Primary Reviewer: _____ **Signature:** _____
[Insert Name of Organization] **Date:** _____
Secondary Reviewer: _____ **Signature:** _____
[Insert Name of Organization] **Date:** _____

Template version 08/2011

DATA EVALUATION RECORD

STUDY TYPE: Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250

PC CODE: *(if applicable)* **DP BARCODE:** *(if applicable)*

TXR#: *(if applicable)* **CAS No.:** **[#]**

TEST MATERIAL (PURITY): *(use name of material tested as referred to in the study (common agency name in parenthesis))*

SYNONYMS: *(Other names and codes)*

CITATION: Author *(up to 3, see SOP for exact format)*. ([Study Year]). Title. Laboratory name and location. Laboratory report number, study completion date. MRID *(if applicable) (no hyphen)*. Unpublished. *(OR if published, list Journal name, vol.:pages)*

TEST ORDER #: [Test Order Recipient or the Consortium No.] *(e.g., EDSP-PC Code-###)*

SPONSOR: *(Name of Study Sponsor)*

EXECUTIVE SUMMARY: In an estrogen receptor (ER) binding assay (MRID *(if applicable)* [number]), uterine cytosol from Sprague-Dawley rats was used as the source of estrogen receptors (ER) to conduct Saturation Binding Experiments and Competitive Binding Experiments in this assay. The Saturation Binding Experiment was conducted to demonstrate that the ER isolated from rat uterine cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radio-labeled reference estrogen prior to routinely conducting ER Competitive Binding Experiments. The Competitive Binding Experiment was conducted to measure the binding of a single concentration of [^{3}H]-17β-estradiol ([1] nM) in the presence of increasing concentrations of [test chemical] (logarithmic increase from [10^{-10} to 10^{-3}] M). [Ethanol or DMSO or water] was used as a vehicle at a final concentration of [#]%. The assay included norethylnodrel as a weak positive control, octyltriethoxysilane as a negative control, and 17-β-estradiol as the natural ligand reference material.

Provide a brief summary of the results and a concise discussion. In particular, mention the classification of the test compound (interactive, equivocal, not interactive, or equivocal up to the limit of concentrations tested) and its Relative Binding Affinity (RBA)% (average and range). Discuss any major deficiencies, failure to meet performance criteria, or any problems encountered in this study. Example text is included below.

In the Saturation Binding Experiments, the maximum binding capacity (B_{max}) was [#] fmol/100 µg protein and the dissociation constant (K_d) was [#] nM. *Indicate whether or not the results from the Saturation Binding Experiments were acceptable, and state whether these values fell within the expected ranges and were consistent across runs and if Scatchard plots were linear. Include non-specific binding as a percent of total binding.*

For the Competitive Binding Experiments, the estimated log IC_{50} for [test chemical] was [#] compared to 17β-estradiol [#] and the positive control [#]. The test substance was classified as [interactive, equivocal, non-interactive, or equivocal up to the limit of concentrations tested]. The Relative Binding Affinity (RBA) for the test material was [#]% compared to the positive control ([1 nM]17β-estradiol).

The study [satisfies/does not satisfy] the Test Order requirement for an Estrogen Receptor Binding Assay Using Rat Uterine Cytosol. (OCSPP 890.1250) *(If it does not satisfy the requirement, concisely list only major deficiencies or refer to deficiency section.)*

COMPLIANCE: Signed and dated GLP and Quality Assurance statements [were/were not] provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Facility: *Name of the Facility*
Location: *Location of the Facility*
Study Director: *Name*
Other Personnel: *Name and study responsibility*
Study Period: *Study start and end dates*

2. Test substance: *Common name as used by Agency*
Description: *e.g. technical, nature, color, molecular weight*
Source: *include catalog #*
Lot/Batch #: *include expiration date*
Purity: %
Solubility:
Volatility:
Stability:
Storage conditions:
CAS #: *CAS # or* Not available
Molecular weight:
Structure: *Insert Structure or state Not available*

3. Non-labeled ligand: 17β-estradiol
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #
Batch #:
Purity: %
CAS #: 50-28-2

4. Radioactive ligand: [^{3}H]-17β-estradiol
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #:
Batch #:
Radiochemical purity: %
Specific activity: MBq/mg
Concentration of stock: Ci/mmol

5. Positive control: Norethynodrel
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #
Batch #:
Purity:
CAS # : 68-23-5

6. Negative control: Octyltriethoxysilane
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #
Batch #:
Purity:
CAS # : 2943-75-1

7. Solvent/vehicle control: [Ethanol, Water, or Dimethyl sulfoxide (DMSO)]
Justification for choice of

solvent:
Final Concentration? %

B. METHODS

1. Preparation of Rat Uterine Cytosol (RUC): *If Rat Uterine Cytosol is from a commercial source, note supplier. Otherwise, describe the procedures, including information regarding: the number of animals and age; days between ovariectomy and harvest; details of homogenizing buffer and homogenizing protocol; and protein concentration (required to be 1-4 mg/mL). The following example text may be altered as necessary to apply to specific methods used by performing laboratory. Note any deviations from standard protocol and provide justification. Example text is included below and should be altered to apply to the specific methods using by the laboratory.*

A total of [#] female Sprague Dawley rats were ovariectomized [#] days prior to being euthanized. Animals were [#] days old at the time of euthanasia. The uteri were weighed, placed in ice-cold TEDG (Tris, EDTA, DTT, glycerol) + PMSF (phenylmethylsulfonyl fluoride) buffer and [used immediately *or* rapidly frozen in liquid nitrogen for storage at -[#]ºC until use]. Uteri were homogenized in buffer, centrifuged for 10 min at 2500 × *g* at 4ºC. Supernatant was transferred and centrifuged for 60 minutes at 105,000 × *g*, discarding the resulting pellets. Protein concentration of the cytosol was determined to be [[#] mg/mL] using a protein kit compatible with DTT in the TEDG buffer (e.g., BioRad Protein Assay Kit). Cytosol was divided into aliquots ([#] mL) for [immediate use OR storage at -80ºC until use].

2. Saturation (radioligand) Binding Experiment: A Saturation Binding Experiment measuring total and non-specific binding of [^{3}H]-17β-estradiol was performed to demonstrate that the estrogen receptor (ER) was present in reasonable concentrations and had the appropriate affinity for the native ligand. The conditions for the saturation binding are summarized in Table 1.

TABLE 1. Summary of Conditions for Saturation Binding Experiment [a]

Source of receptor		Rat uterine cytosol
Concentration of radioligand (as serial dilutions)		[#]-[#] nM
Concentration of non-labeled ligand (100X [radioligand])		[#]-[#] nM
Concentration of receptor		Sufficient to bind [#]-[#]% of radioligand at [#] nM[a]
Temperature		[#]ºC
Incubation time		[#] hours
Composition of assay buffer	Tris	[#] mM (pH [#])
	EDTA	[#] mM
	Glycerol	[#]%
	Phenylmethylsulfonyl fluoride	[#] mM
	DTT	[#] mM

a Data were obtained from page [#] of the study report.

Describe the methods used for conducting the Saturation Binding Experiment, including information regarding: the specific activity of the stock solution and whether or not it was

adjusted for decay; concentrations of [^{3}H]-17β-estradiol and non-radiolabeled 17β-estradiol; protein concentration; and number of runs; incubation time, temperature, conditions; separation of bound ligand from free ligand; and quantitation via scintillation counting. Note that the constituents of the tubes for total and non-specific binding can be depicted in a table and referenced. Example text is included below and should be altered to apply to the specific methods using by the laboratory.

On the day of the assay, the specific activity of the stock solution [^{3}H]-17β-estradiol [was/was not] adjusted for decay over time, and serial dilutions in TEDG + PMSF buffer were prepared to achieve the final concentrations of [#, #, #, #, #, #, #, and #] nM. Solutions of non-labeled 17β-estradiol were prepared in a similar manner to achieve concentrations that were 100-fold greater than each respective radiolabeled concentration to result in final concentrations of [#, #, #, #, #, #, #, and #] nM. For each batch of cytosol, the optimal protein concentration was determined by testing serial amounts of protein per tube, using [#] nM radiolabeled estradiol, until a concentration was reached that bound [#-#]% of the total radioactivity added *(Note: typically 50 ± 10 µg protein per tube)*. Each assay consisted of three non-concurrent runs, and each run contained three concurrent replicates at each concentration, resulting in the [#] samples depicted in Table 2.

TABLE 2. Saturation Binding Experiment Run [a]

Total binding [b]	Non-specific binding [c]	Radioligand alone [d]	Assay Components
Tubes 1-24	Tubes 25-48	Tubes 49-72	
350 µL	300 µL	---	TEDG + PMSF buffer
50 µL	50 µL	50 µL	[^{3}H]-17β-estradiol (8 serial dilutions) [e]
---	50 µL	---	Non-labeled 17β-estradiol (8 serial dilutions, 100x each respective labeled concentration) [f]
100 µL	100 µL	---	Uterine cytosol (diluted to appropriate conc.)
500 µL	500 µL	50 µL	Total volume in each assay tube

a Data were obtained from page [#] of the study report.
b Total binding = [^{3}H]-17β-estradiol bound to ER
c Non-specific binding = [^{3}H]-17β-estradiol and 100-fold greater non-labeled bound to ER
d Total [^{3}H]-17β-estradiol alone for dpm determination at each concentration
e Final concentrations of [^{3}H]-17β-estradiol = [0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, and 3] nM.
f Final concentrations of non-labeled 17β-estradiol = [3, 6, 8, 10, 30, 60, 100, and 300] nM.

Tubes were incubated with gentle vortexing for [#] hours at [#] °C. To separate bound from free estradiol, hydroxyapatite (HAP) slurry was added to each tube and vortexed ([#] number times with [#]-minute intervals). Subsequently, the contents of each tube were washed three times as follows: TEDG +PMSF buffer was added, vortexed, centrifuged for [#] min at [#] x *g*, and the supernatant decanted and discarded. Ethanol was added to the HAP pellet remaining in each tube to extract the [^{3}H]-17β-estradiol, followed by vortexing, and centrifugation for [#] min at [#] x *g*. An aliquot of supernatant was radioassayed by scintillation counting. The temperature was maintained at [#]ºC throughout the assay prior to extraction with ethanol.

3. **Competitive Binding Experiment:** A summary of the experiment conditions for the Competitive Binding Experiment is included in Table 3.

TABLE 3. Summary of Conditions for Competitive Binding Experiment [a]		
Source of receptor		Rat Uterine Cytosol
Concentration of radioligand		[#] nM
Concentration of receptor		Sufficient to bind [#-#]% of radioligand [b]
Concentration of test substance (as serial dilutions)		10^{-10} to 10^{-3} mM [c]
Temperature		[#] °C
Incubation time		[#] hours
Composition of assay buffer	Tris	[#] mM (pH [#])
	EDTA	[#] mM
	Glycerol	[#]%
	Phenylmethylsulfonyl fluoride	[#] mM
	DTT	[#] mM

a Data were obtained from page [#] of the study report.
b Receptor concentration may need to be adjusted by adding volume.
c Selection of the test substance concentrations (range and spacing) may be adjusted depending on solubility, affinity of the test chemical for the receptor, or other factors.

Describe the methods used for conducting the Competitive Binding Experiment, including information regarding: selection of solvent, including test for solubility; the specific activity of the stock solution and whether or not it was adjusted for decay; protein concentration; number of runs; incubation time, temperature, conditions; separation of bound ligand from free ligand; quantitation via scintillation counting; and concentrations of the test material, positive control, negative control, and non-radiolabeled 17β-estradiol. Note that the concentration selection for each of these components can be depicted in a table and referenced. Example text is included below and should be altered to apply to the specific procedures of the laboratory.

A solubility test [was/was not] performed to determine the appropriate solvent ([water, ethanol, or DMSO]) using examination for precipitation using [20x magnification (*e.g.*, dissecting microscope) *or* plate-reading spectrophotometry at [x] nm]. On the day of the assay, the specific activity of the stock solution [^{3}H]-17β-estradiol [was/was not] adjusted for decay over time, and diluted in TEDG + PMSF buffer to achieve a final concentration of [#] nM. For each batch of cytosol, the optimal protein concentration was determined by testing serial amounts of protein per tube, using 1.0 nM radiolabeled estradiol, until a concentration was reached that bound [#]% of the total radioactivity added [Note: typically 50 ± 10 µg protein per tube]. Serial dilutions of the test substance, positive control ([norethynodrel]), negative control ([octyltriethoxysilane]), and reference material (non-labeled 17β-estradiol) were prepared to achieve the concentrations shown in Table 4. Each assay consisted of three runs, and each run contained three replicates at each concentration, resulting in a total of [#] samples.

TABLE 4. Molar (M) concentrations in Competitive Binding Assay Run [a] [b]			
Test substance	Positive control	Negative control	Reference Chemical
	Norethynodrel	Octyltriethoxysilane	Non-labeled 17β-estradiol
Tubes 1-24 [c]	Tubes 25-48 [c]	Tubes 49-72 [c]	Tubes 72-96 [c]
10^{-10}	$10^{-8.5}$	10^{-10}	Solvent control [d]
10^{-9}	$10^{-7.5}$	10^{-9}	10^{-11}
10^{-8}	10^{-7}	10^{-8}	10^{-10}
10^{-7}	$10^{-6.5}$	10^{-7}	$10^{-9.5}$
10^{-6}	10^{-6}	10^{-6}	10^{-9}
10^{-5}	$10^{-5.5}$	10^{-5}	$10^{-8.5}$
10^{-4}	$10^{-4.5}$	10^{-4}	10^{-8}
10^{-3}	10^{-4}	10^{-3}	10^{-7}

a Data were obtained from page [#] of the study report.

b Each tube contains: 10µL of either the test substance, positive control, negative control, solvent control, or non-labeled 17β-estradiol; 390 µL of TEDG + PMSF buffer with [^{3}H]-17β-estradiol; and 100 µL of uterine cytosol (with ER), for a total of 500 µL.

c Each concentration of each chemical was run in triplicate, for a total of 96 tubes per run.

d Solvent is [either water, ethanol (<3%), or DMSO (<10%)]. *(Note: Unless it is water, the solvent used for the test chemical should also be used for the positive control, negative control, and the non-labeled 17β-estradiol. If water is the solvent for the test chemical, then the controls should be run in ethanol).*

The procedures for assay incubation, separation of bound from free estradiol (washing with buffer and extraction with ethanol), followed by scintillation counting of bound [^{3}H]-17β-estradiol were similar to those used in the Saturation Binding Experiment (*or describe methods if different and provide justification*).

C. DATA ANALYSIS:

List parameters that were analyzed and the statistical methods used for both the Saturation and Competitive Binding Experiments; include a statement that indicates whether the reviewers consider these analyses to be appropriate. If inappropriate, provide the reason and propose alternative analyses. Detail methods in the data analysis, such as nonlinear regression for the estimation of B_{max} and K_d; Scatchard plot; outlier determination, ligand depletion, and nonlinear curve fitting for the estimation of log(IC_{50}). When software is used for data analysis, report the software title, version number, and source (company, city, state and country if outside U.S.). Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory.

For the Saturation Binding Experiment, total binding and non-specific binding data were modeled via non-linear regression using [Graph Pad Prism v. 5 (GraphPad Software, Inc., La Jolla, CA)], incorporating automatic outlier elimination according to the method of Motulsky and Brown (2006)[1] implemented by using the [ROUT procedure in Prism v. 5] with a Q value of 1.0. Receptor binding data plots were corrected for ligand depletion using the method of Swillens (1995)[2]. For the competitive binding assay, similar methods of nonlinear regression were used to fit a curve (for 17β-estradiol, the positive control, and the test substance) to the Hill equation

1 Motulsky, H.J. and Brown, R.E. (2006) Detecting outliers when fitting data with nonlinear regression- a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinformatics, Vol 7, pp 123-142.
2 Swillens, S. (1995) Interpretation of binding curves obtained with high receptor concentrations: practical aid for computer analysis. *Molec. Pharmacol.* 47(6):1197-1203.

formula which incorporated log IC_{50} as a parameter to be estimated. For parameters reported from the Saturation Binding Experiment (K_d and B_{max}) and Competitive Binding Experiment (log IC_{50} and RBA), mean and standard deviation were calculated for each run and mean and standard error were calculated for the composite three runs using [Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA)].

1. Definitions

a. Classification of test material: Classification of the test material is based on the average of three runs. Each run was first individually classified as follows:

Interactive = lowest point on the fitted curve within the range of the data is less than 50% (i.e., >50% of the radiolabeled estradiol has been displaced from the ER).

Not interactive = there are usable data points at or above 10^{-6}M and either the lowest point on the fitted response curve within the range of the data is above75% (i.e., <25% of the radiolabeled estradiol has been displaced from the ER) or a binding curve cannot be fitted and the lowest average percent binding among concentration groups in the data is above 75%.

Equivocal up to the limit of concentrations tested = If there are no data points at or above a test chemical concentration of 10^{-6}M and either a binding curve can be fit but ≤50% of the radiolabeled estradiol has been displaced from the ER or a binding curve cannot be fit and the lowest average percent binding among concentration groups in the data is >50%.

Equivocal = A run is classified as equivocal if it does not fall into any of the categories above.

The categorical classification of each run was assigned a numerical value as follows:

Run Classification	Numerical Value
Interactive	2
Equivocal	1
Not interactive	0
Equivocal up to the limit of concentrations tested	"missing"

The values for each run were then averaged across runs and the chemical classified using the following ranges:

Test Material Classification	Numerical Range
Interactive	average ≥1.5
Equivocal	0.5≥ average <1.5
Not interactive	average <0.5
Equivocal up to the limit of concentrations tested	"missing"

b. Descriptors for receptor binding:

B_{max}: maximum specific binding number (fmol ER/100 µg cytosol protein) measures the concentration of active receptor sites
K_d: dissociation constant (nM), measures the affinity of the receptor for its natural ligand
IC_{50}: concentration of the test substance at which 50% of the radioligand is displaced from the receptor
Relative Binding Affinity (RBA %): IC_{50} of 17β-estradiol ÷ IC_{50} of test substance × 100

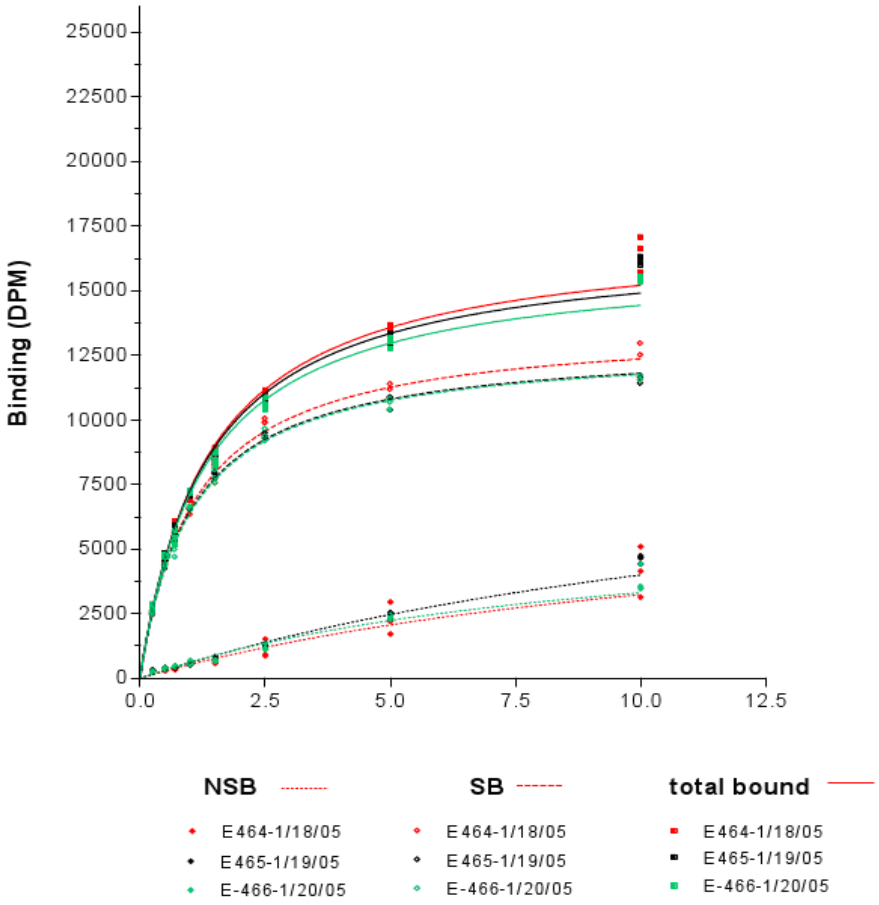
II. RESULTS

A. SATURATION BINDING EXPERIMENT

Provide a graph from the report of total, specific, and non-specific binding across the range of concentrations tested, such as the example below. The example graph should include the following information:

- *Each data point is plotted for total and non-specific binding, but not for specific binding, along with the fitted curves for total, specific, and non-specific binding. Total binding and non-specific binding are fit simultaneously (i.e., shares the non-specific binding parameter).*
- *Accounts for ligand depletion using the method of Swillens (1995)*
- *Separate runs are denoted using a different symbol and/or color (e.g., red, black, and green).*

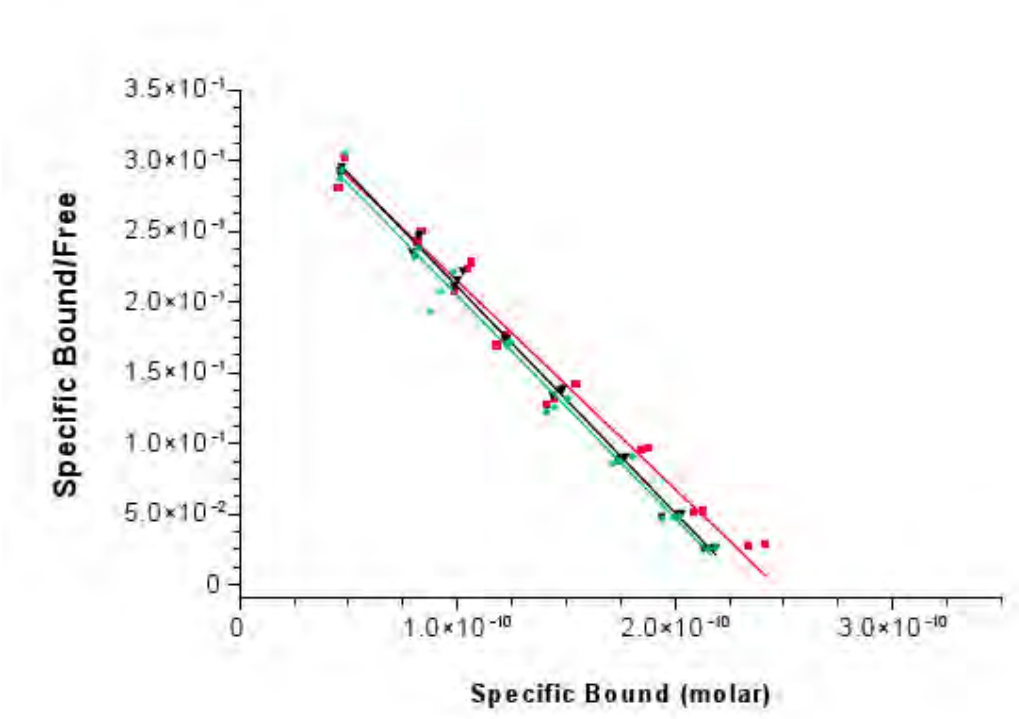
Figure 1. Binding of [^{3}H]-17β-estradiol to the Estrogen Receptor during the Saturation Binding Experiment.



Include a Scatchard plot illustrating the binding of [^{3}H]-17β-estradiol to the ER

- *Provide a figure plotting the specific bound/free by specific bound (nM).*
- *The line fit to the data is based on Swillens correction for ligand depletion.*
- *Each of the three runs is differentiated by color (e.g., red, black, and green in figure below).*

Figure 2. Scatchard Plot of the Binding of [^{3}H]-17β-estradiol to the Estrogen Receptor.



Example text is included below and should be altered to apply to specific procedures used by performing laboratory and results from the assay runs.

Saturation Binding Experiment parameters are presented in Table 5. The K_d for [^{3}H]-17β-estradiol was [mean (± SE)], and the estimated B_{max} was [mean (± SE)] for the prepared rat uterine cytosol. The K_d for each run was within the expected range of 0.03 to 1.5 nM. The data produced a linear Scatchard plot. Confidence in these numbers is [high/low] due to the goodness of fit and the [small/large] variation among runs. *Note if statistical analyses were used to compare consistency among runs. Some form of the following table is mandatory.*

TABLE 5. Saturation Binding Experiment of 17β-estradiol with Estrogen Receptor from Rat Uterine Cytosol [a]

Parameter	Run 1	Run 2	Run 3	Runs 1-3
R^2 (unweighted)				(range)
B_{max} (nM)				(Mean ± SE)
B_{max} (fmol/100 µg protein)				(Mean ± SE)
K_d (nM)				(Mean ± SE)

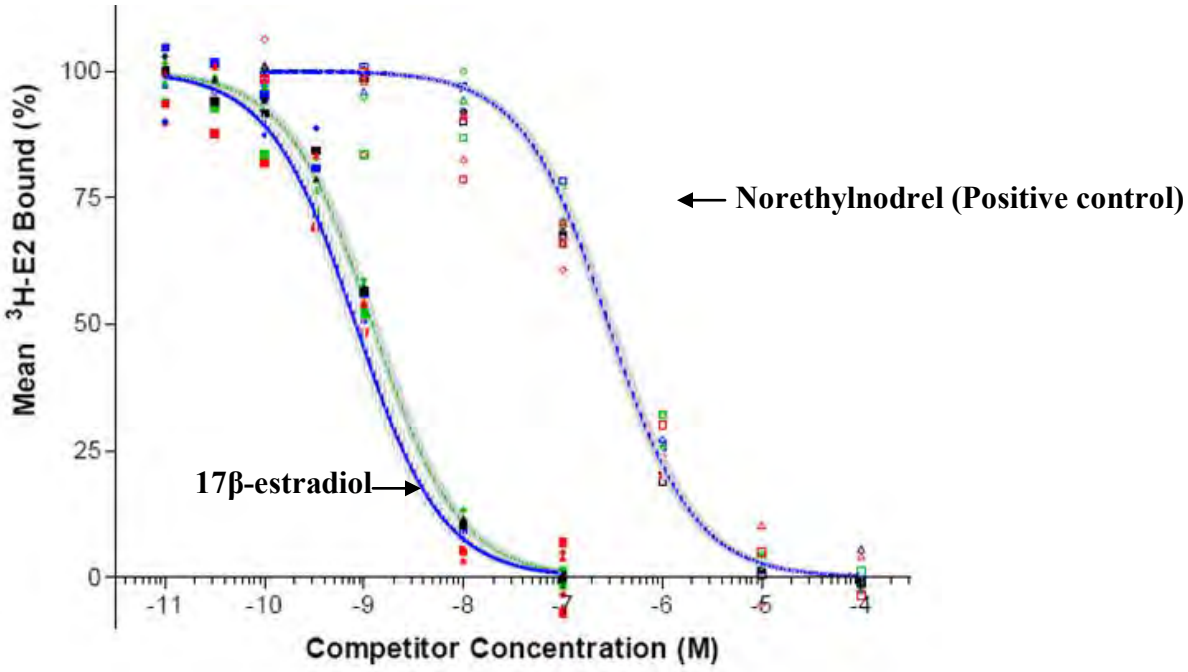
a Data were obtained from page [#] of the study report.
R^2 = Goodness of fit for curve calculated for specific binding

B. COMPETITIVE BINDING EXPERIMENT

The study report should include a separate graph for each run, which plots the data points and the unconstrained curve fitted to the Hill equation for the reference standard (17β-estradiol), positive control (norethylnodrel), and negative control (octyltriethyoxysilane) on

the same graph as the test material. Include a graph of one of these runs as a figure similar to the example below (Note that the example figure below does not depict the negative control or test material, which must also be included). Do not use correction for ligand depletion, but do exclude outliers according to the method of Motulsky and Brown (2006)[3] using a Q-value of 1.0.

Figure 3. Percentage E2 Bound to the Estrogen Receptor in the Presence of Test Compound.



Include another graph which plots the curves for the test chemical (without the data points or other indicators of variability) for all runs. This graph does not need to include the data points or fitted curves for estradiol or the positive or negative controls.

Example text is included below and should be altered to apply to specific procedures used by performing laboratory and results from the assay runs.

Competitive Binding Experiment parameters are presented in Table 6. The estimated mean log IC_{50} was [#] for the test material compared to 17β-estradiol [#] and the positive control [#]. The mean RBA was [#]% for the test material compared to [#]% for the positive control. Confidence in these numbers is [high/low] due to the [small/large] variation. Repeat runs were unnecessary. *If repeat runs were necessary, provide an explanation. Some form of the following table is mandatory.*

[3] Motulsky, H.J. and Brown, R.E. (2006) Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. *BMC Bioinformatics* 7:123-142.

TABLE 6. Competitive Binding Assay of [Test Chemical] with Estrogen Receptor from Rat Uterine Cytosol [a]

Parameter		Run 1 [b]	Run 2 [b]	Run 3 [b]	Mean ± SE [c]
r^2 (unweighted),	17β-estradiol	#			NA
	Positive control	#			NA
	Test substance	#			NA
Log IC_{50} (nM),	17β-estradiol				
	Positive control				
	Test substance				
IC_{50} (nM),	17β-estradiol				
	Positive control				
	Test substance				
Log RBA (%),	Positive control				
	Test substance				
RBA (%),	Positive control				
	Test substance				

a Data were obtained from page [#] of the study report.
r^2 = Goodness of fit
RBA (%) = relative binding affinity
b The mean and standard deviation are reported for the concurrent replicates within each run.
c The range is reported for r^2, and the mean ± SEM is reported for the remaining parameters.
NA Not applicable. r^2 is more appropriately expressed as a range, as opposed to a mean.

TABLE 7. Binding Classification of **[Test Chemical]** with Estrogen Receptor [a]

Run	1	2	3	Mean [c]	Binding Classification [d]
Classification category value [b]					

a Data were obtained from page [#] of the study report.
b Classification category value: Interactive = 2; Equivocal = 1; Not interactive = 0; Equivocal up to the limit of concentrations tested ("missing", i.e., not included in calculation of mean).
c Mean of three runs expressed to the tenths place
d Interactive = mean ≥1.5; Equivocal = 0.5≤ mean <1.5; Not interactive = mean <0.5

C. PERFORMANCE CRITERIA: To ensure that the competitive binding assay functioned properly, each run was evaluated using the following criteria: [*Enter value and place an "X" in the appropriate column indicating whether or not each criterion was met.]*

Criterion [a]	Tolerance Limit(s)	Value	Yes	No
17β-estradiol fitted curve parameters				
Log_e residual SD	≤2.35			
Top (% binding) [b]	94 to 111			
Bottom (% binding)	-4 to 1			
(Hill) Slope ($log_{10}(M)^{-1}$)	-1.1 to -0.7			
Weak Positive control (norethynodrel) fitted curve parameters				
Log_e residual SD`	≤2.60			
Top (% binding) [bd]	90 to 110			
Bottom (% binding)	-5 to 1			
(Hill) Slope ($log_{10}(M)^{-1}$)	-1.1 to -0.7			
Solvent concentration				
Ethanol	≤3%			
DMSO	≤10%			
Negative control (octyltriethoxysilane) does not displace more than 25% of [^{3}H]-17β-estradiol from the ER on average across all concentrations	≤25%			

a Data were obtained from page [#] of the study report.

b If the top plateau for estradiol is significantly above the upper performance criterion, then curves for all chemicals in the run may be normalized using binding of estradiol at the lowest concentration in the reference curve as 100%.

NA Not applicable

Additionally, the curve for the reference material showed that increasing concentrations of unlabeled 17β-estradiol displaced [^{3}H]-17β-estradiol in a manner consistent with one-site binding, as indicated by a descent from [#]% to [#]% binding over approximately an [#]-fold increase in concentration of the test chemical (i.e., covering approximately 2 log units).

[Test chemical] was tested over a concentration range that fully defined the top of the curve. The percent binding at this top plateau [#]% was within 25 percentage points of the value for solvent control [#]% [and/or] the lowest concentration of the estradiol standard [#]%. Examination across the runs indicated consistency of the Hill slope, placement along the X-axis, and top and bottom plateaus.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: *Provide a brief paragraph of the investigators' conclusions.*

B. REVIEWER COMMENTS: *Briefly summarize the results and discuss the following:*

- *Did the Saturation Binding Experiment demonstrate that the estrogen receptor (ER) was present in a reasonable concentration (as indicated by B_{max} and functioning with appropriate affinity for the natural ligand (as determined by K_d)?*

- *Although not strict performance criteria, did the Saturation Binding Experiment perform as expected based on the protocol, regarding: values for K_d ranging from 0.03-1.5 nM and B_{max} ranging from 10-150 fmol/100 µg protein; linear Scatchard plot; consistent runs (K_d and B_{max} similar); and non-specific binding <20% total binding?*

- *For the Competitive Binding Experiment, discuss the estimated log IC_{50} for the test material compared to 17-β-estradiol and the positive control and the RBA for the test material compared to the positive control.*

- *For the Competitive Binding Assay, discuss the classification of the test substance (interactive, equivocal, non-interactive, or equivocal up to the limit of concentrations tested) and its RBA% (average and range)*

- *For the Competitive Binding Assay, were the performance criteria met? If any of the performance criteria were not met, include any justification or reason(s) and discuss how the failure of the assay run(s) to meet these criteria impact the study.*

C. STUDY DEFICIENCIES: *List each deviation from the protocol and classify the deviation as major or minor. Also report any rationale provided by the investigator's for the deviation. Similarly list, classify, and discuss all other deficiencies with the conduct, results, and reporting of the study. Discuss the possibility of resolving the deficiencies and what would be required. Major deficiencies may be presented and discussed in paragraph form, whereas minor deficiencies can be presented in a bulleted list.*