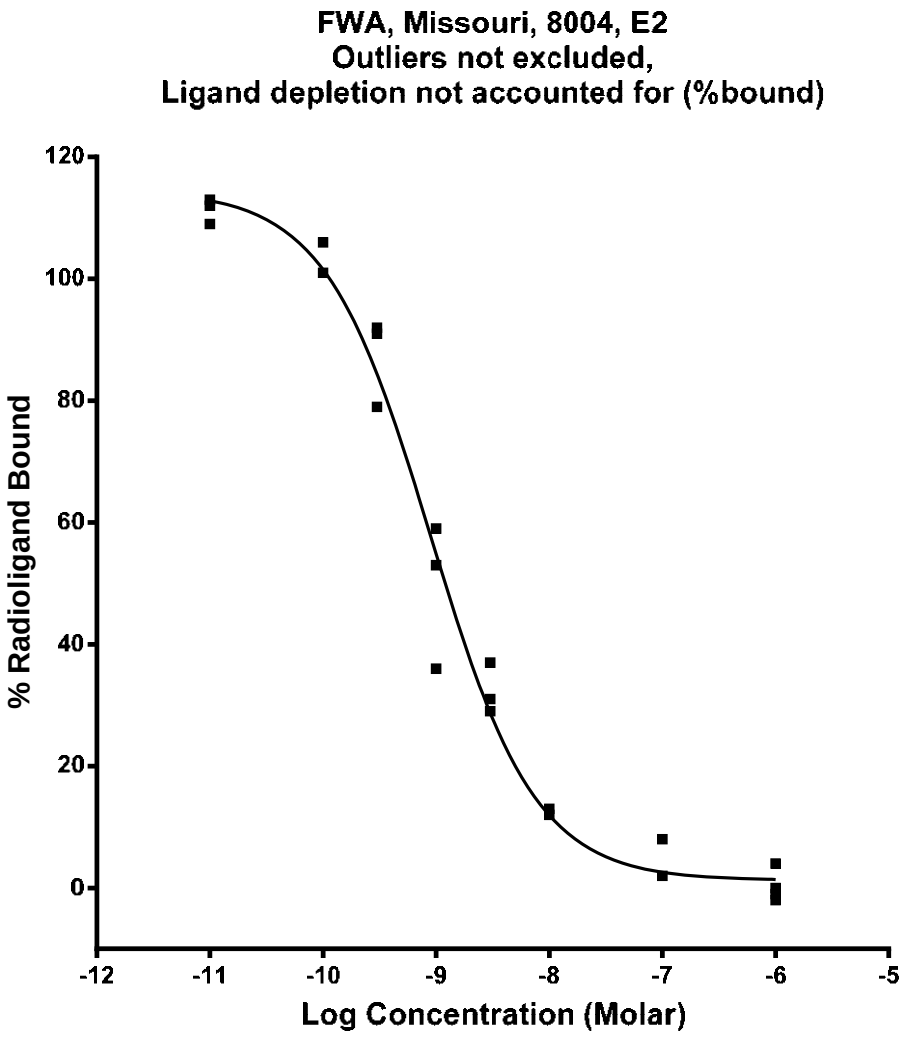
-5
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8004, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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-12
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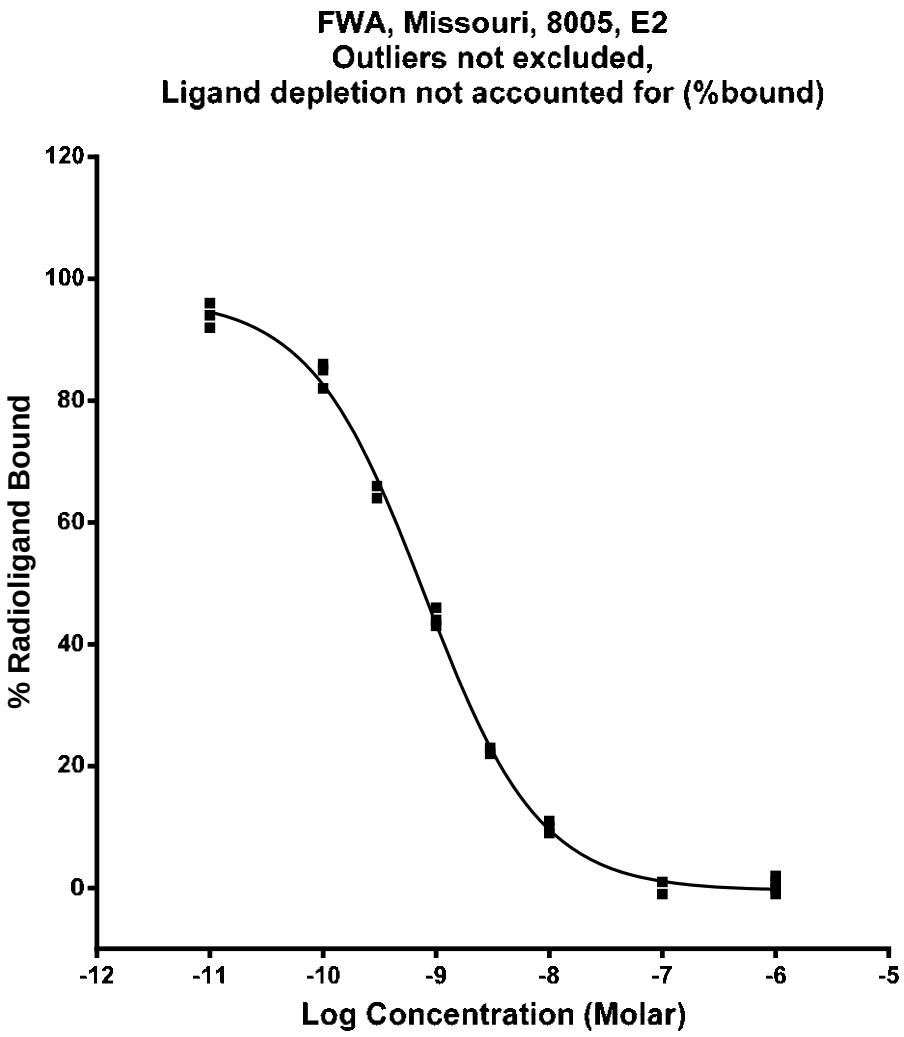
-5
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8005, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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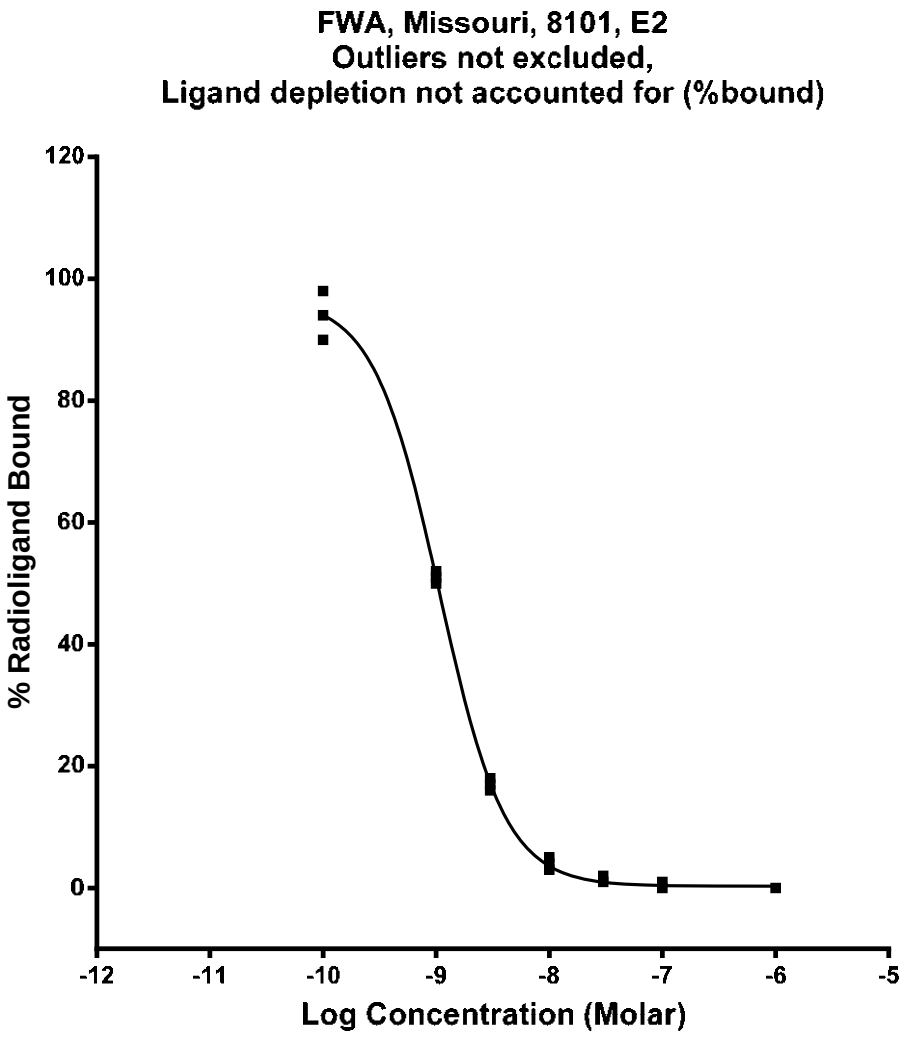
-6
-5
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8101, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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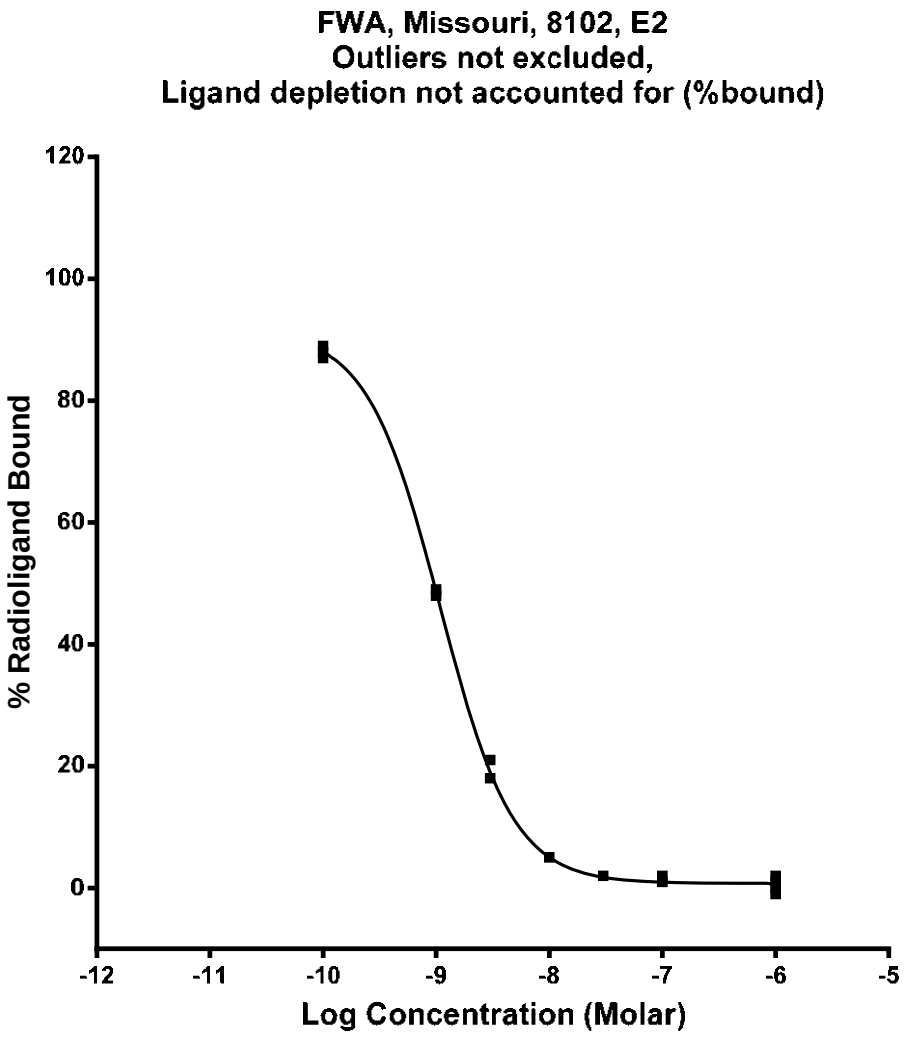
-5
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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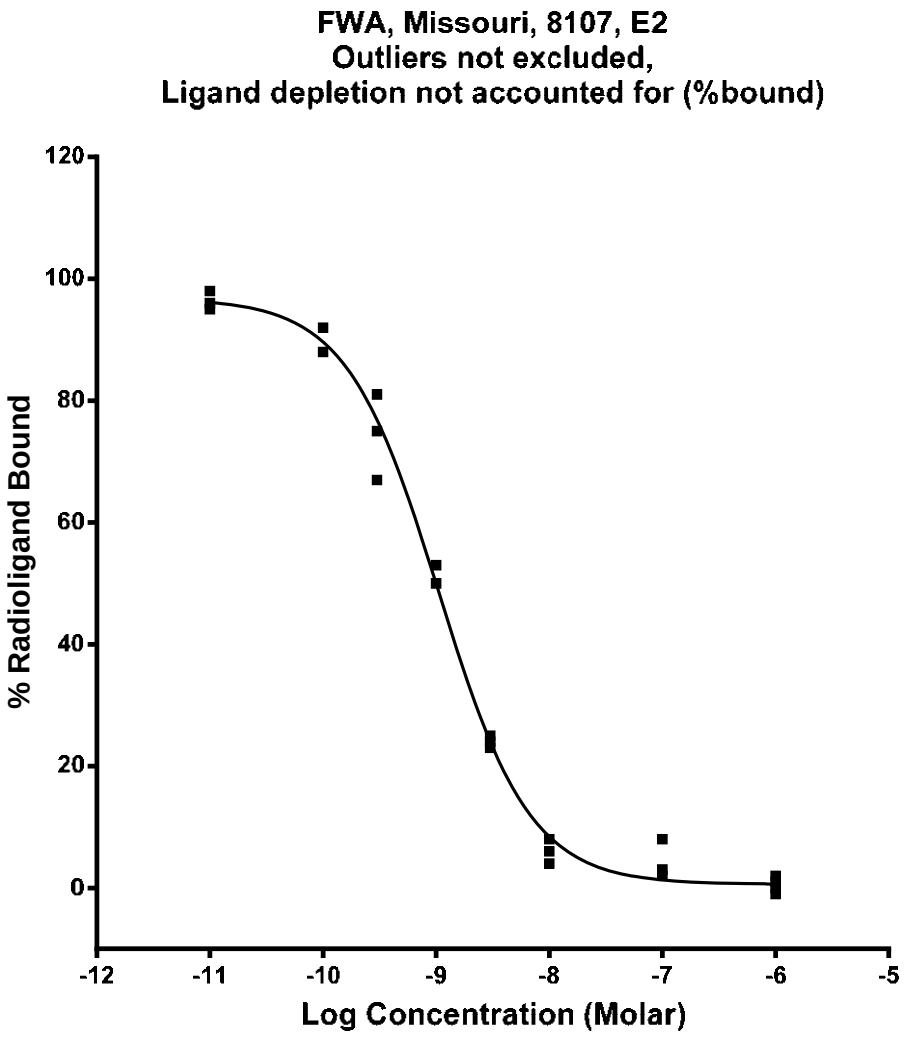
-5
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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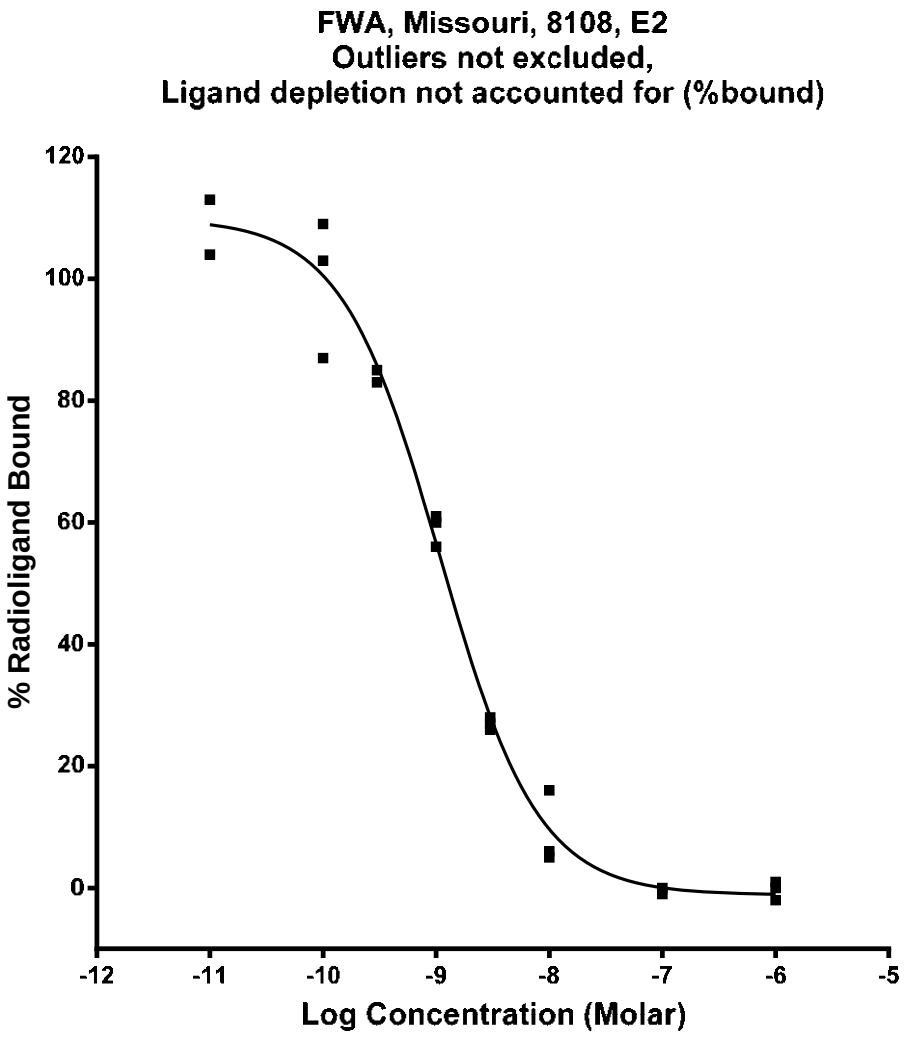
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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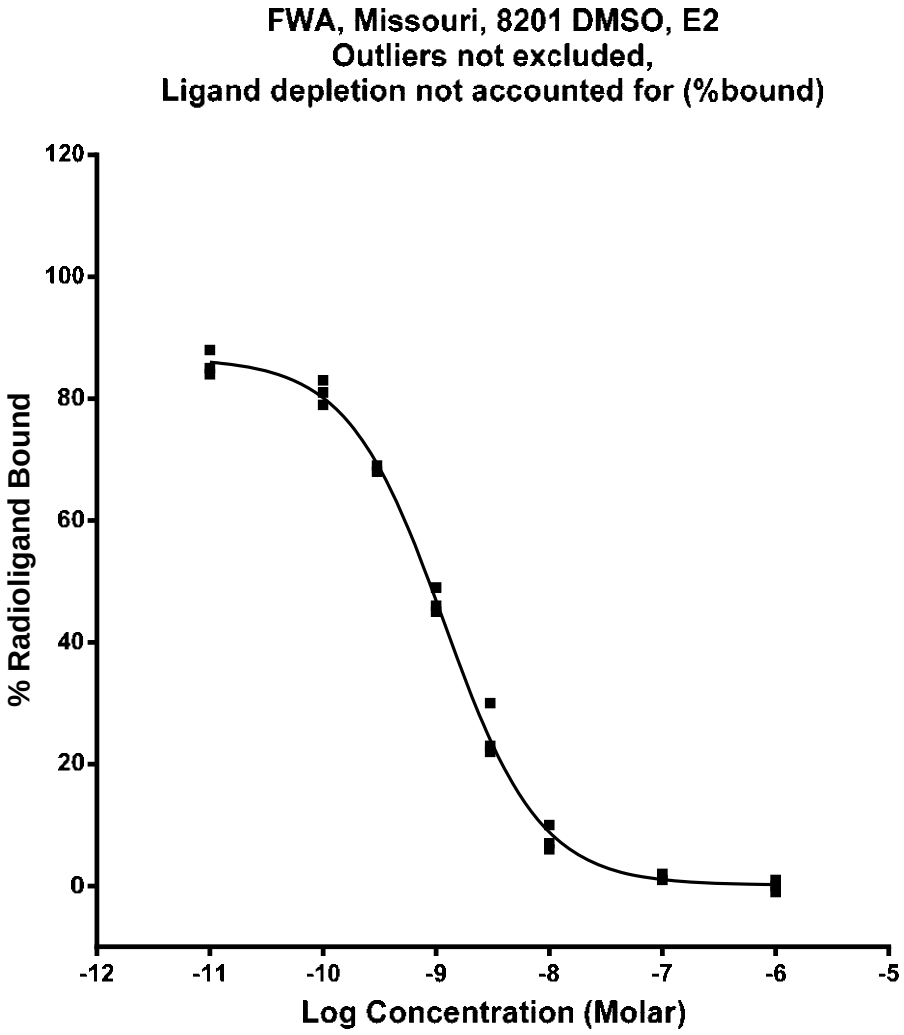
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8201 DMSO, E2
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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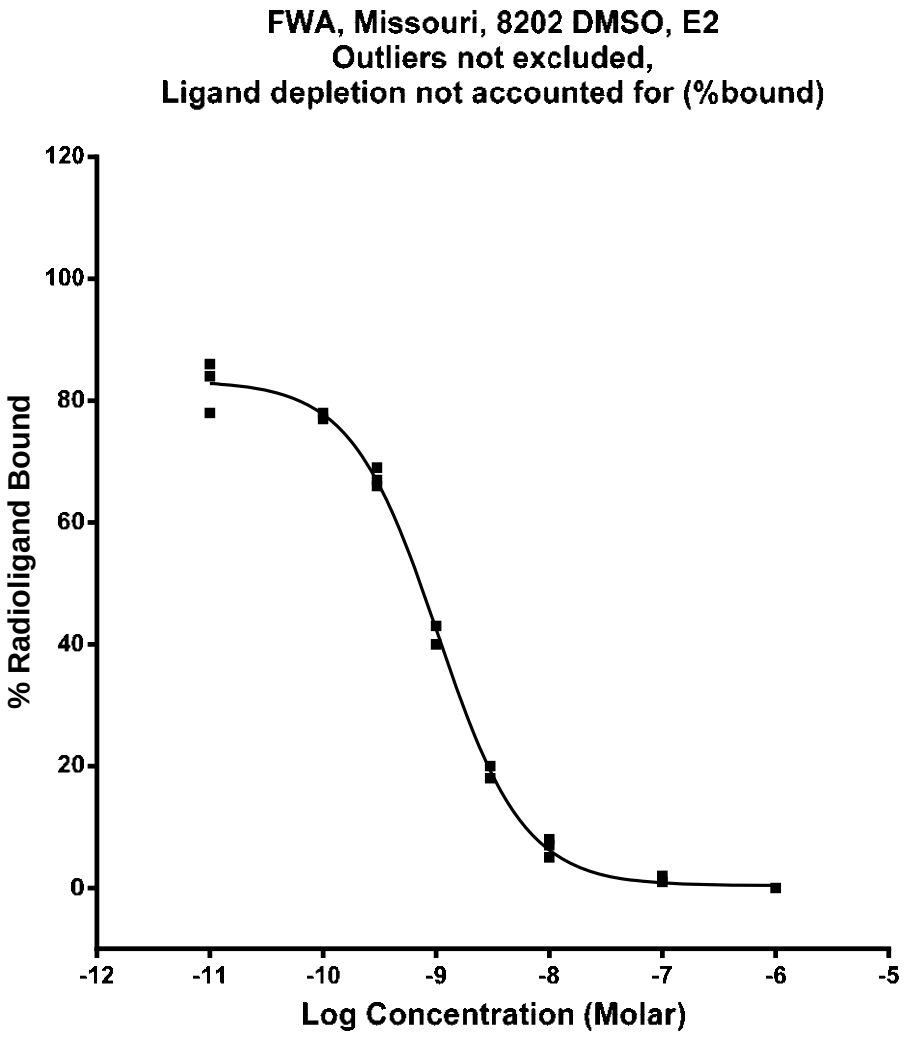
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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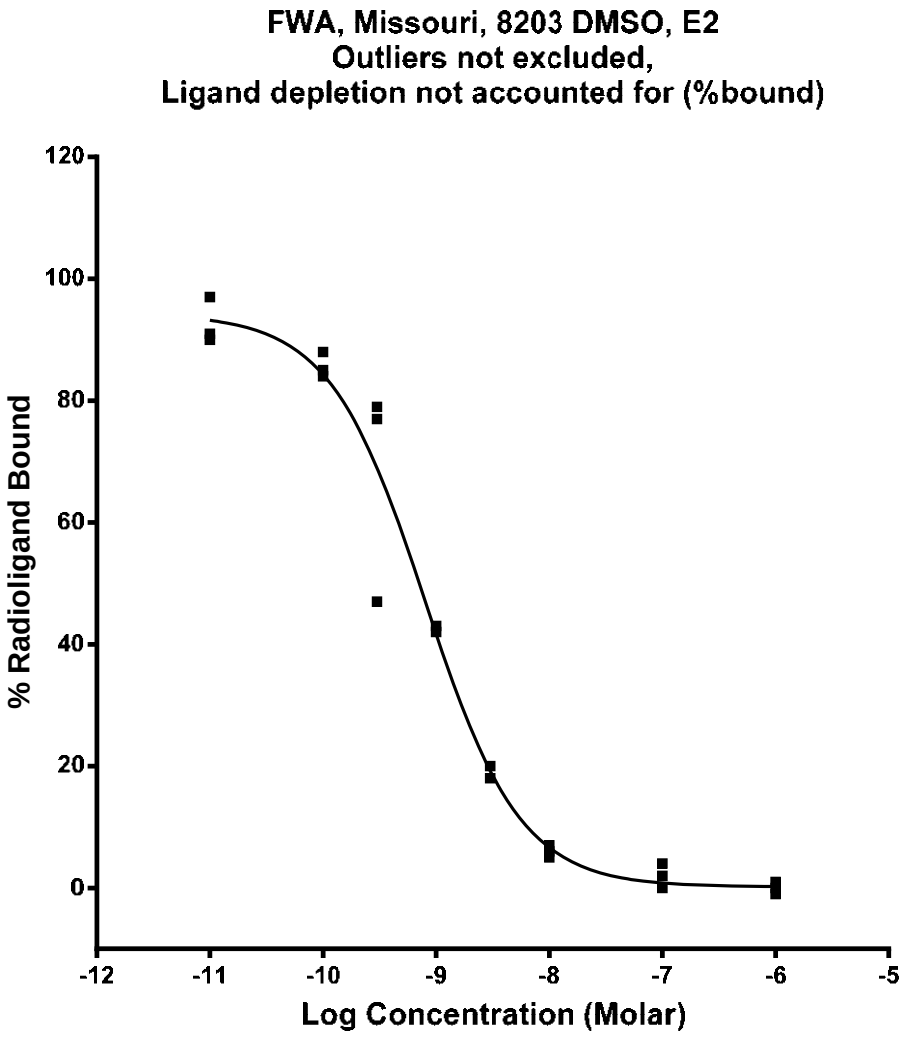
Log Concentration (Molar)

Subtask 3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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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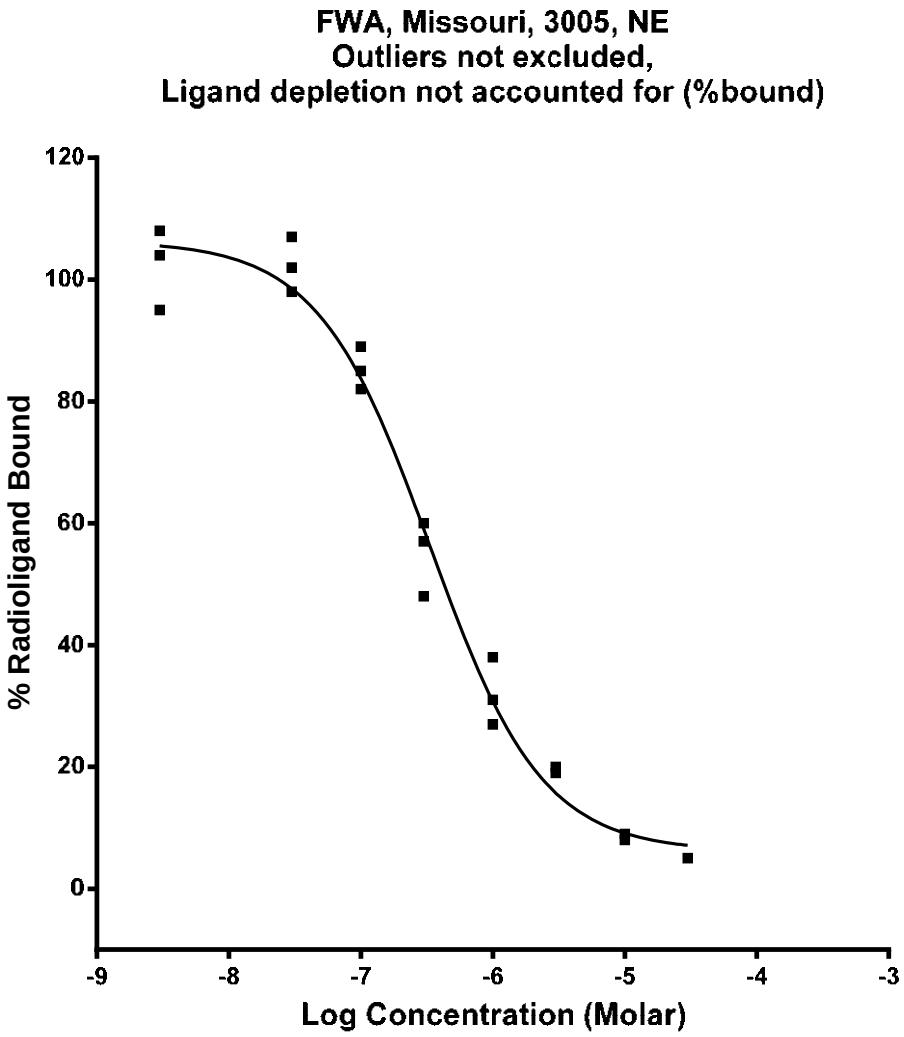
-6
-5
Log Concentration (Molar)

FW Assay, (Missouri Lab) Control Norethynodrel (NE) 30 Nov 2014

Begin Here

Subtask 1


FWA, Missouri, 3005, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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0
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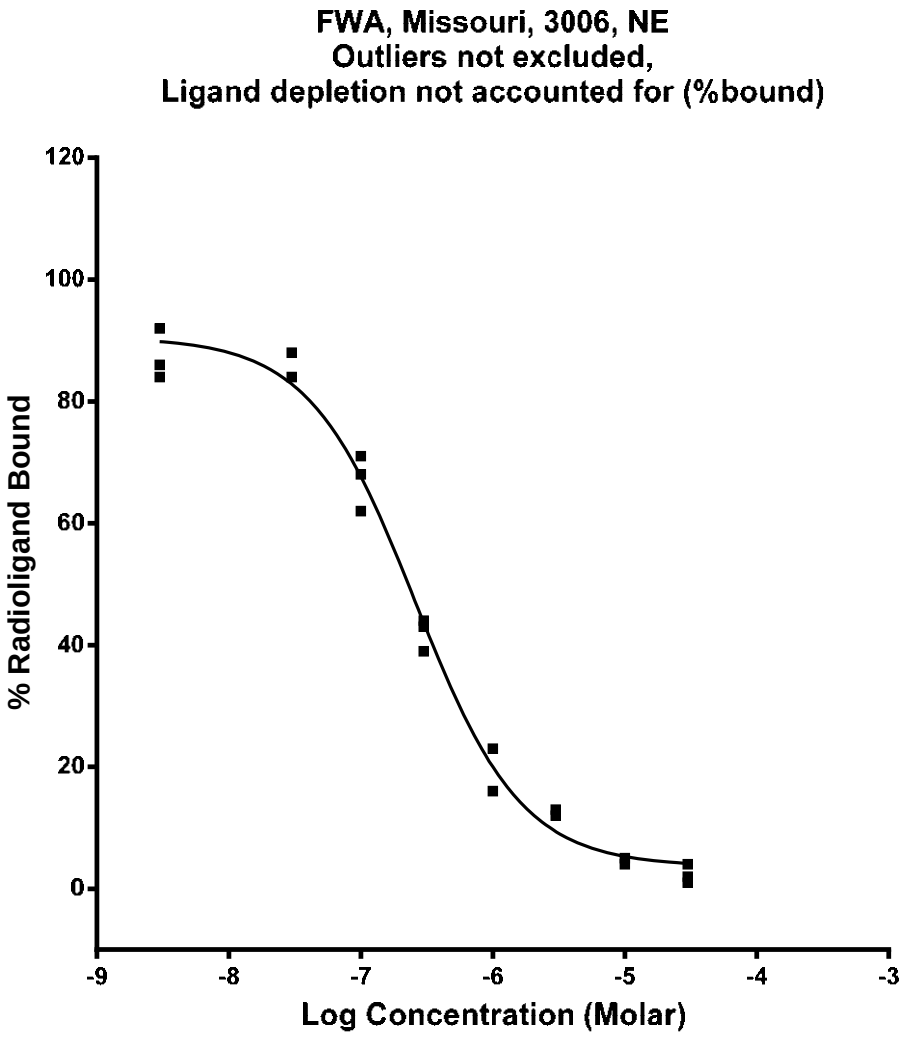
-4
-3
Log Concentration (Molar)

Subtask 1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Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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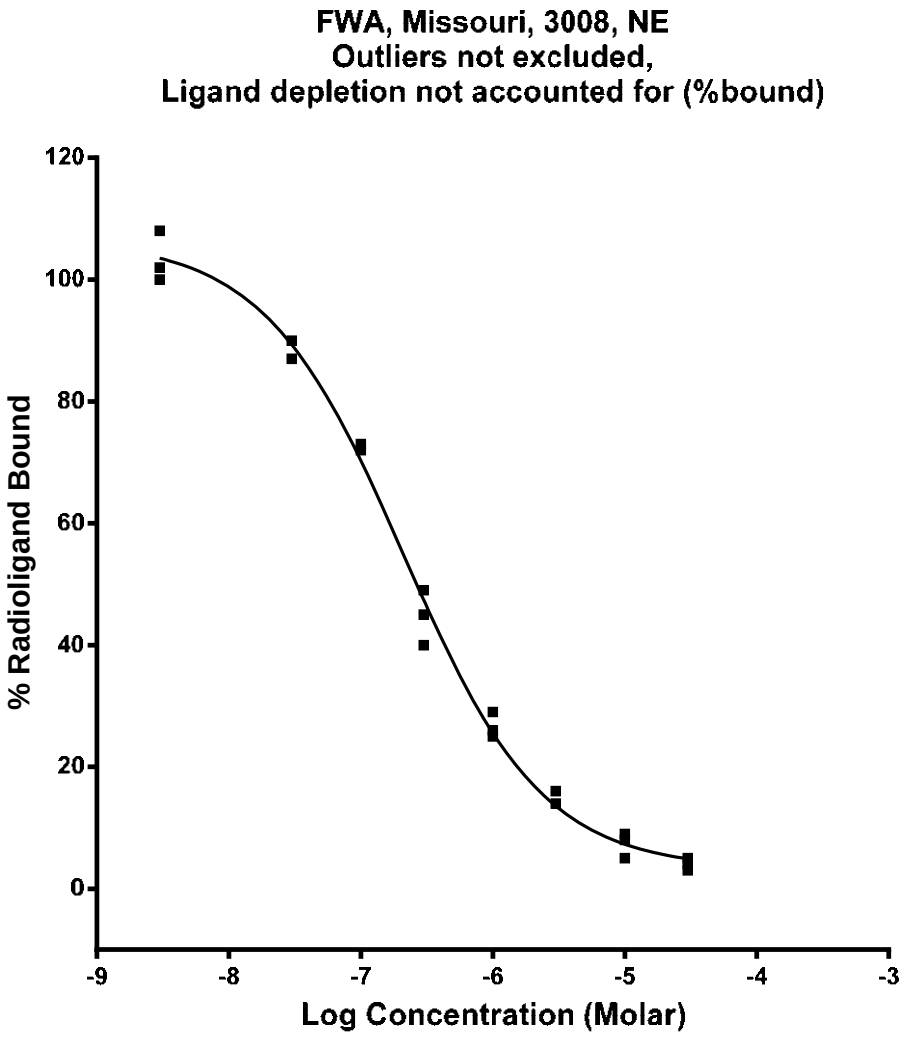
-5
-4
-3
Log Concentration (Molar)

Subtask 1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% Radioligand Bound
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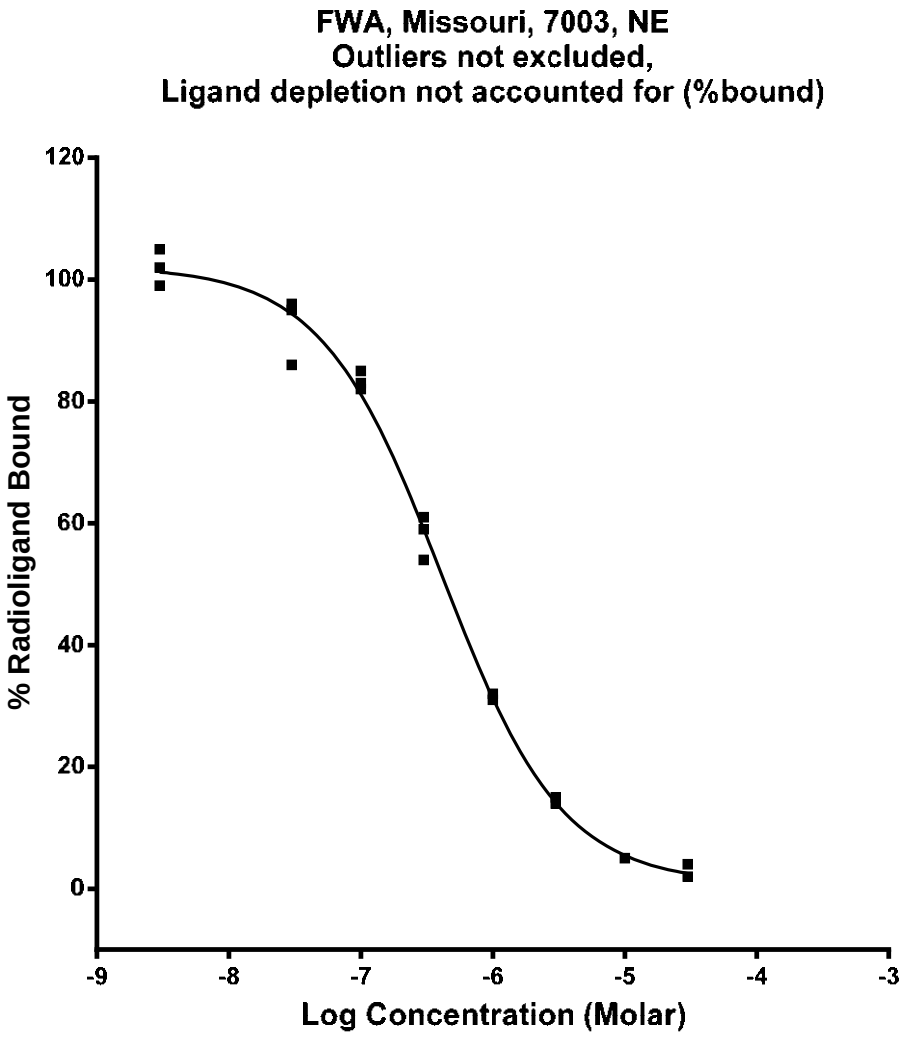
-3
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7003, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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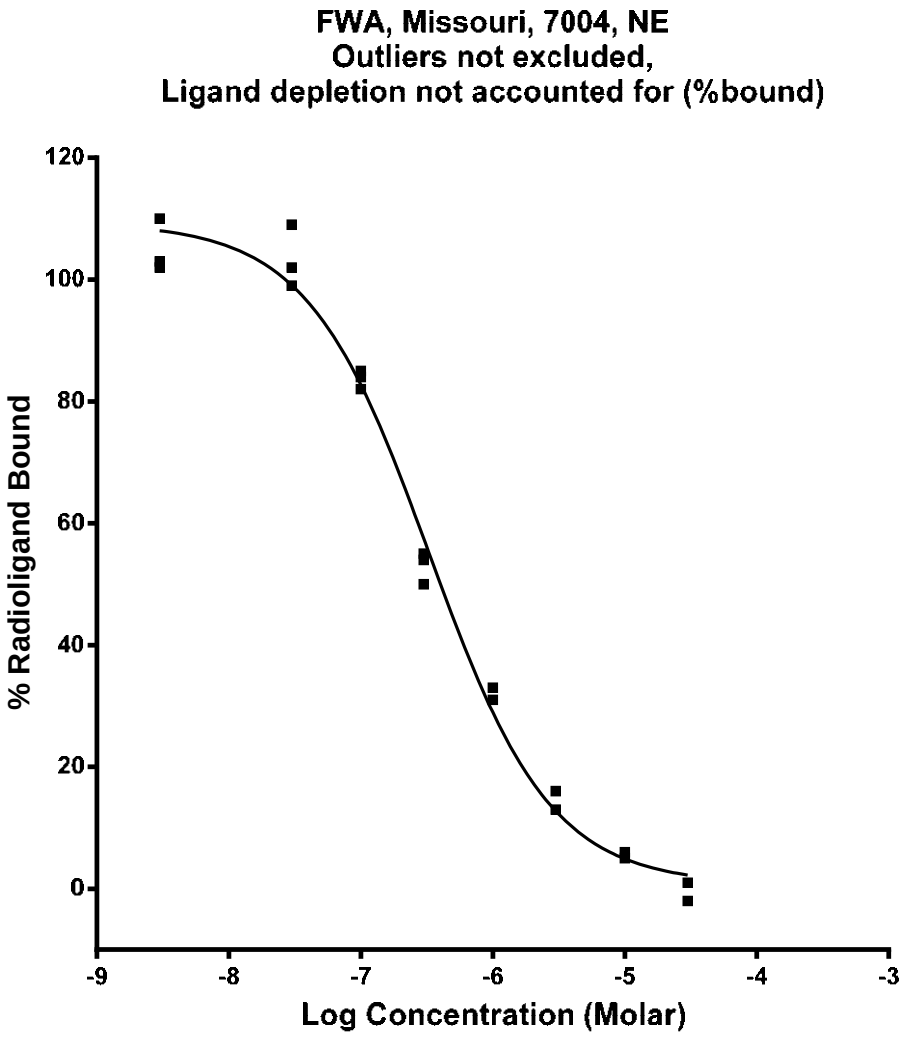
-4
-3
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7004, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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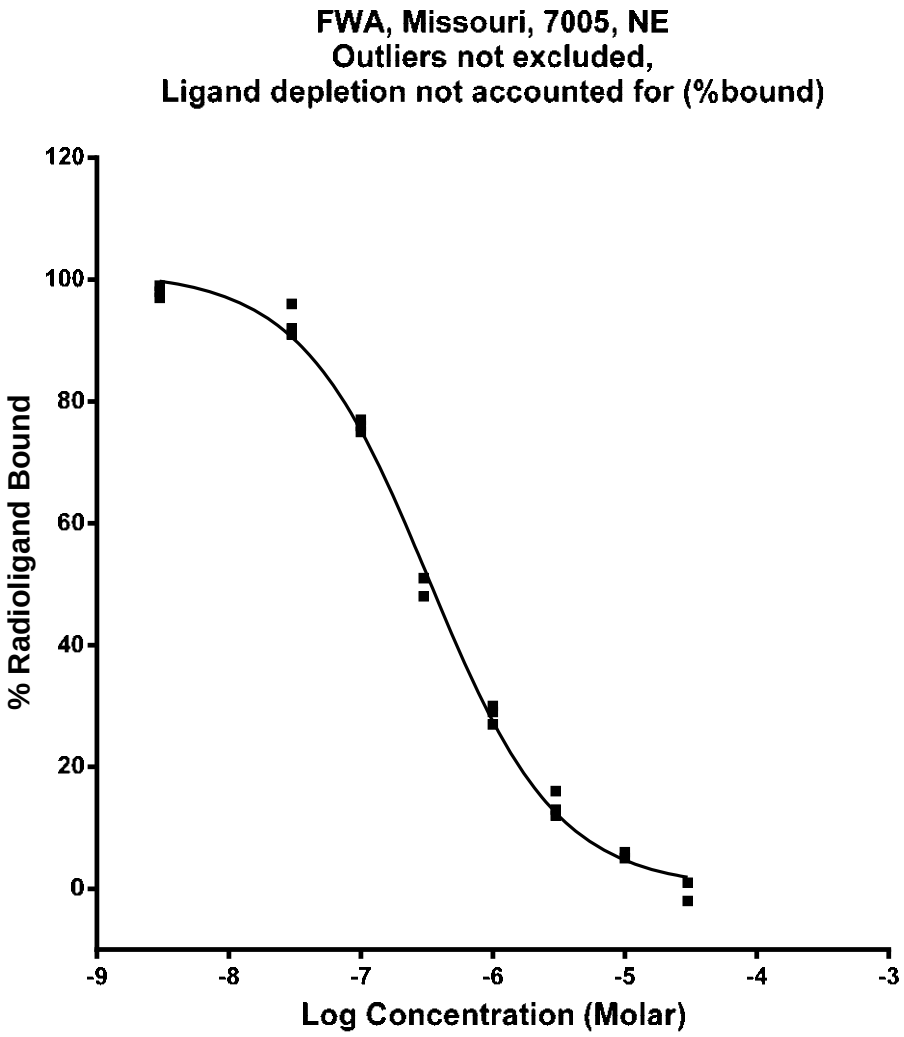
-3
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7005, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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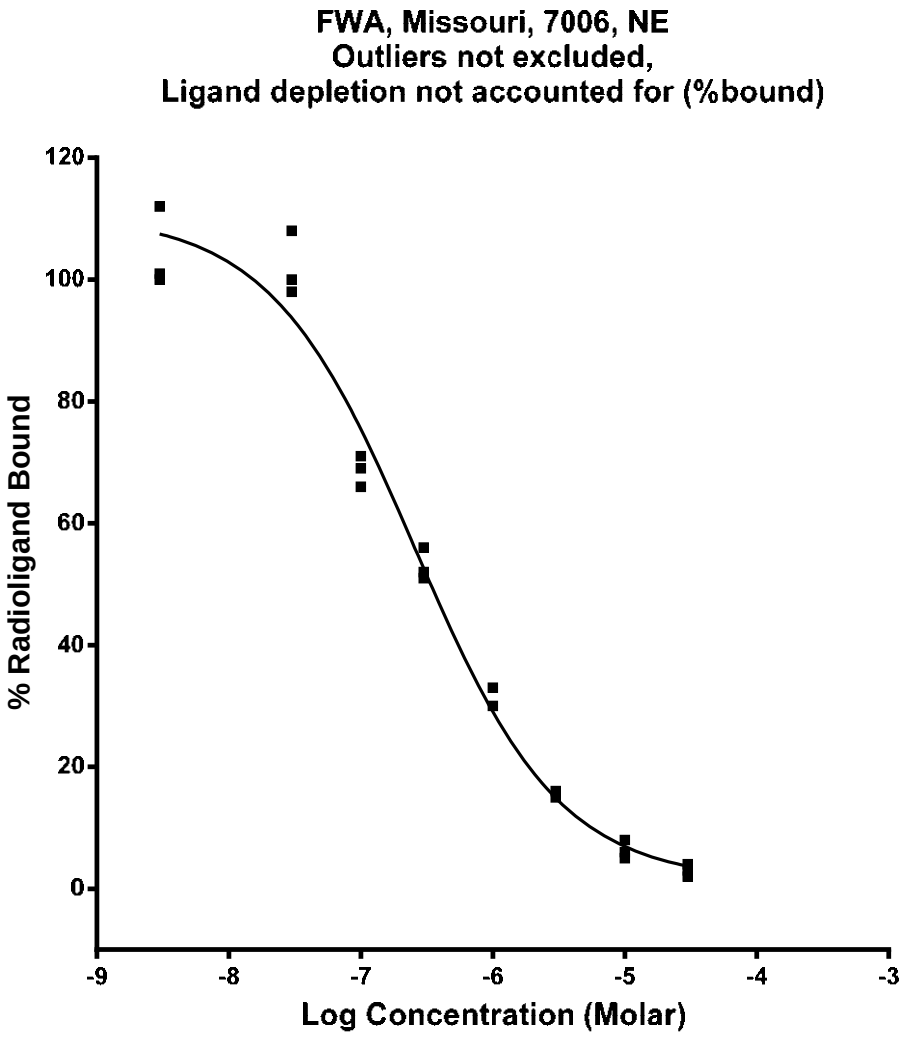
-3
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7006, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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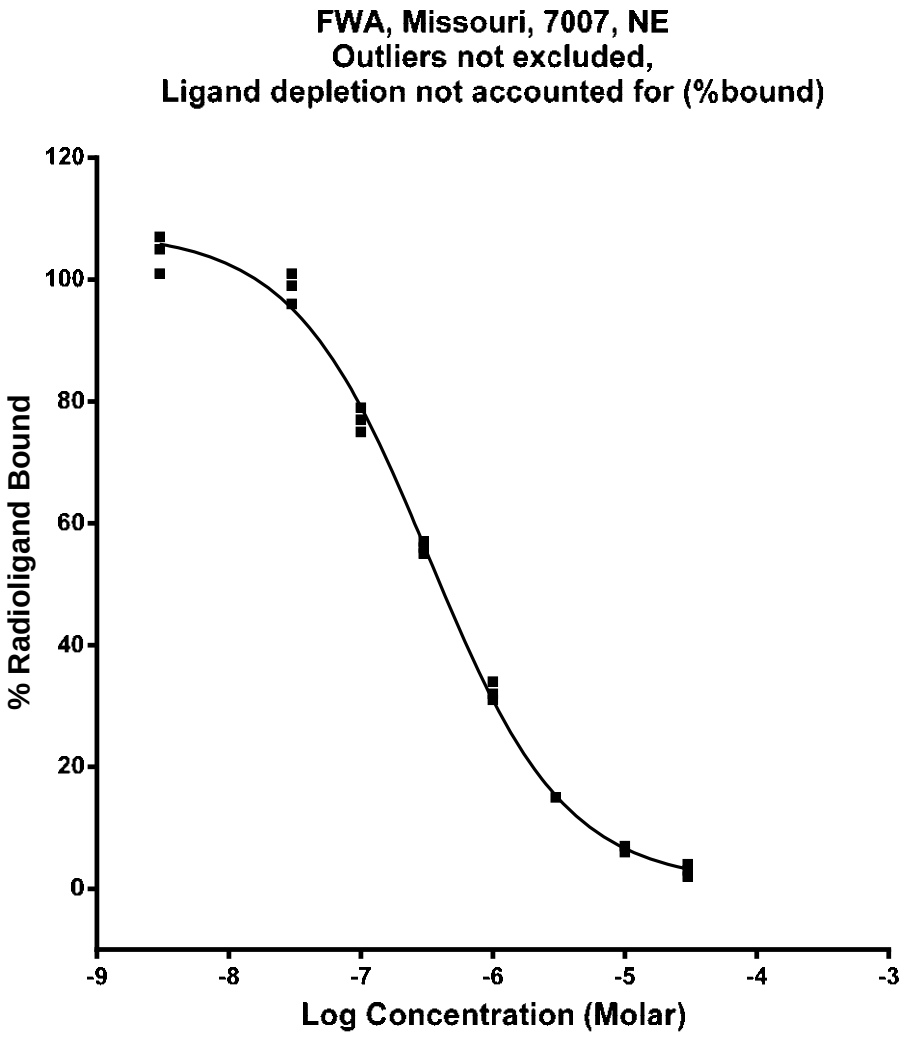
Log Concentration (Molar)

Subtask 1


FWA, Missouri, 7007, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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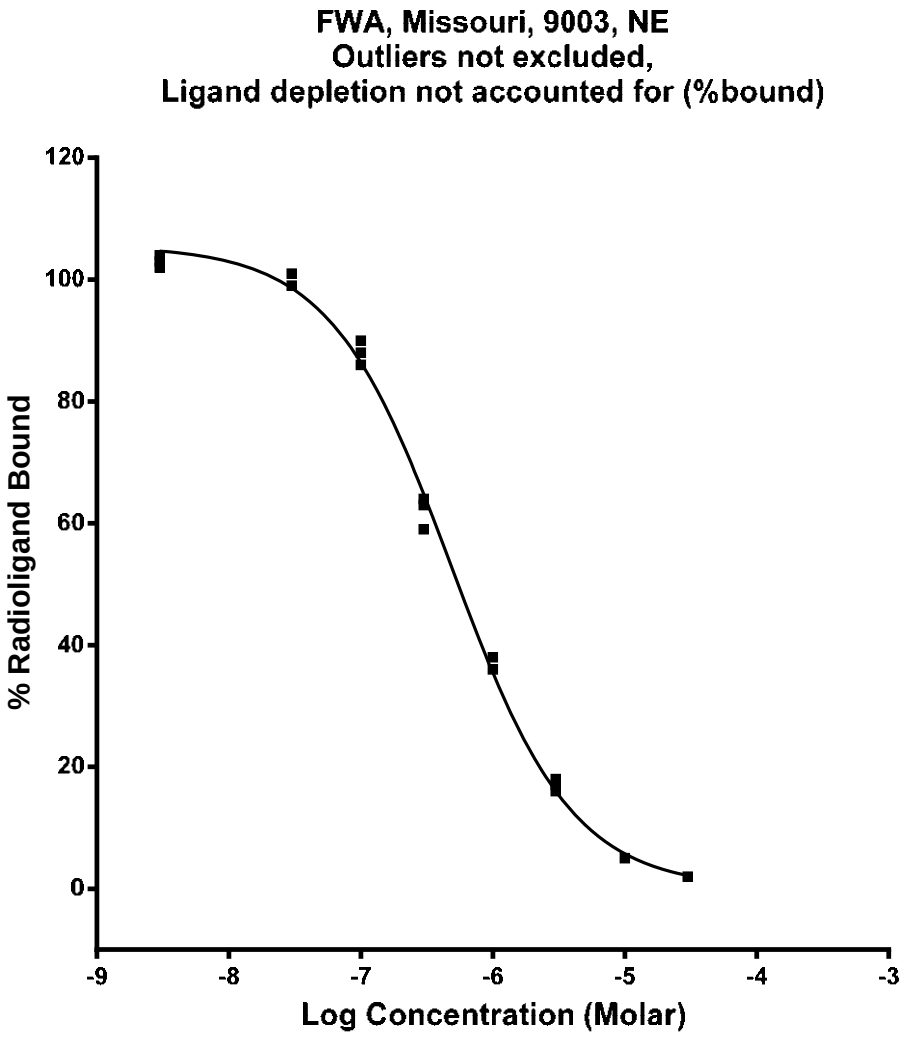
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9003, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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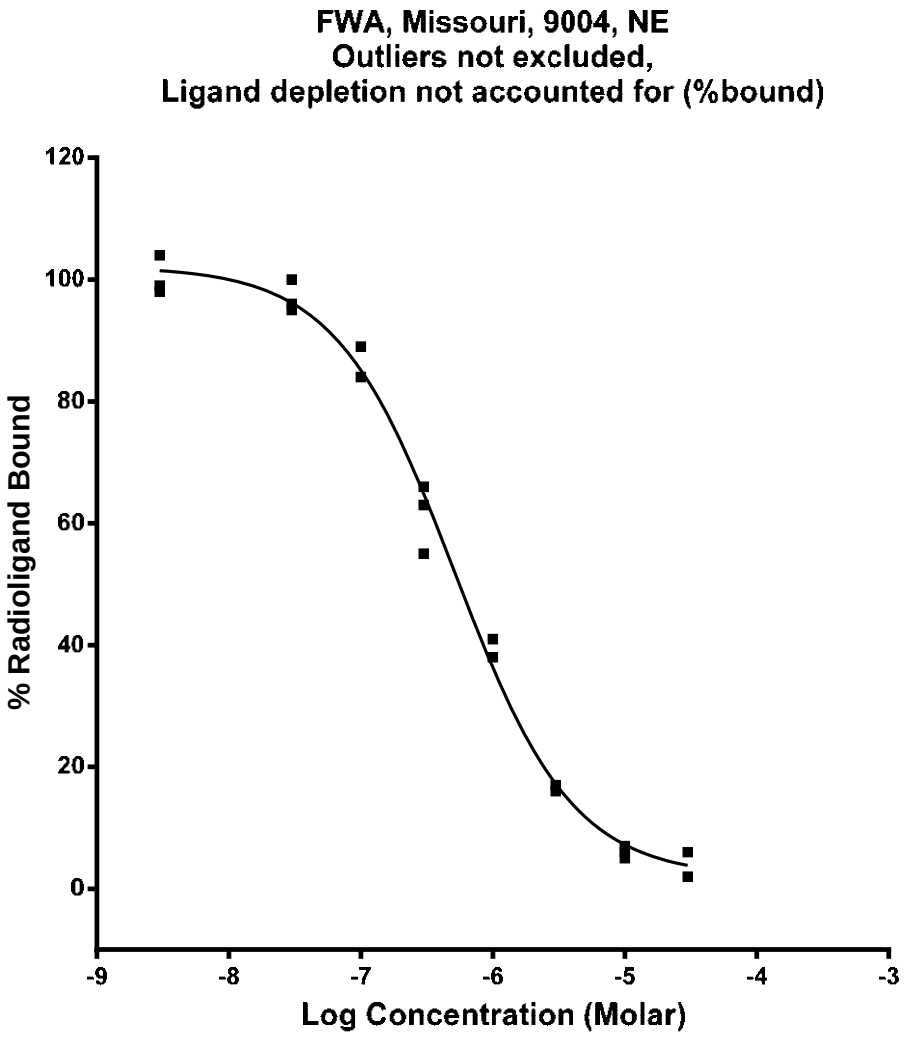
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9004, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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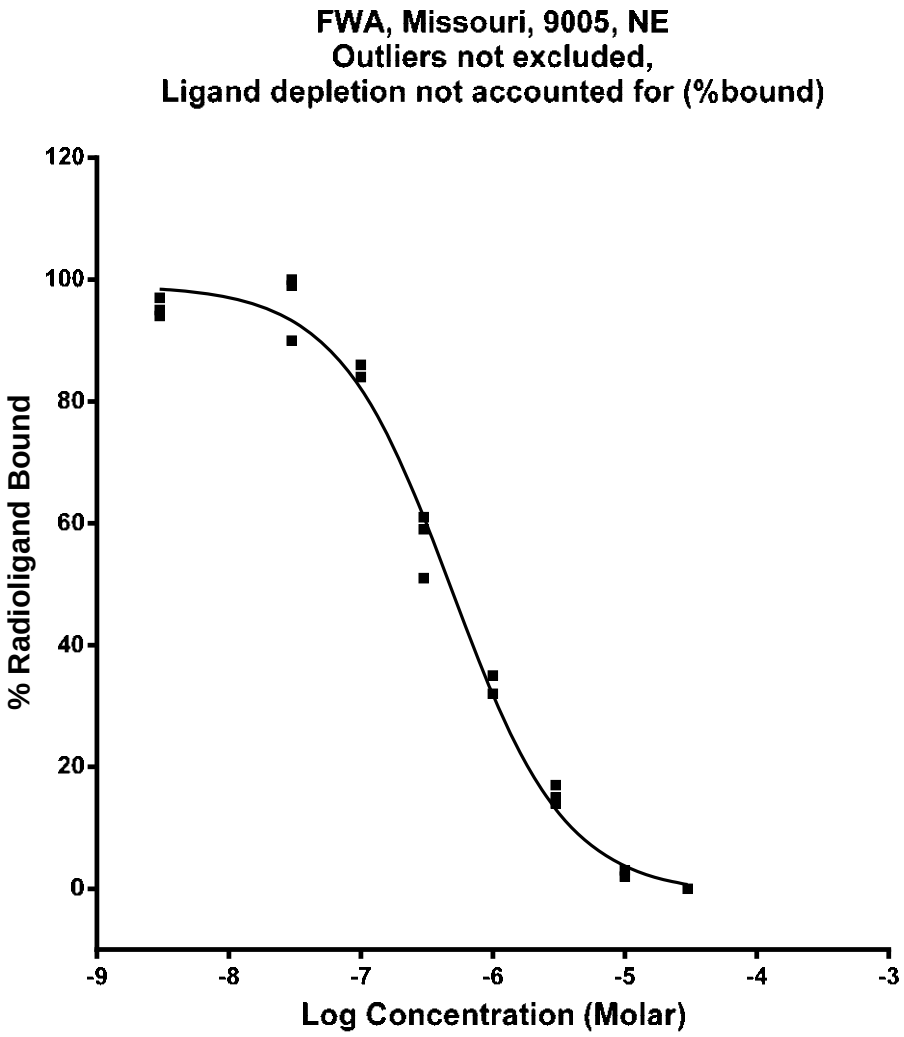
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9005, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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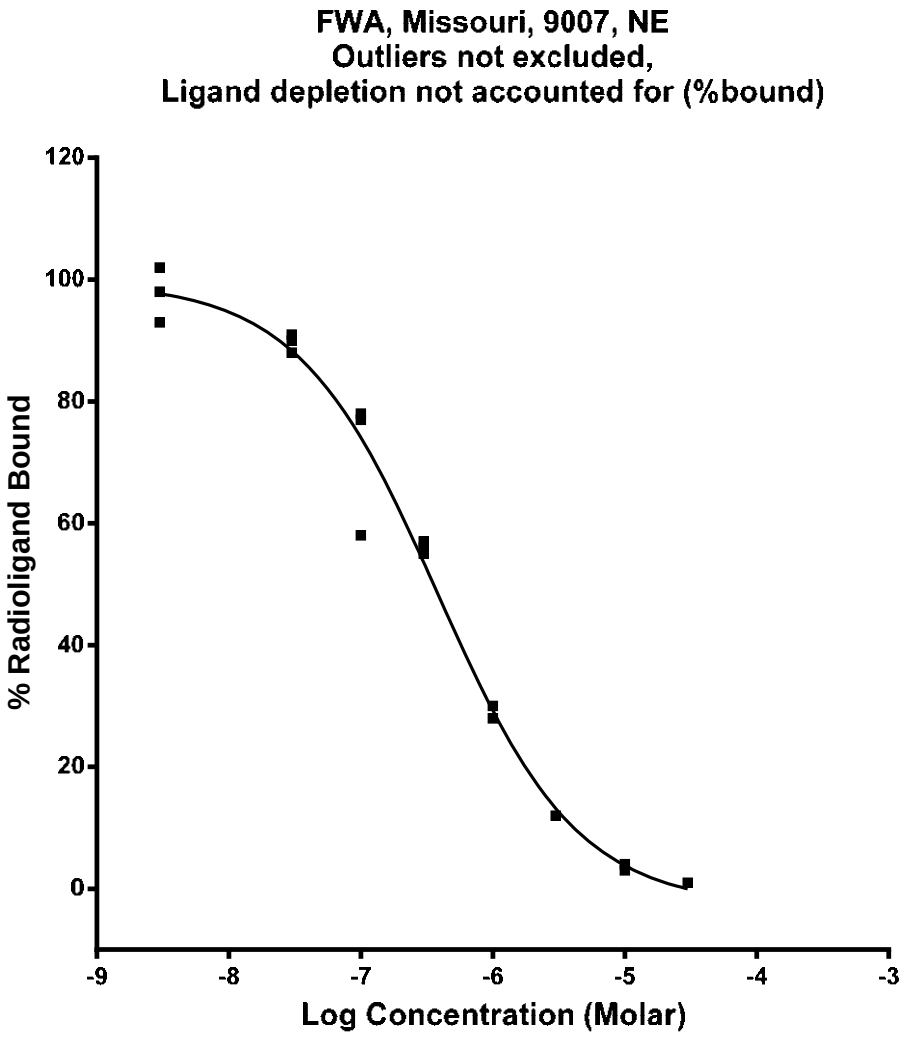
Log Concentration (Molar)

Subtask 2


FWA, Missouri, 9007, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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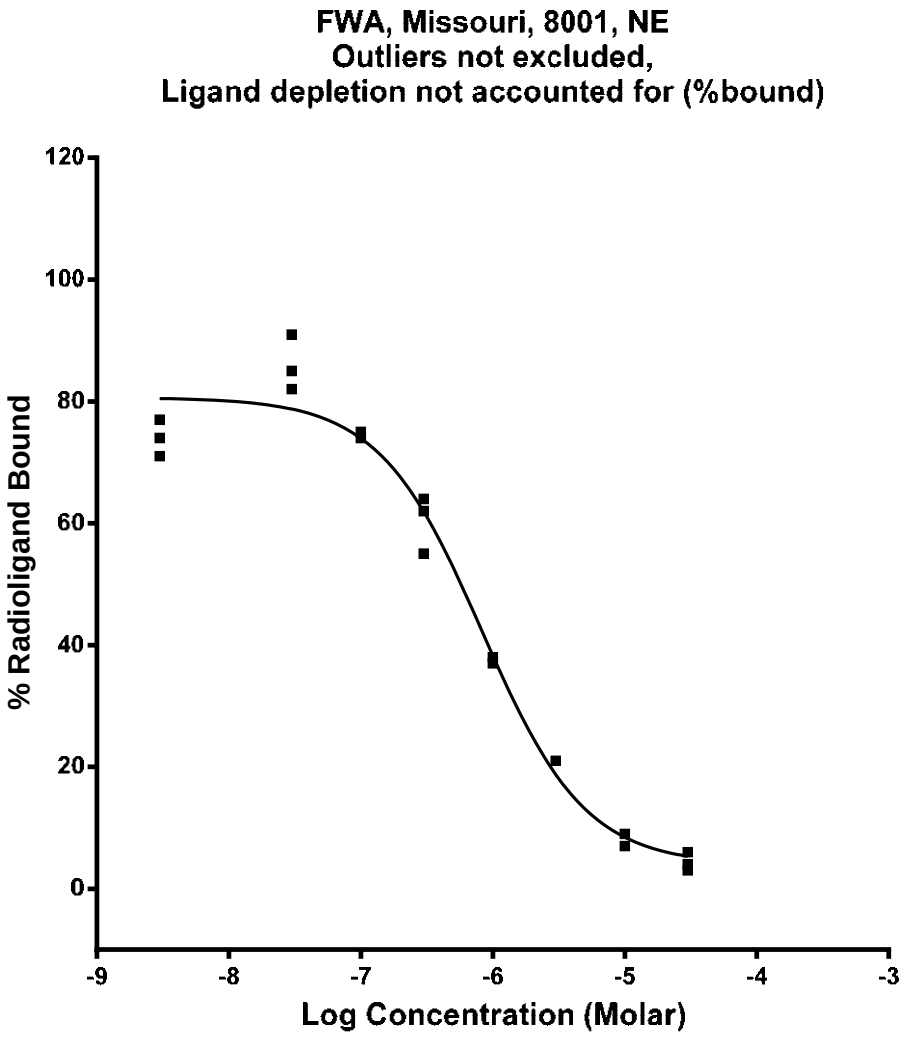
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8001, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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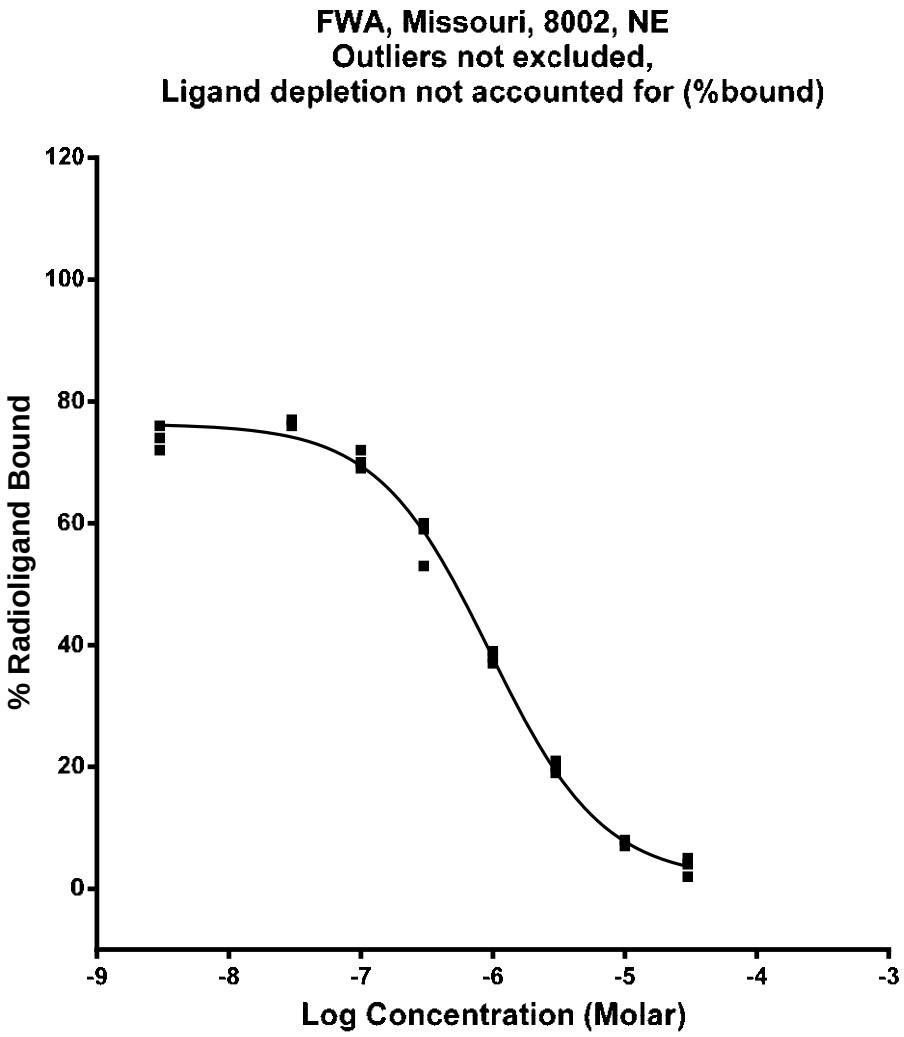
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8002, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
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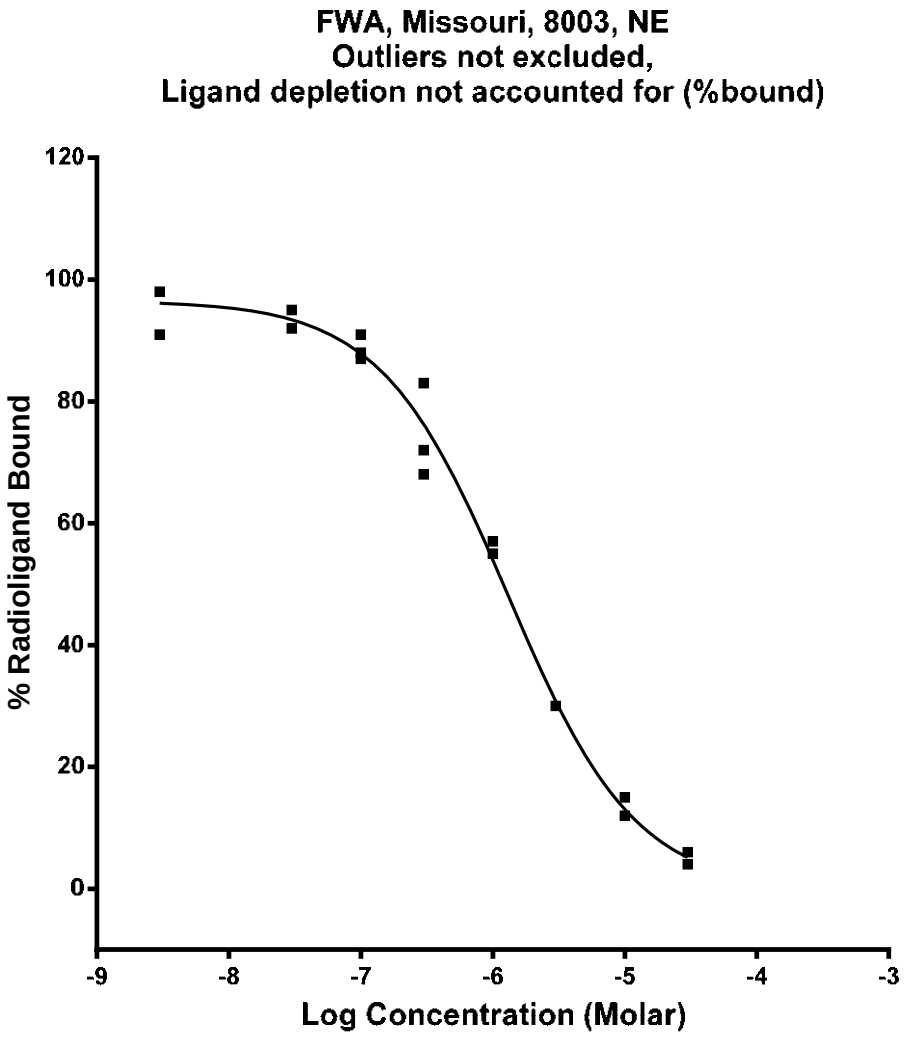
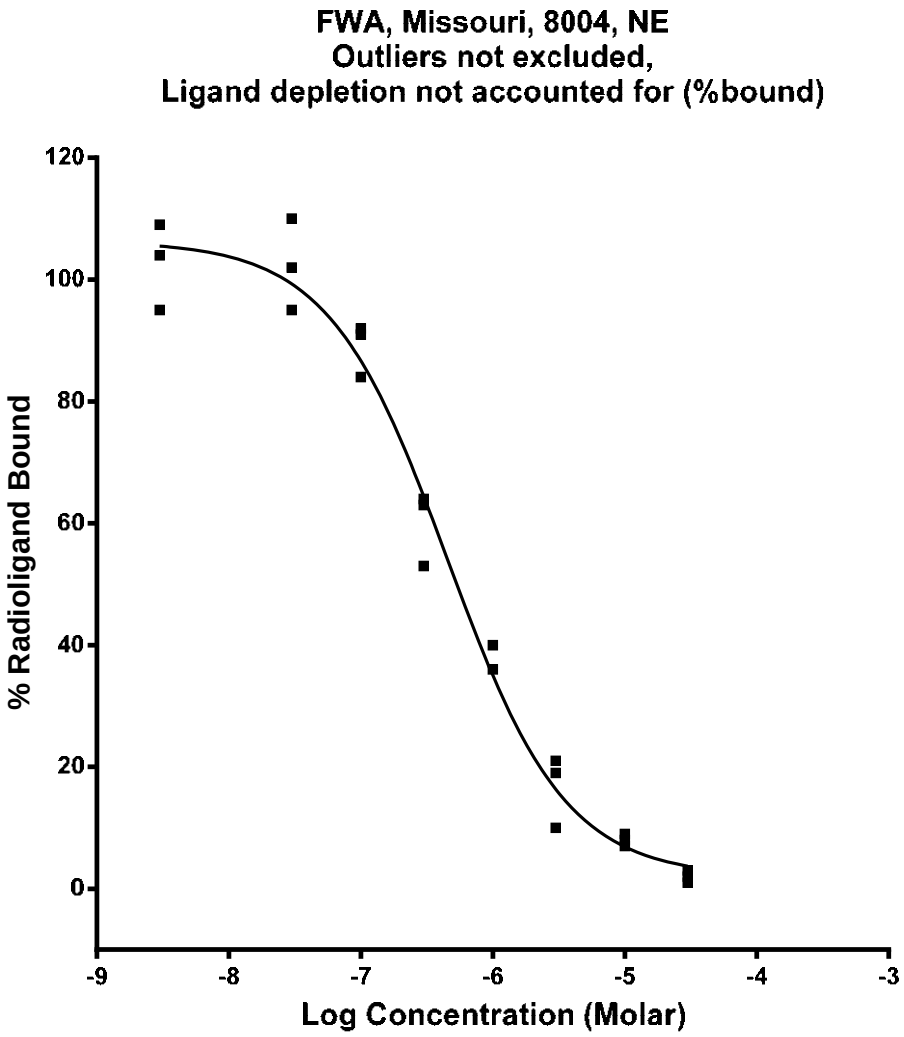
Log Concentration (Molar)

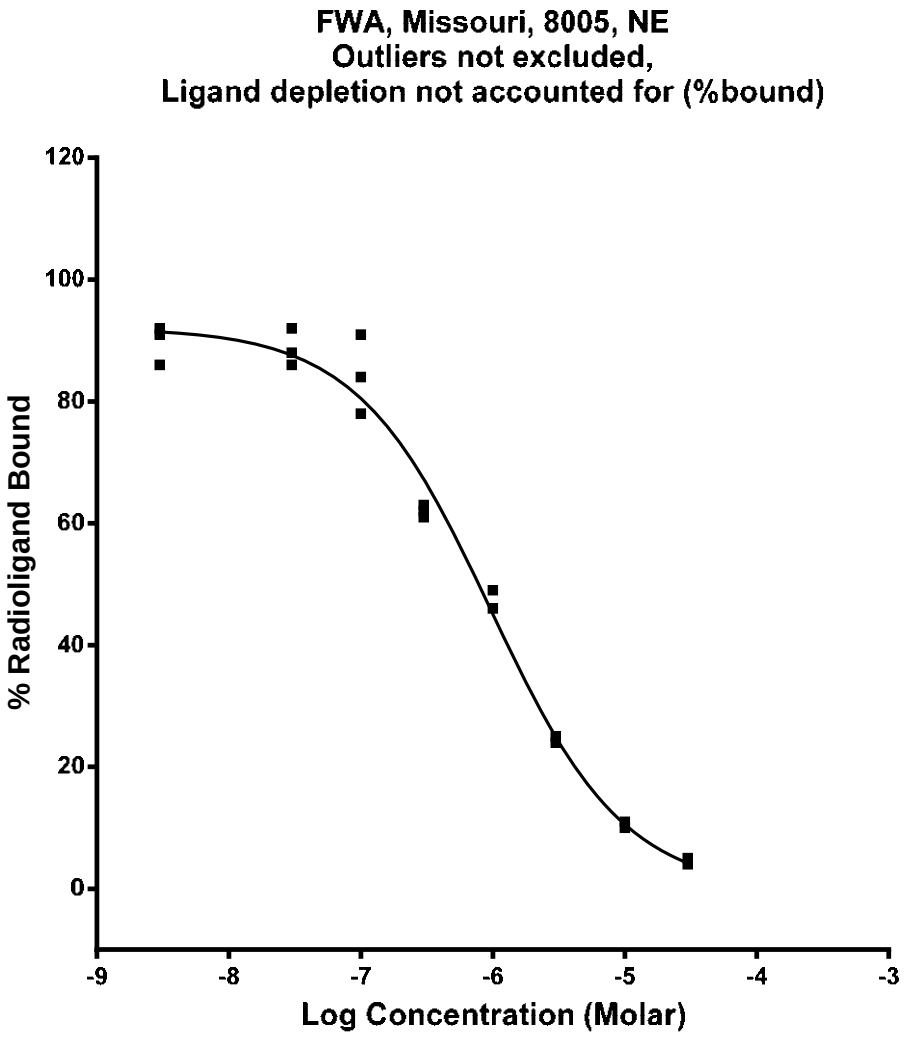
Subtask 3


FWA, Missouri, 8003, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

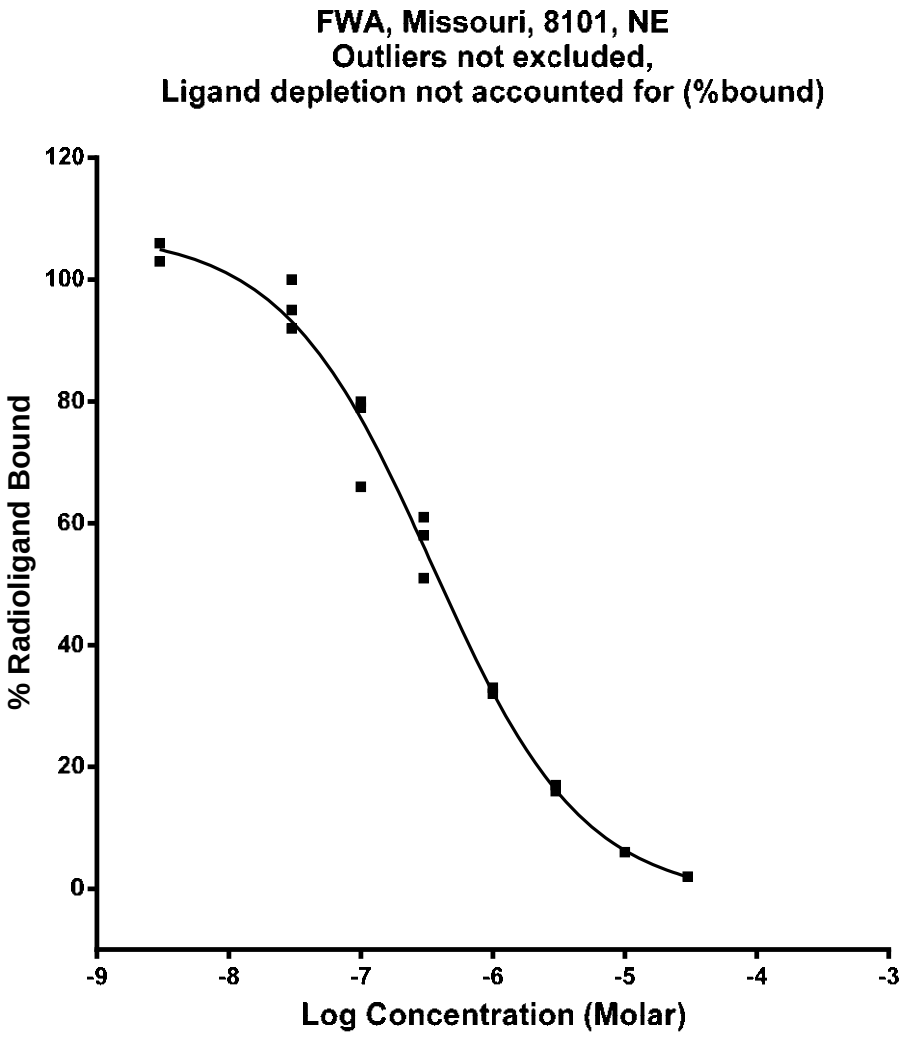
Subtask 3


FWA, Missouri, 8004, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

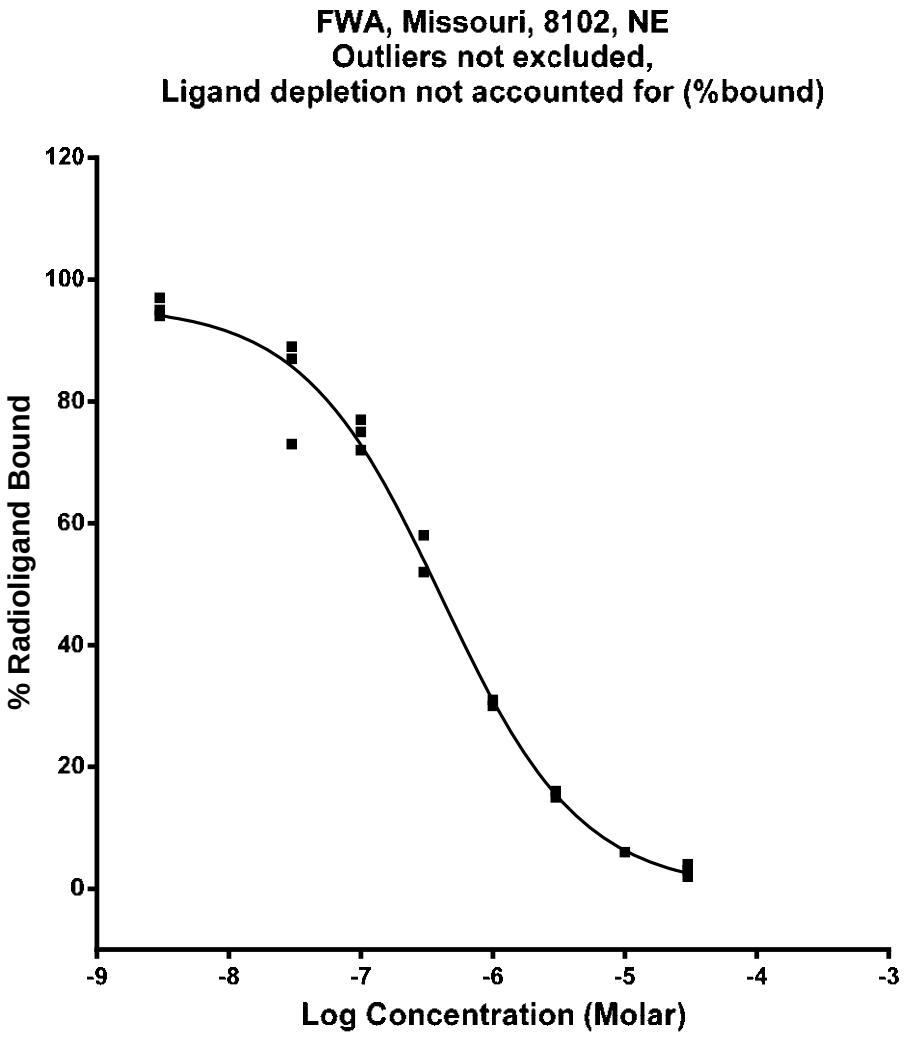
Subtask 3


FWA, Missouri, 8005, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

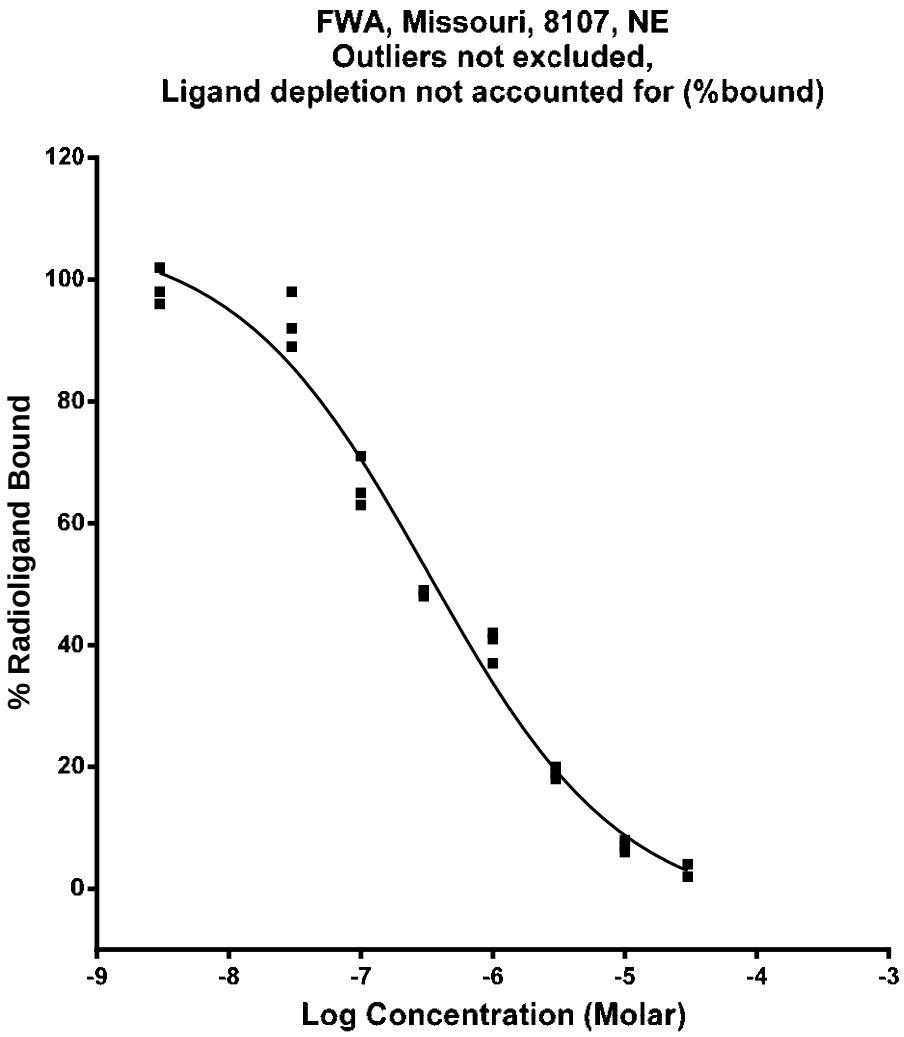
Subtask 3


FWA, Missouri, 8101, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

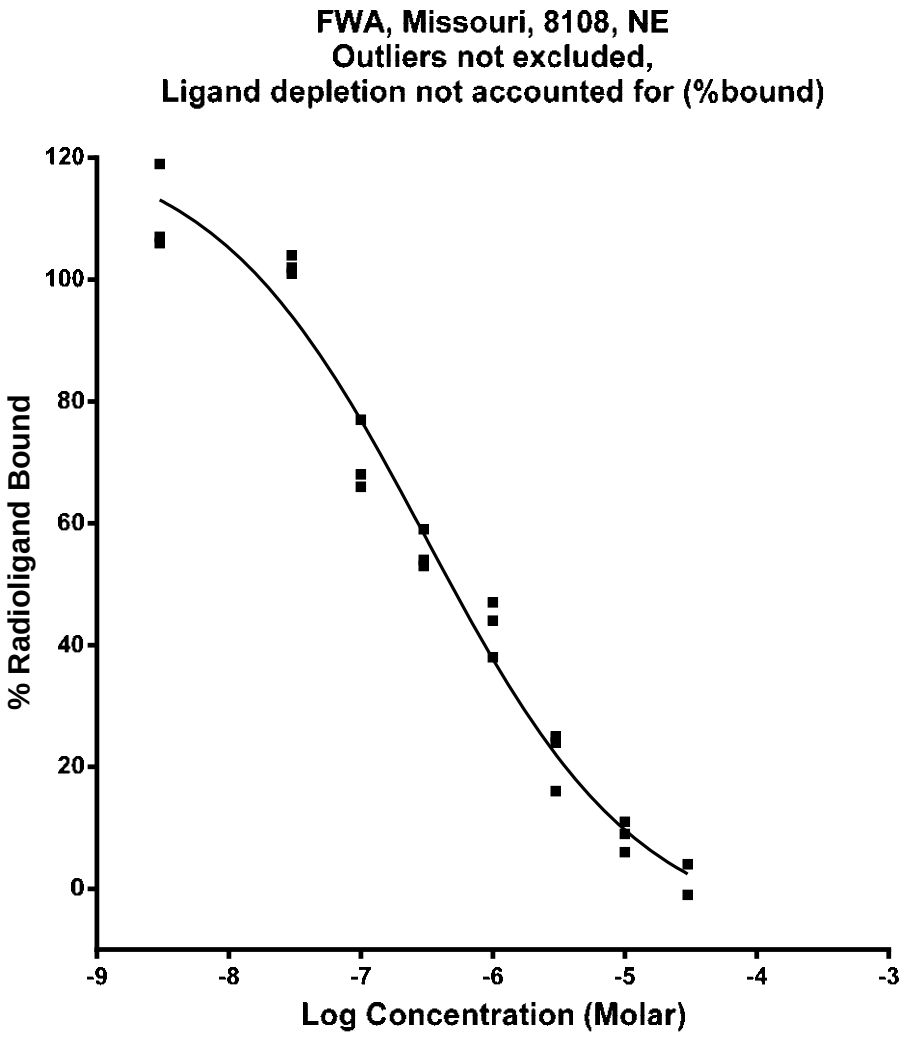
Subtask 3


FWA, Missouri, 8102, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

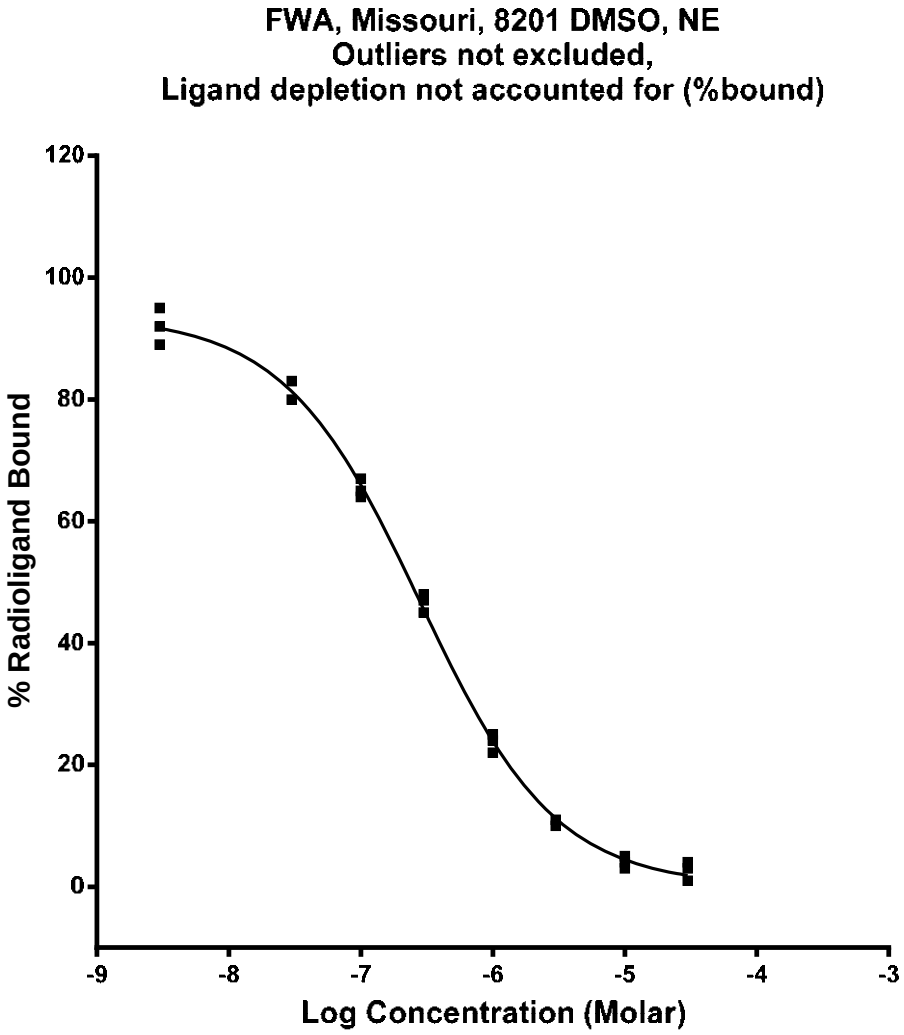
Subtask 3


FWA, Missouri, 8107, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

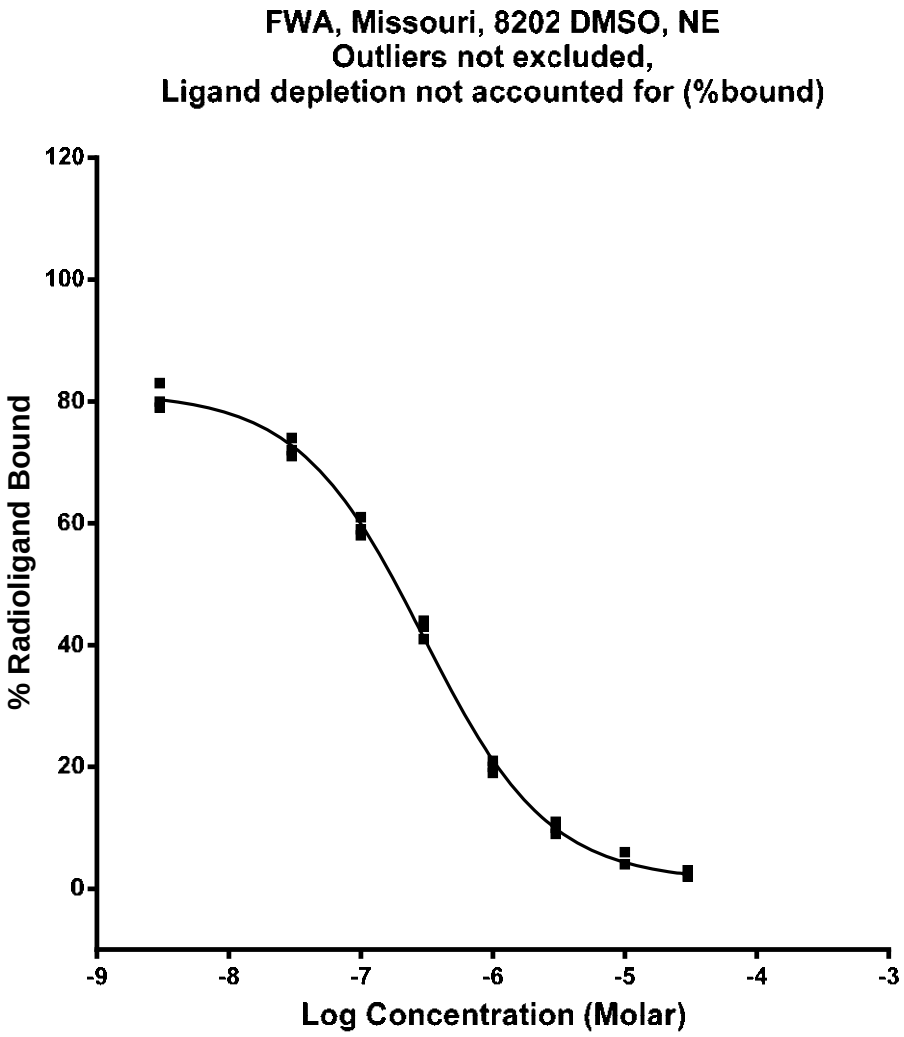
Subtask 3


FWA, Missouri, 8108, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

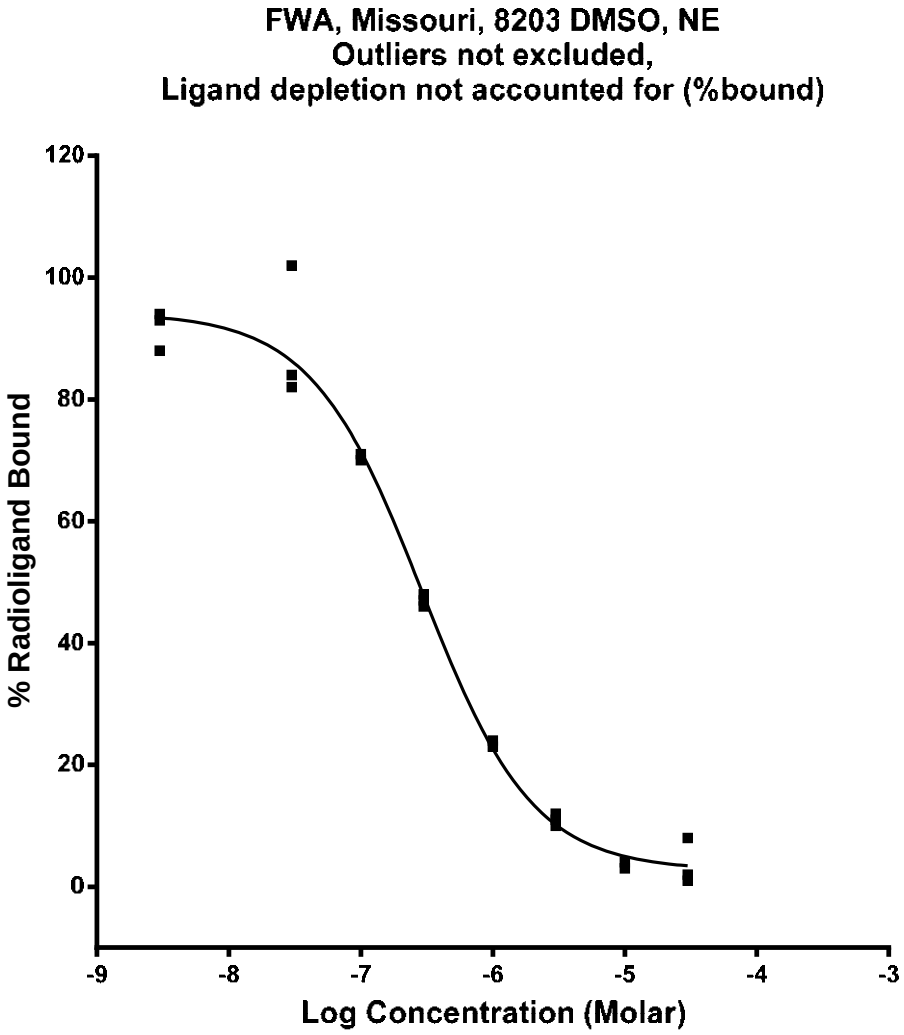
Subtask 3


FWA, Missouri, 8201 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8202 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)

Subtask 3


FWA, Missouri, 8203 DMSO, NE
Outliers not excluded,
Ligand depletion not accounted for (%bound)
% Radioligand Bound
120
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
Log Concentration (Molar)