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: Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150

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 Unpublished.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 androgen receptor (AR) binding assay (MRID *(if applicable)* [number]), ventral prostate cytosol from Sprague Dawley rats was used as the source of AR to conduct a Saturation Binding Experiment and a Competitive Binding Experiment. The Saturation Binding Experiment was conducted to demonstrate that the AR isolated from rat prostate cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radio-labeled reference androgen (R1881) prior to routinely conducting AR Competitive Binding Experiments. The Competitive Binding Assay was conducted to measure the binding of a single concentration of [^{3}H]-R1881 ([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 assay concentration of [#]%. The assay included dexamethasone as a weak positive control, and R1881 as the ligand reference standard.

Provide a brief summary of the results and a concise discussion. In particular, mention the classification of the test compound (binder, equivocal, non-binder, or un-testable) with IC_{50} for binders and its 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 Experiment, 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 Experiment 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 Experiment, the estimated log IC_{50} for [test chemical] was [#] compared to R-1881 alone [#] and the positive control [#]. The test substance was classified as [binder, equivocal, non-binder, or un-testable]. The Relative Binding Affinity (RBA) for the test material was [#] % compared to the R1881 positive control ([#]%).

The study [satisfies/does not satisfy] the Test Order requirement for an Androgen Receptor Binding Assay (Rat Prostate Cytosol). (OCSPP 890.1150) *(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: *[Structure] or* Not available

3. Non-labeled ligand: R1881

Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #:
Batch #:
Purity: %
CAS #: 965-93-5

4. Radioactive ligand: [^{3}H]-R1881

Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog and Batch #:
Date of production: *Date for which the specific activity was certified*
Date of use: *Date*
Radiochemical purity: %
Specific activity: MBq/mg
Concentration of stock: Ci/mmol

5. Positive control: Dexamethasone

Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalog #
Batch #:
Purity:
CAS # : 50-02-2

6. Solvent/vehicle control: [Ethanol, Water, or Dimethyl sulfoxide (DMSO)]

Justification for choice of solvent:
Final Concentration:

B. METHODS

1. Preparation of Rat Ventral Prostate Cytosol: *Identify the source of rat prostate cytosol (i.e., the supplier), including information on the strain and age (at necropsy) of rats from which prostate glands were taken, and the time between castration and prostate excision. Describe the procedures for isolation of the cytosol, including: the number of animals; details of homogenizing buffer and homogenizing protocol; protein determination method and concentration; and storage conditions of AR (if applicable). 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.*

A total of [#] male Sprague Dawley rats were castrated at [#] days of age and were euthanized on the following day. The ventral prostates were excised immediately after termination, weighed, and placed in ice-cold TEDG (Tris, EDTA, DTT, glycerol) + PMSF (phenylmethylsulfonyl fluoride) buffer, homogenized, and centrifuged for [30] min at [30,000] × *g* at [4]ºC. Supernatant was pooled, discarding the resulting pellets. Protein concentration of the cytosol was determined to be [#] mg/mL using a commercially available protein kit compatible with DTT in the TEDG buffer (e.g., BioRad Protein Assay Kit, Richmond, CA). 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]-R1881 was performed to demonstrate that the AR was present in reasonable concentrations and had the appropriate affinity for the R1881 ligand. The conditions for the Saturation Binding Experiment are summarized in Table 1.

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

Source of receptor		Rat prostate cytosol
Concentration of radioligand (as serial dilutions)		[#]-[#] nM
Concentration of non-labeled ligand (100X [radioligand])		[#]-[#] nM nM
Optimization of receptor concentration		Sufficient to bind [#]-[#]% of radioligand at 0.25 nM
Temperature		[#]ºC
Incubation time		[#] hours
Composition of assay buffer (TEDG)	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 radioligand stock solution, date of production and date of use, and whether or not it was adjusted for decay; concentrations of [^{3}H]-R1881 and non-radiolabeled R1881; protein concentration and the protein assay used; 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 used by the laboratory.

On the day of the assay, the specific activity of the stock solution [^{3}H]-R1881 [was/was not] adjusted for decay over time, and serial dilutions in TEDG buffer were prepared to achieve the final concentrations in cytosol of [#, #, #, #, #, #, #, and #] nM to determine total binding. To determine non-specific binding, solutions of non-labeled R1881 were prepared in a similar manner to achieve concentrations that were 100-fold greater than each respective radiolabeled concentration, resulting in final concentrations in cytosol of [#, #, #, #, #, #, #, and #] nM. In the absence of cytosol, the radiation found in [7.5, 15, 21, 30, or 45] µL of [10] nM [^{3}H]-R1881 and [7.5, 15, or 30] µL of [100] nM [^{3}H]-R1881 was measured. For each batch of cytosol, the optimal protein concentration was determined by calculating specific binding to differing amounts of protein per tube, using [0.25] nM radiolabeled R1881. The optimal protein concentration was determined to be [#] mg protein/assay tube, which resulted in the binding of [25-35]% of the total radioactivity added. Cytosolic protein used in this assay was thawed fresh for this experiment at [4]°C and maintained at [4]°C during the binding assay. Each run contained three concurrent replicates at each concentration, resulting in the [72] samples depicted in Table 2.

TABLE 2. Saturation Binding Experiment Run [a] [b]

Total Binding	Non-Specific Binding		Radioligand alone	
Tubes 1-24 [c]	Tubes 25-48 [d]		Tubes 49-72 [e]	
[^{3}H]-R1881 Final conc. (nM)	[^{3}H]-R1881 Final conc. (nM)	R1881 Final conc. (nM)	[^{3}H]-R1881 Initial conc. (nM)	[^{3}H]-R1881 (µL)
0.25	0.25	25	10	7.5
0.50	0.50	50	10	15
0.70	0.70	70	10	21
1.00	1.00	100	10	30
1.50	1.50	150	10	45
2.50	2.50	250	100	7.5
5.00	5.00	500	100	15
10.00	10.00	1000	100	30

a Data were obtained from page [#] of the study report.
b Each concentration was run in triplicate for a total of 72 samples.
c Tubes 1-24 contained 50 µL of triamcelenone acetate and 7.5-45 µL [^{3}H]-R1881. Samples were dried, and 300 µl of prostate cytosol were added.
d Tubes 25-48 contained 50 µL of triamcelenone acetate and 7.5-45 µL [^{3}H]-R1881. R1881 was added in a 100-fold molar excess of [^{3}H]-R1881 in a volume of 7.5-45 µL. Samples were dried, and 300 µl of prostate cytosol were added.
e Tubes 49-72 contained only 7.5, 15, 21, 30, or 45 µL of 10 nM [^{3}H]-R1881 or 7.5, 15, 21, or 30 µL of 100 nM [^{3}H]-R1881 without cytosol or other components to determine the total counts added.

Following addition of triamcelenone acetate, [^{3}H]-R1881, and/or R1881, the tubes were dried, dissolved in diluted prostate cytosol (300 µL), and incubated in a rotor for 20 hours at 4°C.

Samples were maintained at temperatures of 1-4°C except during whole rack vortexing. To separate bound from free R1881, hydroxyapatite (HAP) slurry was added to each tube and vortexed once every 5 minutes for 20 minutes. The samples were then centrifuged, and the supernatant was aspirated and discarded. The samples were washed 3 to 4 times in 50 mM TRIS buffer. Following the last wash and decanting of the Tris buffer pellets were then extracted by additional of [x] ml ethanol. The samples were vortexed 3 times at 5 minute intervals. Samples were maintained on ice at all times between vortexing. Each ethanol supernatants was then decanted into a scintillation vial, and the radiation was quantified by liquid scintillation counting. A total of [#] runs were performed.

3. **Competitive Binding Experiment:** A summary of the assay 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 ventral prostate cytosol
Concentration of radioligand		[#] nM
Optimization of receptor concentration		Sufficient to bind [#]% of 1.0 nM radioligand [b]
Concentration of test substance (as serial dilutions)		[10^{-10} to 10^{-3}] mM [c]
Incubation 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.
b Receptor concentration may need to be adjusted.
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.

Provide a brief synopsis of the methods. Be sure to report:

- *Concentration range and spacing of the test substance, reference chemical, weak positive control, and solvent controls, with justification if deviating from recommended range and spacing.*
- *Dilution schemes used for preparing the concentrations of R1881, weak positive, and test chemical.*
- *Notes on any abnormalities or problems during the conduction of the assay (for example, problems during separation of free radiolabeled R1881 from bound, or the analysis of bound R1881 or the weak positive).*
- *Address ligand depletion, which should be minimal. The recommended ratio of total binding in the absence of competitor to the total amount of [^{3}H]-R1881 added per assay tube should be no greater than 10-15%.*

Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory.

The Competitive Binding Experiment was performed according to the protocol provided in the EPA Test Guidelines OPPTS 890.1150. The Competitive Binding Experiment measures the binding of a single concentration of [^{3}H]-R1881 (adjusted specific activity of [#] MBq/mg) to the AR in the presence of increasing concentrations of a test substance.

Ethanol/water/DMSO was used as a vehicle, and no precipitation was observed at [#] M by monitoring absorbance at [650] nM with a spectrophotometer. Results from the Saturation Binding Experiment demonstrated that [1.0] mg/ml or mg/assay tube of cytosolic protein contains enough receptor to bind [no more than 10-15%] of the [^{3}H]-R1881.

Dilutions of the test substance, reference standard (R1881), weak positive control (dexamethasone), and solvent control (ethanol) were prepared to achieve the concentrations shown in Table 4. Each assay consisted of [three] independent runs on [three] different days, and each run contained three replicates at each concentration, resulting in a total of 81 samples per run.

TABLE 4. Competitor Final Molar (M) Concentrations in Competitive Binding Assay [a] [b]

Solvent Control	**Reference standard**	**Weak positive control**	**Test Chemical**	None
[Ethanol]	R1881	Dexamethasone	[#]	
Tubes 1-3 and 70-72	Tubes 4-21 and 73-75 [c]	Tubes 22-45	Tubes 46-69	Tubes 76-81
	1×10^{-6}	1×10^{-3}	1×10^{-3}	
	1×10^{-7}	1×10^{-4}	1×10^{-4}	
	1×10^{-8}	1×10^{-5}	1×10^{-5}	
	1×10^{-9}	1×10^{-6}	1×10^{-6}	
	1×10^{-10}	1×10^{-7}	1×10^{-7}	
	1×10^{-11}	1×10^{-8}	1×10^{-8}	
	--	1×10^{-10}	1×10^{-10}	

a Data were obtained from page [#] of the study report.
b Each concentration of each chemical was run in triplicate for a total of 81 tubes per run. Tubes 1-75 contained 50 µL of triamcelenone acetate and 30 µL [^{3}H]-R1881. Samples were dried, and 300 µl of prostate cytosol were added. Tubes 1-75 also contained 10µL of the solvent control, reference standard (non-radiolabeled R-1881), weak positive control, or test substance, with the exception of Tubes 4-6 and 73-75 that contained 30 µL of non-radiolabeled R1881 (used to evaluate non-specific binding). Tubes 76-81 contained only 30 µl of [^{3}H]-R1881.
c Tubes 4-6 and 73-75 were used to evaluate non-specific binding by adding 100X of cold (non-radiolabeled) R1881.

The procedures for assay incubation, separation of bound from free R1881 (washing with buffer and extraction with ethanol), followed by scintillation counting of bound [^{3}H]-R1881 were similar to those used in the Saturation Binding Experiment. A total of [#] runs were performed as described.

4. **Data Analysis:** *Detail the methods used 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 IC_{50}. When software is used for data analysis, report the software title, version number, and source (company, location). If the runs are compared to each other statistically, designate the statistical method that was used and the software employed.*

Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory.

The maximal binding capacity (B_{max}), dissociation constant (K_d), and inhibition concentration (IC_{50}) were calculated using nonlinear regression analysis using Graph Pad Prism v. 5 (GraphPad Software, Inc., La Jolla, CA). Scatchard plots were also plotted for the binding data (GraphPad Software, Inc., La Jolla, CA). Automatic outlier elimination for binding data was performed using the method of Motulsky and Brown (2006)[1] with a Q value of 1.0, implemented by using the ROUT procedure of Prism 5. Receptor binding data plots were corrected for ligand depletion using the method of Swillens (1995)[2] using Prism 5. Mean and standard deviation were calculated for each run of the Saturation and Competitive Binding Experiments using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA), and mean and standard error were calculated for the composite three runs using Excel.

5. Definitions

a. Classification of test material

If the data fit a 4-parameter nonlinear regression model, the test chemical is classified as:

Binder: The average curve for the test chemical across runs crosses 50% of radioligand bound.

Equivocal: The average lowest portion of curves across runs is between 50% and 75% radioligand binding (*i.e.* radioligand displacement is at least 25% but less than 50%) , or the curve falls outside the range for the weak positive control (-0.6 to -1.4).

Non-Binder: The average lowest portion of curves across runs is greater than 75% activity (*i.e.* less than 25% displacement of radioligand), or the data do not fit the model.

Untestable: If the test compound is not soluble above 1×10^{-6} M and the binding curve does not cross 50%, the chemical is judged to be untestable.

b. Descriptors for receptor binding

B_{max}: maximal binding capacity

K_d: dissociation constants

IC_{50}: Concentration of the test substance at which 50% of radioligand is displaced from the AR by the competitor

Relative Binding Affinity (RBA): IC_{50} of R1881 × 100 ÷ IC_{50} of test substance

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[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.

II. RESULTS

A. SATURATION BINDING EXPERIMENT: *Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory.*

Saturation Binding Experiment parameters are presented in Table 5. The dissociation constant (K_d) for [^{3}H]-R1881 was [mean (±SE)], and the estimated B_{max} was [mean (±SE)] for the single batch of prostate cytosol that was prepared. The K_d was within the range reported in the EPA validation program. Confidence in these numbers is [high/low] according to the goodness of fit (r^2 = [#-#]) and the [small/large] variation among runs. *(The following table is mandatory.)*

TABLE 5. Saturation Binding Experiment of R-1881 with Androgen Receptor from Rat Prostate Cytosol[a]

Parameter	Run 1 [b]	Run 2 [b]	Run 3 [b]	Mean Runs 1-3 [c]
R^2 (unweighted?)				
B_{max} (nM)				
B_{max} (fmol/100 µg protein)				
K_d (nM)				

a Data were obtained from page [#] of the study report.
b The mean and standard deviation are reported for the concurrent replicates of each run.
c The range of R^2 is reported and the mean ± SEM is reported for the other parameters.
R^2= Goodness of fit for curve calculated for specific binding

Figure 1 illustrates the non-specific, specific, and total binding curves for [^{3}H]-R1881 to the androgen receptor. The specific binding reached a plateau, and non-specific binding was less than 20% of total binding. Figure 2 is a Scatchard plot that illustrates the binding of [^{3}H]-R1881 to the androgen receptor. The data fit results in a linear plot.

Figures similar to the examples below are mandatory. Provide a graph from the study report depicting total, specific, and non-specific binding across the range of concentrations tested, including the following information:

- *Each data point should be plotted for total, non-specific binding and specific binding. The fitted curves for total, specific, and non-specific binding for each run should be included, plotting binding (dpm) by concentration of R1881 (nM).*
- *Each run should be differentiated by a different color.*

For the example Scatchard plot:

- *Provide a figure plotting the specific bound/free by specific bound (molar).*
- *Each run should be differentiated by a different color.*

Figure 1. Binding of [^{3}H]-R1881 to the Androgen Receptor during the Saturation Binding Experiment.

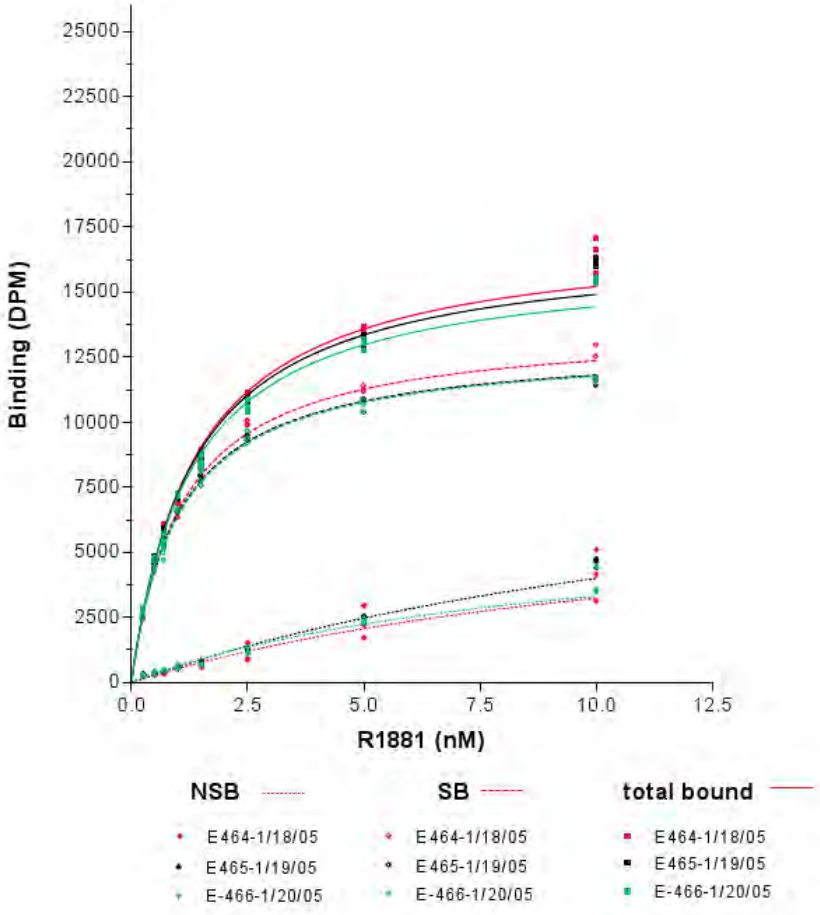
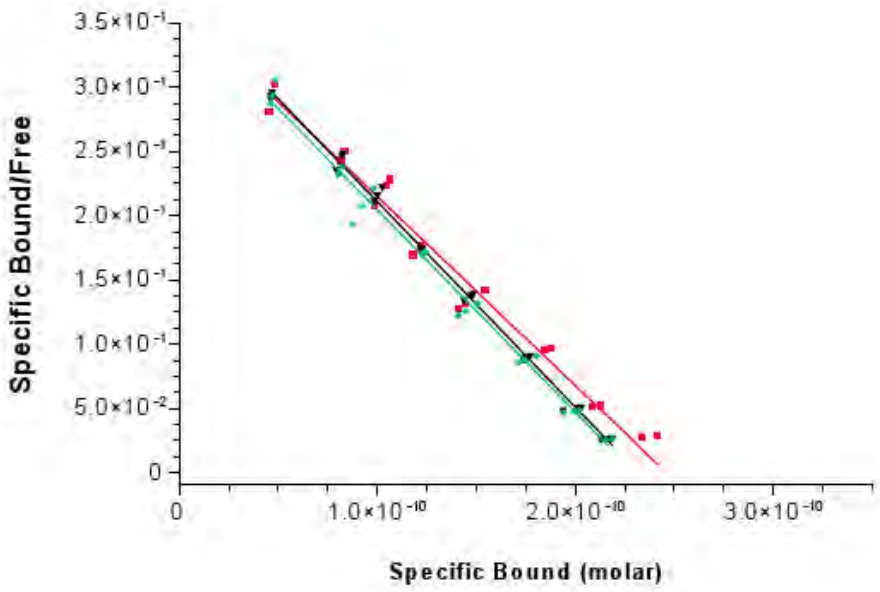


Figure 2. Scatchard Plot of the Binding of [^{3}H]-R1881 to the Androgen Receptor.



B. <u>COMPETITIVE BINDING EXPERIMENT</u>: *Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory.*

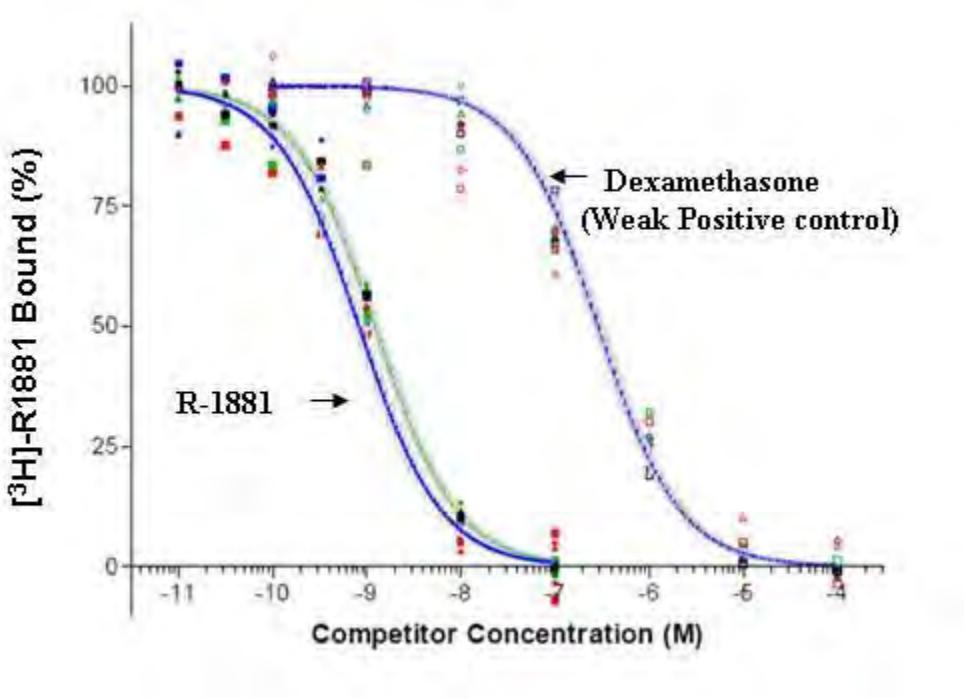
Competitive Binding Experiment parameters are presented in Table 6. The estimated mean log IC_{50} was [#] for the test material compared to that of R-1881 of [#] and the weak positive control of [#]. The mean RBA was [#]% for the test material compared to [#]% for the positive control. Confidence in these numbers is [high/low] due to the [small/large] variation. No precipitation of the test compound was observed absorbance @650nm. The solvent control responses indicated no drift in the study assay. *If repeat runs were necessary, provide an explanation. Some form of the following table is mandatory.*

TABLE 6. Competitive Binding Assay of [test chemical] with AR from Rat Prostate Cytosol [a]					
Parameter		Run 1 [b]	Run 2 [b]	Run 3 [b]	Mean ± SE[c]
r^2 (unweighted)	R1881	#			NA
	Positive control	#			NA
	Test substance	#			NA
Log IC_{50} (nM)	R1881				
	Positive control				
	Test substance				
IC_{50} (nM)	R1881				
	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.
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 = Goodness of fit (r^2 is more appropriately expressed as a range, as opposed to a mean).
RBA (%) = relative binding affinity

- *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 (R-1881) and weak positive control (dexamethasone) 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 test material, which must also be included).* The preferred graph shows percent R1881 Bound (y-axis) vs log molar competitor concentration (x-axis) because it allows for an easier visual representation of the IC_{50}.
- Include a second graph which plots the curve of the mean (± SEM) across all 3 runs. This graph should not include plots for the non-radiolabeled reference standard (R1881) or the weak positive (dexamethasone).
- Similar graphs for inert R1881 and the weak positive controls are required to be reported in the study report, but are only necessary in the DER if there is large variation or anomalous results that the additional graphs would illustrate.

Figure 3. Percentage R1881 Bound to the Androgen Receptor in the Presence of Test Compound.



C. PERFORMANCE CRITERIA: To ensure that the Competitive Binding Assay was functioning 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.*

TABLE 7. Criterion [a]	Tolerance Limit(s) [b]	Value	Yes	No
Ligand depletion is minimal. The recommended ratio of total binding in the absence of competitor to total amount of [^{3}H]-R1881 added per assay tube.	≤15%			
Test chemical Top (% binding)	80 to 115			
R1881 fitted curve parameters				
Top (% binding)	82 to 114			
Bottom (% binding)	-2.0 to 2.0			
Hill Slope	-1.2 to -0.8			
Weak positive control (dexamethasone) fitted curve parameters				
Top (% binding)	87 to 106			
Bottom (% binding)	-12 to 12			
Hill Slope	-1.4 to -0.6			
Saturation Binding Experiment K_d (nM)	0.8121 to 0.9698			
Non-specific binding (%)	8.1-10.0			

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

b These values represent ranges from the validation study. *(It is suggested that an additional run be made when a run does not fall within these ranges, particularly if that run differs from the other 2 runs.)*

The following text is required, but should be adjusted to accurately report the results of a particular study. Example text follows.

Additionally, the curve for the reference material showed that increasing concentrations of unlabeled R1881 displaced [^{3}H]-R1881 in a manner consistent with one-site binding, as indicated by a descent from 90% to 10% binding over approximately an 81-fold increase in concentration of R1881 (i.e., covering approximately 2 log units). 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. <u>INVESTIGATOR'S CONCLUSIONS</u>: *Provide a brief paragraph of the investigators' conclusions.*

B. <u>REVIEWER COMMENTS</u>: *Briefly summarize the results and discuss the following:*

- *Did the Saturation Binding Experiment demonstrate that the AR was present in a reasonable concentration (as indicated by B_{max}) and functioning with appropriate affinity for the R1881 ligand (as determined by K_d)?*
- *Although not strict performance criteria, did the Saturation Binding Experiment perform as expected based on the validation tests, regarding: values for K_d ranging from* 0.685 to 1.57 *nM and B_{max} ranging from 7 to 16 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 R1881 and the weak positive control and the RBA for the test material compared to the weak positive control.*
- *For the Competitive Binding Assay, discuss the classification of the test substance (binder, equivocal, non-binder, or un-testable) 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. <u>STUDY DEFICIENCIES:</u> *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 to do so. Provide a classification statement that indicates if this study was an acceptable guideline study. Major deficiencies are typically presented and discussed in paragraph form, whereas minor deficiencies can be presented in a bulleted list.*