Appendix 6

Final report from the second interlaboratory validation study

Estrogen Receptor Binding Assay using Rat Uterine Cytosol (ER-RUC assay)

Lab X

Note: The analyses, summary, and conclusions in this report were prepared by the individual laboratory. For reasons described in the Integrated Summary Report (ISR) for the ER-RUC assay, data were normalized in a different way for the final analysis that is presented in the ISR. Thus the analyses, summary, and conclusions in this report may differ from those in the ISR.

Final Report

Task Order 6:
Second Inter-laboratory Validation of the Estrogen Receptor Binding Assay (Rat Uterine Cytosol)

Task 7 – Test Coded Chemicals

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Quality Assurance Statement

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This study was audited by the Sciences and Engineering – Quality Assurance Unit and the results of the inspections and audits were reported to the Study Director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Data Audit	February 18-22 and 26-29, and March 3, 2008	March 3, 2008
Report Audit	March 21, 2008	March 21, 2008
Data Audit	April 2-4, 7, 8, 11 and 14-18, 2008	April 18, 2008
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Data Audit	May 12-16 and 19, 2008	May 19, 2008
Report Audit	May 16 and 19-20, 2008	May 20, 2008
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1.0 Introduction

This report describes the estrogen receptor binding assays conducted in RTI's DMPK laboratory for Task 7 "Test Coded Chemicals" of EDSP Task Order 6. The first 23 test chemicals assayed in the rat uterine cytosol estrogen receptor (RUC ER) assay on Task 7 are included in this report. A separate report will be issued for the additional 20 chemicals assayed as part of the Task 7 option. This report also describes the preparation of cytosol and the saturation binding assays conducted on each batch of cytosol. Data and results from the testing of the first 5 test chemicals were included in a previously issued report, which is appended to this report.

2.0 Materials and Methods

2.1 Cytosol Isolation Procedure

Rat uteri were collected from ovariectomized rats, flash frozen in liquid nitrogen and stored at ca. -70 to -80°C. The frozen uteri were transferred to an ice cold TEDG-PMSF buffer at a ratio of 0.1 g tissue to 1 mL buffer. After the uteri had thawed, they were homogenized using a Polytron (PT35/10, Brinkmann) homogenizer with 3 to 5 bursts of about 5 sec per burst. The homogenizer probe was prechilled in an ice cold buffer and was chilled between each burst. The tissue samples were kept chilled throughout the entire isolation process. The homogenates were transferred to chilled centrifuge tubes and were centrifuged at 2500 *g* for 10 min at 4°C using an Allegra X-15R centrifuge. The supernatants were transferred to chilled ultracentrifuge tubes and centrifuged at ca. 105000 *g* for 60 min at 4°C using a Beckman Model L5-50 with an 80Ti rotor. The supernatants (cytosol) were combined, divided into volumes appropriate for the conduct of assays, flash frozen in liquid nitrogen and stored in an ultracold freezer at <-70°C. Cytosol preparations were identified by their preparation date. Two cytosol preparations were made and used in this work.

2.2 Protein Assay Procedure

Cytosol was diluted in TEDG + PMSF buffer (1:5 or 1:10) and was assayed for protein content using a BioRad Protein Assay kit (Catalog # 500-0002). A standard curve (range 0.22 to 0.89 mg/mL) was prepared using bovine serum albumin. Standards, QC samples and cytosol samples (100 μL) were aliquoted in triplicate into tubes. Diluted dye reagent (5 mL of a 1:5 dilution of stock) was added to each tube and the samples were mixed by vortexing. Samples were allowed to sit at room temperature at least 5 min, but less than 1 h prior to being transferred into disposable cuvettes. Absorbance at 595 nm was measured using a BioSpec mini spectrophotometer. The concentration of protein in the cytosol samples was calculated based on the standard curve.

2.3 Saturation Binding Assays

The saturation binding assay was conducted in triplicate for each lot of cytosol preparation used. The assays were conducted as described in the protocol and as reported in the Saturation Binding reports.

2.4 Competitive Binding Assays

Competitive Binding assays were conducted as described in the protocol and as reported in the Competitive Binding reports. The maximum concentration of ethanol used in any assay tube was maintained below 3% (See Appendix A for calculations). Assay worksheets showing the methods used for preparation of reference and control chemical dilutions and the volumes of reagents used in each assay tube are presented in Appendix B. Test chemicals were assayed in groups of 3 or 4 and were assayed in the competitive binding assay along with estradiol standard and norethynodrel positive control. The negative control, R1881, noted in the protocol was not used in competitive binding assays for Sets 2-6 per instructions from EPA after review of the data from Set 1. Each set of test chemicals was assayed in at least 3 independent runs of the assay.

3.0 Results

3.1 Protein Assay

Two cytosol preparations were assayed for protein content. The first (1/23/08) contained between 2.2 mg protein/mL while the second (3/19/08) contained 2.3 mg protein/mL. Protein assay results are presented in Appendix C.

3.2 Saturation Binding Assays

Saturation binding assays were conducted in triplicate for both of the cytosol preparations (1/23/08 and 3/19/08). The assay results for Sat 1-3 are included in the report for Set 1 (Appendix F) and assay results for Sat 4-6 are presented in Appendix D. The assay results were evaluated against the points outlined in Section 8.6.3 of the protocol and summary data are presented in Table 1 below.

Table 1. Evaluation of Saturation Binding Assay Results

Assay ID	Cytosol Prep	Plateau reached ?	Linear Scatchard ?	K_d (nM)	RSE of K_d (%)	B_{max} (fmole/ 100 µg)	RSE of B_{max} (%)	NSB acceptable? [a]
Sat 1	1/23/08	Y	Y	0.1508	10.9%	71.03	3.2%	Y
Sat 2	1/23/08	Y	Y	0.1466	12.5%	58.02	3.9%	Y
Sat 3	1/23/08	Y	Y	0.1813	18.9%	59.84	5.6%	Y
Average				0.1596				
Sat 4	3/19/08	Y	Y	0.6457	6.8%	52.09	2.7%	Y
Sat 5	3/19/08	Y	Y	1.1810	28.8%	84.96	13.1%	Y
Sat 6	3/19/08	Y	Y	0.3691	7.0%	57.16	2.4%	Y
Average (4,5,6)				0.7319				
Average (4.6)				0.5074				

[a]NSB is acceptable if it is <50% of total binding.

Saturation binding assay data (Sat 1-3) from the assays using the 1/23/08 cytosol preparation all have low relative standard errors (RSE) and are consistent between assays. Average Kd for these assays is 0.1596 nM, which is within the 0.05 to 0.5 nM range for literature values that the protocol indicates is reasonable. Saturation binding data from Sat 4 and Sat 6 also have low RSE and fairly good consistency between assays. Average Kd for these assays is 0.5074 nM, which is comparable with the expected range listed in the protocol. Data from Sat 5 have high RSE (above the 20% recommended limit for Kd) and the Kd and Bmax for this assay are notably higher than for the other assays using this cytosol preparation. For these reasons, the data from Sat 5 will not be included in the average Kd value used for assessment of the competitive assays.

3.3 Competitive Binding Assays

At least 3 runs of the competitive binding assay were completed for each set of test chemicals. A summary of the assays conducted is presented in Table 2. Assay reports for each assay are presented in Appendix E. Data for the performance criteria parameters are presented in Table 3. In our laboratory, one technician did the assay set up, through placing the tubes on the rotator, and a second technician did the tasks associated with the second day of the assay, i.e., separating bound from free and sampling for scintillation counting. Plots of the standards (estradiol and norethynodrel) for each run are presented in Appendix G. Plots of the competitive binding curves for each test chemical are presented in Appendix H. Plots showing both the standard and test chemicals are presented in Appendix I.

Table 2. Summary of Assays Conducted

Set #	Test Chemicals	Assay ID	Assay Date	Cytosol Prep
2	4, 5, 16, 22	S2R1	2/21/2008	1/23/08
		S2R2	2/26/2008	1/23/08
		S2R3	2/27/2008	1/23/08
		S2R4	3/3/2008	1/23/08
3	9, 14, 17, 20	S3R1	2/28/2008	1/23/08
		S3R2	3/4/2008	1/23/08
		S3R3	3/5/2008	1/23/08
4	1, 3 ,8, 23	S4R1	3/6/2008	1/23/08
		S4R2	3/11/2008	1/23/08
		S4R3	3/13/2008	1/23/08
		S4R4	3/18/2008	1/23/08
		S4R5	4/3/2008	1/23/08
5	2, 10, 18	S5R1	3/17/2008	1/23/08
		S5R2	3/27/2008	1/23/08
		S5R3	3/31/2008	1/23/08
6	6, 7, 13	S6R1	4/1/2008	3/19/08
		S6R2	4/7/2008	3/19/08
		S6R3	4/8/2008	3/19/08
		S6R4	6/9/2008	3/19/08

Table 3. Performance Criteria

Criterion	Standard	Assay ID Set 2					Assay ID Set 3		
		Run 1	Run 2	Run 3	Run 4	Run 1_norm	Run 1	Run 2	Run 3
Maximum solvent concentration in assay									
Ethanol	< 3%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%
DMSO	< 10%	NA	NA	NA	NA	NA	NA	NA	NA
Ligand depletion	< 10%	0.97	5.48	5.19	5.82	0.97	4.74	4.51	3.50
17β-Estradiol									
Binding Curve Descent	90-10% over approximately 2 log units	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IC50 (nM)	about 1 nM + Kd	**10.754**	0.830	0.834	0.814	0.723	0.999	1.086	0.943
Curve fit parameters:									
Top	90<Top<110	**732**	106	**111**	110	101	**112**	**119**	102
Bottom	-5<Bottom<1	**-9**	-0	-1	-1	-1	0	-1	-1
Slope	-1.1<Slope<-0.7	-0.9	-1.1	-1.0	-1.0	-0.9	-1.1	-1.0	**-1.2**
Within run SD	≤5.0	**19.3**	2.8	4.8	3.3	2.7	4.9	2.5	2.9
Norethynodrel									
Curve fit parameters:									
Top	90<Top<110	**723**	106	**115**	**112**	100	99	**111**	97
Bottom	-5<Bottom<1	**-28**	-2	-3	-5	-4	-4	**2**	**-11**
Slope	-1.1<Slope<-0.7	-0.9	-0.8	-0.9	-0.8	-0.9	-0.8	-1.1	-1.0
Within run SD	≤5.7	**40.0**	**6.2**	4.6	4.8	5.5	3.4	5.3	3.9

Table 3. Performance Criteria (continued)

Criterion	Standard	Assay ID								
		Set 4								
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 1_norm	Run 3_norm	Run 4_norm	Run 5_norm
Maximum solvent concentration in assay										
Ethanol	< 3%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%	2.99%
DMSO	< 10%	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ligand depletion	< 10%	3.02	3.54	2.46	0.55	3.48	3.02	2.46	0.55	3.48
17β-Estradiol										
Binding Curve Descent	90-10% over approximately 2 log units	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
IC50 (nM)	about 1 nM + Kd	**1.503**	1.325	**1.711**	**12.781**	1.305	0.893	1.022	0.953	0.704
Curve fit parameters:										
Top	90<Top<110	**136**	**111**	**132**	**777**	**137**	104	96	102	101
Bottom	-5<Bottom<1	-1	0	-1	-1	-3	-1	-1	0	-2
Slope	-1.1<Slope<-0.7	-0.9	-1.2	-1.1	-1.0	-0.8	-0.9	-1.1	-1.0	-0.8
Within run SD	≤5.0	**5.1**	4.5	**6.0**	**87.5**	4.0	3.9	4.4	**11.5**	2.9
Norethynodrel										
Curve fit parameters:										
Top	90<Top<110	**127**	109	**128**	**706**	**125**	97	93	92	92
Bottom	-5<Bottom<1	-1	0	-1	-1	-3	-2	-2	1	-2
Slope	-1.1<Slope<-0.7	-0.8	**-1.2**	-0.9	-1.1	-0.8	-0.8	-0.9	-1.1	-0.8
Within run SD	≤5.7	5.0	4.2	**6.3**	**55.6**	5.1	3.8	4.6	**7.3**	3.7

Table 3. Performance Criteria (continued)

Criterion	Standard	Assay ID Set 5				Assay ID Set 6				
		Run 1	Run 2	Run 3	Run 3_norm	Run 1	Run 2	Run 3	Run 2_norm	Run 4
Maximum solvent concentration in assay										
Ethanol	< 3%	2.99	2.99	2.99	2.99	2.99%	2.99%	2.99%	2.99%	2.99%
DMSO*	< 10%	1.0	1.0	1.0	1.0	NA	NA	NA	NA	NA
Ligand depletion	< 10%	3.05	1.76	4.25	4.25	2.19	4.13	2.79	4.13	4.39
17β-Estradiol										
Binding Curve Descent	90-10% over approximately 2 log units	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IC50 (nM)	about 1 nM + Kd	1.121	**2.044**	1.081	0.656	1.385	1.103	1.301	0.748	8.90
Curve fit parameters:										
Top	90<Top<110	**114**	**113**	**138**	102	**122**	**126**	**134**	102	110
Bottom	-5<Bottom<1	-1	-2	1	0	-2	-1	-5	-1	-1
Slope	-1.1<Slope<-0.7	-1.0	-1.0	-1.1	-1.1	-0.9	-1.0	-0.7	-1.0	-1.0
Within run SD	≤5.0	4.1	4.8	**6.1**	4.5	4.1	**5.5**	**6.8**	4.5	3.5
Norethynodrel										
Curve fit parameters:										
Top	90<Top<110	105	106	**132**	97	104	**123**	110	100	98
Bottom	-5<Bottom<1	-1	-2	1	0	-2	**-5**	-2	-4	-3
Slope	-1.1<Slope<-0.7	-0.9	-1.1	-0.9	-0.9	-0.9	-0.7	-0.9	-0.7	-0.9
Within run SD	≤5.7	4.4	**7.2**	**5.9**	4.3	**7.0**	**11.2**	**7.3**	**9.1**	3.7

*DMSO was used as the solvent for TC18 at the direction of the Contractor Task Leader.

3.3.1 Set 2: Test Chemicals 4, 5, 16, and 22

A total of four runs were conducted for this set. For Run 1, the solvent tubes (i.e., total binding tubes) had unexplained low DPMs. DPMs in standard and positive control tubes were similar to those found in other assays. Because the percent of radioligand bound is calculated using the total binding tubes as 100%, the low DPMs in the total binding tubes resulted in an apparently very high percent of radioligand bound for the standard and test chemical samples. Since the percent of radioligand bound at the lowest concentration (10 pM) of estradiol should be approximately equal to the total binding tubes (i.e., 100%), the data can be normalized by using the DPM for the 10 pM estradiol tubes in place of the total binding DPM. The results of this analysis are labeled as 'Norm' or normalized.

Performance Criteria. With the exception of the un-normalized Run 1 data, data for all runs in this set met performance criteria or were just outside the acceptable ranges. The criterion for Top was not met for estradiol in Run 3 and for norethynodrel for Runs 3 and 4, though these values were all just outside the acceptable range (all <115). The normalized Run 1 data met all performance criteria. The within run SD for norethynodrel for Run 2 was just slightly higher than the 5.7% criterion limit.

Test Chemical Results. With the exception of Test Chemical (TC) 22, it was possible to fit the data from each test chemical run to the IC50 equation. No IC50 could be calculated for TC22 in Runs 1-3 because Bottom was >50%. Concentrations of each test chemical used in the assay were changed for Run 4 to eliminate excess points where the radioligand was not displaced and also to eliminate concentrations that did not maintain solubility in the assay tubes. Using these adjusted concentrations, data were obtained for TC22 in Run 4 that allowed for the calculation of IC50. For most of the chemicals the curves for Runs 2-4 are similar, the exception being Run 3 of TC16, which is displaced to the left. Similar IC50, Ki and RBA are found across Runs 2-4 for each chemical.

3.3.2 Set 3: Test Chemicals 9, 14, 17, 20

A total of 3 runs were conducted for this set. None of the assays were normalized.

Performance Criteria. There were a few Top values that were outside the acceptable ranges, the most notable of which was a 119% obtained for estradiol Top in Run 2. The Bottom of the norethynodrel curve for Run 3 was below the acceptable range at -10.93. The central portions of each standard and positive control curve were overlaid across the runs.

Test Chemical Results. Test chemicals 9, 14, and 20 did not displace more than 50% of the radioligand in any run and so no IC50 could be calculated. Run to run variation in the IC50, Ki and RBA for TC17 was low.

3.3.3 Set 4: Test Chemicals 1, 3, 8, and 23

A total of 5 runs were conducted for this set. In Runs, 1, 3, and 5, low total binding DPMs caused the Top of the estradiol and norethynodrel curves to run about 135 and 125, respectively. For Run 4, the total binding counts were very low and led to extremely high percent of radioligand bound for all standards and chemicals. The data for Runs 1, 3, 4, and 5 were normalized as described for Set 2

above. This report includes normalized and un-normalized results for all runs except Run 2, which was not normalized.

Performance Criteria. For the un-normalized Runs 1, 3, and 4 data sets, other than the Top values, most performance criteria were met. The exception was the within run SD for Run 3 (estradiol and norethynodrel), which were both just slightly higher than the limit. For the un-normalized Run 4, within run SDs were far above (i.e., > 10-fold) the limit. In the normalized data sets, all performance criteria were met except for the within run SDs for Run 4-norm, which are still high at 11.46 and 7.28, respectively, for estradiol and norethynodrel.

Test Chemical Results. The Competitive Binding reports (Appendix E) present IC50, Ki and RBA for each test chemical calculated using both normalized and un-normalized data. With the exception of the Run 4 data, these values are similar across all runs, whether or not the data are normalized. The TC8 data appear to have the most run to run variance.

3.3.4 Set 5: Test Chemicals 2, 10, and 18

A total of 3 runs were conducted for this set. Data from Run 3 are presented normalized and un-normalized.

Performance Criteria. The Top of the estradiol curve is >110 for all runs but is only markedly higher than the limit for Run 3 (138.1). The Top of the norethynodrel curve for Run 3 is also elevated (131.7). Other performance criteria are met except that the within run SD for Run 3 is slightly high for estradiol and norethynodrel and that for Run 2 is slightly high for norethynodrel. The normalized Run 3 data set met all performance criteria.

Test Chemical Results. IC50s were calculated for TC2 and TC10. TC18 did not displace more than 20% of the radioligand at any soluble concentration, so no IC50 could be calculated. RBA for each chemical were similar across runs.

3.3.5 Set 6: Test Chemicals 6, 7, and 13

A total of 4 runs were made with this set. Data from Run 2 are presented both normalized and un-normalized.

Performance Criteria. The Top of the estradiol curve ranges from 110- 134 across the 4 runs, with that for Run 3 being the highest. The Top of the norethynodrel curve is 123 in Run 2. Within run SDs are out of range on most data sets, but are generally <7.5.

Test Chemical Results. Despite the fact that there is some scatter at the top of the curves the central portion of the test chemical and standard and control curves show little run to run variation. This leads to similar IC50, Ki and RBA across the runs.

4.0 Discussion

4.1 Characterization of the Cytosol Batches

Saturation binding assay were conducted on each batch of cytosol to establish ER specificity and saturation. The receptor was saturable in all cases with low non-specific binding and linear Scatchard plots. Values for Kd and Bmax were determined for each cytosol preparation. The average Kd for the first batch of cytosol (1/23/08), which was used for the assay of Sets 1-5 was 0.1596 nM and that for the second batch of cytosol (3/19/08) was 0.5074 nM and both are comparable to the 0.05 to 0.5 nM range for literature values that the protocol indicates is reasonable. Relative standard errors for Kd and Bmax were less than 20% for all accepted saturation assays.

4.2 Evaluation of Reference Ligand and Positive Control Data

IC50 data for all runs of the estradiol standard and IC50, Ki and RBA for all positive control runs are summarized in Table 4. The highlighted values are not included in any averages. The formula describes the relationship between IC50 and Kd: IC50 = [radioligand] + Kd. In the present assays, [radioligand] = 1 nM and the Kd for the cytosol used in Sets 1-5 was 0.1596 nM and that for the cytosol used in Set 6 was 0.5074 nM. Therefore, the expected IC50 for estradiol in Sets 1-5 should be 1.16 nM and that in Set 6 should be 1.5 nM. The experimentally determined average IC50s for estradiol were 1.12 ± 0.37 nM for Set 1-5 and 1.26 ± 0.12 nM for Set 6, both of which are comparable to the expected values..

The average IC50 for norethynodrel over the un-normalized runs was 943 ± 318 nM and the average RBA was 1.24E-3 ± 1.83E-4 (14.8% CV).

Table 4. Standard and Control Parameter Summary

Set	Run	Estradiol	Norethynodrel		
		IC50	IC50	Ki	RBA
1	1	0.73	552	76	1.34E-03
	2	0.97	996	137	9.73E-04
	3	0.69	652	90	1.06E-03
2	1*	10.75	7979	1098	1.35E-03
	2	0.83	589	81	1.41E-03
	3	0.83	688	95	1.20E-03
	4	0.81	636	87	1.29E-03
	1_Norm	0.72	624	86	1.17E-03
3	1	1.00	787	108	1.26E-03
	2	1.09	879	121	1.26E-03
	3	0.94	924	127	1.00E-03
4	1	1.50	1248	172	1.20E-03
	2	1.32	1314	181	1.00E-03
	3	1.71	1736	239	9.77E-04
	4*	12.78	10752	1480	1.20E-03
	5	1.30	886	122	1.48E-03
	1-Norm	0.89	678	93	1.32E-03
	3-Norm	1.02	923	127	1.10E-03
	4-Norm	0.95	965	133	1.00E-03
	5-Norm	0.70	457	63	1.55E-03
5	1	1.12	952	131	1.17E-03
	2	2.04	1619	223	1.26E-03
	3	1.08	898	124	1.20E-03
	3_Norm	0.66	480	66	1.38E-03
6	1	1.38	947	319	1.45E-03
	2	1.10	863	291	1.26E-03
	3	1.30	931	313	1.38E-03
	2_Norm	0.75	512	172	1.45E-03
	4	1.24	761	256	1.66E-03
All Sets	Average[a]	1.15	943	165	1.24E-03
	SD[a]	0.34	318	80	1.83E-04
	Average[b]	1.00	812	140	1.26E-03
	SD[b]	0.32	277	75	1.88E-04
Sets 1-5	Average[a]	1.12			
	SD[a]	0.37			
	Average[b]	0.96			
	SD[b]	0.32			
Set 6	Average[a]	1.26			
	SD[a]	0.12			
	Average[b]	1.17			
	SD[b]	0.29			

*Data are excluded from averages
[a]Excludes normalized data
[b]Includes normalized data and excludes matched un-normalized sets.

5.0 Conclusion

Each test chemical was classified according to instructions in the protocol and the average RBA (and range) was calculated (as applicable). The results are presented in Table 5. Of particular note are TC9 and TC14. Both of these chemicals seemed to have some interaction with the estrogen receptor, with TC9 displacing about 50% of the radioligand and TC14 displacing about 30%. No curve could be fitted using Prism for either chemical. The protocol (Section 9.7.4) instructs:

> "Due to solubility constraints (for example), some test substances might induce a significant reduction in binding of the radioligand to the ER but without reducing it below 50%. Until additional information becomes available about the significance of this category of dose response curves, substances with acceptable binding curves which reach 25% displacement but not 50% at the highest concentration with acceptable data should be classified as 'equivocal'."

Since no curve could be fitted for these two chemicals, they have been classified as 'negative'. TC22 is classified as positive, but it is important to note that a curve could be fitted for only one run.

Table 5. Classification of Test Substances

Test Chemical	Classification	Average RBA	RBA range	
1[a]	Positive	1.10E+00	1.15E+00	1.05E+00
1[b]	Positive	1.13E+00	1.20E+00	1.07E+00
2	Positive	1.65E+00	1.78E+00	1.48E+00
2[c]	Positive	1.72E+00	1.91E+00	1.48E+00
3[a]	Positive	5.90E+00	8.32E+00	4.90E+00
3[b]	Positive	6.09E+00	8.91E+00	4.90E+00
4[d]	Positive	3.45E+00	4.90E+00	2.57E+00
4[e]	Positive	2.77E+00	4.90E+00	7.08E-01
5[d]	Positive	9.71E-03	1.00E-02	9.35E-03
5[e]	Positive	9.31E-03	1.00E-02	8.13E-03
6	Positive	1.35E-03	1.45E-03	1.29E-03
6[f]	Positive	1.34E-03	1.41E-03	1.29E-03
6[h]	Positive	1.43E-03	1.70E-03	1.29E-03
7	Positive	4.16E-05	5.13E-05	3.63E-05
7[f]	Positive	4.05E-05	5.13E-05	3.39E-05
7[h]	Positive	3.97E-05	5.13E-05	3.16E-05
8[a]	Positive	1.25E-03	2.51E-03	5.01E-04
8[b]	Positive	2.98E-03	6.92E-03	7.96E-04

Table 5. Classification of Test Substances (continued)

Test Chemical	Classification	Average RBA	RBA range	
9	Negative	NA		
10	Positive	1.03E-05	1.78E-05	2.88E-06
10[c]	Positive	1.05E-05	1.78E-05	3.24E-06
11	Positive	8.12E-04	8.81E-04	7.21E-04
12	Positive	2.33E-02	2.86E-02	1.56E-02
13	Positive	2.16E-02	2.57E-02	1.82E-02
13[f]	Positive	2.08E-02	2.57E-02	1.82E-02
13[h]	Positive	2.38E-02	2.75E-02	1.82E-02
14	Negative	NA		
15	Positive	1.50E-04	1.58E-04	1.41E-04
16[d]	Positive	1.97E04	3.31E04	1.23E-04
16[e]	Positive	2.82E-04	5.37E-04	1.23E-04
17	Positive	2.93E-04	3.39E-04	2.09E-04
18	Negative	NA		
19	Positive	5.07E-06	5.50E-06	4.63E-06
20	Negative	NA		
21	Negative	NA		
22[g]	Positive	2.19E-05	2.19E-05	2.19E-05
23[a]	Positive	1.79E-04	1.95E-04	1.58E-04
23[b]	Positive	1.88E-04	2.75E-04	1.26E-04

[a]Set 4 – Run 4 data was excluded

[b]Set 4 – Includes R2, R1-norm, R3-norm, R4-norm and R5-norm

[c]Set 5 - Includes R1, R2 and R3-Norm

[d]Set 2 – Run 1 data was excluded

[e]Set 2 – Includes R1-Norm, R2, R3, R4

[f]Set 6 – Includes R1, R2norm, R3

[g]Only Run 4 had RBA

[h]Set 6 – Includes R1, R3, R4

In running these 23 test chemicals in the RUC-ER assay, we have found numerous instances where the performance criteria (especially for TOP) seem to be overly strict and would cause the disqualification of assays that otherwise appear acceptable. Even for assays where some of the performance criteria were not met, it was possible to determine with a fairly small run to run variance, the IC50, Ki and RBA of the test chemicals. The assay provides data that can be used to identify chemicals that interact with the estrogen receptor and would allow ranking of a series of chemicals for binding affinity.

Appendix A

Solvent Concentration Calculations

Calculation of Maximum Solvent Concentration in Assay Tubes

Concentration of Ethanol in Assay Buffer

1. The 100 mM PMSF Stock Solution is prepared in 100 % ethanol.
2. The TEDG + PMSF buffer contains 1 mL of the PMSF stock per 100 mL buffer,
   therefore, the TEDG + PMSF buffer contains 1% ethanol.

Concentration of Ethanol in 50 nM [^{3}H]E2 solution.

1. 50 nM [^{3}H]E2 solution is prepared by diluting 5.4 μL [^{3}H]E2 stock (100% ethanol) to 1.0 mL with assay buffer.

=(5.4/1000 + 994.6/1000*0.01)*100
1.53%

Concentration of Ethanol in Master Mix

1. Master mix is prepared by combining 380 μL of assay buffer with 10 μL of 50 nM [^{3}H]E2 solution

=((380*0.01)+(10*0.0153))/390*100
1.01%

Total ethanol in assay tubes (total binding)

1. The total binding tubes contain 10 μL ethanol, 390 μL of master mix and 100 μL cytosol (in assay buffer) in a final volume of 500 μL

=(10/500 + (390* 0.0101/500)+ (100*0.01/500))*100
2.99%

2. Other tubes that have fairly high ethanol concentrations include P1 and NSB

The P1 dilution added to the tubes is 50% ethanol, so effectively 5 μL ethanol is added. As above
=(5/500 + (390* 0.0101/500)+ (100*0.01/500))*100
1.99%

The NSB dilution added to the tubes is 10% ethanol, so effectively 1 μL ethanol is added. As above:
=(1/500 + (390* 0.0101/500)+ (100*0.01/500))*100
1.19%

Other dilutions in each series approach a final ethanol concentration of 1%.

Concentration of DMSO in Assay Tubes

1. At most 10 μL of DMSO was used in a 500 μL incubation.

so, 10/500*100 = 2% max

Appendix B

Standard and Test Chemical Dilutions

Estradiol and Control Solution Preparation

Date: _____

Assay Run ID _____

Balance Cal b ID _____

Ethanol supplier/lot # _____

Pipette	Serial Number
L20	
L200	
L1000	

Preparation of [^{3}H]17b-estradiol

[^{3}H]17b-estradiol

Lot #	3589221	
Label SA	110	Ci/mmol
Certification Date	06/28/07	
Label concentration	1	mCi/mL

1) Calculate the SA for the date of use.

Date of use	03/06/08
Days	252
% Remaining	0.962
New SA	105.8129

2) Calculate the concentration of the [^{3}H]17β-estradiol stock

SA (Ci/mmol)	105.8129
Stock concentration (mCi/mL)	1
Concentration (nM)	9450.644

3) For Competitive Assay

Want concentration in assay tube - 1 nM
Prepare stock at 50 nM

In 2 mL, need:

nmol/L = 0.10 nmol/ Y L
Y L = 0.10 nmol * L/nmol
Y µL = 0.10 nmol/ nM * 1E6
Y µL = 10.6

Dilute Y uL of stock to 2 mL

	µL stock
	µL buffer added
	mL total volume

Signature _____ Date _____

QC Initials/Date _____

Estradiol and Control Solution Preparation

Date: _____

Preparation of 17b-estradiol stock (non-radiolabeled)

Use amber glassware
Solvent: EtOH

17b-estradiol
Lot # 086K1611

Stock 1 - 1 mM
Weigh 6.74 - 6.88 mg 17β-estradiol into a 25 mL volumetric flask
Partially fill with ethanol. Mix until dissolved. Then q.s. with additonal ethanol

	mg estradiol
	mL final solution

Stock 2 - 50 µM
Dilute Stock 1 1:20 in ethanol (i.e., Dilute 0.5 mL of 1 mM stock to 10 mL)

	mL Stock 1
	mL final solution

Note: If necessary, adjust the dilution so that 50 µM is achieved

Preparation of Weak Positive Control (norethynodrel) Stock

Norethynodrel
Want stock concentration of 10 mM
Lot # G
MW = 298.4 g/mol

Weight 28.35 - 31.33 mg norethynodrel into a 10 mL volumetric flask.
Partially fill with ethanol. Mix until dissolved. Then q.s. with additional ethanol

	mg norethynodrel
	mL final solution

Preparation of Test Chemical Stock

Want stock concentration of 100 mM
The table calculates mass needed to prepare 1 mL at 100 mM.

TC Code	MW	mg needed	Actual mg	Final volume* (mL)	Solvent
27	346.46	34.65			
38	346.46	34.65			
39	298.42	29.84			
44	298.42	29.84			

*Adjust volume so that 1) target concentration is achieved, or, if solubility is a problem, 2) so that a solution is achieved
If a concentration other than target is prepared, make necessary adjustments to the test chemical dilution scheme to maintain target concentrations

Signature _____ Date _____

QC Initials/Date _____

Dilution of Standards, Controls and Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Non-radiolabeled 17β-estradiol

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (nM)	Final concentration in Assay (M)
NSB1	900	--	100	Stock (50 µM)	1	5000	1.00E-07
S2	900	--	100	NSB1	1	500	1.00E-08
S3	600	--	277	S2	0.877	158.0	1.00E-8.5
S4	900	--	100	S2	1	50.0	1.00E-09
S5	900	--	100	S3	1	15.8	1.00E-9.5
S6	900	--	100	S4	1	5	1.00E-10
S7	900	--	100	S6	1	0.5	1.00E-11

Norethynodrel

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
P1	200	200	400	Stock (10 mM)	0.80	5000	1.00E-04
P2	400	400	150	Stock (10 mM)	0.95	1580	1.00E-4.5
P3	900	--	100	P2	1.0	158.0	1.00E-5.5
Intermed	900	--	100	P1	1.0	500.0	--
P4	900	--	100	Intermed	1.0	50.0	1.00E-06
P5	900	--	100	P3	1.0	15.8	1.00E-6.5
P6	900	--	100	P4	1.0	5	1.00E-07
P7	900	--	100	P5	1.0	1.58	1.00E-7.5
P8	900	--	100	P7	1.0	0.158	1.00E-8.5

Test Chemicals

Tube #*	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
1	500		500	Stock (100 mM)	1.0	50000	1.00E-03
2	900		100	1	1.0	5000	1.00E-04
3	900		100	2	1.0	500.0	1.00E-05
4	900		100	3	1.0	50.0	1.00E-06
5	900		100	4	1.0	5.0	1.00E-07
6	900		100	5	1.0	0.5	1.00E-08
7	900		100	6	1.0	0.05	1.00E-09
8	900		100	7	1.0	0.005	1.00E-10

For test chemicals, label them nTC1 through nTC8
where n=test chemical code

Signature _____ Date _____

QC Initials/Date _____

Dilution of Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Test Chemical 4

Tube #*	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
Prediln 1	500	500	Stock (1 mM)	1.0	500	1 00E-05
Prediln 2	900	100	Prediln 1	1.0	50	1 00E-06
1	900	100	Prediln 2	1.0	5 0	1 00E-07
2	900	100	1	1.0	0 5	1 00E-08
3	900	100	2	1.0	0.05	1 00E-09
4	700	300	3	1.0	0.015	3 00E-10
5	900	100	3	1.0	0.005	1 00E-10
6	900	100	4	1.0	0 0015	3 00E-11
7	900	100	5	1.0	0 0005	1 00E-11
8	900	100	7	1.0	0 00005	1 00E-12

Test Chemical 5

Tube #*	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
1	500	500	Stock (10 mM)	1.0	5000	1 00E-04
2	700	300	1	1.0	1500	3 00E-05
3	900	100	1	1.0	500	1 00E-05
4	700	300	2	1.0	150.0	3 00E-06
5	900	100	2	1.0	50 0	1 00E-06
6	900	100	5	1.0	5 0	1 00E-07
7	900	100	6	1.0	0 5	1 00E-08
8	900	100	7	1.0	0.05	1 00E-09

Test Chemical 16

Tube #*	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1	500	500	Stock (10 mM)	1.0	5000	1 00E-04
2	700	300	1	1.0	1500	3 00E-05
3	900	100	1	1.0	500	1 00E-05
4	500	500	3	1.0	250	5 00E-06
5	800	200	3	1.0	100	2 00E-06
6	900	100	3	1.0	50	1 00E-06
7	900	100	6	1.0	5	1 00E-07
8	900	100	7	1.0	0 5	1 00E-08

Test Chemical 22

Tube #*	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1	500	500	Stock (50 mM)	1.0	25000	5 00E-04
2	800	200	Stock (50 mM)	1.0	10000	2 00E-04
3	900	100	Stock (50 mM)	1.0	5000	1 00E-04
4	700	300	4	1.0	1500	3 00E-05
5	900	100	4	1.0	500	1 00E-05
6	900	100	3	1.0	50	1 00E-06
7	900	100	6	1.0	5	1 00E-07
8	900	100	7	1.0	0 5	1 00E-08

Signature _____ Date _____

QC Initials/Date _____

Dilution of Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Test Chemical 9

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
1	350	500	150	Stock (100 mM)	1.0	15000	3.00E-04
2	---	200	100	1	1.0	5000	1.00E-04
3	400	500	100	1	1.0	1500 0	3.00E-05
4	500	400	100	2	1.0	500.0	1.00E-05
5	900	--	100	4	1.0	50.0	1.00E-06
6	900	--	100	5	1.0	5.0	1.00E-07
7	900	--	100	6	1.0	0.5	1.00E-08
8	900	--	100	7	1.0	0.05	1.00E-09

Test Chemical 14

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	500	--	500	Stock (10 mM)	1.0	5000	1.00E-04
2	300	400	300	1	1.0	1500	3.00E-05
3	400	500	100	1	1.0	500	1.00E-05
4	400	500	100	2	1.0	150.0	3.00E-06
5	900	--	100	3	1.0	50.0	1.00E-06
6	900	--	100	5	1.0	5.0	1.00E-07
7	900	--	100	6	1.0	0.5	1.00E-08
8	900	--	100	7	1.0	0.05	1.00E-09

Test Chemical 17

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	500	--	500	Stock (10 mM)	1.0	5000	1.00E-04
2	300	400	300	1	1.0	1500	3.00E-05
3	500	400	100	1	1.0	500	1.00E-05
4	500	--	500	3	1.0	250	5.00E-06
5	800	--	200	3	1.0	100	2.00E-06
6	900	--	100	3	1.0	50	1.00E-06
7	900	--	100	6	1.0	5	1.00E-07
8	900	--	100	7	1.0	0.5	1.00E-08

Test Chemical 20

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	500	--	500	Stock (10 mM)	1.0	5000	1.00E-04
2	500	400	100	1	1.0	500	1.00E-05
3	500	400	100	2	1.0	50	1.00E-06
4	700	--	300	3	1.0	15	3.00E-07
5	900	--	100	3	1.0	5	1.00E-07
6	900	--	100	5	1.0	0.50	1.00E-08
7	900	--	100	6	1.0	0.05	1.00E-09
8	900	--	100	7	1.0	0.005	1.00E-10

*Adjust composi ion as necessary based on actual concentra ion of stock to prepare target concentration

Signature _____ Date _____

QC Initials/Date _____

Dilution of Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Test Chemical 1

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
Predilution*	450	500	50	Stock (10 mM)	1.0	500	1.00E-05
1	400	500	100	Predilution	1.0	50	1.00E-06
2	900	--	100	1	1.0	5.0	1.00E-07
3	900	--	100	2	1.0	0.5	1.00E-08
4	700	--	300	3	1.0	0.15	3.00E-09
5	900	--	100	3	1.0	0.050	1.00E-09
6	900	--	100	4	1.0	0.015	3.00E-10
7	900	--	100	5	1.0	0.005	1.00E-10
8	900	--	100	7	1.0	0 0005	1.00E-11

Test Chemical 3

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
Prediln 1*	400	500	50	Stock (10 mM)	1.0	500	1.00E-05
Prediln 2	900	--	100	Prediln 1	1.0	50	1.00E-06
1	900	--	100	Prediln 2	1.0	5	1.00E-07
2	900	--	100	1	1.0	0.5	1.00E-08
3	900	--	100	2	1.0	0.1	1.00E-09
4	700	--	300	3	1.0	0.0	3.00E-10
5	900	--	100	3	1.0	0.0	1.00E-10
6	900	--	100	4	1.0	0 0015	3.00E-11
7	900	--	100	5	1.0	0 0005	1.00E-11
8	900	--	100	7	1.0	0.00005	1.00E-12

Test Chemical 8

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
Predilution*	500	--	500	Stock (10 mM)	1.0	5000	1.00E-04
1	500	400	200	Predilution	1.0	1000	2.00E-05
2	500	400	100	Predilution	1.0	500	1.00E-05
3	250	250	500	2	1.0	250	5.00E-06
4	400	400	200	2	1.0	100	2.00E-06
5	600	300	100	2	1.0	50	1.00E-06
6	700	--	300	5	1.0	15	3.00E-07
7	900	--	100	5	1.0	5	1.00E-07
8	900	--	100	7	1.0	0.5	1.00E-08

Test Chemical 23

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	500	--	500	Stock (100 mM)	1.0	50000	1.00E-03
2	300	400	300	1	1.0	15000	3.00E-04
3	500	400	100	1	1.0	5000	1.00E-04
4	900	--	100	2	1.0	1500	3.00E-05
5	900	--	100	3	1.0	500	1.00E-05
6	900	--	100	4	1.0	150	3.00E-06
7	900	--	100	5	1.0	50	1.00E-06
8	900	--	100	7	1.0	5.0	1.00E-07

*Adjust composition as necessary based on actual concentration of stock to prepare target concentration

Signature _____ Date _____

QC Initials/Date _____

Dilution of Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Test Chemical 2

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
Predilution*	450	500	50	Stock (10 mM)	1.0	500	1.00E-05
1	400	500	100	Predilution	1.0	50	1.00E-06
2	900	--	100	1	1.0	5.0	1.00E-07
3	900	--	100	2	1.0	0.5	1.00E-08
4	700		300	3	1.0	0.15	3.00E-09
5	900	--	100	3	1.0	0.050	1.00E-09
6	700	--	300	5	1.0	0.015	3.00E-10
7	900	--	100	5	1.0	0.005	1.00E-10
8	900	--	100	7	1.0	0.0005	1.00E-11

Test Chemical 10

Tube #*	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	concentration (µM)	concentration in Assay (M)
1*	--	--	500	Stock (50 mM)	0.5	50000	1.00E-03
2	300	400	300	1	1.0	15000	3.00E-04
3	500	400	100	1	1.0	5000	1.00E-04
4	500	400	100	3	1.0	500.0	1.00E-05
5	900	--	100	4	1.0	50.0	1.00E-06
6	900	--	100	5	1.0	5.0	1.00E-07
7	900	--	100	6	1.0	0.5	1.00E-08
8	900	--	100	7	1.0	0.05	1.00E-09

Test Chemical 18

Tube #	µL buffer	µL DMSO	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	450	500	50	Stock (10 mM)	1.0	500	1.00E-05
2	400	300	300	1	1.0	150	3.00E-06
3	900	--	100	1	1.0	50	1.00E-06
4	900	--	100	3	1.0	5	1.00E-07
5	700	--	300	4	1.0	1.50	3.00E-08
6	900	--	100	4	1.0	0.50	1.00E-08
7	900	--	100	6	1.0	0.0500	1.00E-09
8	900	--	100	7	1.0	0.0050	1.00E-10

*Adjust composition as necessary based on actual concentration of stock to prepare target concentration

Signature _____ Date _____

QC Initials/Date _____

Dilution of Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Test Chemical 6

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
1	400	450	150	Stock (10 mM)	1.0	1500	3.00E-05
2	400	550	50	Stock (10 mM)	1.0	500	1.00E-05
3	900	--	100	1	1.0	150.0	3.00E-06
4	900	--	100	2	1.0	50.0	1.00E-06
5	900		100	3	1.0	15.00	3.00E-07
6	900	--	100	4	1.0	5.000	1.00E-07
7	900	--	100	6	1.0	0.500	1.00E-08
8	900	--	100	7	1.0	0.050	1.00E-09

Test Chemical 7

Tube #*	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	500	--	500	Stock (100 mM)	0.5	50000	1.00E-03
2	400	500	100	1	1.0	5000	1.00E-04
3	0	500	500	2	1.0	2500	5.00E-05
4	400	400	200	2	1.0	1000.0	2.00E-05
5	900	--	100	2	1.0	500.0	1.00E-05
6	900	--	100	5	1.0	50.0	1.00E-06
7	900	--	100	6	1.0	5.0	1.00E-07
8	900	--	100	7	1.0	0.5	1.00E-08

Test Chemical 13

Tube #	µL buffer	µL ethanol	µL Sample	Sample	Total Volume (mL)	Soln concentration	Final concentration in
1*	450	500	50	Stock (10 mM)	1.0	500	1.00E-05
2	400	500	100	1	1.0	50	1.00E-06
3	700	--	300	2	1.0	15	3.00E-07
4	900	--	100	2	1.0	5	1.00E-07
5	900	--	100	3	1.0	1.50	3.00E-08
6	900	--	100	4	1.0	0.50	1.00E-08
7	900	--	100	6	1.0	0.0500	1.00E-09
8	900	--	100	7	1.0	0.0050	1.00E-10

*Adjust composition as necessary based on actual concentration of stock to prepare target concentration

Signature _____ Date _____

QC Initials/Date _____

Appendix C

Protein Assay Results

	A	B	C	D	E	F	G	H	I	J	K	L
1	Standards:	0.89	0.74	0.59	0.44	0.3	0.22	Blk	Protein stock (mg/10 mL)			
2		1 092	0.917	0.938	0 885	0.810	0.742	0.546	14.8			
3		1.125	0.927	0.973	0.754	0.822	0.734	0.549				
4		0 819	1.026	0.805	0 927	0.744	0.700	0.527				
5	Samples:	1:5	1:10	0.59	0.3							
6		0 842	0.731	1.005	0.799							
7		0.799	0.745	0.836	0 828							
8		0 864	0.661	1.033	0.758							
9	mg Protein		µL Standard		mg Protein		A_{raw}	A_{adj}	Curve			
10	per µL		Used		Measured				Output	Variables	Regression results	
11	0.00089		100		0.0890		1 012	0.471	0.0863	m, b	0.242	-0.028
12	0.00074		100		0.0740		0 957	0.416	0.0729	se_m, se_b	0.017	0.006
13	0.00059		100		0.0590		0 905	0.365	0.0605	r^2, se_y	0.981	0.004
14	0.00044		100		0.0440		0 855	0.315	0.0484	F, df	202	4
15	0.00030		100		0.0300		0.792	0.251	0.0331	ss_{reg}, ss_{resid}	0.003	0.000
16	0.00022		100		0.0220		0.725	0.185	0.0169			
17		Blank	0.541		r^2=	0.981					Regression results are calculated using the function L NEST	
18					m=	0.242						
19					b=	-0.028						
20		A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL	
21	1:5	0 842	0.301	0.045	100	80	400		2.26E-03	2.17E-03	2.2	
22	1:5	0.799	0.258	0.035	100	80	400		1.74E-03			
23	1:5	0 864	0.323	0.050	100	80	400		2.52E-03			
24	1:10	0.731	0.190	0.018	100	40	400		1.83E-03	1.38E-03	1 38	
25	1:10	0.745	0.204	0.022	100	40	400		2.17E-03			
26	1:10	0 661	0.120	0.001	100	40	400		1.37E-04			
27	0.59	1 005	0.464	0.085	100	1	1		8.46E-04	6.41E-04	0 64	
28	0.59	0 836	0.295	0.044	100	1	1		4.37E-04			
29	0.59	1 033	0.492	0.091		1	1					
30	0.3	0.799	0.258	0.035	100	1	1		3.47E-04	3.83E-04	0 38	
31	0.3	0 828	0.287	0.042	100	1	1		4.18E-04			
32	0.3	0.758	0.217	0.025		1	1					
33												
34												
35												
36												
37												
38												
39												
40												
41												

	A	B	C	D	E	F	G	H	I	J	K	L
1	Standards:	0.89	0.74	0.59	0.44	0.3	0.22	Blk	Protein stock (mg/10 mL)			
2		1.494	1.407	0.961	0 822	0.799	0.725	0.509	14.8			
3		1.491	1.396	0.919	0 833	0.805	0 691	0.530				
4		1.491	1.384	0.777	0 870	0.723	0.758	0.476				
5	Samples:	1:5	1:10	0.59	0.3							
6		0 847	0.758	0.975	0.772							
7		0.798	0.773	0.887	0.778							
8		0 860	0.683	0.980	0.701							
9	mg Protein		µL Standard		mg Protein		A_{raw}	A_{adj}	Curve			
10	per µL		Used		Measured				Output	Variables	Regression results	
11	0.00089		100		0.0890		1.492	0.987	0.0871	m, b	0.072	0.016
12	0.00074		100		0.0740		1 396	0.891	0.0802	se_m, se_b	0.014	0.008
13	0.00059		100		0.0590		0 886	0.381	0.0434	r^2, se_y	0.873	0.010
14	0.00044		100		0.0440		0 842	0.337	0.0402	F, df	27	4
15	0.00030		100		0.0300		0.776	0.271	0.0354	ss_{reg}, ss_{resid}	0.003	0.000
16	0.00022		100		0.0220		0.725	0.220	0.0317			
17		Blank	0.505		r^2=	0.873					Regression results are calculated using the function L NEST	
18					m=	0.072						
19					b=	0.016						
20		A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL	
21	1:5	0 847	0.342	0.048	100	100	500		2.39E-03	2.26E-03	2.3	
22	1:5	0.798	0.293	0.037	100	100	500		1.84E-03			
23	1:5	0 860	0.355	0.051	100	100	500		2.54E-03			
24	1:10	0.758	0.253	0.028	100	50	500		2.77E-03	2.32E-03	2 32	
25	1:10	0.773	0.268	0.031	100	50	500		3.11E-03			
26	1:10	0 683	0.178	0.011	100	50	500		1.07E-03			
27	0.59	0 975	0.470	0.077	100	1	1		7.68E-04	7.05E-04	0.71	
28	0.59	0 887	0.382	0.057	100	1	1		5.69E-04			
29	0.59	0 980	0.475	0.078	100	1	1		7.79E-04			
30	0.3	0.772	0.267	0.031	100	1	1		3.08E-04	2.59E-04	0 26	
31	0.3	0.778	0.273	0.032	100	1	1		3.22E-04			
32	0.3	0.701	0.196	0.015	100	1	1		1.48E-04			

Appendix D

Saturation Assay Reports

Saturation Binding Report

Assay Date	3/20/2008
Assay ID	SAT4

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol	
Rat Strain	Sprague Dawley	
Rat Age	103 days	
Ovariectomy Date	3/4/2008	
Uterus removal Date	3/11/2008	
Cytosol Prep Date	3/19/2008	
Cytosol Prep ID	3/19/2008	
Protein Content	2.3 mg/mL	
Protein Assay Date	3/19/2008	
RUC Storage Info	-70 °C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF
Protein Concentration Used	50 μg/tube
Assay volume	0.5 mL
Incubation Time	17.92 h
Incubation Temp.	4 °C
Notes on problems:	None

Results

Counter Data File	21MR1659.P00 & 24MR1824.P00
Excel Filename	Sat_#4_3_20_08.xls
PRISM Filename	RTI Sat_#4_3_20_08.pzf

Assay Date	3/20/2008	
Elapsed days since SA determination	266	
Adjusted SA	105.59	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	0.6457	nM
SE Kd	0.044	
Estimated Bmax	52.09	fmol/100 μg
SE Bmax	1.39	

Discussion

		Enter Yes or No
1	Did specific binding curve reach a plateau?	Yes
2	Is the Scatchard plot linear?	Yes
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	Yes - Slightly high
4	Standard errors for Kd and Bmax acceptable?	
	ratio SE:parameter should be ≤20%	
	SE:Kd 6.81	
	SE:Bmax 2.67	
5	Non-specific binding should be < 50% of total binding?	Yes

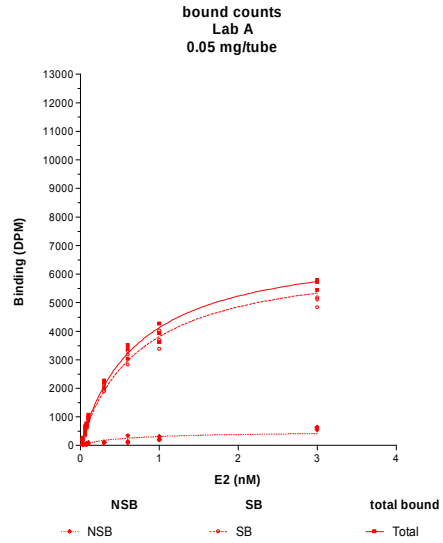
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

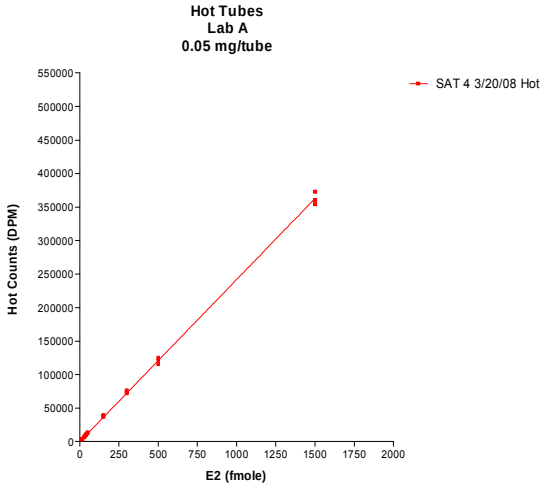
All acceptable? Yes

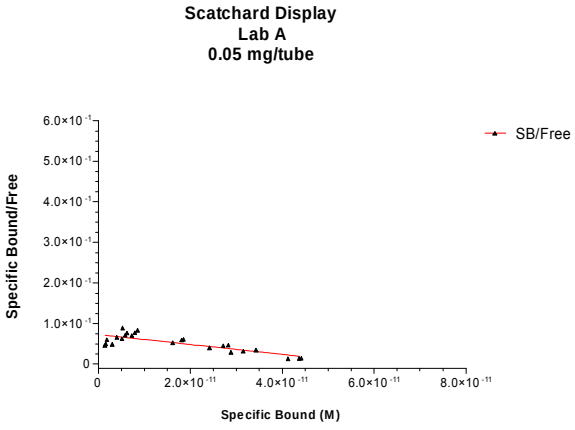
Conclusion

Note any reasons why confidence in the results should be high or low.

Run is acceptable.


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
Hot Counts (DPM)
E2 (fmole)
SAT 4 3/20/08 Hot


Scatchard Display
Lab A
0.05 mg/tube
Specific Bound/Free
Specific Bound (M)
SB/Free

Raw file

Filename:21MR1659.P00 & 24MR1824.P00

Via	Tim	DPM				tSIE
1	10	17.94	20.54	0	21.42	478.6
2	5	140.7	58.7	0	13.47	488.41
3	5	174.45	70.9	0	12.82	483.91
4	5	150.61	61.74	0	12.78	484.06
5	5	345.62	138.3	0	12.21	475.82
6	5	267.39	107.1	0	12.33	474.15
7	5	445.64	181.54	0	12.52	474.28
8	5	520.64	213.94	0	12.08	473.74
9	5	434.62	178.7	0	12.55	475.28
10	5	490.9	201.86	0	12.46	480.61
11	5	620.58	262.46	0	11.95	475.18
12	5	672.26	273.46	0	12.28	476.16
13	5	717.71	301.78	0	12.47	477.28
14	5	1337.07	550.7	0	12.26	479.55
15	5	1486.08	603.94	0	12.21	473.08
16	5	1518.77	622.3	0	12.35	477.39
17	5	2254.8	915.86	0	12.36	472.98
18	5	2343.08	949.18	0	12.43	473.34
19	5	2021.61	810.66	0	12.56	474.46
20	5	2623.83	1058.3	0	12.3	474.78
21	5	2416.37	986.7	0	12.46	473.67
22	5	2841.82	1151.26	0	12.36	472.74
23	5	3857.15	1575.7	0	12.35	474.9
24	5	3819.96	1560.66	0	12.21	475.25
25	5	3633.41	1473.66	0	12.27	473.16
26	5	6.46	1.3	0	25.83	482.58
27	5	37.47	14.62	0	17.18	475.68
28	5	31.21	11.38	0	15.55	474.22
29	5	14.9	7.98	0	9.59	475.62
30	5	36.69	15.1	0	11.55	472.89
31	5	26.46	12.5	0	11.78	476.29
32	5	34.62	14.94	0	12.63	477.17
33	5	44.43	19.06	0	15.43	475.9
34	5	23.88	10.94	0	8.88	480.75
35	5	32.18	14.5	0	8.45	474.59
36	5	44.3	17.1	0	12.73	478.71
37	5	66.61	25.46	0	12.29	468.72
38	5	43.4	19.3	0	13.31	474.93
39	5	81.51	33.9	0	11.4	474.82
40	5	69.22	29.94	0	13.09	474.26
41	5	64.03	26.46	0	11.9	473.84
42	5	233.32	96.1	0	12.6	474.75
43	5	65.79	28.7	0	10.89	475.6
44	5	95.56	43.34	0	11.48	473.88
45	5	209.21	88.1	0	12.75	472.73
46	5	138.66	57.9	0	12.3	473.74
47	5	133.19	52.5	0	13.81	475.39
48	5	434.04	173.46	0	12.77	474.39
49	5	416.22	163.7	0	12.23	474.17
50	5	369.99	152.38	0	12.03	474.43

File w/ backgrounds stripped out

Sample IDs

Type	Col	Tube	DPM	Rounded DPM
H	1	1	140.70	141
H	1	2	174.45	175
H	1	3	150.61	151
H	2	1	345.62	346
H	2	2	267.39	267
H	2	3	445.64	446
H	3	1	520.64	521
H	3	2	434.62	435
H	3	3	490.90	491
H	4	1	620.58	621
H	4	2	672.26	672
H	4	3	717.71	718
H	5	1	1337.07	1337
H	5	2	1486.08	1486
H	5	3	1518.77	1519
H	6	1	2254.80	2255
H	6	2	2343.08	2343
H	6	3	2021.61	2022
H	7	1	2623.83	2624
H	7	2	2416.37	2416
H	7	3	2841.82	2842
H	8	1	3857.15	3857
H	8	2	3819.96	3820
H	8	3	3633.41	3633
HC	1	1	37.47	37
HC	1	2	31.21	31
HC	1	3	14.90	15
HC	2	1	36.69	37
HC	2	2	26.46	26
HC	2	3	34.62	35
HC	3	1	44.43	44
HC	3	2	23.88	24
HC	3	3	32.18	32
HC	4	1	44.30	44
HC	4	2	66.61	67
HC	4	3	43.40	43
HC	5	1	81.51	82
HC	5	2	69.22	69
HC	5	3	64.03	64
HC	6	1	233.32	233
HC	6	2	65.79	66
HC	6	3	95.56	96
HC	7	1	209.21	209
HC	7	2	138.66	139
HC	7	3	133.19	133
HC	8	1	434.04	434
HC	8	2	416.22	416
HC	8	3	369.99	370

Original Hot vials counted on 3/24/08 File: 24MR1824.P00

1	10	20.4	26.90	0	20.04	476.57	
2	5	372881.81	168574.25	0	13.84	565.2	
3	5	360075.09	163153.73	0	13.91	569.28	
4	5	354406.34	160306.17	0	13.85	566.45	
5	5	125142.11	56578.54	0	13.8	566.5	
6	5	121051.72	54664.66	0	13.84	566.19	
7	5	115703.99	52724.02	0	13.84	571.74	
8	5	76558.29	34767.98	0	13.86	570.16	
9	5	73529.67	33159.42	0	13.82	564.2	
10	5	72578.37	32824.46	0	13.81	565.57	
11	5	39347.48	17793.70	0	13.82	566.65	
12	5	37409.76	16924.98	0	13.83	567.43	
13	5	37493.55	16899.94	0	13.83	566.59	
14	5	13393.56	6025.94	0	13.8	563.57	
15	5	12938.23	5837.10	0	13.81	564.96	
16	5	12751.37	5726.10	0	13.85	564.93	
17	5	10662.08	4820.10	0	13.75	566.45	
18	5	10373.16	4652.54	0	13.81	564.21	
19	5	9899.95	4476.50	0	13.83	567.41	
20	5	7997.49	3617.78	0	13.77	565	
21	5	7474.47	3399.94	0	13.7	565.13	
22	5	7418.8	3359.10	0	13.8	566.02	
23	5	3982.15	1816.34	0	13.71	569.03	
24	5	3907.77	1777.50	0	13.91	568.55	
25	5	3807.55	1700.94	0	13.9	564.01	
26	5	221735	82143.14	0	12.18	434.29	
27	5	0	0.00	0	0	481.22	

File w/ backgrounds stripped out

Sample IDs

Type	Conc	Tube		DPM	Rounded DPM
H		1	1	3982.15	3982
H		1	2	3907.77	3908
H		1	3	3807.55	3808
H		2	1	7997.49	7997
H		2	2	7474.47	7474
H		2	3	7418.80	7419
H		3	1	10662.08	10660
H		3	2	10373.16	10370
H		3	3	9899.95	9900
H		4	1	13393.56	13390
H		4	2	12938.23	12940
H		4	3	12751.37	12750
H		5	1	39347.48	39350
H		5	2	37409.76	37410
H		5	3	37493.55	37490
H		6	1	76558.29	76560
H		6	2	73529.67	73530
H		6	3	72578.37	72580
H		7	1	125142.11	125100
H		7	2	121051.72	121100
H		7	3	115703.99	115700
H		8	1	372881.81	372900
H		8	2	360075.09	360100
H		8	3	354406.34	354400

Saturation Binding Report

Assay Date	3/24/2008
Assay ID	SAT5

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol	
Rat Strain	Sprague Dawley	
Rat Age	103 days	
Ovariectomy Date	3/4/2008	
Uterus removal Date	3/11/2008	
Cytosol Prep Date	3/19/2008	
Cytosol Prep ID	3/19/2008	
Protein Content	2.3 mg/mL	
Protein Assay Date	3/19/2008	
RUC Storage Info	-70 °C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF
Protein Concentration Used	50 μg/tube
Assay volume	0.5 mL
Incubation Time	18.92 h
Incubation Temp.	4 °C
Notes on problems:	None

Results

Counter Data File 25MR1840.P00
Excel Filename Sat_#5_3_24_08.xls
PRISM Filename RTI Sat_#5_3_24_08.pzf

Assay Date	3/24/2008	
Elapsed days since SA determination	270	
Adjusted SA	105.53	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	1.1810	nM
SE Kd	0.3397	
Estimated Bmax	84.96	fmol/100 μg
SE Bmax	11.11	

Discussion

		Enter Yes or No	
1	Did specific binding curve reach a plateau?	Yes	
2	Is the Scatchard plot linear?	Yes	
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	No	High
4	Standard errors for Kd and Bmax acceptable?		
	ratio SE:parameter should be ≤20%		
	SE:Kd 28.76		
	SE:Bmax 13.08		
5	Non-specific binding should be < 50% of total binding?	Yes	

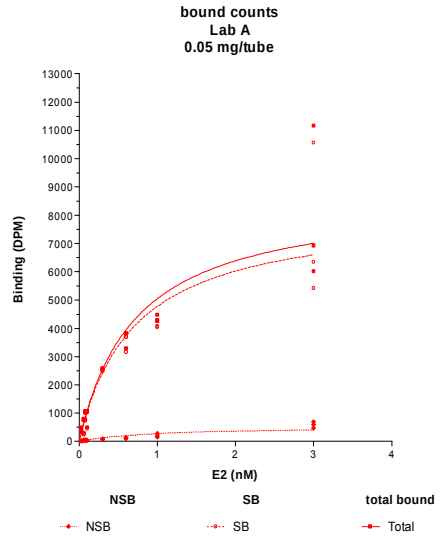
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

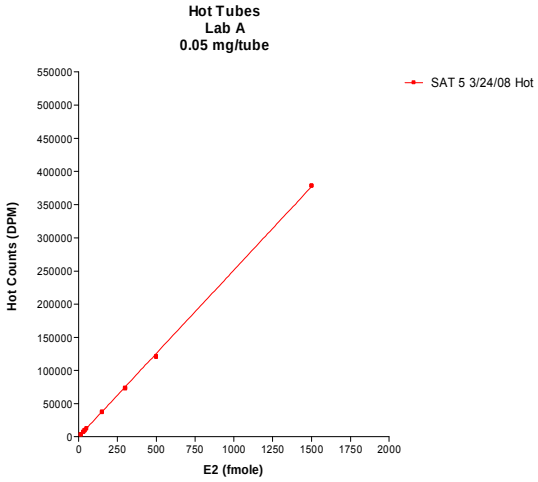
All acceptable? No

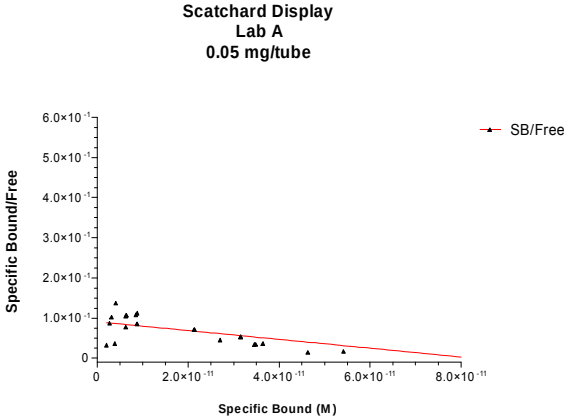
Conclusion

Note any reasons why confidence in the results should be high or low.

Run is questionable. High Kd, high CVs for Kd and Bmax


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
SAT 5 3/24/08 Hot
Hot Counts (DPM)
E2 (fmole)


Scatchard Display
Lab A
0.05 mg/tube
SB/Free
Specific Bound/Free
Specific Bound (M)

Raw file

Filename: 25MR1840.P00

Vial #	Time	DPM				tSIE
1	10	18.2	20.1	0	21.81	476.21
2	5	225.54	93.9	0	12.32	480.08
3	5	332.98	152.98	0	13 9	478.72
4	5	258.56	103.54	0	12.07	475.1
5	5	193.51	76.78	0	11 5	475.72
6	5	530.98	207.14	0	12.27	471.45
7	5	521.71	207.5	0	12.52	473.5
8	5	515.58	206.94	0	12.41	469.41
9	5	714.22	280.9	0	12.4	472.08
10	5	694.42	276.74	0	12.41	468.5
11	5	336.59	131.74	0	12.62	472.41
12	5	713.57	288.54	0	12.34	470.91
13	5	715.58	299.38	0	12.16	471.75
14	5	1721.07	690.74	0	12.4	472.14
15	5	1722.14	699.14	0	12.38	470.09
16	5	1724.07	698.54	0	12.28	471.13
17	5	2545.54	1031.74	0	12.37	471.94
18	5	2546 9	1030.14	0	12.29	472.15
19	5	2194.37	884.38	0	12.35	468.67
20	5	2865.65	1145.82	0	12.26	467.96
21	5	2986.85	1208.74	0	12.36	473.72
22	5	2840 2	1137.7	0	12.39	469.38
23	5	4622.73	1884.9	0	12.38	476.58
24	5	4012.4	1600.9	0	12.42	469.12
25	5	7445.87	2998.3	0	12.37	469.03
26	5	0	0	0	317.91	478.53
27	5	16.37	9 54	0	8.99	474.56
28	5	8.91	5 54	0	12.68	472.53
29	5	15.83	6.74	0	14.75	471.95
30	5	29.55	11.5	0	15.49	471.57
31	5	34.15	14.78	0	12.11	472.39
32	5	26.46	11.74	0	12.42	472.89
33	5	40.12	15.5	0	13.81	473.11
34	5	17.63	10.5	0	10.51	471.06
35	5	19.03	9.78	0	9.09	473.85
36	5	27.1	13.98	0	10.98	474.93
37	5	30.58	13.22	0	11.65	471.23
38	5	33.86	15.34	0	12.87	474.69
39	5	60.09	25.9	0	14.49	473.82
40	5	50.35	21.9	0	13.04	471.15
41	5	52.05	22.74	0	11.15	474.05
42	5	93.38	38.34	0	10.81	470.63
43	5	71.92	32.9	0	11.35	469.99
44	5	80.6	33.5	0	10 5	470.91
45	5	135.68	54.34	0	12.16	470.32
46	5	104.21	47.74	0	9.88	475.85
47	5	188.91	73.98	0	12.18	471.55
48	5	317.21	133.94	0	11.88	472.29
49	5	465.09	184.54	0	12.34	468.72
50	5	399.76	161.82	0	12.31	475.25
51	5	1.98	1 54	0	22.05	480.37
52	5	3915.34	1761.94	0	13.73	564.7
53	5	4037.01	1822.66	0	13.82	566.04
54	5	4006.68	1801.74	0	13.71	562.05
55	5	7613.48	3447.7	0	13.82	565.19
56	5	7866.95	3570.42	0	13.81	565.5
57	5	7908.43	3562.5	0	13.84	565.39
58	5	10214.76	4598.58	0	13.77	559.19
59	5	10204 9	4569.34	0	13.79	563.41
60	5	10448.65	4724.5	0	13 8	565.91
61	5	12959.35	5838.1	0	13.73	562.4
62	5	13024.05	5879.1	0	13.86	562.68
63	5	13225.82	5939.38	0	13.89	563.06
64	5	37060.93	16715.34	0	13.69	562.99
65	5	37912.13	17028.5	0	13.69	560.57
66	5	37819.68	17071.1	0	13.69	562.22
67	5	72961.88	33023.3	0	13.79	565.77
68	5	74047.09	33364.82	0	13.75	562.51
69	5	73895.59	33508.62	0	13.74	566.65
70	5	120650.89	54378.78	0	13 8	563.01
71	5	121035.84	54539.58	0	13.75	562.83
72	5	121905.46	54919.94	0	13.76	562.83
73	5	378768.25	170346.44	0	13.85	563.09
74	5	378616.13	170734.78	0	13.85	564.12
75	5	379135.53	170634.47	0	13.82	562.58
76	5	0	0	0	0	479.33
77	5	219685.13	82210.9	0	12.18	437.82

File w/ backgrounds stripped out

Sample Type	Ds Conc	Tube	DPM	Rounded DPM
H		1	1	225.54	226
H		1	2	332.98	333
H		1	3	258.56	259
H		2	1	193.51	194
H		2	2	530.98	531
H		2	3	521.71	522
H		3	1	515.58	516
H		3	2	714.22	714
H		3	3	694.42	694
H		4	1	336.59	337
H		4	2	713.57	714
H		4	3	715.58	716
H		5	1	1721.07	1721
H		5	2	1722.14	1722
H		5	3	1724.07	1724
H		6	1	2545.54	2546
H		6	2	2546.90	2547
H		6	3	2194.37	2194
H		7	1	2865.65	2866
H		7	2	2986.85	2987
H		7	3	2840.20	2840
H		8	1	4622.73	4623
H		8	2	4012.40	4012
H		8	3	7445.87	7446
HC		1	1	16.37	16
HC		1	2	8.91	9
HC		1	3	15.83	16
HC		2	1	29.55	30
HC		2	2	34.15	34
HC		2	3	26.46	26
HC		3	1	40.12	40
HC		3	2	17.63	18
HC		3	3	19.03	19
HC		4	1	27.10	27
HC		4	2	30.58	31
HC		4	3	33.86	34
HC		5	1	60.09	60
HC		5	2	50.35	50
HC		5	3	52.05	52
HC		6	1	93.38	93
HC		6	2	71.92	72
HC		6	3	80.60	81
HC		7	1	135.68	136
HC		7	2	104.21	104
HC		7	3	188.91	189
HC		8	1	317.21	317
HC		8	2	465.09	465
HC		8	3	399.76	400
Hot		1	1	3915.34	3915
Hot		1	2	4037.01	4037
Hot		1	3	4006.68	4007
Hot		2	1	7613.48	7613
Hot		2	2	7866.95	7867
Hot		2	3	7908.43	7908
Hot		3	1	10214.76	10210
Hot		3	2	10204.90	10200
Hot		3	3	10448.65	10450
Hot		4	1	12959.35	12960
Hot		4	2	13024.05	13020
Hot		4	3	13225.82	13230
Hot		5	1	37060.93	37060
Hot		5	2	37912.13	37910
Hot		5	3	37819.68	37820
Hot		6	1	72961.88	72960
Hot		6	2	74047.09	74050
Hot		6	3	73895.59	73900
Hot		7	1	120650.89	120700
Hot		7	2	121035.84	121000
Hot		7	3	121905.46	121900
Hot		8	1	378768.25	378800
Hot		8	2	378616.13	378600
Hot		8	3	379135.53	379100

Saturation Binding Report

Assay Date	3/25/2008
Assay ID	SAT6

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol	
Rat Strain	Sprague Dawley	
Rat Age	103 days	
Ovariectomy Date	3/4/2008	
Uterus removal Date	3/11/2008	
Cytosol Prep Date	3/19/2008	
Cytosol Prep ID	3/19/2008	
Protein Content	2.3 mg/mL	
Protein Assay Date	3/19/2008	
RUC Storage Info	-70 °C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF
Protein Concentration Used	50 μg/tube
Assay volume	0.5 mL
Incubation Time	18.78 h
Incubation Temp.	4 °C
Notes on problems:	None

Results

Counter Data File 26MR1818.P00
Excel Filename Sat_#6_3_25_08.xls
PRISM Filename RTI Sat_#6_3_25_08.pzf

Assay Date	3/25/2008	
Elapsed days since SA determination	271	
Adjusted SA	105.51	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	0.3691	nM
SE Kd	0.0258	
Estimated Bmax	57.16	fmol/100 μg
SE Bmax	1.37	

Discussion

		Enter Yes or No
1	Did specific binding curve reach a plateau?	Yes
2	Is the Scatchard plot linear?	Yes
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	Yes
4	Standard errors for Kd and Bmax acceptable?	
	ratio SE:parameter should be ≤20%	
	SE:Kd 6.99	
	SE:Bmax 2.40	
5	Non-specific binding should be < 50% of total binding?	Yes

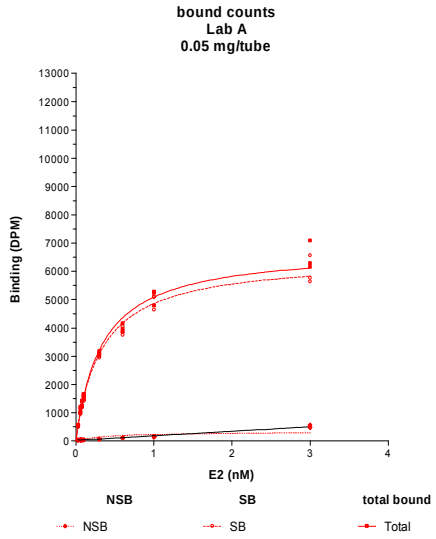
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

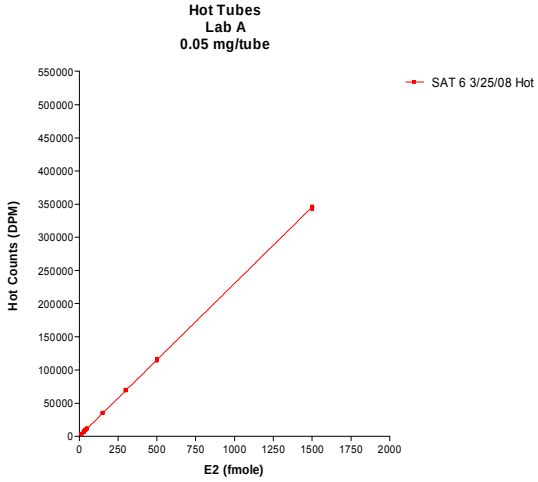
All acceptable? Yes

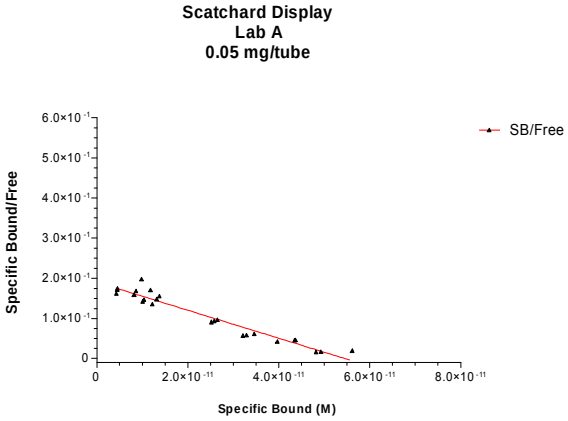
Conclusion

Note any reasons why confidence in the results should be high or low.

The run is acceptable


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
Hot Counts (DPM)
E2 (fmole)
SAT 6 3/25/08 Hot


Scatchard Display
Lab A
0.05 mg/tube
Specific Bound/Free
Specific Bound (M)
SB/Free

Raw file

Filename: 26MR1818.P00

Via	Tim	DPM				tSIE
1	10	19.72	23.32	0	23 02	485.48
2	5	358.03	147.28	0	12.14	485.22
3	5	380.1	156.52	0	12.16	485.31
4	5	797.85	331.32	0	12 36	482.16
5	5	374.85	156.32	0	12 21	476 8
6	5	668.35	278.6	0	12 26	488.09
7	5	701.58	286.12	0	11 96	475.4
8	5	816.27	338.76	0	12.2	481 5
9	5	948.68	388.68	0	12.18	481.08
10	5	837.26	340.88	0	12.14	479.51
11	5	1058 2	434.28	0	11 85	481.49
12	5	1106.51	450.88	0	12 37	478.89
13	5	980.89	396.16	0	12 39	475.55
14	5	2115.66	866.12	0	12.3	480.19
15	5	2013.82	828.32	0	12 36	478.95
16	5	2062.74	845.2	0	12 28	478.57
17	5	2583.11	1050.36	0	12 31	477.42
18	5	2646.23	1070.8	0	12.3	475.08
19	5	2777.67	1123.52	0	12 51	477.93
20	5	3514 5	1431.72	0	12 51	479.29
21	5	3499.11	1426.08	0	12.3	479.26
22	5	3199.38	1302.92	0	12.45	477.59
23	5	4727.03	1952.92	0	12.4	485.65
24	5	4108.53	1661.4	0	12.47	476 3
25	5	4191.51	1704.08	0	12.47	478.05
26	5	7.68	0.08	0	11 68	478 8
27	5	27.37	11.88	0	14 29	481.24
28	5	13.97	5.12	0	2 81	479 8
29	5	32.16	12.08	0	8 03	483.44
30	5	14.67	6.16	0	8.2	481.32
31	5	42.75	17.72	0	10 82	483.03
32	5	34 3	12.88	0	6.44	479.05
33	5	17.58	9.12	0	2 61	482 8
34	5	23 8	9.48	0	10.48	478.57
35	5	36.03	14.08	0	11 86	480.86
36	5	30.84	13.56	0	6 31	478.92
37	5	33.46	14	0	9 96	478.73
38	5	27.63	13.12	0	5 99	478.73
39	5	45.1	21.72	0	9.48	477.46
40	5	40.92	18.32	0	5.1	482.91
41	5	52.86	23.36	0	8.19	481.51
42	5	84.58	35.32	0	9.76	479.07
43	5	77.25	28.32	0	12 85	478.43
44	5	65.4	28.12	0	10 94	478.98
45	5	106 8	43.08	0	11 88	481.69
46	5	105.28	47.96	0	9.76	478.41
47	5	88.97	37.56	0	10 02	482.88
48	5	381.59	155.04	0	12.13	479
49	5	318.96	127.36	0	12 25	476.85
50	5	331.65	136.96	0	12 36	480.16
51	5	0	0	0	0	478.36
52	5	3624.42	1636.48	0	13.7	568.75
53	5	3673.19	1657.08	0	13.75	568.16
54	5	3602.39	1656.88	0	13.71	570.43
55	5	6884.57	3131.52	0	13 54	565.75
56	5	7147.65	3264.76	0	13.75	571.56
57	5	7029.68	3210.08	0	13 59	567.82
58	5	9399.76	4278.28	0	13 64	568.29
59	5	9649.34	4386.32	0	13.79	569.04
60	5	9563.15	4328	0	13.8	566.83
61	5	11911.01	5407.92	0	13.72	566.92
62	5	12108.56	5526.88	0	13.74	570.1
63	5	12000.22	5458.12	0	13.75	571.04
64	5	35137.39	15993 8	0	13 83	570.67
65	5	35434.77	16148 6	0	13.73	569.48
66	5	35414.81	16129 8	0	13.73	568.55
67	5	69464.13	31426 8	0	13.75	565.55
68	5	70309	31914.52	0	13.79	568.44
69	5	70070.95	31953.28	0	13.8	571.16
70	5	114103.41	52082.24	0	13 81	571.76
71	5	115496.77	52627.96	0	13.78	570.32
72	5	116795.23	53181.12	0	13.8	570.61
73	5	342863.84	155908.22	0	13 86	570.43
74	5	346193.56	159480.61	0	14	582.21
75	5	346341.28	157339.56	0	13 91	570.58
76	5	0	0	0	0	470.7
77	5	221036.22	82281.48	0	12.2	436.23

File w/ backgrounds stripped out

Sample IDs

Type	Conc	Tube	DPM	Rounded DPM
H		1	1 358 03	358
H		1	2 380.10	380
H		1	3 374 85	375
H		2	1 797 85	798
H		2	2 668 35	668
H		2	3 701 58	702
H		3	1 816 27	816
H		3	2 948 68	949
H		3	3 837 26	837
H		4	1 1058 20	1058
H		4	2 1106 51	1107
H		4	3 980 89	981
H		5	1 2115 66	2116
H		5	2 2013 82	2014
H		5	3 2062.74	2063
H		6	1 2583.11	2583
H		6	2 2646 23	2646
H		6	3 2777 67	2778
H		7	1 3514 50	3515
H		7	2 3499.11	3499
H		7	3 3199 38	3199
H		8	1 4727 03	4727
H		8	2 4108 53	4109
H		8	3 4191 51	4192
HC		1	1 27.37	27
HC		1	2 13.97	14
HC		1	3 32.16	32
HC		2	1 14.67	15
HC		2	2 42.75	43
HC		2	3 34.30	34
HC		3	1 17.58	18
HC		3	2 23.80	24
HC		3	3 36.03	36
HC		4	1 30.84	31
HC		4	2 33.46	33
HC		4	3 27.63	28
HC		5	1 45.10	45
HC		5	2 40.92	41
HC		5	3 52.86	53
HC		6	1 84.58	85
HC		6	2 77.25	77
HC		6	3 65.40	65
HC		7	1 106 80	107
HC		7	2 105 28	105
HC		7	3 88.97	89
HC		8	1 381 59	382
HC		8	2 318 96	319
HC		8	3 331 65	332
Hot		1	1 3624.42	3624
Hot		1	2 3673.19	3673
Hot		1	3 3602 39	3602
Hot		2	1 6884 57	6885
Hot		2	2 7147 65	7148
Hot		2	3 7029 68	7030
Hot		3	1 9399.76	9400
Hot		3	2 9649 34	9649
Hot		3	3 9563.15	9563
Hot		4	1 11911 01	11910
Hot		4	2 12108 56	12110
Hot		4	3 12000 22	12000
Hot		5	1 35137 39	35140
Hot		5	2 35434.77	35430
Hot		5	3 35414 81	35410
Hot		6	1 69464.13	69460
Hot		6	2 70309 00	70310
Hot		6	3 70070 95	70070
Hot		7	1 114103.41	114100
Hot		7	2 115496.77	115500
Hot		7	3 116795 23	116800
Hot		8	1 342863 84	342900
Hot		8	2 346193 56	346200
Hot		8	3 346341 28	346300

Appendix E

Competitive Assay Reports

Competitive Binding Report

Test Chemicals Set 2

Tested Chemicals 4, 5, 16, 22
Assay Dates 2/21, 2/26, 2/27, and 3/3/2008

Radioactive Ligand

Name [^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier Perkin Elmer (Boston)
Catalog # NET-517
Batch # 3589221
Specific Ac ivity 110 Ci/mmol
SA date 6/28/2007
Concentra ion (mCi/mL) 1
Tested Concentrations (nM) 1

Radioinert Ligand

Name 17β-Estradiol
Supplier Battelle Sequim
Lot # 086K1611
CAS # 50-28-2
Purity ≥98%
Concentration in standard curve tubes (nM) 0.01, 0.1, 0.316, 1, 3.16, 10, 100 (All runs)

Estrogen Receptor

Type Rat Uterine Cytosol
Rat Strain Sprague Dawley
Rat Age 94 days
Ovariectomy Date 1/14/2008
Uterus removal Date 1/22/2008
Cytosol Prep Date 1/23/2008
Cytosol Prep ID 1/23/2008
Protein Content 2.20 mg/mL
Protein Assay Date 1/23/2008
RUC Storage Info -70 °C (UCF027)

Positive Control

Name Norethynodrel
Supplier Battelle Sequim
Lot # G
CAS # 68-23-5
Purity 100
Concentration in assay tubes (nM) 3.16, 31.6, 100, 316, 1000, 3160, 31600, 100000 (All runs)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4
	18.42	18.42	18.58	18.58

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	23FE0741.P00	28FE0534	29FE0505	05MR0503
Excel (xls)	S2R1_2_21_08	S2R2_2_26_08	S2R3_2_27_08	S2R4_3_3_08
Prism (.pzf)	RTI_Set 2			

	Run 1	Run 2	Run 3	Run 4
Assay Date	2/21/2008	2/26/2008	2/27/2008	3/3/2008
Elapsed days since SA determination	238	243	244	249
Adjusted SA (Ci/mmol)	106.04	105.96	105.94	105.86

standard curve- Run 1			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	14.42	-8.74	-9.22
-7.0	-9.87	-13.57	26.98
-8.0	47.78	82.25	38.61
-8.5	155 32	138.65	123 96
-9.0	326 96	303.40	288 05
-9.5	507 00	496.28	525.72
-10.0	630 89	619.32	578 31
-11.0	706 82	740.27	725.12

standard curve- Run 2			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.44	0.65	1.24
-7.0	-0.43	-0.16	-0.86
-8.0	6.80	6.36	6.64
-8.5	19.72	19.82	16.16
-9.0	43.82	42.15	45.52
-9.5	78.18	77.08	74.92
-10.0	95.43	96.63	92.32
-11.0	105.16	110.40	97.98

standard curve- Run 3			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7 0	0.69	1.28	0.44
-7 0	-0.58	-0.93	-0.90
-8 0	6.38	5.26	5.51
-8 5	20.83	18.93	20.12
-9 0	46.57	43.91	45.88
-9 5	73.35	73.60	76.45
-10 0	92.25	100.40	103.95
-11 0	112.87	96.63	117.00

weak positive- Run 1			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.35	-1.73	-3.35
-5.5	122 34	133.37	123 27
-6.0	329 27	247.03	266.17
-6.5	514 58	493.33	431.49
-7.0	570 53	618.27	596.40
-7.5	609.44	761.72	657.18
-8.5	748 89	714.60	692.10

weak positive- Run 2			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.60	0.32	2.02
-4.5	1.69	2.23	1.86
-5.5	17.84	16.92	16.43
-6.0	44.26	41.37	41.37
-6.5	59.55	63.23	60.07
-7.0	80.32	57.20	89.58
-7.5	95.30	97.67	91.28
-8.5	104.79	102.37	107.61

weak positive- Run 3			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4 0			
-4 5	0.50	1.13	1.04
-5 5	16.60	16.57	16.45
-6 0	41.75	40.58	42.66
-6 5	69.60	69.77	71.05
-7 0	88.45	85.96	102.15
-7 5	104.20	104 03	104.58
-8 5	105.55	115 59	122.41

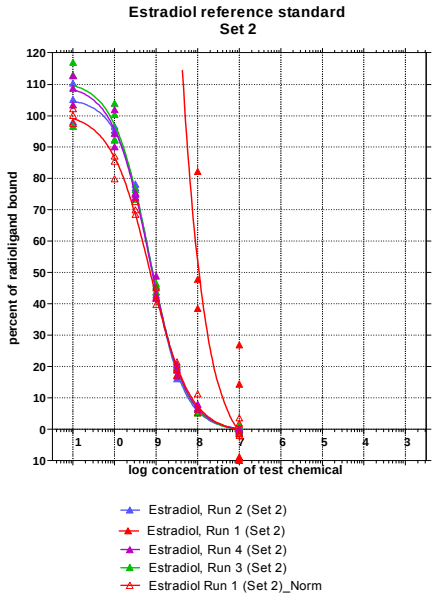
standard curve- Run 4			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.08	0.25	-0.03
-7.0	0.23	-0.35	-0.02
-8.0	6.41	7.84	7.93
-8.5	20 53	19.07	17.32
-9.0	48 90	41.90	42.85
-9.5	75 25	75.35	74.00
-10.0	94 35	101.89	90.09
-11.0	108 82	103.49	112.65

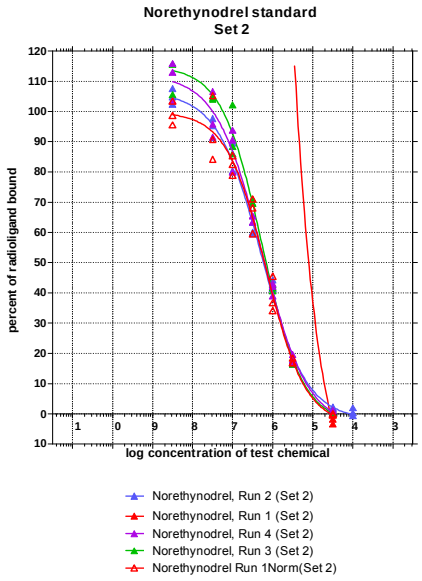
standard curve- Run 1 Norm			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	1.99	-1.21	-1.27
-7.0	-1.36	-1.87	3.73
-8.0	6.60	11.36	5.33
-8.5	21.45	19.15	17.12
-9.0	45.16	41.90	39.78
-9.5	70.02	68.54	72.61
-10.0	87.13	85.53	79.87
-11.0	97.62	102.24	100.15

weak positive- Run 4			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.91	0.96	1.18
-5.5	19.63	17.60	17.09
-6.0	42 20	43.02	38.96
-6.5	63 34	65.51	65.36
-7.0	79 93	90.56	93.85
-7.5	91 53	95.79	106 57
-8.5	103.49	113.02	115 91

weak positive- Run 1 Norm			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.05	-0.24	-0.46
-5.5	16.90	18.42	17.02
-6.0	45.48	34.12	36.76
-6.5	71.07	68.13	59.59
-7.0	78.79	85.39	82.37
-7.5	84.17	105.20	90.76
-8.5	103.43	98.69	95.58

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Method	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-7 97	-7.85	-8.08	1	1.480	1.934	1.133	NA	NA
	2	-9 08	-9.05	-9.11	1	0.114	0.123	0.106	NA	NA
	3	-9 08	-9.02	-9.13	1	0.115	0.130	0.101	NA	NA
	4	-9 09	-9.05	-9.13	1	0.112	0.122	0.103	NA	NA
	1_Norm	-9.14	-9.10	-9.18	1	0.100	0.110	0.090	NA	NA
Norethynodrel	1	-5.10	-4.87	-5.33	1	1098.14	1861.53	647 80	1.35E-03	-2.87
	2	-6 23	-6.18	-6.28	1	81.08	90.57	72.59	1.41E-03	-2.85
	3	-6.16	-6.10	-6.22	1	94.69	109.30	82.03	1.20E-03	-2.92
	4	-6 20	-6.12	-6.27	1	87.49	103.22	74.16	1.29E-03	-2.89
	1_Norm	-6 21	-6.12	-6.29	1	85.84	103.22	71.38	1.175E-03	-2.93


Estradiol reference standard
Set 2
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 2 (Set 2)
Estradiol, Run 1 (Set 2)
Estradiol, Run 4 (Set 2)
Estradiol, Run 3 (Set 2)
Estradiol Run 1 (Set 2)_Norm


Norethynodrel standard
Set 2
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 2 (Set 2)
Norethynodrel, Run 1 (Set 2)
Norethynodrel, Run 4 (Set 2)
Norethynodrel, Run 3 (Set 2)
Norethynodrel Run 1Norm(Set 2)

Competitive Binding Report

Test Chemicals Set 2

Tested Chemicals 4, 5, 16, 22
Assay Dates 2/21, 2/26, 2/27, and 3/3/2008

Test Chemical

Name Test Chemical 4
Code TC 4
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1, 2, 3)
0.001, 0.01, 0.03, 0.1, 0.3, 1, 10, 100, (Run 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0 5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.42	18.42	18.58	18.58

Incubation Temp. 4 °C
Kd for E2 (from satura ion binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	23FE0741	28FE0534	29FE0505	05MR0503
Excel (.xls)	S2R1_2_21_08	S2R2_2_26_08	S2R3_2_27_08	S2R4_3_3_08
Prism (.pzf)	RTI_Set 2			

Any precipitation of test chemicals? If yes, describe Level 1 was precipitated for run 2 and run 3

Test Chemical 4- Run 1

concentration (logM)	y1-U1	y2-U1	y3-U1
-3 0			
-4 0	-6.09	-17.49	-10.12
-5 0	-15.07	-21.39	-19.78
-6 0	-18.41	-9.96	-15.00
-7 0	-1.63	-6.25	-8.17
-8 0	81.71	70.01	70.69
-9 0	342.73	395.95	358.93
-10 0	682.00	624.37	639.73

Test Chemical 4- Run 2

concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0			
-4.0	6.07	5.82	5.31
-5.0	-1.27	-1.43	-1.02
-6.0	-1.56	-0.26	-0.68
-7.0	-1.10	-0.71	-0.65
-8.0	1.93	0.78	1.54
-9.0	19.87	16.34	14.65
-10.0	67.61	71.24	71.21

Test Chemical 4- Run 3

concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0			
-4.0			
-5.0	-1.43	-1.38	-1.21
-6.0	-0.96	-0.73	-1.93
-7.0	-0.87	3.17	-1.01
-8.0	0.91	2.07	2.32
-9.0	18.72	17.81	15.85
-10.0	49.37	68.74	66.31

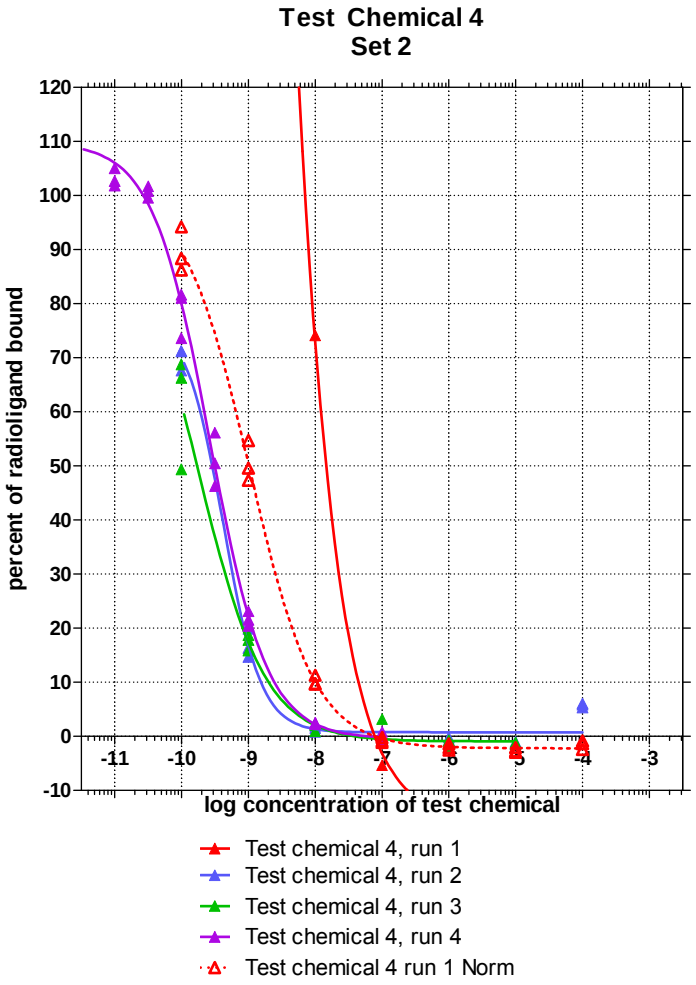
Test Chemical 4- Run 4

concentration (logM)	y1-U1	y2-U1	y3-U1
-7 0	0.79	-0.13	-0.81
-8 0	2.45	2.23	2.48
-9 0	23.13	21.56	20.46
-9 5	46.29	56.15	50.47
-10 0	81.07	73.63	81.67
-10 5	99.58	100.82	101.69
-11 0	101.84	105.03	102.69
-12 0	109.64	104.86	118.08

Test Chemical 4- Run 1 Norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0			
-4.0	-0.84	-2.41	-1.40
-5.0	-2.08	-2.95	-2.73
-6.0	-2.54	-1.37	-2.07
-7.0	-0.22	-0.86	-1.13
-8.0	11.28	9.67	9.76
-9.0	47.33	54.68	49.57
-10.0	94.19	86.23	88.35

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 4	1	-7.83	-7.71	-7.96	1	2.01	2.68	1.51	7.24E-01	-0.14
	2	-9.54	-8.86	-10 21	1	0.04	0.19	0.01	2 88E+00	0.46
	3	-9.77	-9.50	-10 03	1	0.02	0.04	0.01	4 90E+00	0.69
	4	-9.50	-9.45	-9.55	1	0.04	0.05	0.04	2 57E+00	0.41
	1_Norm	-8.99	-8.94	-9.04	1	0.14	0.16	0.13	7.08E-01	-0.15



Competitive Binding Report

Test Chemicals Set 2

Tested Chemicals 4, 5, 16, 22
Assay Dates 2/21, 2/26, 2/27, and 3/3/2008

Test Chemical

Name Test Chemical 5
Code TC 5
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1, 2 and 3)
0.1, 1, 10, 30, 100, 1000, 10000, 100000 (Run 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.42	18.42	18.58	18.58

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	23FE0741	28FE0534	29FE0505	05MR0503
Excel (.xls)	S2R1_2_21_08	S2R2_2_26_08	S2R3_2_27_08	S2R4_3_3_08
Prism (.pzf)	RTI_Set 2			

Any precipitation of test chemicals? If yes, describe Level 1 was cloudy for run 1, run 2 and run 3

Test Chemical 5- Run 1

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	-0.97	-5 50	2.03
-5.0	-2.17	-9 05	0.22
-6.0	72.60	149.79	62.80
-7.0	320.65	316 86	326.33
-8.0	676.74	611.12	681.79
-9.0	610.70	715 24	635.94
-10 0	615.75	645 61	677.38

Test Chemical 5- Run 2

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	0.91	0.25	1.70
-5.0	1.10	1.14	1.72
-6.0	10.63	9.32	11.95
-7.0	45.18	44.97	44.76
-8.0	85.90	91.88	84.18
-9.0	88.72	92.66	93.31
-10 0	96.94	94.38	102.18

Test Chemical 5- Run 3

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	2.06	1 27	0.82
-5.0	1.67	0 98	1.77
-6.0	10.31	8 99	9.51
-7.0	50.28	41 30	40.86
-8.0	91.75	92 36	91.09
-9.0	87.04	97 99	83.63
-10.0	74.59	87 87	81.86

Test Chemical 5- Run 4

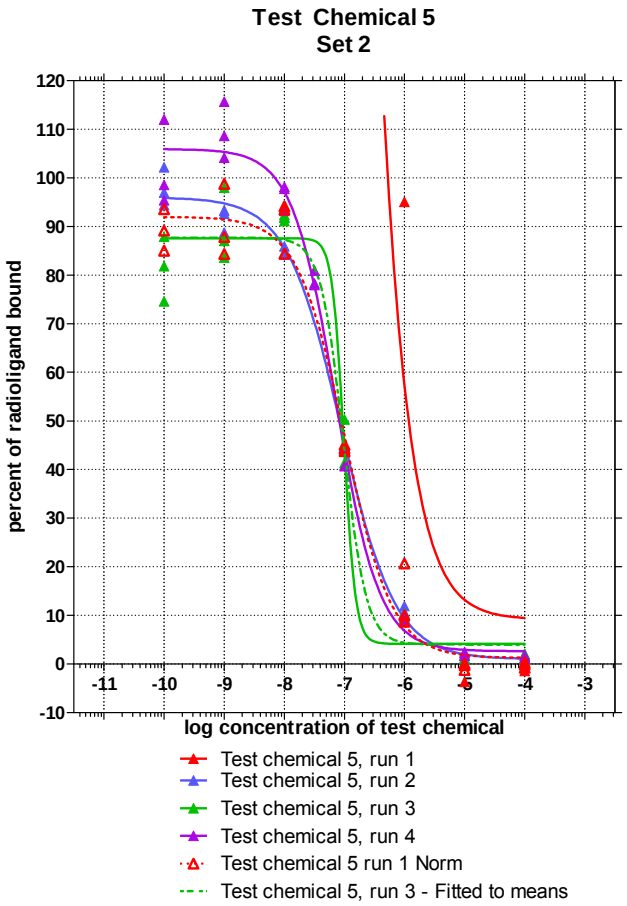
concentration (logM)	y1-U2	y2-U2	y3-U2
-4.0	1.17	1 28	1.74
-5.0	2.41	1.76	2.16
-6.0	9.77	8 69	9.83
-7.0	40.71	44 69	43.85
-7.5	80.92	78 29	77.99
-8.0	98.16	97.78	93.18
-9.0	115.69	104.13	108.64
-10 0	98.56	95 39	112.03

Test Chemical 5- Run 1 Norm

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	-0.13	-0.76	0.28
-5.0	-0.30	-1.25	0.03
-6.0	10.03	20.69	8.67
-7.0	44.28	43.76	45.07
-8.0	93.46	84.40	94.16
-9.0	84.34	98.78	87.83
-10 0	85.04	89.16	93.55

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 5	1	-5 93	-5.55	-6.30	1	162.20	385.50	68.24	9.12E-03	-2.04
	2	-7 08	-7.02	-7.14	1	11.46	13.20	9.96	1.00E-02	-2.00
	3	-7 03			1	12 93			8.91E-03	-2.05
	4	-7 08	-7.02	-7.15	1	11.40	13.20	9.84	9.77E-03	-2.01
	1_Norm	-7 05	-6.94	-7.16	1	12 24	15.89	9.43	8.13E-03	-2.09
	3*	-7 05	-6.65	-7.45	1	12 23	30.82	4.85	9.35E-03	-2.03

* The Prism fit for Run 3 using data from each individual tube was returned as "ambiguous". The fit was rerun using average values for each concentration and a better fit was obtained. This line reports the result of the fit using mean values.



Competitive Binding Report

Test Chemicals Set 2

Tested Chemicals 4, 5, 16, 22
Assay Dates 2/21, 2/26, 2/27, and 3/3/2008

Test Chemical

Name Test Chemical 16
Code TC 16
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1, 2, and 3)
10, 100, 1000, 2000, 5000, 10000, 30000, 100000 (Run 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.42	18.42	18.58	18.58

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	23FE0741	28FE0534	29FE0505	05MR0503
Excel (.xls)	S2R1_2_21_08	S2R2_2_26_08	S2R3_2_27_08	S2R4_3_3_08
Prism (.pzf)	RTI_Set 2			

Any precipitation of test chemicals? If yes, describe Level 1 was precipitated for run 2 and run 3

Test Chemical 16- Run 1

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0	-15 89	-13.05	-11.87
-5.0	32 07	35.90	22.79
-6.0	447 69	406.88	439.49
-7.0	653 61	629.84	586.51
-8.0	614 27	662.23	664.12
-9.0	666 86	647.72	646.04
-10.0	644.14	650.03	547.60

Test Chemical 16- Run 2

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0	-0.08	0 96	-0.68
-5.0	29.37	30 33	30.72
-6.0	93.96	97 90	93.83
-7.0	95.87	93 65	101.56
-8.0	102.70	99 83	98.14
-9.0	90.70	107 54	107.90
-10.0	103.98	100 04	95.30

Test Chemical 16- Run 3

concentration (logM)	y1-U3	y2-U3	y3-U3
-3 0			
-4 0	0.30	0 38	0.47
-5 0	22.92	27 36	24.23
-6 0	57.44	77 56	56.05
-7 0	77.75	71 82	79.92
-8 0	68.72	70 85	77.67
-9 0	79.92	89.17	77.23
-10 0	69.52	94.19	85.74

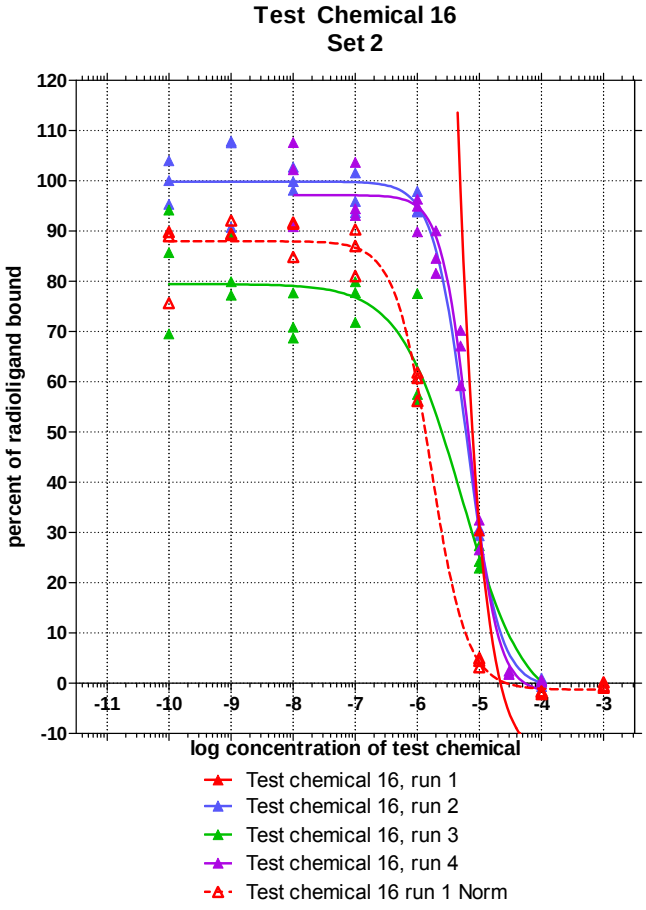
Test Chemical 16- Run 4

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.0	0 53	0.58	0.20
-4.5	2 38	1.73	1.78
-5.0	32.47	26.51	30.40
-5.3	70 27	67.10	59.21
-5.7	84 54	81.57	90.06
-6.0	94 97	89.84	96.31
-7.0	93.13	94.42	103.69
-8.0	90 84	102.24	107.64

Test Chemical 16- Run 1 Norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0	0.13	-0 92	-0.61
-4.0	-2.19	-1 80	-1.64
-5.0	4.43	4 96	3.15
-6.0	61.83	56.19	60.70
-7.0	90.27	86 99	81.00
-8.0	84.84	91.46	91.72
-9.0	92.10	89.46	89.22
-10.0	88.96	89.77	75.63

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 16	1	-5.11	-4.89	-5.33	1	1062 98	1767.05	639.44	1.38E-03	-2.86
	2	-5.22	-5.13	-5.30	1	834.94	1022.03	682.09	1.38E-04	-3.86
	3	-5.60	-5.38	-5.83	1	344.23	578.31	204.89	3.31E-04	-3.48
	4	-5.18	-5.14	-5.22	1	909.34	1000.00	826.91	1.23E-04	-3.91
	1_Norm	-5.87	-5.80	-5.94	1	186.51	218.99	158.85	5.37E-04	-3.27



Competitive Binding Report

Test Chemicals Set 2

Tested Chemicals 4, 5, 16, 22
Assay Dates 2/21, 2/26, 2/27, and 3/3/2008

Test Chemical

Name Test Chemical 22
Code TC 22
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1, 2 and 3)
10, 100, 1000, 10000, 30000, 100000, 200000, 500000 (Run 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.42	18.42	18.58	18.58

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: For run 2, 22TC6 and 22TC7-Possible wrong dilution used.

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	23FE0741	28FE0534	29FE0505	05MR0503
Excel (xls)	S2R1_2_21_08	S2R2_2_26_08	S2R3_2_27_08	S2R4_3_3_08
Prism (.pzf)	RTI_Set 2			

Any precipitation of test chemicals? If yes, describe Level 1 was precipited for all runs

Test Chemical 22- Run 1

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-4 0			
-5 0			
-6 0			
-7 0			
-8 0	538.76	540.66	565 69
-9 0	637.41	648.35	655 50
-10 0	621.85	661.39	637 20

Test Chemical 22- Run 2

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-4 0	63.93	67.17	71 01
-5 0	101.74	99.44	87.10
-6 0	106.41	91.96	103.12
-7 0	96.65	104.11	97.12
-8 0	92.74	92.71	99 05
-9 0	82.28	103.77	96 31
-10 0	89.84	94.46	95 95

Test Chemical 22- Run 3

concentration (logM)	y1-U4	y2-U4	y3-U4
-3.0			
-4.0	56.80	46 60	65.92
-5.0	76.12	72 87	62.37
-6.0	84.90	68 61	66.92
-7.0	88.09	58 90	73.46
-8.0	61.12	67 86	76.59
-9.0	75.76	94 30	89.26
-10.0	65.58	58 88	65.67

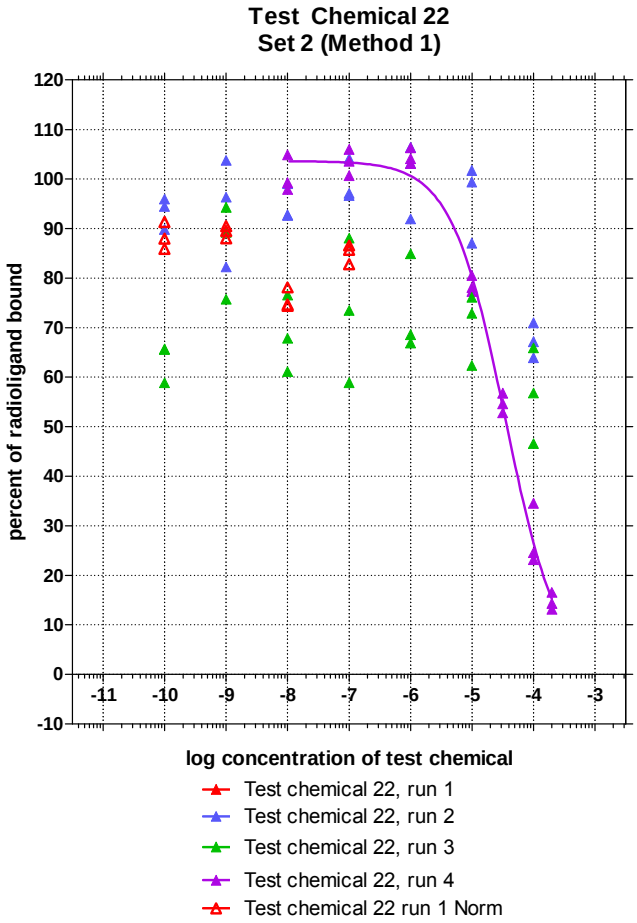
Test Chemical 22- Run 4

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 3			
-3.7	13.15	16.56	14.32
-4 0	23.15	24.55	34.53
-4 5	54.65	52.81	56.75
-5 0	78.11	77.29	80.53
-6 0	106.33	104.13	103.14
-7 0	105.98	100.72	103 59
-8 0	97.91	99.28	104 91

Test Chemical 22- Run 1 Norm

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-4 0			
-5 0			
-6 0			
-7 0	82.80	85.71	86.58
-8 0	74.41	74.67	78.13
-9 0	88.03	89.54	90.53
-10 0	85.88	91.34	88.00

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 22	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA
	4	-4.43	-4.37	-4.49	1	5124.49	5938.64	4421 96	2.19E-05	-4.66
	1_Norm	NA	NA	NA	1	NA	NA	NA	NA	NA



Competitive Binding Report

Test Chemicals Set 3

Tested Chemicals	9, 14, 17, 20
Assay Dates	2/28, 3/4, and 3/5/2008

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol	
Supplier	Perkin Elmer (Boston)	
Catalog #	NET-517	
Batch #	3589221	
Specific Ac ivity	110	Ci/mmol
SA date	6/28/2007	
Concentra ion (mCi/mL)	1	
Tested Concentrations (nM)	1	

Radioinert Ligand

Name	17β-estradiol
Supplier	Battelle Sequim
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in standard curve tubes (nM)	0.01, 0.1, 0.316, 1, 3.16, 10, 100 (All Runs)

Estrogen Receptor

Type	Rat Uterine Cytosol		
Rat Strain	Sprague Dawley		
Rat Age	94	days	
Ovariectomy Date	1/14/2008		
Uterus removal Date	1/22/2008		
Cytosol Prep Date	1/23/2008		
Cytosol Prep ID	1/23/2008		
Protein Content	2 20	mg/mL	
Protein Assay Date	1/23/2008		
RUC Storage Info	-70	°C	(UCF027)

Positive Control

Name	Norethynodrel
Supplier	Battelle Sequim
Lot #	G
CAS #	68-23-5
Purity	100
Concentration in assay tubes (nM)	3.16, 31.6, 100, 316, 1000, 3160, 31600, 100000 (All Runs)

Test Conditions

Buffer	TEDG + PMSF		
Protein Concentration Used	50	µg/tube	
Assay volume	0.5	mL	
Solvent	Ethanol		
Max Solvent Concentration	2 99%	(present in total binding tubes)	

Incubation Time (h)	Run 1	Run 2	Run 3
	19:08	18:42	18:42

Incubation Temp.	4	°C
Kd for E2 (from saturation binding assays)	0.1596	nM

Notes on problems: For run 2, P2 tube 3-may be short on protein

Results			
Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	01MR0555	06MR0502	07MR0425
Excel (xls)	S3R1_2_28_08	S3R2_3_4_08	S3R3_3_5_08
Prism (.pzf)	RTI_Set 3		

	Run 1	Run 2	Run 3
Assay Date	2/28/2008	3/4/2008	3/5/2008
Elapsed days since SA determination	245	250	251
Adjusted SA (Ci/mmol)	105.94	105.85	105.84

standard curve- Run 1			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	1.99	-0.39	-0 30
-7.0	-0.87	-1.32	0 89
-8.0	7.25	7.32	6 54
-8.5	24 50	20.69	21.87
-9.0	49.66	47.44	46.87
-9.5	86.43	88.68	77.41
-10.0	109 95	94.97	99.50
-11.0	103.18	119.42	110.37

standard curve- Run 2			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	0.34	0.05	-0 02
-7.0	0.13	-0.28	-0 23
-8.0	7.88	8.00	7.39
-8.5	24.55	22.95	21.29
-9.0	55.82	53.22	47.27
-9.5	87.63	87.21	87.30
-10.0	98.26	102.32	103.00
-11.0	118.31	123.41	116.03

standard curve- Run 3			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-7.20	-7.30	-8.39
-7.0	14.97	13.92	-6.00
-8.0	3.70	3.10	0.58
-8.5	25.04	21.63	18.27
-9.0	47.57	50.13	43.05
-9.5	83.53	73.89	79.71
-10.0	94.43	98.72	93.27
-11.0	101 09	100.02	100.82

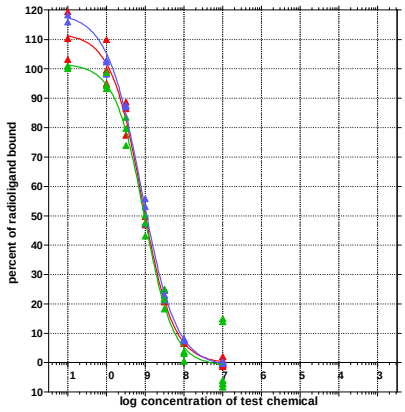
weak positive- Run 1			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	1.28	0.90	3.40
-5.5	17 84	19.38	19.81
-6.0	49 84	46.69	47.35
-6.5	68.18	68.48	70.43
-7.0	78.79	79.18	87.27
-7.5	95.42	84.63	98.24
-8.5	101.65	96.05	100.60

weak positive- Run 2			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	2.33	2.20	7.47
-5.5	22.26	19.03	18.42
-6.0	45.87	41.91	49.80
-6.5	77.98	85.97	77.33
-7.0	89.48	100.40	96.21
-7.5	111.51	103.59	112.94
-8.5	101.05	119.90	112.10

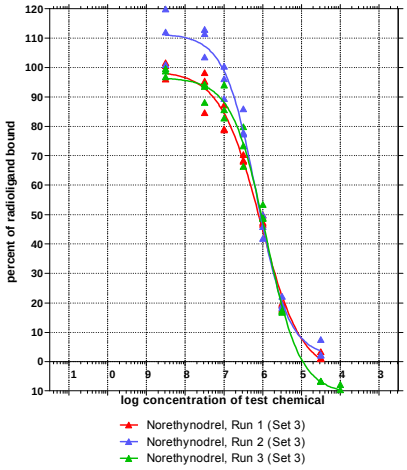
weak positive- Run 3			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-7.79	-10.32	-9.53
-4.5	-6.62	-6.66	-6.81
-5.5	16.77	17.09	17.44
-6.0	53.44	48.97	48.64
-6.5	79.85	73.42	66.39
-7.0	82.79	94.15	85.63
-7.5	94.39	88.14	93.55
-8.5	99.60	98.81	96.86

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Method	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-9.00	-8.95	-9.05	1	0.138	0.156	0.122	NA	NA
	2	-8.96	-8.93	-9.00	1	0.150	0.163	0.137	NA	NA
	3	-9.03	-8.94	-9.11	1	0.130	0.157	0.107	NA	NA
Norethynodrel	1	-6.10	-6.04	-6.17	1	108.3	126.2	92.8	1.26E-03	-2.90
	2	-6.06	-5.98	-6.13	1	121.0	142.6	102.6	1.26E-03	-2.90
	3	-6.03	-5.98	-6.09	1	127.1	142.9	113.1	1.00E-03	-3.00

Estradiol reference standard
Set 3



Norethynodrel standard
Set 3



Competitive Binding Report

Test Chemicals Set 3

Tested Chemicals: 9, 14, 17, 20
Assay Dates: 2/28, 3/4, and 3/5/2008

Test Chemical

Name: Test Chemical 9
Code: TC9
Supplier: Battelle Sequim
Lot #: N/A
CAS #: N/A
Purity: N/A
Concentration in assay tubes (nM): 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
1, 10, 100, 1000, 10000, 30000, 100000, 300000 (Run 2 and 3)

Test Conditions

Buffer: TEDG + PMSF
Protein Concentration Used: 50 µg/tube
Assay volume: 0.5 mL
Solvent: Ethanol
Max Solvent Concentration: 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	19:08	18:42	18:42

Incubation Temp.: 4 °C
Kd for E2 (from saturation binding assays): 0.1596 nM
Notes on problems: None

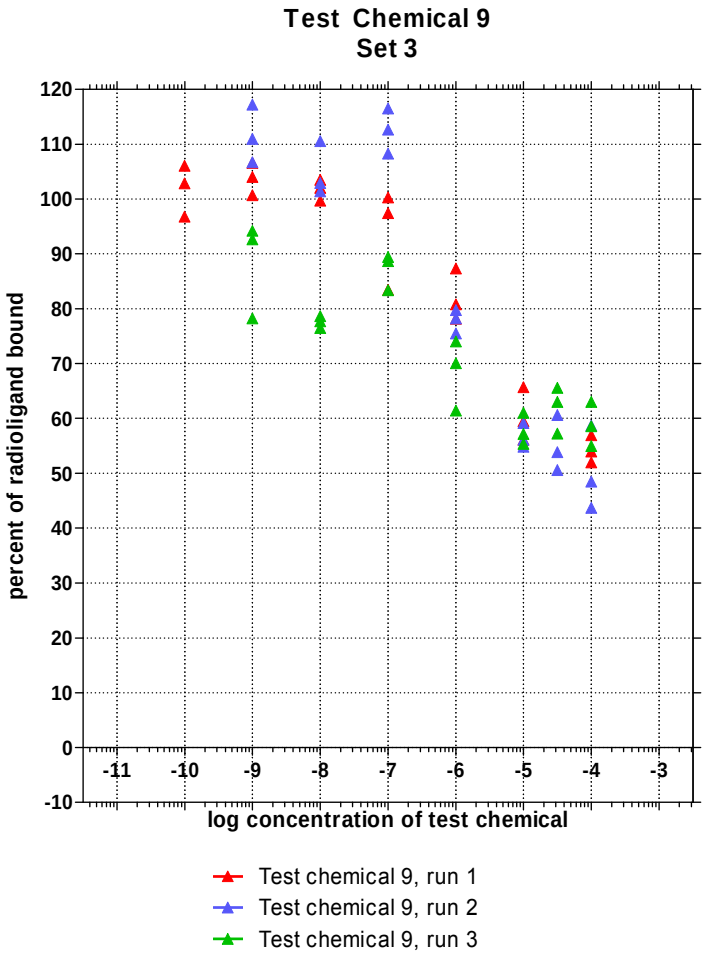
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	2/28/2008	3/4/2008	3/5/2008
Excel (.xls)	245	250	251
Prism (.pzf)	105.94	105.85	105.84

Any precipitation of test chemicals? If yes, describe: Level 1 was precipitated for all runs

Test Chemical 9- Run 1				Test Chemical 9- Run 2				Test Chemical 9- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0				-3.5				-3.5			
-4.0	56.97	53.98	51.97	-4.0	58.71	43.66	48.47	-4.0	58.56	63.04	54.93
-5.0	59.46	65.72	56.10	-4.5	60.63	50.55	53.87	-4.5	65.60	63.04	57.21
-6.0	80.77	78.16	87.33	-5.0	54.87	56.11	59.13	-5.0	57.17	55.40	61.03
-7.0	100.30	97.46	83.43	-6.0	75.51	78.24	79.73	-6.0	70.07	74.03	61.45
-8.0	102.01	103.48	99.68	-7.0	112.62	108.30	116.45	-7.0	83.39	89.45	88.70
-9.0	106.63	104.02	100.66	-8.0	102.94	101.38	110.54	-8.0	77.66	78.64	76.50
-10.0	106.06	96.77	102.85	-9.0	117.23	110.99	106.67	-9.0	78.22	92.66	94.20

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 9	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA


Test Chemical 9
Set 3
percent of radioligand bound
log concentration of test chemical
Test chemical 9, run 1
Test chemical 9, run 2
Test chemical 9, run 3

Competitive Binding Report

Test Chemicals Set 3

Tested Chemicals: 9, 14, 17, 20
Assay Dates: 2/28, 3/4, and 3/5/2008

Test Chemical

Name: Test Chemical 14
Code: TC14
Supplier: Battelle Sequim
Lot #: N/A
CAS #: N/A
Purity: N/A
Concentration in assay tubes (nM): 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
1, 10, 100, 1000, 3000, 10000, 30000, 100000 (Run 2 and 3)

Test Conditions

Buffer: TEDG + PMSF
Protein Concentration Used: 50 μg/tube
Assay volume: 0.5 mL
Solvent: Ethanol
Max Solvent Concentration: 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	19:08	18:42	18:42

Incubation Temp.: 4 °C
Kd for E2 (from saturation binding assays): 0.1596 nM
Notes on problems: None

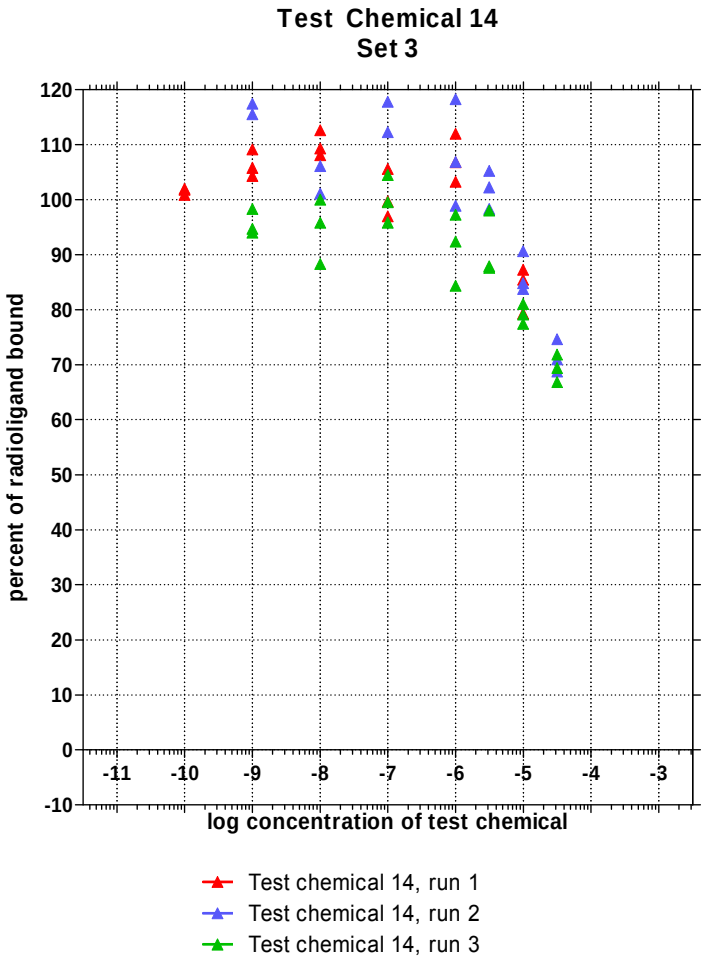
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	2/28/2008	3/4/2008	3/5/2008
Excel (.xls)	245	250	251
Prism (.pzf)	105.94	105.85	105.84

Any precipitation of test chemicals? If yes, describe: Level 1 was precipitated for run 1

Test Chemical 14- Run 1				Test Chemical 14- Run 2				Test Chemical 14- Run 3			
concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0				-4.0				-4.0			
-4.0				-4.5	68.75	74.63	70.99	-4.5	66.86	69.37	71.84
-5.0	87.27	85.47	79.30	-5.0	84.83	83.73	90.65	-5.0	79.11	77.43	81 06
-6.0	106.78	103.27	111.99	-5.5	98.32	105.21	102.22	-5.5	87.63	98.02	87.91
-7.0	99.68	96.95	105.58	-6.0	118.24	106.84	98.91	-6.0	97.23	84.37	92.38
-8.0	108.10	109.29	112.65	-7.0	112.26	117.79	121.39	-7.0	95.83	104.45	99.51
-9.0	109.11	104.29	105.79	-8.0	101.12	106.12	122.30	-8.0	100.02	95.78	88.33
-10.0	100.87	101.98	101.83	-9.0	115.54	122.66	117.40	-9.0	98.30	94.01	94.67

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 14	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA


Test Chemical 14
Set 3
percent of radioligand bound
log concentration of test chemical
Test chemical 14, run 1
Test chemical 14, run 2
Test chemical 14, run 3

Competitive Binding Report

Test Chemicals Set 3

Tested Chemicals: 9, 14, 17, 20
Assay Dates: 2/28, 3/4, and 3/5/2008

Test Chemical

Name: Test Chemical 17
Code: TC17
Supplier: Battelle Sequim
Lot #: N/A
CAS #: N/A
Purity: N/A
Concentration in assay tubes (nM): 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
10, 100, 1000, 2000, 5000, 10000, 30000, 100000 (Run 2 and 3)

Test Conditions

Buffer: TEDG + PMSF
Protein Concentration Used: 50 μg/tube
Assay volume: 0.5 mL
Solvent: Ethanol
Max Solvent Concentration: 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	19:08	18:42	18:42

Incubation Temp.: 4 °C
Kd for E2 (from saturation binding assays): 0.1596 nM
Notes on problems: None

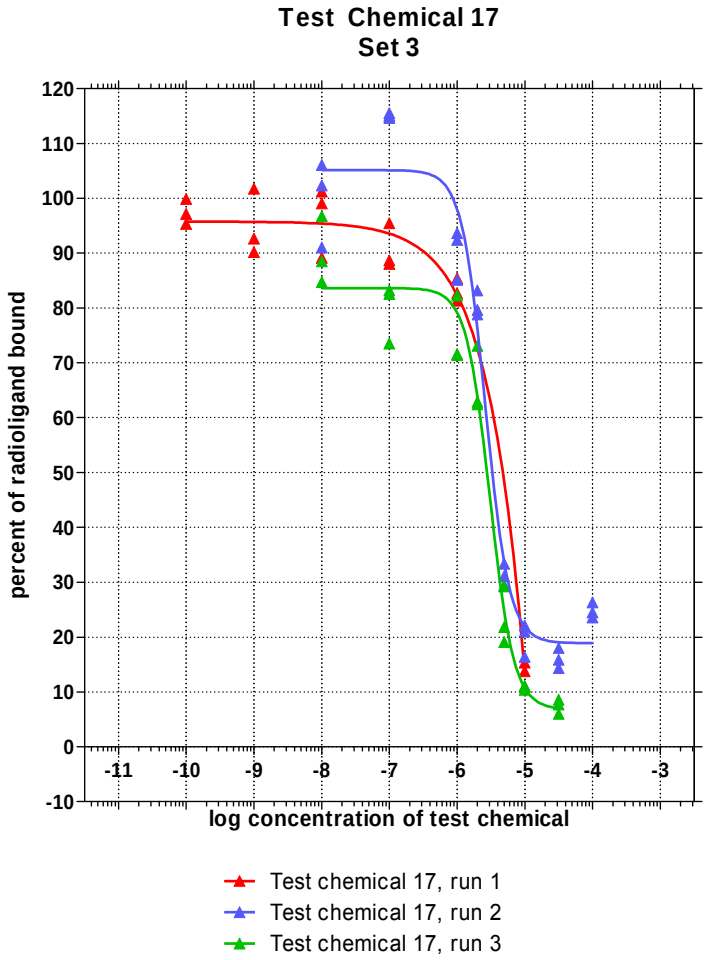
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	2/28/2008	3/4/2008	3/5/2008
Excel (.xls)	245	250	251
Prism (.pzf)	105.94	105.85	105.84

Any precipitation of test chemicals? If yes, describe: Level 1 was precipitated for run 1

Test Chemical 17- Run 1				Test Chemical 17- Run 2				Test Chemical 17- Run 3			
concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0				-4.0	24.47	26.33	23.58	-4.0			
-4.0				-4.5	14.37	18.00	15.87	-4.5	8.60	7.72	6.05
-5.0	15.35	13.76	10.96	-5.0	22.07	21.03	16.41	-5.0	11.12	10.95	10.43
-6.0	82.68	85.41	81.34	-5.3	33.36	31.12	29.43	-5.3	21.83	29.24	19 08
-7.0	88.02	88.68	95.45	-5.7	78.79	83.21	79.64	-5.7	62.90	73.10	62.43
-8.0	89.16	101.20	99.05	-6.0	93.61	85.09	92.41	-6.0	71.42	71.56	82 27
-9.0	90.18	92.60	101.74	-7.0	114.63	115.48	115.06	-7.0	73.52	83.21	82.55
-10.0	95.30	99.89	97.10	-8.0	91.01	106.06	102.32	-8.0	84.74	88.47	96.72

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 17	1	-5.32	-5.24	-5.41	1	653.30	791.59	539.16	2.09E-04	-3.68
	2	-5.48	-5.40	-5.56	1	456.17	543.14	383.12	3.31E-04	-3.48
	3	-5.56	-5.49	-5.62	1	382.43	443.01	330.13	3.39E-04	-3.47


Test Chemical 17
Set 3
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
Test chemical 17, run 1
Test chemical 17, run 2
Test chemical 17, run 3

Competitive Binding Report

Test Chemicals Set 3

Tested Chemicals 9, 14, 17, 20
Assay Dates 2/28, 3/4, and 3/5/2008

Test Chemical

Name Test Chemical 20
Code TC20
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000
0.1, 1, 10, 100, 300, 1000, 10000, 100000 (Run 2 and 3)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	19:08	18:42	18:42

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

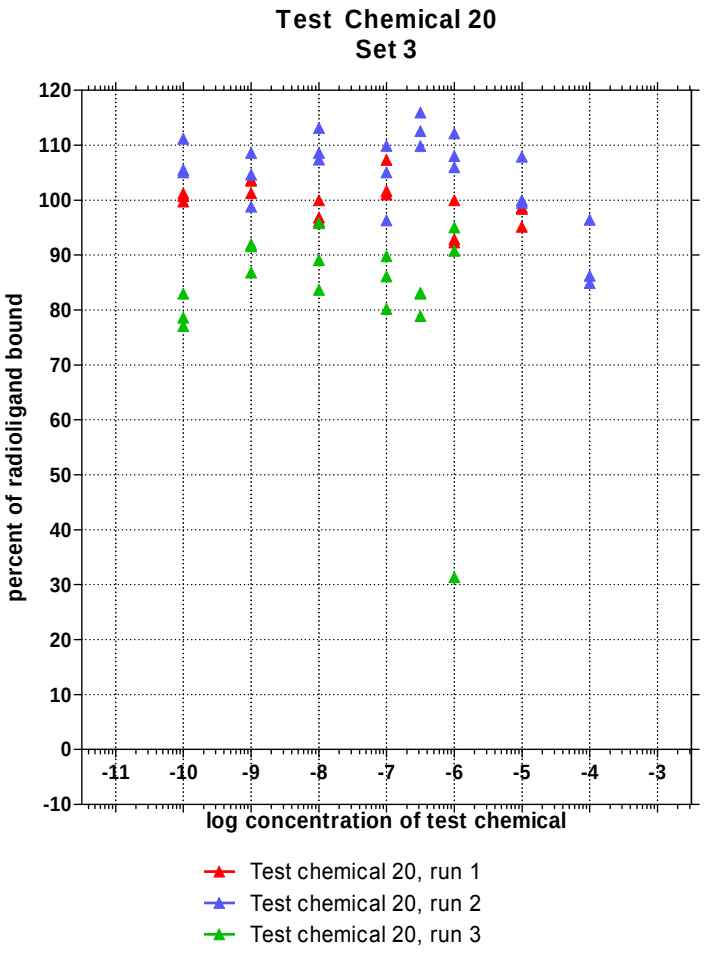
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	2/28/2008	3/4/2008	3/5/2008
Excel (.xls)	245	250	251
Prism (.pzf)	105.94	105.85	105.84

Any precipitation of test chemicals? If yes, describe: Level 1 was precipitated for run 1

Test Chemical 20- Run 1				Test Chemical 20- Run 2				Test Chemical 20- Run 3			
concentration (logM)	y1-U4	y2-U4	y3-U4	concentration (logM)	y1-U4	y2-U4	y3-U4	concentration (logM)	y1-U4	y2-U4	y3-U4
-3.0				-4.0	96.44	84.90	86.26	-4.0			
-4.0				-5.0	99.95	107.87	99.52	-5.0			
-5.0	98.42	98.72	95.12	-6.0	112.16	108.04	105.99	-6.0	95.04	31.38	90.75
-6.0	100.00	92.90	92.27	-6.5	112.59	115.97	109.82	-6.5	83.07	78.87	83.02
-7.0	101.05	107.35	101.71	-7.0	96.34	109.89	105.02	-7.0	80.18	89.82	86.09
-8.0	96.11	100.00	96.83	-8.0	107.39	113.17	108.65	-8.0	95.78	83.72	89.08
-9.0	103.57	101.26	103.66	-9.0	98.81	108.65	104.63	-9.0	91.96	86.79	91.59
-10.0	100.75	99.77	101.20	-10.0	105.50	105.05	111.16	-10.0	77.10	78.59	83.02

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 20	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA


Test Chemical 20
Set 3
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
Test chemical 20, run 1
Test chemical 20, run 2
Test chemical 20, run 3

Competitive Binding Report

Test Chemicals Set 4

Tested Chemicals 1, 3, 8, 23
Assay Dates 3/6, 3/11, 3/13, 3/18 and 4/3/2008

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	1

Radioinert Ligand

Name	17β-estradiol
Supplier	Battelle Sequim
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in standard curve tubes (nM)	0.01, 0.1, 0.316, 1, 3.16, 10, 100 (All runs)

Estrogen Receptor

Type	Rat Uterine Cytosol
Rat Strain	Sprague Dawley
Rat Age	94 days
Ovariectomy Date	1/14/2008
Uterus removal Date	1/22/2008
Cytosol Prep Date	1/23/2008
Cytosol Prep ID	1/23/2008
Protein Content	2.20 mg/mL
Protein Assay Date	1/23/2008
RUC Storage Info	-70 C (UCF027)

Positive Control

Name	Norethynodrel
Supplier	Battelle Sequim
Lot #	G
CAS #	68-23-5
Purity	100
Concentration in assay tubes (nM)	3.16, 31.6, 100, 316, 1000, 3160, 31600, 100000 (All runs)

Test Conditions

Buffer	TEDG + PMSF
Protein Concentration Used	50 μg/tube
Assay volume	0.5 mL
Solvent	Ethanol
Max Solvent Concentration	2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	Run 5
	18.13	18.00	18.42	18.33	18.50

Incubation Temp.	4 C
Kd for E2 (from saturation binding assays)	0.1596 nM
Notes on problems:	None

Results

Filenames	Run 1	Run 2	Run 3	Run 4	Run 5
Counter Data (.P00)	08MR0437	13MR0359	15MR0355	20MR0825	05AP0250
Excel (.xls)	S4R1_3_6_08	S4R2_3_11_08	S4R3_3_13_0	S4R4_3_18_08	S4R5_4_3_08
Prism (.pzf)	RTI_Set 4				

	Run 1	Run 2	Run 3	Run 4	Run 5
Assay Date	3/6/2008	3/11/2008	3/13/2008	3/18/2008	4/3/2008
Elapsed days since SA determination	252	257	259	264	280
Adjusted SA (Ci/mmol)	105.81	105.73	105.70	105.62	105.36

standard curve- Run 1

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	0.58	-0.46	0.96
-7.0	-0.08	-0.46	-0.54
-8.0	13.92	11.83	10.15
-8.5	38.04	32.71	31.82
-9.0	65.59	53.04	59.18
-9.5	102.06	92.89	87.87
-10.0	125.62	127.62	114.35
-11.0	135.86	131.92	125.88

standard curve- Run 2

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.01	-0.01	0.02
-7.0	0.24	0.36	-0.59
-8.0	11.14	7.92	9.20
-8.5	30.67	24.54	21.19
-9.0	62.87	54.61	55.71
-9.5	98.83	92.97	84.71
-10.0	108.77	104.52	105.32
-11.0	102.37	112.32	114.68

standard curve- Run 3

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	1.89	-0.27	0.57
-7.0	-0.09	-2.03	-0.06
-8.0	11.53	10.55	9.55
-8.5	35.62	31.25	28.16
-9.0	74.31	58.48	62.15
-9.5	119.83	121.66	108.30
-10.0	121.22	104.06	105.46
-11.0	143.38	135.21	133.88

standard curve- Run 4

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-3.26	-5.52	17.85
-7.0	5.76	-0.65	-14.17
-8.0	76.51	61.36	76.95
-8.5	232.14	156.27	193.45
-9.0	447.28	260.73	297.50
-9.5	690.09	623.40	515.81
-10.0	806.15	593.55	695.24
-11.0	890.15	806.15	595.03

standard curve- Run 5

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.91	0.53	-0.40
-7.0	0.22	0.49	0.06
-8.0	9.07	14.22	9.25
-8.5	29.25	32.78	22.80
-9.0	58.76	51.32	56.36
-9.5	96.48	96.29	95.83
-10.0	113.21	104.94	104.85
-11.0	128.88	141.27	135.21

standard curve- Run 1_norm

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	0.44	-0.35	0.73
-7.0	-0.06	-0.35	-0.42
-8.0	10.61	9.01	7.73
-8.5	28.99	24.92	24.25
-9.0	49.98	40.42	45.10
-9.5	77.78	70.79	66.96
-10.0	95.73	97.25	87.14
-11.0	103.54	100.53	95.93

standard curve- Run 3_norm

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	1.37	-0.20	0.41
-7.0	-0.07	-1.48	-0.04
-8.0	8.38	7.67	6.95
-8.5	25.91	22.73	20.48
-9.0	54.05	42.53	45.21
-9.5	87.15	88.49	78.77
-10.0	88.17	75.69	76.70
-11.0	104.28	98.34	97.38

standard curve- Run 4_norm

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.43	-0.72	2.34
-7.0	0.75	-0.08	-1.86
-8.0	10.02	8.03	10.07
-8.5	30.39	20.46	25.33
-9.0	58.56	34.14	38.95
-9.5	90.35	81.62	67.53
-10.0	105.55	77.71	91.03
-11.0	116.55	105.55	77.91

standard curve- Run 5_norm

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.67	0.39	-0.29
-7.0	0.16	0.36	0.05
-8.0	6.72	10.53	6.85
-8.5	21.65	24.26	16.87
-9.0	43.49	37.98	41.71
-9.5	71.40	71.27	70.92
-10.0	83.79	77.66	77.59
-11.0	95.38	104.55	100.07

weak positive- Run 1

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	0.48	2.59	1.53
-4.5	2.20	2.60	3.68
-5.5	26.67	27.16	28.30
-6.0	67.74	52.01	55.39
-6.5	83.51	78.49	91.51
-7.0	97.14	105.85	100.98
-7.5	116.66	121.42	128.59
-8.5	122.34	119.88	130.59

weak positive- Run 2

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	2.28	1.60	1.86
-5.5	25.97	28.95	26.46
-6.0	61.31	56.00	54.19
-6.5	87.95	90.06	86.56
-7.0	100.34	108.90	111.39
-7.5	99.92	106.96	104.48
-8.5	100.34	112.19	114.34

weak positive- Run 3

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.85	-1.18	0.09
-4.5	2.45	3.58	8.90
-5.5	30.81	29.35	32.00
-6.0	76.90	67.79	64.56
-6.5	96.94	96.91	96.66
-7.0	107.48	117.86	116.60
-7.5	110.27	113.37	138.06
-8.5	130.34	130.34	131.03

weak positive- Run 4

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	10.74	12.95	5.91
-5.5	179.08	158.56	174.73
-6.0	374.32	347.06	413.38
-6.5	588.03	410.80	538.65
-7.0	604.24	679.77	676.45
-7.5	650.66	751.62	660.61
-8.5	627.82	692.67	809.10

weak positive- Run 5

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.71	1.61	1.71
-5.5	23.64	21.62	22.03
-6.0	52.02	48.69	42.87
-6.5	77.76	76.51	68.42
-7.0	105.45	104.98	103.09
-7.5	110.72	113.77	114.27
-8.5	129.85	129.76	110.25

weak positive- Run 1 -Norm

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	0.37	1.98	1.17
-4.5	1.68	1.98	2.80
-5.5	20.32	20.70	21.56
-6.0	51.62	39.64	42.21
-6.5	63.64	59.82	69.73
-7.0	74.03	80.66	76.96
-7.5	88.90	92.53	98.00
-8.5	93.23	91.36	99.52

weak positive- Run 3_norm

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.61	-0.86	0.07
-4.5	1.78	2.60	6.47
-5.5	22.41	21.35	23.27
-6.0	55.93	49.30	46.96
-6.5	70.51	70.48	70.30
-7.0	78.17	85.73	84.80
-7.5	80.20	82.46	100.41
-8.5	94.80	94.80	95.30

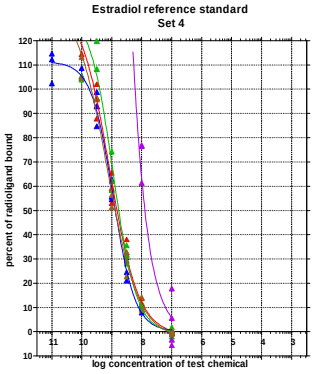
weak positive- Run 4_norm

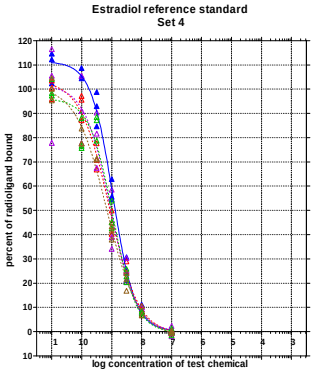
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-1.17	0.70	9.19
-4.5	1.41	1.70	0.77
-5.5	23.45	20.76	22.88
-6.0	49.01	45.44	54.12
-6.5	76.99	53.79	70.52
-7.0	79.11	89.00	88.57
-7.5	85.19	98.41	86.49
-8.5	82.20	90.69	105.93

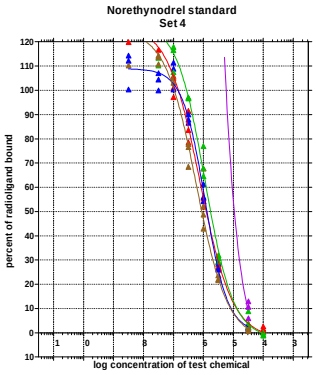
weak positive- Run 5_norm

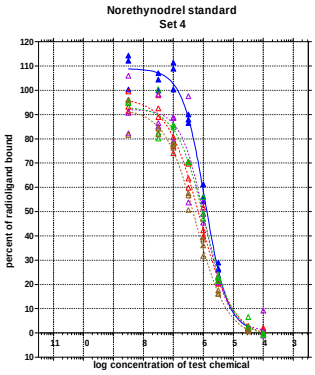
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.53	1.19	1.27
-5.5	17.49	16.00	16.31
-6.0	38.50	36.03	31.72
-6.5	57.55	56.63	50.64
-7.0	78.04	77.70	76.29
-7.5	81.94	84.20	84.57
-8.5	96.10	96.03	81.60

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Method	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-8.82	-8.76	-8.89	1	0.207	0.239	0.179	NA	NA
	2	-8.88	-8.83	-8.93	1	0.182	0.204	0.163	NA	NA
	3	-8.77	-8.67	-8.87	1	0.235	0.296	0.187	NA	NA
	4	-7.89	-7.44	-8.34	1	1.759	4.955	0.625	NA	NA
	5	-8.88	-8.82	-8.95	1	0.180	0.208	0.155	NA	NA
Estradiol - Norm	1	-9.05	-8.99	-9.11	1	0.12	0.14	0.11	NA	NA
	3	-8.99	-8.90	-9.08	1	0.14	0.17	0.11	NA	NA
	4	-9.02	-8.88	-9.16	1	0.13	0.18	0.09	NA	NA
	5	-9.15	-9.09	-9.21	1	0.10	0.11	0.08	NA	NA
Norethynodrel	1	-5.90	-5.83	-5.98	1	171.8	205.6	143.5	1.20E-03	-2.92
	2	-5.88	-5.82	-5.94	1	180.8	207.3	157.8	1.00E-03	-3.00
	3	-5.76	-5.68	-5.85	1	239.0	290.7	196.4	9.77E-04	-3.01
	4	-4.97	-4.47	-5.46	1	1479.8	4615.5	474.5	1.20E-03	-2.92
	5	-6.05	-5.98	-6.12	1	121.9	143.8	103.3	1.48E-03	-2.83
Norethynodrel - Norm	1	-6.17	-6.10	-6.24	1	93.37	109.65	79.50	1.32E-03	-2.88
	3	-6.03	-5.96	-6.11	1	127.04	150.81	107.01	1.10E-03	-2.96
	4	-6.02	-5.88	-6.15	1	132.76	180.81	97.48	1.00E-03	-3.00
	5	-6.34	-6.27	-6.41	1	62.83	73.29	53.87	1.55E-03	-2.81


Estradiol reference standard
Set 4
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 1 (Set 4)
Estradiol, Run 2 (Set 4)
Estradiol, Run 3 (Set 4)
Estradiol, Run 4 (Set 4)
Estradiol, Run 5 (Set 4)


Estradiol reference standard
Set 4
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 1-Norm (Set 4)
Estradiol, Run 2 (Set 4)
Estradiol, Run 3-Norm (Set 4)
Estradiol, Run 4-Norm (Set 4)
Estradiol, Run 5 - Norm


Norethynodrel standard
Set 4
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 1
Norethynodrel, Run 2
Norethynodrel, Run 3
Norethynodrel, Run 4
Norethynodrel, Run 5


Norethynodrel standard
Set 4
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 1-N
Norethynodrel, Run 2
Norethynodrel, Run 3-Norm
Norethynodrel, Run 4-N
Norethynodrel, Run 5 Norm

Competitive Binding Report

Test Chemicals Set 4

Tested Chemicals 1, 3, 8, 23
Assay Dates 3/6, 3/11, 3/13, 3/18 and 4/3/2008

Test Chemical

Name Test Chemical 1
Code TC1
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
0.01, 0.1, 0.3, 1, 3, 10, 100, 1000 (Run 2, 3, 4 and 5)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	Run 5
	18.13	18.00	18.42	18.33	18.50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4	Run 5
Counter Data (.P00)	08MR0437	13MR0359	15MR0355	20MR0825	05AP0250
Excel (.xls)	S4R1_3_6_08	S4R2_3_11_08	S4R3_3_13_08	S4R4_3_18_08	S4R5_4_3_08
Prism (.pzf)	RTI_Set 4				

Any precipitation of test chemicals? If yes, describe For Run-1, level 1 tubes were precipitated.

Test Chemical 1- Run 1

concentration (logM)	y1-U1	y2-U1	y3-U1
-3 0			
-4 0			
-5 0	-1.36	-2.21	-2.22
-6 0	-1.49	-2.51	-0.78
-7 0	-0.27	0.53	0 55
-8 0	14 28	11.38	9.70
-9 0	59 95	56.06	57.13
-10 0	108.46	102.11	103.75

Test Chemical 1- Run 2

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-1.49	-1 57	-1.40
-7 0	-0.26	1.16	1.09
-8 0	9.20	9 87	9.64
-8 5	27.71	26.72	26.76
-9 0	54 61	52.12	52.76
-9 5	74.76	96.09	88.42
-10.0	95.41	104.18	91.41
-11.0	97 65	111.01	104.73

Test Chemical 1- Run 3

concentration (logM)	y1-U1	y2-U1	y3-U1
-6.0	-1.79	-2 90	-2.22
-7.0	1.84	-0 55	0.86
-8.0	9.77	10.79	8.60
-8.5	35.74	32.10	35.26
-9.0	55.68	61 27	64.68
-9.5	86.84	91 59	106.91
-10.0	97.79	114.76	104.44
-11.0	107.61	117.61	115.90

Test Chemical 1- Run 4

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-14.08	-5.17	-14.45
-7 0	-1.31	-7.30	-3.60
-8 0	60 59	63.35	63 61
-8 5	175 88	188.44	173 56
-9 0	372 85	303.95	336 37
-9 5	552 29	576.60	547 86
-10 0	569 23	628.19	506 97
-11 0	576 60	715.51	634.45

Test Chemical 1- Run 5

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-1.25	-1.73	-0.92
-7 0	1.42	0.14	1.08
-8 0	7.90	8 35	7.74
-8 5	28.71	24.57	21.02
-9 0	49 57	52.16	48.87
-9 5	84 97	98.61	86.82
-10.0	98 51	108.36	101.15
-11.0	105.40	108.08	116.26

Test Chemical 1- Run 1 norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0			
-4.0			
-5.0	-1.04	-1 68	-1.70
-6.0	-1.13	-1 92	-0.60
-7.0	-0.20	0.40	0.42
-8.0	10.88	8 67	7.39
-9.0	45.69	42.72	43.54
-10.0	82.66	77 81	79.06

Test Chemical 1- Run 3 norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-1.30	-2.11	-1.61
-7 0	1.34	-0.40	0 62
-8 0	7.11	7.85	6 26
-8 5	26 00	23.35	25 65
-9 0	40 50	44.56	47 05
-9 5	63.16	66.62	77.76
-10 0	71.13	83.47	75 96
-11 0	78 27	85.54	84 30

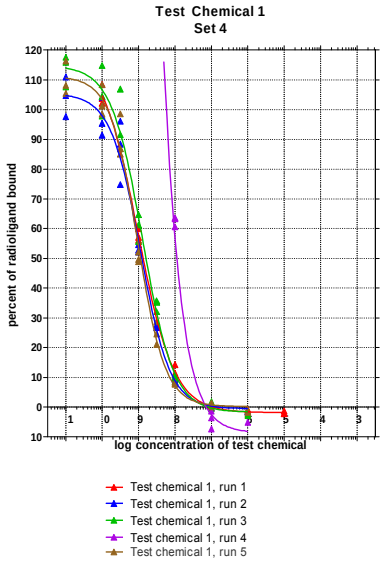
Test Chemical 1- Run 4 norm

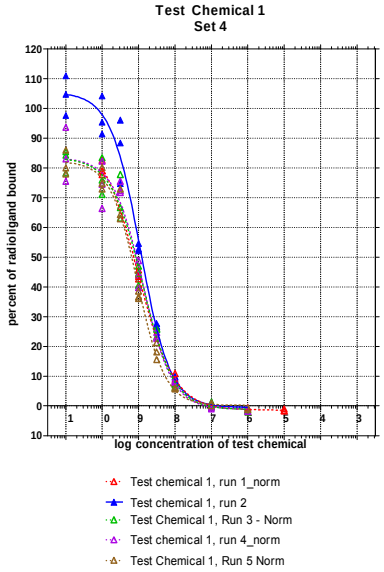
concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-1.84	-0 68	-1.89
-7 0	-0.17	-0 96	-0.47
-8 0	7.93	8 29	8.33
-8 5	23 03	24.67	22.72
-9 0	48 82	39.80	44.04
-9 5	72 31	75.49	71.73
-10.0	74 53	82.25	66.38
-11.0	75.49	93.68	83.07

Test Chemical 1- Run 5 norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-6.0	-0.92	-1 28	-0.68
-7.0	1.05	0.10	0.80
-8.0	5.84	6.18	5.73
-8.5	21.25	18.18	15.55
-9.0	36.68	38 60	36.17
-9.5	62.89	72 98	64.25
-10.0	72.91	80.19	74.86
-11.0	78.01	79 99	86.04

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 1	1	-8.87	-8.84	-8 91	1	0.18	0.20	0.17	1.12E+00	0.05
	2	-8.91	-8.85	-8 98	1	0.17	0.20	0.15	1.07E+00	0.03
	3	-8.79	-8.73	-8 86	1	0.22	0.26	0.19	1.05E+00	0.02
	4	-7.95	-7.74	-8.16	1	1.55	2.53	0.95	1.15E+00	0.06
	5	-8.94	-8.89	-9 00	1	0.16	0.18	0.14	1.15E+00	0.06
Test Chemical 1 - Norm	1	-9.13	-9.09	-9.17	1	0.10	0.11	0.09	1 20E+00	0.08
	3	-9.07	-9.00	-9.15	1	0.12	0.14	0.10	1 20E+00	0.08
	4	-9.05	-8.96	-9.14	1	0.12	0.15	0.10	1 07E+00	0.03
	5	-9.19	-9.13	-9 24	1	0.09	0.10	0.08	1.10E+00	0.04


Test Chemical 1
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 1, run 1
Test chemical 1, run 2
Test chemical 1, run 3
Test chemical 1, run 4
Test chemical 1, run 5


Test Chemical 1
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 1, run 1_norm
Test chemical 1, run 2
Test Chemical 1, Run 3 - Norm
Test chemical 1, run 4_norm
Test Chemical 1, Run 5 Norm

Competitive Binding Report

Test Chemicals Set 4

Tested Chemicals 1, 3, 8, 23
Assay Dates 3/6, 3/11, 3/13, 3/18 and 4/3/2008

Test Chemical

Name Test Chemical 3
Code TC3
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
0.001, 0.01, 0.03, 0.1, 0 3, 1, 10, 100 (Run 2, 3, 4 and 5)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	Run 5
	18.13	18.00	18.42	18.33	18.50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: For Run 3, 3TC5-tube 1 possible pipetting error.

Results

Filenames	Run 1	Run 2	Run 3	Run 4	Run 5
Counter Data (.P00)	08MR0437	13MR0359	15MR0355	20MR0825	05AP0250
Excel (xls)	S4R1_3_6_08	S4R2_3_11_08	S4R3_3_13_08	S4R4_3_18_08	S4R5_4_3_08
Prism (pzf)	RTI_Set 4				

Any precipitation of test chemicals? If yes, describe For Run-1, level 1 tubes were precipitated.

Test Chemical 3- Run 1			
concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	5.31	5.11	5.67
-5.0	-1.01	-1.92	-1.42
-6.0	-0.84	-1.76	-1.22
-7.0	-0.82	-1.34	1.35
-8.0	6.88	2.20	1.95
-9.0	22.54	21.03	19.14
-10 0	84.59	88.38	78.75

Test Chemical 3- Run 2			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	-0.17	-1.11	-0.96
-8.0	1.98	3 27	1.55
-9.0	20.82	19 63	14.93
-9.5	41.50	46 69	48.08
-10.0	78.21	78 51	68.35
-10.5	106.12	96.47	100.64
-11.0	94.74	99.75	109.66
-12.0	105.19	100.47	102.03

Test Chemical 3- Run 3			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	5.10	-2.67	-2.24
-8.0	1 69	1.80	2.15
-9.0	23.89	19.26	19.59
-9.5	55.48	55.09	49.11
-10.0	94.95	74.88	71.46
-10.5	86.21	107.99	98.55
-11.0	114.57	84.06	100.83
-12.0	102.29	112.55	107.67

Test Chemical 3- Run 4			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	-8.22	-14.48	-15.68
-8.0	11.40	3.15	2.74
-9.0	115.93	127.61	102.67
-9.5	256.42	258.04	335.27
-10 0	464.59	385.38	444.33
-10 5	678.30	697.09	650.29
-11 0	703.35	649.93	592.08
-12 0	777.78	629.66	684.56

Test Chemical 3- Run 5			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	-1.13	-1.43	-1.58
-8.0	1.52	1 65	0.35
-9.0	11.58	12.48	11.58
-9.5	35.25	34 93	28.85
-10.0	63.71	63 57	52.89
-10.5	87.51	84 00	78.50
-11.0	85.62	86 27	82.43
-12.0	92.97	88.44	93.34

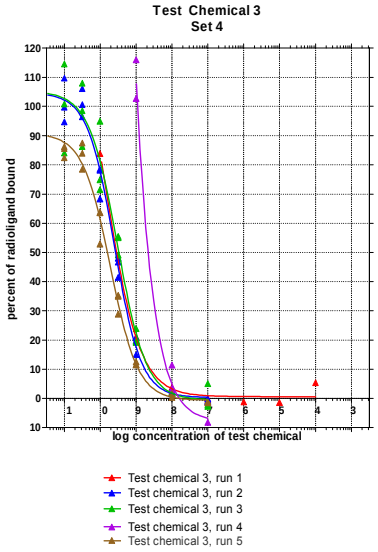
Test Chemical 3- Run 1 norm			
concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0			
-4.0	4 04	3.90	4.32
-5.0	-0.77	-1.46	-1.08
-6.0	-0 64	-1.34	-0.93
-7.0	-0 63	-1.02	1.03
-8.0	5 25	1.67	1.48
-9.0	17.18	16.02	14.59
-10.0	64.46	67.35	60.01

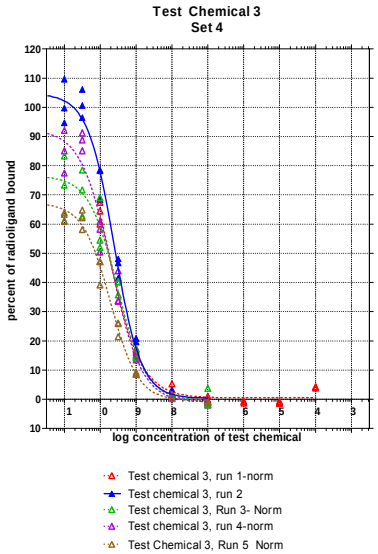
Test Chemical 3- Run 3 norm			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	3.71	-1.94	-1.63
-8.0	1.23	1.31	1.56
-9.0	17.38	14.01	14.25
-9.5	40.35	40.07	35.72
-10 0	69.06	54.46	51.97
-10 5	62.70	78.54	71.68
-11 0	83.33	61.14	73.34
-12 0	74.40	81.86	78.31

Test Chemical 3- Run 4 norm			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	-1.08	-1.90	-2.05
-8.0	1.49	0.41	0.36
-9.0	15.18	16.71	13.44
-9.5	33.57	33.78	43.90
-10.0	60.83	50.46	58.18
-10.5	88.81	91 27	85.14
-11.0	92.09	85 09	77.52
-12.0	101.83	82.44	89.63

Test Chemical 3- Run 5 norm			
concentration (logM)	y1-U2	y2-U2	y3-U2
-7.0	-0 83	-1.06	-1.17
-8.0	1.12	1.22	0.26
-9.0	8 57	9.23	8.57
-9.5	26.09	25.85	21.35
-10.0	47.15	47.05	39.15
-10.5		62.17	58.10
-11.0	63 36	63.84	61.00
-12.0	68 80	65.45	69.08

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 3	1	-9 54	-9.31	-9.77	1	0.04	0.07	0.02	5.25E+00	0.72
	2	-9 57	-9.51	-9.63	1	0.04	0.04	0.03	4.90E+00	0.69
	3	-9.48	-9.37	-9.58	1	0.05	0.06	0.04	5.13E+00	0.71
	4	-8 66	-8.38	-8.95	1	0.30	0.58	0.16	5.89E+00	0.77
	5	-9 80	-9.74	-9.85	1	0.02	0.02	0.02	8.32E+00	0.92
Test Chemical 3 - Norm	1	-9.75	-9.56	-9.95	1	0.02	0.04	0.02	5.01E+00	0.70
	3	-9.77	-9.65	-9.89	1	0.02	0.03	0.02	6.03E+00	0.78
	4	-9.77	-9.67	-9.86	1	0.02	0.03	0.02	5.62E+00	0.75
	5	-10.13	-10.06	-10 20	1	0.01	0.01	0.01	8.91E+00	0.95


Test Chemical 3
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 3, run 1
Test chemical 3, run 2
Test chemical 3, run 3
Test chemical 3, run 4
Test chemical 3, run 5


Test Chemical 3
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 3, run 1-norm
Test chemical 3, run 2
Test chemical 3, Run 3- Norm
Test chemical 3, run 4-norm
Test Chemical 3, Run 5 Norm

Competitive Binding Report

Test Chemicals Set 4

Tested Chemicals 1, 3, 8, 23
Assay Dates 3/6, 3/11, 3/13, 3/18 and 4/3/2008

Test Chemical

Name Test Chemical 8
Code TC8
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
10, 100, 300, 1000, 2000, 5000, 10000, 20000 (Run 2, 3, 4 and 5)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	Run 5
	18.13	18.00	18.42	18.33	18 50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: For Run 3- 8TC2-tube 2 possible pipetting error.

Results

Filenames	Run 1	Run 2	Run 3	Run 4	Run 5
Counter Data (.P00)	08MR0437	13MR0359	15MR0355	20MR0825	05AP0250
Excel (xls)	S4R1_3_6_08	S4R2_3_11_08	S4R3_3_13_08	S4R4_3_18_08	S4R5_4_3_08
Prism (pzf)	RTI_Set 4				

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 8- Run 1

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0			
-5.0	2.76	5.92	9.98
-6.0	80.49	71.68	69.74
-7.0	94.53	38.25	46.79
-8.0	86.54	96.53	83.57
-9.0	81.52	93.86	75.73
-10 0	92.94	76.86	82.54

Test Chemical 8- Run 2

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	4.13	4.54	3.48
-5.0	8.24	10 05	9.17
-5.3	25.96	23 31	21.24
-5.7	42.60	41 88	42.85
-6.0	51.87	57 94	57.52
-6.5	85.59	77.41	85.04
-7.0	94.53	104.10	88.63
-8.0	83.99	95.75	96.93

Test Chemical 8- Run 3

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	9.01	7.54	8.69
-5.0	13.85	11 97	9.02
-5.3	31.59	29.87	27.85
-5.7	46.91	51.98	45.01
-6.0	50.54	51.50	57.72
-6.5	85.26	78.68	75.19
-7.0	81.27	69.37	85.26
-8.0	72.34	92.48	101.21

Test Chemical 8- Run 4

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	44.27	28.72	4.10
-5.0	70.17	60.92	61.07
-5.3	92.97	90.51	93.82
-5.7	153.66	55.98	142.24
-6.0	193.64	122.97	150.08
-6.5	298.42	296.10	254.47
-7.0	437.33	461.65	367.69
-8.0	673.51	518.39	572.92

Test Chemical 8- Run 5

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	1.18	1.41	2 03
-5.0	4.36	4.30	5 27
-5.3	11.15	10 99	12.85
-5.7	26.71	28 87	26.60
-6.0	38.98	37 20	37.11
-6.5	62.60	56.41	54.05
-7.0	75.50	75 91	70.41
-8.0	81.04	79 66	91.72

Test Chemical 8- Run 1 norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0	12.98	12.21	12.20
-5.0	2.10	4.51	7.61
-6.0	61.34	54.63	53.14
-7.0	72.04	29.15	35.66
-8.0	65.95	73 56	63.68
-9.0	62.12	71 53	57.71
-10.0	70.83	58 57	62.90

Test Chemical 8- Run 3 norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	6.55	5.48	6.32
-5.0	10.07	8.71	6.56
-5.3	22.98	21.72	20.26
-5.7	34.12	37.81	32.74
-6.0	36.76	37.46	41.98
-6.5	62.01	57.22	54.69
-7.0	59.11	50.45	62.01
-8.0	52.62	67.26	73.62

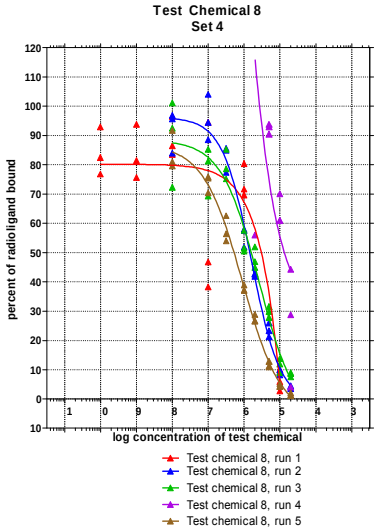
Test Chemical 8- Run 4 norm

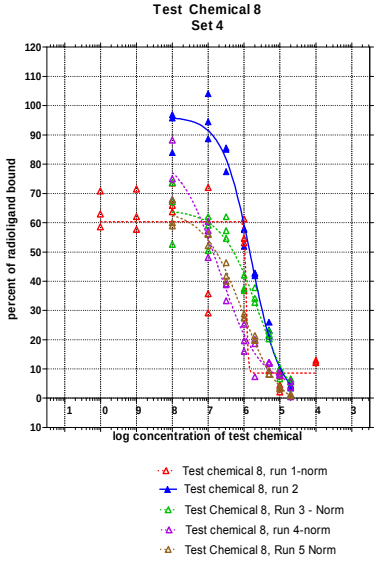
concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	5.80	3.76	0 54
-5.0	9.19	7.98	8 00
-5.3	12.17	11 85	12.28
-5.7	20.12	7.33	18.62
-6.0	25.35	16.10	19.65
-6.5	39.07	38.77	33.32
-7.0	57.26	60.44	48.14
-8.0	88.18	67 87	75.01

Test Chemical 8- Run 5 norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-4.7	0.87	1.04	1.50
-5.0	3.23	3.19	3.90
-5.3	8.25	8.14	9.51
-5.7	19.77	21.37	19.69
-6.0	28.85	27.53	27.47
-6.5	46.33	41.75	40.00
-7.0	55.87	56.18	52.11
-8.0	59.98	58.95	67.88

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 8	1	-5 52	-3.38	-7.65	1	417.35	57119 27	3.05	5.01E-04	-3.30
	2	-5 84	-5.78	-5.90	1	198.98	230.38	171.86	9.12E-04	-3.04
	3	-5 80	-5.68	-5.92	1	218.71	286.66	166.86	1.07E-03	-2.97
	4	-4 88	-4.15	-5.60	1	1825.31	9682.73	344.09	9.77E-04	-3.01
	5	-6 28	-6.22	-6.33	1	72.44	82.42	63.68	2.51E-03	-2.60
Test Chemical 8 - Norm	1	-5 95			2	154.07			7.96E-04	-3.10
	3	-6 29	-6.10	-6.49	1	70.40	110.31	44.94	2.00E-03	-2.70
	4	-6 86	-6.72	-7.00	1	18 94	26.20	13.69	6.92E-03	-2.16
	5	-6.78	-6.69	-6.87	1	22 98	28.34	18.63	4.27E-03	-2.37


Test Chemical 8
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 8, run 1
Test chemical 8, run 2
Test chemical 8, run 3
Test chemical 8, run 4
Test chemical 8, run 5


Test Chemical 8
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 8, run 1-norm
Test chemical 8, run 2
Test Chemical 8, Run 3 - Norm
Test chemical 8, run 4-norm
Test Chemical 8, Run 5 Norm

Competitive Binding Report

Test Chemicals Set 4

Tested Chemicals 1, 3, 8, 23
Assay Dates 3/6, 3/11, 3/13, 3/18 and 4/3/2008

Test Chemical

Name Test Chemical 23
Code TC23
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
100, 1000, 3000, 10000, 30000, 100000, 300000, 1000000 (Run 2, 3, 4 and 5)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	Run 5
	18.13	18.00	18.42	18 33	18 50

Incubation Temp. 4 °C

Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: For Run 2, Level 4-tube 3 may be not enough TC23#4?

Results

Filenames	Run 1	Run 2	Run 3	Run 4	Run 5
Counter Data (.P00)	08MR0437	13MR0359	15MR0355	20MR0825	05AP0250
Excel (xls)	S4R1 3 6	S4R2 3 11 08	S4R3 3 13 08	S4R4 3 18 08	S4R5 4 3 08
Prism (pzf)	RTI_Set 4				

Any precipitation of test chemicals? If yes, describe For Run-2 and 3, level 1 tubes were precipited.

Test Chemical 23- Run 1

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0	1.40	1 26	1.52
-4 0	9.06	9 21	5.31
-5 0	12.64	44 83	49.14
-6 0	81.47	64 97	95.19
-7 0	86.18	44 03	58.36
-8 0	86.43	59 95	81.26
-9 0	82.59	96 94	58.11
-10 0	69.68	63 38	88.07

Test Chemical 23- Run 2

concentration (logM)	y1-U4	y2-U4	y3-U4
-3.0			
-3.5	4 06	3.84	5 82
-4.0	9.75	8.68	9 38
-4.5	22.74	22.27	19.15
-5.0	45.84	43.23	42 93
-5.5	76.15	72.48	71 60
-6.0	94.61	95.83	84 96
-7.0	98.57	89.98	96 68

Test Chemical 23- Run 3

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-3 5	3.77	4.49	13.81
-4 0	11.11	9.72	8.82
-4 5	23 83	24.44	23.66
-5 0	50 22	49.70	48.11
-5 5	75.76	69.43	60.38
-6 0	104.51	85.13	79.06
-7 0	94 95	84.69	110.71

Test Chemical 23- Run 4

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-3 5	12.87	29 23	16.37
-4 0	52.52	33 69	34.69
-4 5	153.99	117.18	137.12
-5 0	271.01	165 26	223.52
-5 5	376.17	438 80	425.17
-6 0	613.82	524 28	543.07
-7 0	560.39	481 54	464.59

Test Chemical 23- Run 5

concentration (logM)	y1-U4	y2-U4	y3-U4
-3.0	14.21	13.67	4 60
-3.5	2 89	3.70	3 94
-4.0	7 21	7.80	8 60
-4.5	25.81	24.11	23 57
-5.0	43.60	41.41	39 66
-5.5	81.23	80.67	78.41
-6.0	108.68	104.06	112.56
-7.0	132.81	117.51	135.26

Test Chemical 23- Run 1 norm

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0	1 07	0.96	1.16
-4 0	6 91	7.02	4.05
-5 0	9 63	34.16	37.45
-6 0	62 08	49.51	72.54
-7 0	65 67	33.56	44.48
-8 0	65 87	45.69	61.93
-9 0	62 94	73.87	44.28
-10 0	53.10	48.30	67.12

Test Chemical 23- Run 3 norm

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0			
-3 5	2.74	3 26	10.04
-4 0	8.08	7 07	6.42
-4 5	17.33	17.78	17.21
-5 0	36.53	36.15	34.99
-5 5	55.11	50 50	43.92
-6 0	76.01	61 92	57.50
-7 0	69.06	61 60	80.52

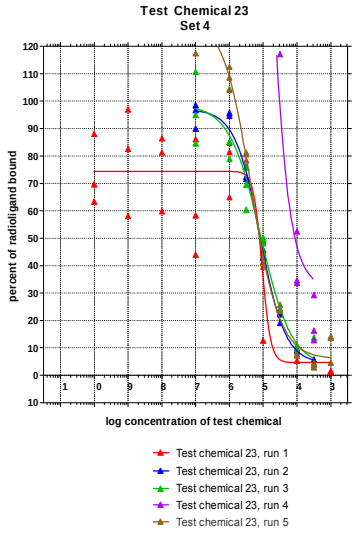
Test Chemical 23- Run 4 norm

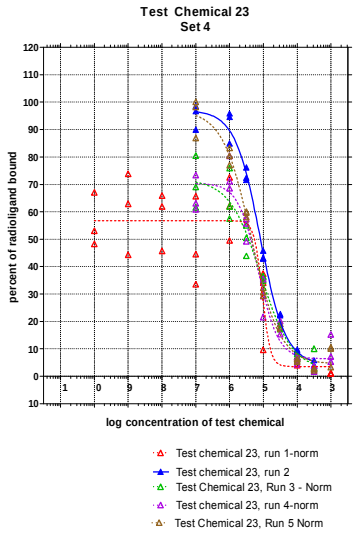
concentration (logM)	y1-U4	y2-U4	y3-U4
-3.0	7.16	5.34	15 20
-3.5	1 69	3.83	2.14
-4.0	6 88	4.41	4 54
-4.5	20.16	15.34	17 95
-5.0	35.48	21.64	29 26
-5.5	49.25	57.45	55 67
-6.0	80.37	68.64	71.10
-7.0	73.37	63.05	60 83

Test Chemical 23- Run 5 norm

concentration (logM)	y1-U4	y2-U4	y3-U4
-3 0	10 52	10.12	3.40
-3 5	2.14	2.74	2.92
-4 0	5 33	5.77	6.36
-4 5	19.10	17.84	17.44
-5 0	32 27	30.65	29.35
-5 5	60.12	59.70	58.03
-6 0	80.43	77.01	83.31
-7 0	98 29	86.97	100.10

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 23	1	-5.11	2.42	-12.64	1	1075.37	36375559358.72	0 00	1 95E-04	-3.71
	2	-5.08	-5 03	-5.13	1	1145.11	1276.50	1027 24	1 58E-04	-3.80
	3	-5.06	-4 93	-5.18	1	1205.91	1603.09	907.14	1 95E-04	-3.71
	4	-4.06	-3.43	-4.68	1	12080.08	50730 68	2876 53	1.48E-04	-3.83
	5	-5.10	-5 03	-5.16	1	1099.33	1275.86	947.23	1 66E-04	-3.78
Test Chemical 23 - Norm	1	-5.15			2	974.37			1 26E-04	-3.90
	3	-5.43	-5 28	-5.59	1	505.82	716.30	357.18	2.75E-04	-3.56
	4	-5.37	-5 23	-5.51	1	588.25	805.40	429.65	2 24E-04	-3.65
	5	-5.34	-5 28	-5.40	1	626.72	715.29	549.12	1 55E-04	-3.81


Test Chemical 23
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 23, run 1
Test chemical 23, run 2
Test chemical 23, run 3
Test chemical 23, run 4
Test chemical 23, run 5


Test Chemical 23
Set 4
percent of radioligand bound
log concentration of test chemical
Test chemical 23, run 1-norm
Test chemical 23, run 2
Test Chemical 23, Run 3 - Norm
Test chemical 23, run 4-norm
Test Chemical 23, Run 5 Norm

Competitive Binding Report

Test Chemicals Set 5

Tested Chemicals 2, 10, 18
Assay Dates 3/17, 3/27, and 3/31/2008

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Ac ivity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	1

Radioinert Ligand

Name	17β-estradiol
Supplier	Battelle Sequim
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in standard curve tubes (nM)	0.01, 0.1, 0 316, 1, 3.16, 10, 100 (All Runs)

Estrogen Receptor

Type	Rat Uterine Cytosol	
Rat Strain	Sprague Dawley	
Rat Age	94 days	
Ovariectomy Date	1/14/2008	
Uterus removal Date	1/22/2008	
Cytosol Prep Date	1/23/2008	
Cytosol Prep ID	1/23/2008	
Protein Content	2.20 mg/mL	
Protein Assay Date	1/23/2008	
RUC Storage Info	-70 °C	(UCF027)

Positive Control

Name	Norethynodrel
Supplier	Battelle Sequim
Lot #	G
CAS #	68-23-5
Purity	100
Concentration in assay tubes (nM)	3.16, 31.6, 1000, 316, 1000, 3160, 31600, 100000 (All Runs)

Test Conditions

Buffer	TEDG + PMSF		
Protein Concentration Used	50 µg/tube		
Assay volume	0.5 mL		
Solvent	Ethanol		
Max Solvent Concentration	2.99% (present in total binding tubes)		
Incubation Time (h)	Run 1	Run 2	Run 3
	18.52	18.83	18.05
Incubation Temp.	4 °C		
Kd for E2 (from saturation binding assays)	0.1596 nM		

Notes on problems: For Run-3, S2-tube 2 possible pipetting error.

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	19MR0137	29MR0119	02AP0023
Excel (.xls)	S5R1_3_17_08	S5R2_3_27_08	S5R3_3_31_08
Prism (.pzf)	RTI_Set 5		

	Run 1	Run 2	Run 3
Assay Date	3/17/2008	3/27/2008	3/31/2008
Elapsed days since SA determination	263	273	277
Adjusted SA (Ci/mmol)	105.63	105.47	105.41

standard curve- Run 1

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.31	-0.76	2.01
-7.0	-0.52	1.23	-1.65
-8.0	10.18	7.58	9.19
-8.5	30 25	25.86	21.42
-9.0	54 54	50.66	50.27
-9.5	88.42	83.33	82.02
-10.0	103 30	95.31	99.86
-11.0	114.12	120.13	104.95

standard curve- Run 2

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.75	-0.65	10.47
-7.0	-0.58	-4.18	-4.32
-8.0	14.33	11.93	15.64
-8.5	41.34	42.38	34.94
-9.0	69.49	63.46	73.66
-9.5	99.86	92.85	89.30
-10.0	107.23	113.35	107.05
-11.0	116.90	108.82	105.89

standard curve- Run 3

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	0.07	0.08	0.51
-7.0	-0.43	-0.37	0.15
-8.0	8.16	8.58	9.09
-8.5	24.50	24.39	22.28
-9.0	48.19	46.76	52.52
-9.5	103.68	93.28	91.02
-10.0	118 31	121.72	124.75
-11.0	146 96	140.51	120.49

weak positive- Run 1

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.32	-0.31	-0.58
-4.5	2.13	10.71	1.25
-5.5	25 08	25.06	22.76
-6.0	51.19	51.19	48.33
-6.5	73 54	71.84	69.32
-7.0	88 91	88.62	104.90
-7.5	99.18	91.52	102.87
-8.5	105 39	103.40	104.76

weak positive- Run 2

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-3.81	-2.85	-3.38
-4.5	0.10	1.27	-0.67
-5.5	29.47	35.47	29.98
-6.0	75.27	62.71	49.88
-6.5	94.71	95.24	84.59
-7.0	105.98	84.95	105.36
-7.5	105.01	102.08	110.07
-8.5	102.52	100.83	112.46

weak positive- Run 3

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	0.63	1.26	0.11
-4.5	1.26	2.31	2.45
-5.5	20.54	19.66	19.05
-6.0	52.45	47.30	48.87
-6.5	76.67	81.68	78.44
-7.0	87.48	113.16	114.73
-7.5	129 05	121.86	120.59
-8.5	128 34	128.03	131.51

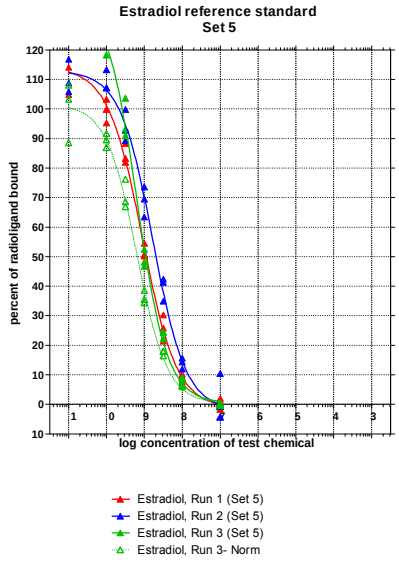
standard curve- Run 3 Norm

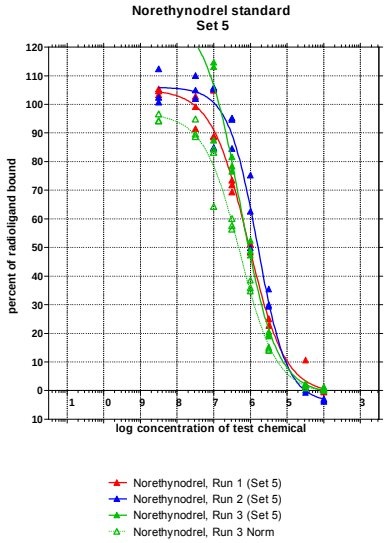
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	0.05	0.06	0.38
-7.0	-0.32	-0.27	0.11
-8.0	6.00	6.31	6.69
-8.5	18 01	17.93	16.39
-9.0	35.44	34.38	38.62
-9.5	76 24	68.59	66.94
-10.0	87 00	89.51	91.74
-11.0	108 07	103.33	88.61

weak positive- Run 3 Norm

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	0.47	0.93	0.08
-4.5	0.93	1.70	1.81
-5.5	15.11	14.46	14.01
-6.0	38.57	34.78	35.94
-6.5	56.38	60.06	57.68
-7.0	64.33	83.21	84.37
-7.5	94.90	89.61	88.68
-8.5	94.37	94.15	96.71

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Me hod	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-8.95	-8.90	-9.00	1	0.154	0.172	0.138	NA	NA
	2	-8.69	-8.63	-8.75	1	0.281	0.324	0 245	NA	NA
	3	-8.97	-8.90	-9.03	1	0.149	0.172	0.129	NA	NA
	3_Norm	-9.18	-9.13	-9.24	1	0.090	0.103	0 079	NA	NA
Norethynodrel	1	-6.02	-5.95	-6.09	1	130.99	152.96	112.18	1.17E-03	-2.93
	2	-5.79	-5.70	-5.88	1	222.78	272.13	182.39	1.26E-03	-2.90
	3	-6.05	-5.97	-6.12	1	123.54	145.93	104.59	1.20E-03	-2.92
	3_Norm	-6.32	-6.25	-6.38	1	66.105	76.899	56.827	1.38E-03	-2.86


Estradiol reference standard
Set 5
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 1 (Set 5)
Estradiol, Run 2 (Set 5)
Estradiol, Run 3 (Set 5)
Estradiol, Run 3- Norm


Norethynodrel standard
Set 5
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 1 (Set 5)
Norethynodrel, Run 2 (Set 5)
Norethynodrel, Run 3 (Set 5)
Norethynodrel, Run 3 Norm

Competitive Binding Report

Test Chemicals Set 5

Tested Chemicals 2, 10, 18
Assay Dates 3/17, 3/27, and 3/31/2008

Test Chemical

Name Test Chemical 2
Code TC2
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
0.001, 0.01, 0.1, 0.3, 1, 10, 100, 1000 (Run 2 and 3)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent E hanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	18.52	18.83	18.05

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	19MR0137	29MR0119	02AP0023
Excel (.xls)	S5R1_3_17_08	S5R2_3_27_08	S5R3_3_31_08
Prism (.pzf)	RTI_Set 5		

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 2- Run 1

concentration (logM)	y1-U1	y2-U1	y3-U1
-3 0			
-4 0	1.06	3.05	3.50
-5 0	-1.61	-2.02	-1.35
-6 0	-1.27	-1.58	-0.88
-7 0	-0.02	-0.80	-0.16
-8 0	7.05	5.80	5.52
-9 0	40.90	39.31	35.87
-10 0	84.40	88.37	86.14

Test Chemical 2- Run 2

concentration (logM)	y1-U1	y2-U1	y3-U1
-6.0	-3.31	-6.07	-6.44
-7.0	-4.88	-2.01	-6.61
-8.0	6.99	8.29	9.22
-9.0	58.29	55.18	59.00
-9.5	87.70	75.13	87.16
-10.0	93.73	105.63	96.22
-11.0	97.64	95.06	100 39
-12.0	88.85	99.41	94.18

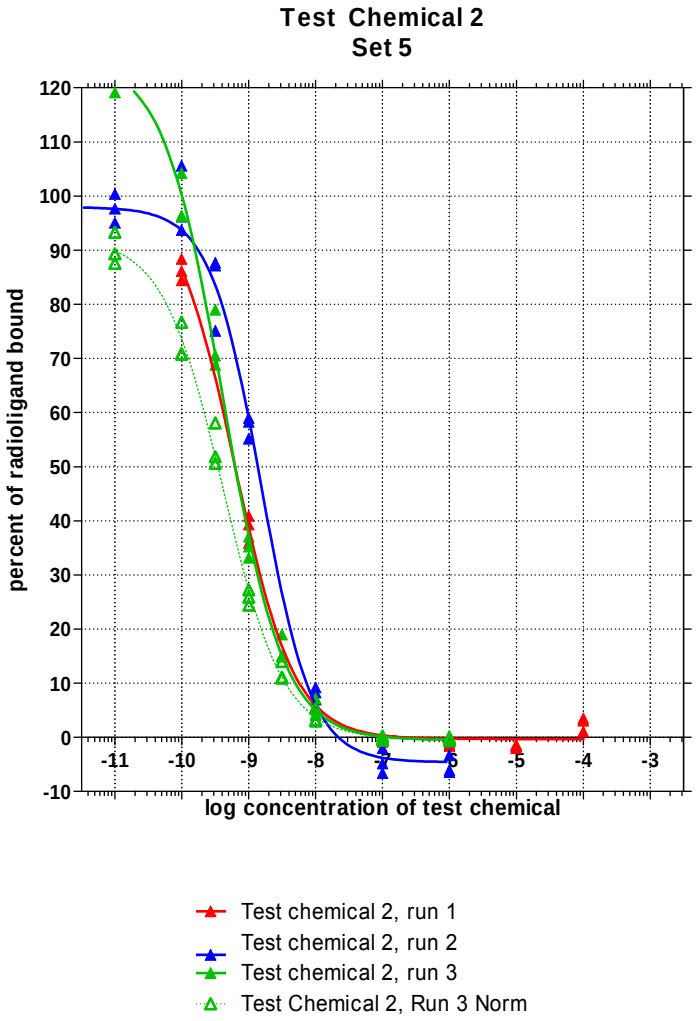
Test Chemical 2- Run 3

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-0.73	-0.04	-0.49
-7 0	-0.44	0.27	-0.61
-8.0	7.19	4.33	4.09
-8.5	19.01	14.89	15.03
-9.0	37.07	35.26	33.18
-9 5	68.82	70.53	78.99
-10 0	96.35	104.26	96.28
-11 0	126.90	121.48	119.13

Test Chemical 2- Run 3_Norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-6 0	-0.53	-0.03	-0.36
-7 0	-0.32	0.20	-0.45
-8 0	5.29	3.18	3.01
-8 5	13.98	10.95	11.05
-9 0	27.26	25.93	24.40
-9 5	50.61	51.86	58.08
-10 0	70.85	76.67	70.80
-11 0	93.32	89.33	87.60

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 2	1	-9.20	-9.15	-9.26	1	0.09	0.10	0.08	1.78E+00	0.25
	2	-8.86	-8.78	-8.94	1	0.19	0.23	0.16	1.48E+00	0.17
	3	-9.20	-9.16	-9.24	1	0.09	0.09	0.08	1.70E+00	0.23
	3-Norm	-9.46	-9.42	-9.50	1	0.05	0.05	0.04	1.91E+00	0.28



Competitive Binding Report

Test Chemicals Set 5

Tested Chemicals 2, 10, 18
Assay Dates 3/17, 3/27, and 3/31/2008

Test Chemical

Name Test Chemical 10
Code TC10
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
1, 10, 100, 1000, 10000, 100000, 300000, 1000000 (Run 2 and 3

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0 5 mL
Solvent Ethanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	18.52	18.83	18.05

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	19MR0137	29MR0119	02AP0023
Excel (.xls)	S5R1_3_17_08	S5R2_3_27_08	S5R3_3_31_08
Prism (.pzf)	RTI_Set 5		

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 10- Run 1

concentration (logM)	y1-U2	y2-U2	y3-U2
-3 0	85.32	84 01	76.88
-4 0	97.78	103.45	92.83
-5 0	57.35	98 26	104.61
-6 0	111.89	104.13	103.11
-7 0	98.31	100 35	108.44
-8 0	104.66	104 81	98.75
-9 0	104.08	96 86	102.48
-10.0	106.99	100 83	85.32

Test Chemical 10- Run 2

concentration (logM)	y1-U2	y2-U2	y3-U2
-3 0	14.02	13.96	11.16
-3 5	31.11	4.71	8 24
-4 0	59.71	64.15	57 94
-5 0	86.01	93.02	99 68
-6 0	108.56	104.92	85 92
-7 0	92.67	108.47	100 83
-8 0	113.26	99.68	98 35
-9 0	79.00	77.13	94 00

Test Chemical 10- Run 3

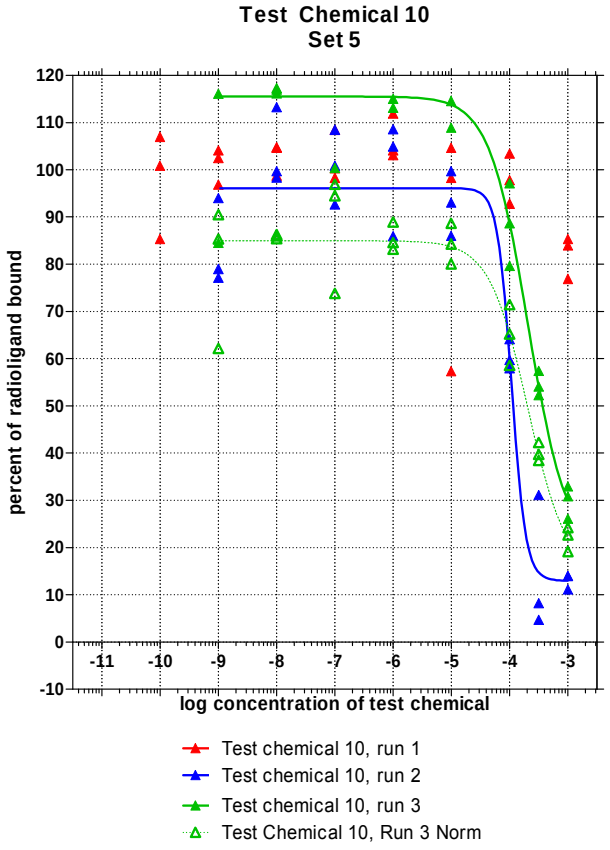
concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	26.07	32 94	30.83
-3.5	52.25	54 06	57.40
-4.0	88.67	97.13	79.63
-5.0	108 90	114 59	120.56
-6.0	113 09	115 00	120.90
-7.0	100 37	131.78	128.54
-8.0	117 32	116.13	116.98
-9.0	123 02	84 51	116.09

Test Chemical 10- Run 3_Norm

concentration (logM)	y1-U2	y2-U2	y3-U2
-3 0	19.17	24 23	22.67
-3 5	38.42	39.75	42.21
-4 0	65.21	71.43	58.56
-5 0	80.08	84 27	88.66
-6 0	83.16	84 57	88.91
-7 0	73.81	96 91	94.52
-8 0	86.27	85.40	86.02
-9 0	90.46	62.15	85.37

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 10	1	*			1				NA	NA
	2	-3 94	-3.78	-4.09	1	15889.15	22805.50	11070.36	1.78E-05	-4.75
	3	-3.43	-3 25	-3.62	1	50676 39	77358.44	33197.37	2 88E-06	-5.54
	3_Norm	-3 69	-3 55	-3.84	1	27955.72	39134.76	19970.03	3 24E-06	-5.49

*No Log(IC50) could be calculated



Competitive Binding Report

Test Chemicals Set 5

Tested Chemicals 2, 10, 18
Assay Dates 3/17, 3/27, and 3/31/2008

Test Chemical

Name Test Chemical 18
Code TC18
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
0.1, 1, 10, 30, 100, 1000, 3000, 10000 (Run 2 and 3)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent DMSO
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	18.52	18.83	18.05

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: For Run-3, 18TC8-tube 3 possible added drop of water.

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	19MR0137	29MR0119	02AP0023
Excel (.xls)	S5R1_3_17_08	S5R2_3_27_08	S5R3_3_31_08
Prism (.pzf)	RTI_Set 5		

Any precipitation of test chemicals? If yes, describe For Run-1, Level 1 and 2 tubes were precipited.

Test Chemical 18- Run 1

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0			
-5.0			
-6.0			
-7.0	93.75	96.03	91.91
-8.0	94 92	89.73	102.48
-9.0	99 57	89.97	79.07
-10.0	94.77	91.91	95.16

Test Chemical 18- Run 2

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0			
-5.5	103.05	98.26	89.92
-6.0			
-7.0			
-7.5	81.93	85.57	95.15
-8.0	84.59	92.05	96.49
-9.0	95.33	87.25	91.43
-10.0	96.13	100.57	97.28

Test Chemical 18- Run 3

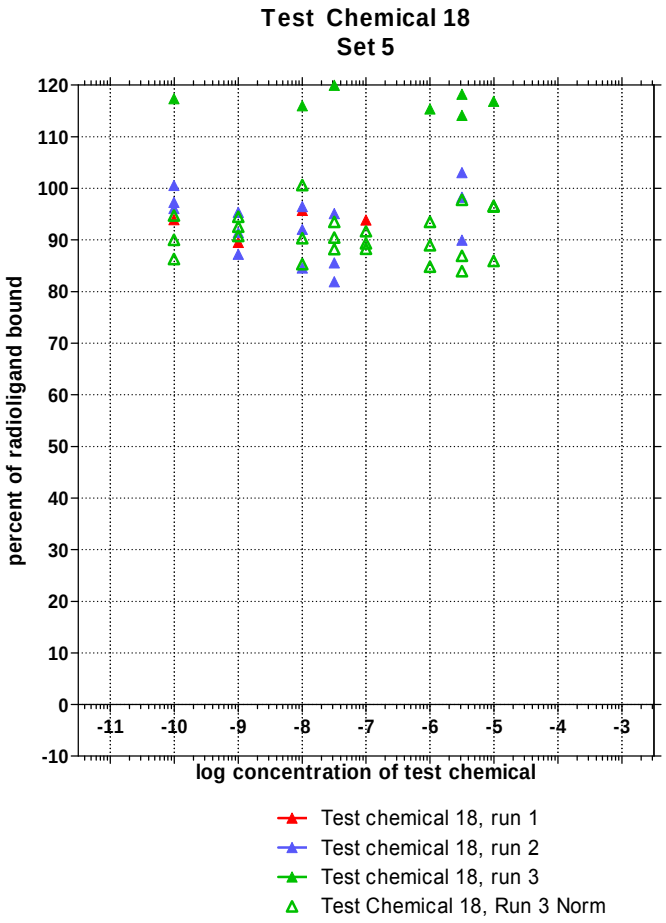
concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	116.88	131.40	131.20
-5.5	118.21	132.94	114.18
-6.0	120.93	127.14	115.34
-7.0	120.08	121.45	124.65
-7.5	127.14	119.98	123.02
-8.0	116.02	136.86	122.84
-9.0	125.91	128.54	123.39
-10.0	122.40	128.81	117.39

Test Chemical 18- Run 3 Norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	85 95	96.63	96.48
-5.5	86 93	97.76	83.97
-6.0	88 93	93.50	84.82
-7.0	88 30	89.31	91.67
-7.5	93 50	88.23	90.46
-8.0	85 32	100.64	90.34
-9.0	92 59	94.52	90.74
-10.0	90 01	94.72	86.32

Ligand	Run	Log(IC50)*	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 18	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA
	3_Norm	NA	NA	NA	1	NA	NA	NA	NA	NA

*No Log(IC50) can be calculated



Competitive Binding Report

Test Chemicals Set 6

Tested Chemicals 6, 7, 13
Assay Dates 4/1, 4/7, 4/8, and 6/9/08

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol	
Supplier	Perkin Elmer (Boston)	
Catalog #	NET-517	
Specific Activity	110	Ci/mmol
Batch #	3589221	Run 1, 2 and 3
SA date	6/28/2007	Run 1, 2 and 3
Batch #	3589791	Run 4
SA date	2/29/2008	Run 4
Concentration (mCi/mL)	1	
Tested Concentrations (nM)	1	

Radioinert Ligand

Name	17β-estradiol
Supplier	Battelle Sequim
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in standard curve tubes (nM)	0.01, 0.1, 0 316, 1, 3.16, 10, 100 (All runs)

Estrogen Receptor

Type	Rat Uterine Cytosol	
Rat Strain	Sprague Dawley	
Rat Age	94	days
Ovariectomy Date	3/4/2008	
Uterus removal Date	3/11/2008	
Cytosol Prep Date	3/19/2008	
Cytosol Prep ID	3/19/2008	
Protein Content	2.3	mg/mL
Protein Assay Date	3/19/2008	
RUC Storage Info	-70 °C	(UCF027)

Positive Control

Name	Norethynodrel
Supplier	Battelle Sequim
Lot #	G
CAS #	68-23-5
Purity	100
Concentration in assay tubes (nM)	3.16, 31.6, 100, 316, 1000, 3160, 31600, 100000 (All runs)

Test Conditions

Buffer	TEDG + PMSF				
Protein Concentration Used	50	μg/tube			
Assay volume	0.5	mL			
Solvent	Ethanol				
Max Solvent Concentration	2	99%	(present in total binding tubes)		
Incubation Time (h)	Run 1	Run 2	Run 3	Run 4	
	18.35	18.07	18.33	18 50	
Incubation Temp.	4	°C			
Kd for E2 (from saturation binding assays)	0.5074	nM			
Notes on problems:	None				

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	03AP0130	09AP0205	10AP0001	11JN0243
Excel (.xls)	S6R1_4_1_08	S6R2_4_7_08	S6R3_4_8_08	S6R4_6_9_08
Prism (.pzf)	RTI_Set 6			

	Run 1	Run 2	Run 3	Run 4
Assay Date	4/1/2008	4/7/2008	4/8/2008	6/9/2008
Elapsed days since SA determination	278	284	285	101
Adjusted SA (Ci/mmol)	105.39	105.29	105 28	108.30

standard curve- Run 1			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-2.02	-2 57	-2.07
-7.0	1.20	1.41	4.05
-8.0	11.73	8 55	8.86
-8.5	30.99	32 54	26.00
-9.0	57.63	61.79	56.67
-9.5	91.45	93 33	90.61
-10 0	104.12	96 25	109.69
-11 0	116.31	129 81	120.00

standard curve- Run 2			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.96	0 23	0.13
-7.0	-0.26	0 83	0.02
-8.0	8.45	9.13	7.66
-8.5	30.03	26.79	19.21
-9.0	54.14	51.19	50.17
-9.5	83.77	88.44	86.18
-10 0	99.08	108.74	121.24
-11 0	123.94	115 67	129.34

standard curve- Run 3			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.28	-0.45	0.91
-7.0	-1.24	-1 28	2.34
-8.0	12.39	11.66	9.81
-8.5	35.19	34 08	25.61
-9.0	50.82	58 06	57.94
-9.5	96.59	87 21	81.29
-10.0	102 97	106.36	94.92
-11.0	121 20	144.55	121.26

weak positive- Run 1			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.42	0 05	-2.36
-4.5	0.77	4 25	0.65
-5.5	21.73	21 27	24.68
-6.0	55.88	49 35	45.36
-6.5	70.77	77 52	70.84
-7.0	82.81	93 05	104.89
-7.5	99.87	101.47	95.49
-8.5	95.49	119 86	94.93

weak positive- Run 2			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.27	-0 39	-0.25
-4.5	2.46	1 55	1.47
-5.5	19.34	22 89	20.88
-6.0	52.90	49 00	46.78
-6.5	72.66	77 29	79.80
-7.0	106.30	51 33	98.46
-7.5	107.03	106 88	119.49
-8.5	127.29	117 34	114.61

weak positive- Run 3			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	1.36	0.45	-0.14
-4.5	1.85	3 96	0.96
-5.5	15.95	28.41	25.67
-6.0	58.00	52.42	36.97
-6.5	72.38	77.78	76.35
-7.0	87.85	97.80	90.32
-7.5	104 98	104.41	114.41
-8.5	110 50	121.03	93.14

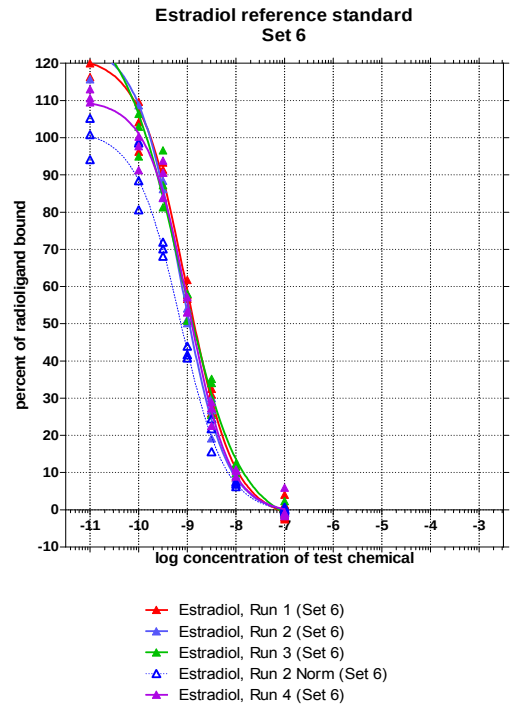
standard curve- Run 2 Norm			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.78	0.19	0.11
-7.0	-0.21	0 67	0.02
-8.0	6.87	7.42	6.23
-8.5	24.42	21.78	15.62
-9.0	44.02	41 62	40.79
-9.5	68.12	71 91	70.08
-10 0	80.57	88.42	98.59
-11 0	100.78	94 05	105.17

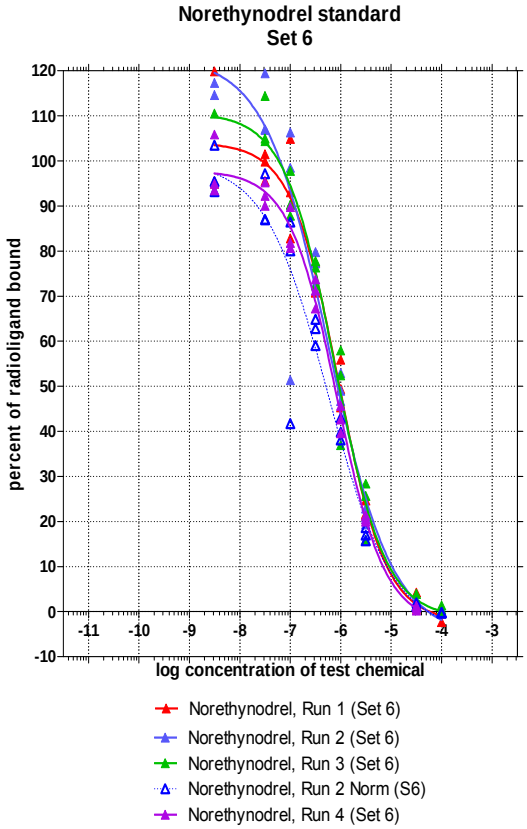
weak positive- Run 2 Norm			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	-0.22	-0 32	-0.20
-4.5	2.00	1 26	1.20
-5.5	15.73	18 61	16.98
-6.0	43.02	39 84	38.04
-6.5	59.08	62 84	64.89
-7.0	86.44	41.74	80.06
-7.5	87.03	86 91	97.16
-8.5	103.51	95.42	93.19

standard curve- Run 4			
concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	5.95	-0 80	-0.89
-7.0	-1.47	-1 08	-1.71
-8.0	8.93	10 54	10.86
-8.5	27.29	28 93	22.54
-9.0	53.05	53 08	57.07
-9.5	90.54	93 85	83.92
-10 0	100.24	91 25	97.71
-11 0	109.53	110 66	113.06

weak positive- Run 4			
concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	0.78	1 28	0.26
-5.5	20.00	20.47	21.33
-6.0	42.63	45 68	39.58
-6.5	73.76	71 23	67.23
-7.0	81.84	80 67	89.76
-7.5	95.24	90 05	92.29
-8.5	105.86	94 92	93.46

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Method	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-8 86	-8.80	-8.92	1	0.466	0 531	0.409	NA	NA
	2	-8 96	-8.90	-9.02	1	0 371	0.425	0.324	NA	NA
	3	-8 89	-8.80	-8.98	1	0.438	0 538	0.356	NA	NA
	2_Norm	-9.13	-9.07	-9.18	1	0 252	0 286	0.222	NA	NA
	4	-8 90	-8.85	-8.96	1	0.419	0.473	0.371	NA	NA
Norethynodrel	1	-6 02	-5.93	-6.12	1	318.72	394.66	257.40	1.45E-03	-2.84
	2	-6 06	-5.90	-6.23	1	290.53	428.23	197.11	1 26E-03	-2.90
	3	-6 03	-5.93	-6.13	1	313.42	394.10	249.27	1 38E-03	-2.86
	2_Norm	-6 29	-6.13	-6.45	1	172.24	247.92	119.66	1.45E-03	-2.84
	4	-6.12	-6.06	-6.18	1	256.17	291.91	224.81	1 66E-03	-2.78


Estradiol reference standard
Set 6
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 1 (Set 6)
Estradiol, Run 2 (Set 6)
Estradiol, Run 3 (Set 6)
Estradiol, Run 2 Norm (Set 6)
Estradiol, Run 4 (Set 6)


Norethynodrel standard
Set 6
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 1 (Set 6)
Norethynodrel, Run 2 (Set 6)
Norethynodrel, Run 3 (Set 6)
Norethynodrel, Run 2 Norm (S6)
Norethynodrel, Run 4 (Set 6)

Competitive Binding Report

Test Chemicals Set 6

Tested Chemicals 6, 7, 13
Assay Dates 4/1, 4/7, 4/8, and 6/9/08

Test Chemical

Name Test Chemical 6
Code TC6
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentra ion in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
1, 10, 100, 300, 1000, 3000, 10000, 30000 (Run 2, 3 and 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.35	18.07	18.33	18.50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.5074 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	03AP0130	09AP0205	10AP0001	11JN0243
Excel (.xls)	S6R1_4_1_	S6R2_4_7_08	S6R3_4_8_08	S6R4_6_9_08
Prism (.pzf)	RTI_Set 6			

Any precipitation of test chemicals? If yes, describe: Level 1 tubes were precipitated in Run 1

Test Chemical 6- Run 1

concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0			
-4.0			
-5.0	20.75	17.76	16.72
-6.0	52.50	54.83	48.66
-7.0	89.57	96.74	84.76
-8.0	111.08	103.42	102.66
-9.0	88.10	93.88	104.05
-10.0	96.18	102.17	95.90

Test Chemical 6- Run 2

concentration (logM)	y1-U1	y2-U1	y3-U1
-4.5	2.67	1.53	1 05
-5.0	6.83	7.30	6.19
-5.5	11.46	17.98	18.99
-6.0	45.58	43.79	37.34
-6.5	80.09	72.62	67.66
-7.0	99.23	96.20	97.22
-8.0	79.95	96.90	107.65
-9.0	107.65	103.42	116.58

Test Chemical 6- Run 3

concentration (logM)	y1-U1	y2-U1	y3-U1
-4 5	4.90	2.12	1.11
-5 0	9.45	9.38	6 22
-5 5	19.46	21.65	21.55
-6 0	50.50	53.23	45.22
-6 5	71.06	79.11	72.21
-7 0	90.32	97.62	96.47
-8 0	86.87	88.59	96.36
-9 0	85.49	100.67	103.09

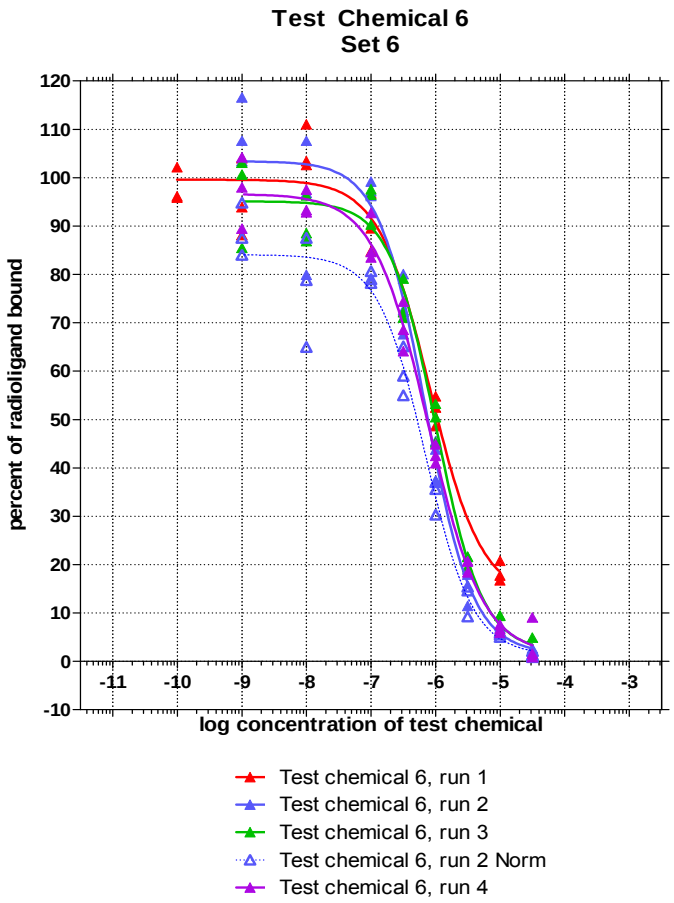
Test Chemical 6- Run 2_Norm

concentration (logM)	y1-U1	y2-U1	y3-U1
-4.5	2.17	1.25	0.86
-5.0	5.56	5.94	5.03
-5.5	9.31	14.62	15.44
-6.0	37.06	35.61	30.36
-6.5	65.13	59.05	55.02
-7.0	80.69	78.23	79.06
-8.0	65.01	78.79	87.53
-9.0	87.53	84.09	94.79

Test Chemical 6- Run 4

concentration (logM)	y1-U1	y2-U1	y3-U1
-4.5	1.63	0.86	9 09
-5.0	7.65	6.62	5 92
-5.5	20.74	20.64	18.39
-6.0	44.87	42.50	40.94
-6.5	68.56	74.41	64.12
-7.0	92.71	83.50	84.63
-8.0	92.81	97.55	93.33
-9.0	89.47	104.20	98.00

Ligand	Run	Log(IC50)	95% CI		Me hod	Ki	95% CI		RBA	log(RBA)
Test Chemical 6	1	-5.97	-5.82	-6.11	1	363.29	504.61	261.55	1.29E-03	-2.89
	2	-6.12	-6.03	-6.21	1	256.91	317.54	207.85	1.45E-03	-2.84
	3	-6.01	-5.94	-6.09	1	325.54	384.21	275.83	1.32E-03	-2.88
	2_Norm	-6.28	-6.19	-6.38	1	175.15	217.71	140.91	1.41E-03	-2.85
	4	-6.13	-6.07	-6.19	1	249.78	285.54	218.50	1.70E-03	-2.77



Competitive Binding Report

Test Chemicals Set 6

Tested Chemicals 6, 7, 13
Assay Dates 4/1, 4/7, 4/8, and 6/9/08

Test Chemical

Name Test Chemical 7
Code TC7
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
10, 100, 1000, 10000, 20000, 50000, 100000, 1000000 (Run 2 , 3 and 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.35	18.07	18.33	18.50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.5074 nM
Notes on problems: For run 1, 7TC1-tube 1 possible less han 100 µl cytosol added.

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	03AP0130	09AP0205	10AP0001	11JN0243
Excel (xls)	S6R1_4_1_	S6R2_4_7_08	S6R3_4_8_08	S6R4_6_9_08
Prism (.pzf)	RTI_Set 6			

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 7- Run 1

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	7.87	4.59	11.95
-4.0	20 00	15.73	18.90
-5.0	84.62	76.55	77.87
-6.0	90.47	95.83	100.50
-7.0	100 57	109.76	99.87
-8.0	101.13	104.19	111.57
-9.0		101.20	105.51
-10.0	102 94	100.64	106.91

Test Chemical 7- Run 2

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	4.67	4 97	5.76
-4.0	5.98	15.05	15.14
-4.3	35.00	35.66	39.49
-4.7	61.50	67.12	60.34
-5.0	83.96	93.40	90.48
-6.0	119.06	120.41	121.68
-7.0	120.92	124.93	113.26
-8.0	120.73	125.62	112.93

Test Chemical 7- Run 3

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	6.25	11.49	20.58
-4.0	18.86	21.32	17.56
-4.3	41.89	48.95	41.53
-4.7	69.50	69.73	68.87
-5.0	85.49	85.55	91.64
-6.0	115.62	111.71	101.42
-7.0	114.82	119.82	112.06
-8.0	110.04	117.12	108.26

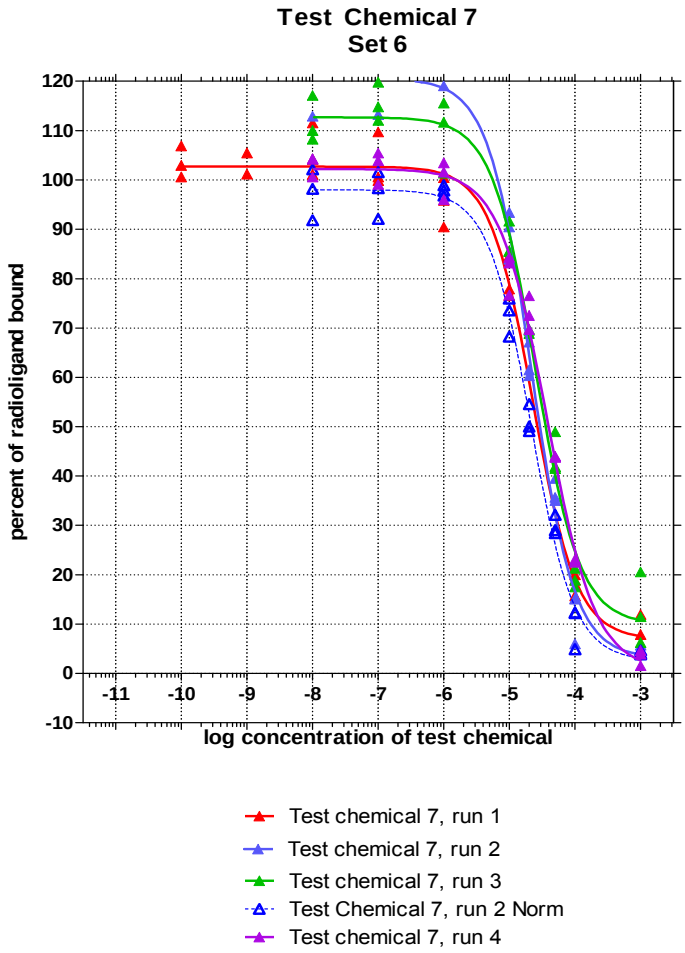
Test Chemical 7- Run 2 Norm

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	3.80	4.04	4.68
-4.0	4.86	12.23	12.31
-4.3	28.46	29.00	32.11
-4.7	50 01	54.57	49.06
-5.0	68 27	75.94	73.57
-6.0	96 81	97.91	98.94
-7.0	98 32	101.58	92.10
-8.0	98.17	102.14	91.83

Test Chemical 7- Run 4

concentration (logM)	y1-U2	y2-U2	y3-U2
-3.0	1.54	3 90	4.65
-4.0	22.51	22.75	23.07
-4.3	43.80	44.06	43.86
-4.7	69.67	76.52	72.52
-5.0	84.34	83.14	76.68
-6.0	103.49	95.99	101.57
-7.0	99.11	105.47	103.94
-8.0	104.27	104.24	100.57

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 7	1	-4.57	-4.46	-4.68	1	9048.20	11544.76	7091 50	5.13E-05	-4.29
	2	-4.53	-4.49	-4.58	1	9855.21	10978.20	8847.10	3.72E-05	-4.43
	3	-4.45	-4.39	-4.51	1	12022.31	13827.64	10452.71	3.63E-05	-4.44
	2_Norm	-4.66	-4.62	-4.71	1	7303.89	8075 08	6606 34	3 39E-05	-4.47
	4	-4.40	-4.37	-4.44	1	13344.70	14525.06	12260.30	3.16E-05	-4.50



Competitive Binding Report

Test Chemicals Set 6

Tested Chemicals 6, 7, 13
Assay Dates 4/1, 4/7, 4/8, and 6/9/08

Test Chemical

Name Test Chemical 13
Code TC13
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)
0.1, 1, 10, 30, 100, 300, 1000, 10000 (Run 2, 3 and 4)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2 99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3	Run 4
	18.35	18.07	18.33	18.50

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.5074 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3	Run 4
Counter Data (.P00)	03AP0130	09AP0205	10AP0001	11JN0243
Excel (xls)	S6R1_4_1_08	S6R2_4_7_08	S6R3_4_8_08	S6R4_6_9_08
Prism (.pzf)	RTI_Set 6			

Any precipitation of test chemicals? If yes, describe Level 1 tubes were precipitated in Run 1

Test Chemical 13- Run 1

concentration (logM)	y1-U3	y2-U3	y3-U3
-3.0			
-4.0	2.70	9.99	2.35
-5.0	-0.87	1.99	1.84
-6.0	2.77	0.19	1.53
-7.0	39 22	38.79	42.34
-8.0	83 02	91.03	81.42
-9.0	84.69	78.50	92.98
-10.0	91.66	94.02	93.54

Test Chemical 13- Run 2

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	0.69	0.19	0.62
-6.0	2.46	1.08	1.43
-6.5	7.53	6.65	4.18
-7.0	28.70	27.93	25 99
-7.5	71.96	71.78	60 26
-8.0	104.77	100.03	106.04
-9.0	115.52	97.66	94.60
-10.0	117.05	110.89	106.23

Test Chemical 13- Run 3

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	-0.47	1.70	0.31
-6.0	1.35	0.19	0.49
-6.5	8.52	6.03	4.46
-7.0	20.77	26.42	32.68
-7.5	67.38	58.12	67.55
-8.0	83.71	95.78	85.66
-9.0	99.52	90.72	86.35
-10.0	98.26	95.15	87.16

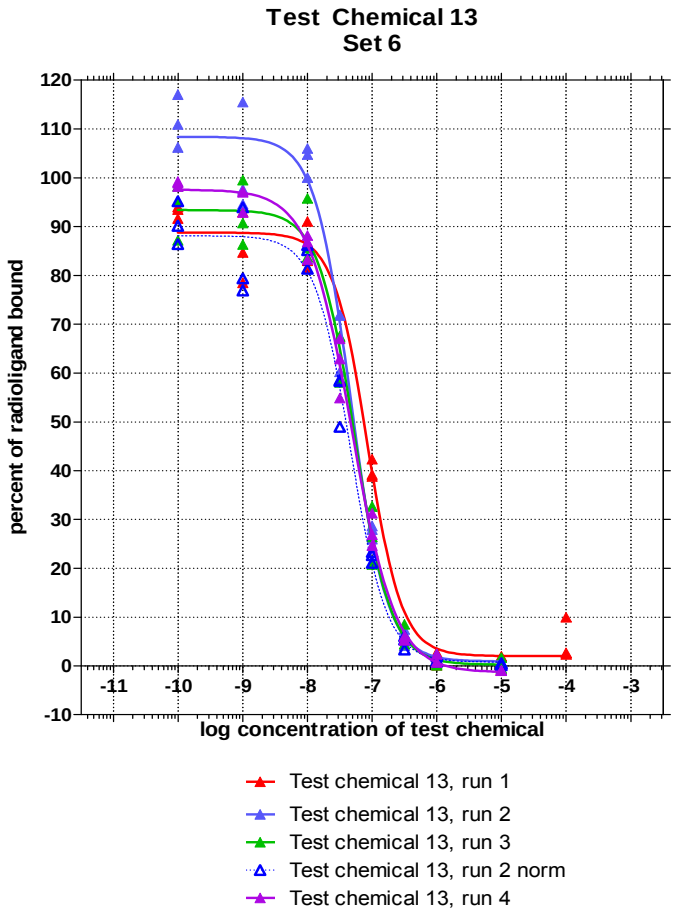
Test Chemical 13- Run 2 Norm

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	0.56	0.16	0.50
-6.0	2.00	0.88	1.16
-6.5	6.12	5.41	3.40
-7.0	23 33	22.71	21.13
-7.5	58 52	58.37	49.00
-8.0	85.19	81.34	86.23
-9.0	93 93	79.41	76.92
-10.0	95.18	90.17	86.38

Test Chemical 13- Run 4

concentration (logM)	y1-U3	y2-U3	y3-U3
-5.0	-0.97	-0.91	-0 94
-6.0	0.74	2.41	0.79
-6.5	6.04	5.28	5 38
-7.0	24.68	31.27	26 90
-7.5	54.87	67.04	62 92
-8.0	88.14	86.90	83 24
-9.0	97.03	97.26	92 91
-10.0	98.20	98.95	99 24

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 13	1	-7.12	-7.03	-7.22	1	25.27	31.73	20.13	1.82E-02	-1.74
	2	-7.28	-7.22	-7.34	1	17.56	20.20	15 27	2.09E-02	-1.68
	3	-7.30	-7.25	-7.36	1	16.69	18.94	14.70	2.57E-02	-1.59
	2_Norm	-7.40	-7.34	-7.46	1	13.34	15.34	11.60	1.86E-02	-1.73
	4	-7.34	-7.30	-7.37	1	15.56	16.84	14 38	2.75E-02	-1.56



Appendix F

Final Report on the First Five Test Chemicals

Final Report

Task Order 6:
Second Inter-laboratory Validation of the Estrogen Receptor Binding Assay (Rat Uterine Cytosol)

Task 7 – Testing Coded Chemicals

Qualifying Laboratory:
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March 31, 2008

RTI INTERNATIONAL

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1.0 Introduction

This report describes the estrogen receptor binding assay of the first set of 5 coded chemicals conducted in RTI's DMPK laboratory as described in Task 6 of the Statement of Work (which includes the EPA protocol for conduct of the assay) and in the Analytical Project Plan for EDSP Laboratory Assay Validation Services Task Order 6 prepared by the Study Director.

2.0 Materials and Methods

2.1 Cytosol Isolation Procedure

Rat uteri were collected from ovariectomized rats, flash frozen in liquid nitrogen and stored in an ultracold freezer at < -70°C. The frozen uteri were transferred to ice cold TEDG-PMSF buffer at a ratio of 0.1 g tissue to 1 mL buffer. After the uteri had thawed, they were homogenized using a Polytron (PT35/10, Brinkmann) homogenizer with 3 to 5 bursts of about 5 sec per burst. The homogenizer probe was prechilled in ice cold buffer and was chilled between each burst. The tissue samples were kept chilled throughout the entire isolation process. The homogenates were transferred to chilled centrifuge tubes and were centrifuged at 2500 *g* for 10 min at 4°C using an Allegra X-15R centrifuge. The supernatants were transferred to chilled ultracentrifuge tubes and centrifuged at ca. 105000 *g* for 60 min at 4°C using a Beckman L5-50B Ultracentrifuge with a 80Ti rotor. The supernatants (cytosol) were combined, flash frozen in liquid nitrogen and stored in an ultracold freezer at < -70°C. Cytosol preparations were identified by their preparation date.

2.2 Protein Assay Procedure

Cytosol was diluted in TEDG + PMSF buffer (1:5 or 1:10) and was assayed for protein content using a BioRad Protein Assay kit (Catalog # 500-0002). A standard curve (range 0.22 to 0.89 mg/mL) was prepared using bovine serum albumin. Standards, QC samples and cytosol samples (100 μL) were aliquoted in triplicate into tubes. Diluted dye reagent (5 mL of a 1:5 dilution of stock) was added to each tube and the samples were mixed by vortexing. Samples were allowed to sit at room temperature at least 5 min, but less than 1 h prior to being transferred into disposable cuvettes. Absorbance at 595 nm was measured using a BioSpec mini spectrophotometer. The concentration of protein in the cytosol samples was calculated based on the standard curve.

2.3 Saturation Binding Assays

Three independent saturation binding assays were conducted for each lot of cytosol preparation used. The assays were conducted as described in the protocol and as reported in the Saturation Binding reports.

2.4 Competitive Binding Assays

Competitive binding assays were conducted as described in the protocol and as reported in the Competitive Binding reports. The maximum concentration of ethanol used in any assay tube was

maintained below 3% (See Appendix A for calculations). Assay worksheets showing the methods used for preparation of reference and control chemical dilutions and the volumes of reagents used in each assay tube are presented in Appendix B. A total of 3 competitive binding assays (runs) were conducted and these are identified as runs 1, 2 and 3, respectively. Each competitive assay contained tubes for the standard (estradiol), weak positive control (norethynodrel), negative control (R1881) and five coded test chemicals (TC 11, 12, 15, 19 and 21). Each level of each control and test chemical was assayed in triplicate tubes per run. In our laboratory, one technician did the assay set up, through placing the tubes on the rotator, and a second technician did the tasks associated with the second day of the assay, i.e., separating bound from free and sampling for scintillation counting.

For run 1, norethynodrel and R1881 stocks were mistakenly prepared at $1/10^{th}$ the target concentration. The actual concentrations used were entered into the data calculation spreadsheets.

3.0 Results and Discussion

3.1 Protein Assay

The cytosol preparation was assayed for protein content and contained 2.2 mg protein/mL. Protein assay results are presented in Appendix C.

3.2 Saturation Binding Assays

Three independent saturation binding assays were conducted The assay results are presented in Appendix D. The assay results were evaluated against the points outlined in Section 8.6.3 of the protocol and summary data are presented in Table 1 below.

Table 1. Evaluation of Saturation Binding Assay Results

Assay ID	Cytosol Prep	Plateau reached?	Linear Scatchard?	K_d (nM)	CV% of K_d (%)	B_{max} (fmole/100 μg)	CV% of B_{max} (%)	NSB acceptable?[a]
Sat 1	1/23/08	Y	Y	0.1508	10.9%	71.03	3.2%	Y
Sat 2	1/23/08	Y	Y	0.1466	12.5%	58.02	3.9%	Y
Sat 3	1/23/08	Y	Y	0.1813	18.9%	59.84	5.6%	Y
Average				0.1596				

[a]NSB is acceptable if it is <50% of total binding.

All saturation binding assays were determined to be acceptable. The average Kd value across the three runs was used in the determination of Ki for the test chemicals in the competitive binding assays.

3.3 Competitive Binding Assays

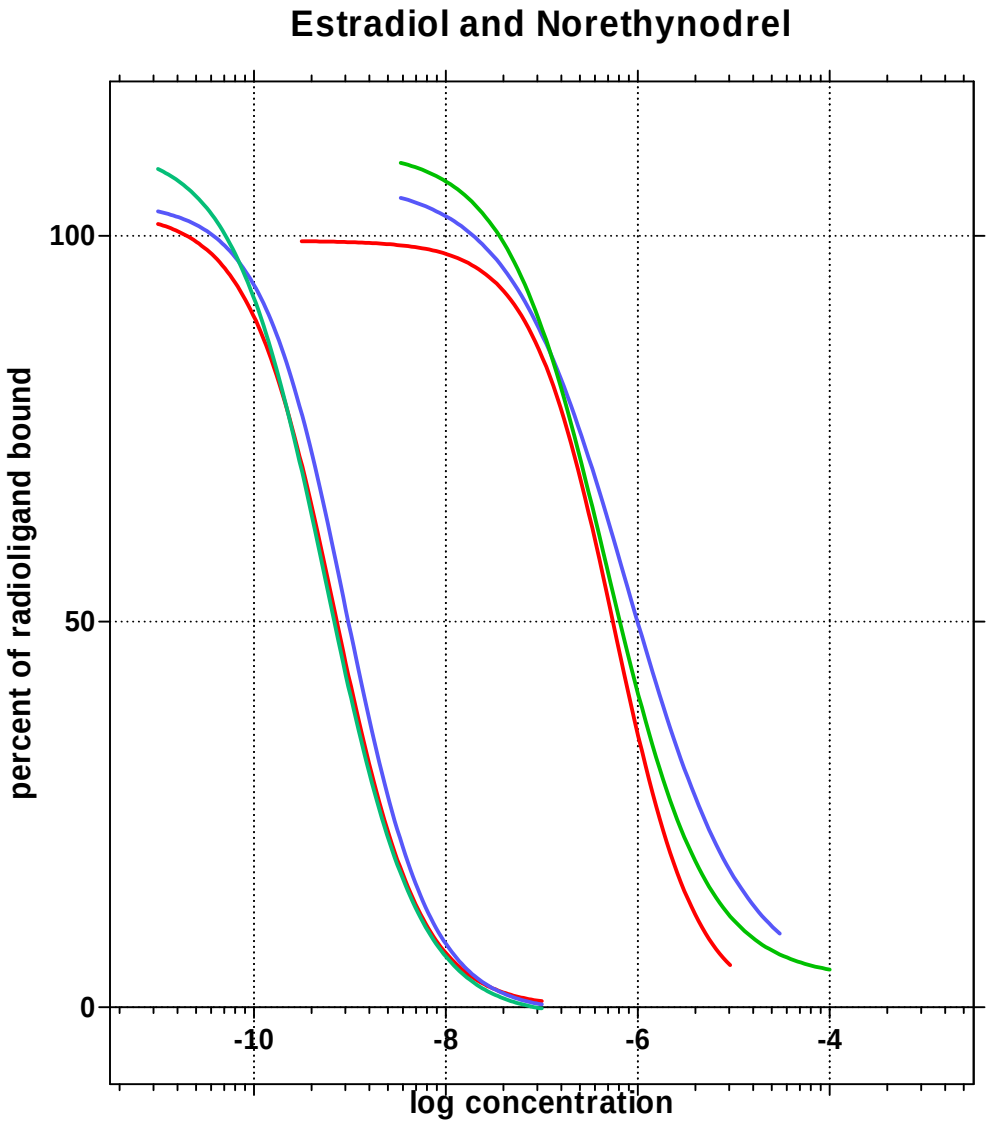
A total of three competitive binding assays were conducted. The assay reports are presented in Appendix E. A summary of the performance criteria parameters is presented in Table 2. All performance criteria were met except for the value of Top for the estradiol and norethynodrel curves for run 3 were 111.5 and 111.1, respectively. In light of the fact that all other criteria were met and these Top values were just outside the acceptable range of 90-110, we concluded that the assay was functioning correctly and all runs were accepted.

Table 2. Performance Criteria Evaluation for Competitive Binding Assays

Criterion	Standard	Assay ID		
		Run 1	Run 2	Run 3
Maximum solvent concentration in assay				
Ethanol	< 3%	2.99%	2.99%	2.99%
DMSO	< 10%	NA	NA	NA
Ligand depletion	< 10%	6.60%	4.98%	5.67%
17β-Estradiol				
Binding Curve Descent	90-10% over approximately 2 log units	Yes	Yes	Yes
IC50 (nM)	about 1 nM + Kd	0.734	0.968	0.689
Curve fit parameters:				
Top	90<Top<110	103.2	104.4	**111.5**
Bottom	-5<Bottom<1	-0.02	-0.62	-1.20
Slope	-1.1<Slope<-0.7	-0.976	-0.990	-0.903
Within run SD	≤5.0	1.90	4.95	2.86
Norethynodrel				
Curve fit parameters:				
Top	90<Top<110	99.4	107.1	**111.1**
Bottom	-5<Bottom<1	-0.13	2.42	3.73
Slope	-1.1<Slope<-0.7	-1.008	-0.712	-0.842
Within run SD	≤5.7	2.88	1.89	4.47
R1881				
Within run SD	≤10	4.57	4.20	6.67

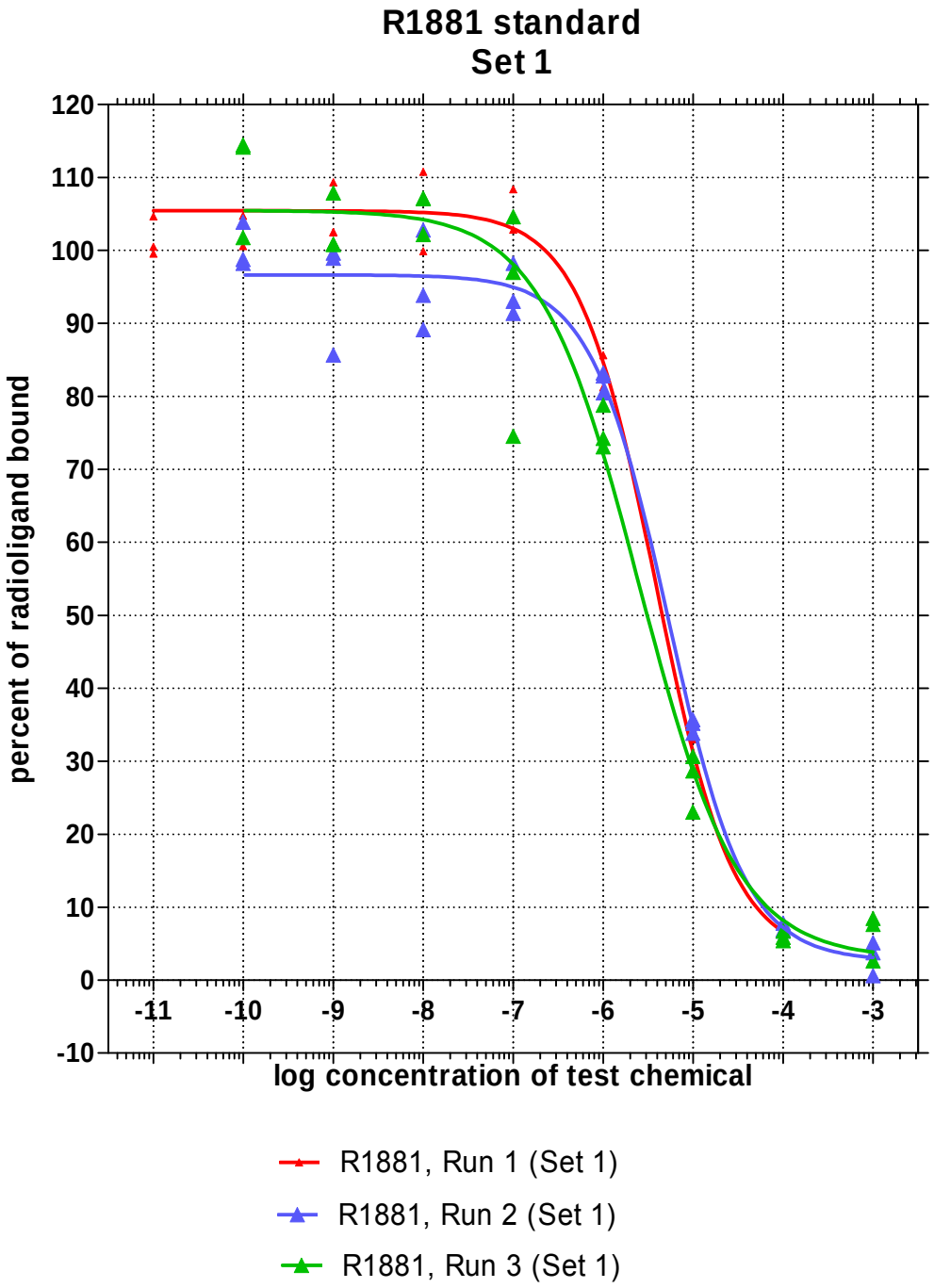
Plots of the curve fits for estradiol and norethynodrel for each run are presented in Figure 1. There is good reproducibility between the runs for estradiol and norethynodrel. Plots of the curve fits for R1881 for each run are presented in Figure 2. Surprisingly, this negative control chemical did show some binding in our assays. It is less potent than the weak positive control (Log(IC50) of ca. -5.3 vs Log(IC50) of ca -6.1 for norethynodrel) and the results were consistent across the three runs.

Figure 1. Curve Fits Estradiol and Norethynodrel for Runs 1-3



Run 1 - Red
Run 2 - Blue
Run 3 - Green

Figure 2. R1881 Curve Fits for Runs 1-3

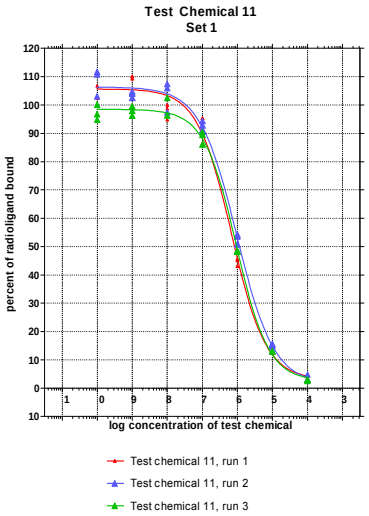


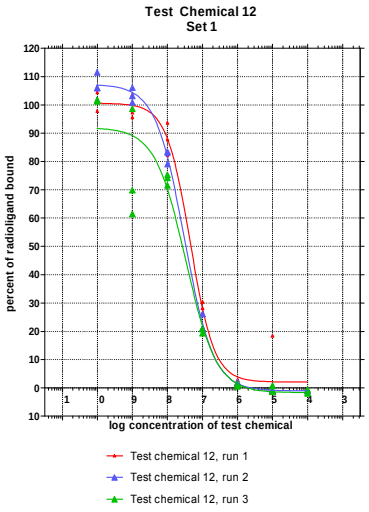
Five test chemicals were evaluated for estrogen receptor binding affinity using the competitive binding assay. The assay results are summarized in Table 3. The curve fits for the test chemicals alone are presented in Figure 3 and those for the test chemicals along with their associated standards and controls are presented in Figure 4.

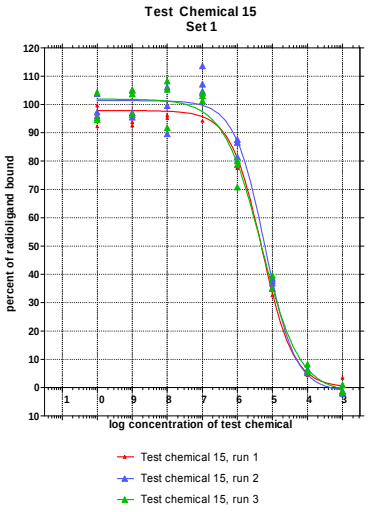
Table 3. Test Chemical Results Summary

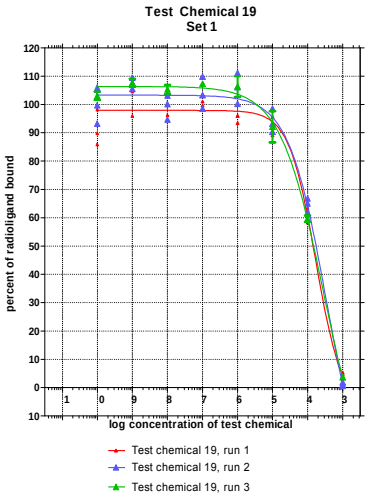
Ligand	Run	Log(IC50)	Log(IC50) – 95% CI		Method	Ki	Ki - 95% CI		RBA	Log (RBA)
Estradiol	1	-9.13	-9.10	-9.16	1	0.101	0.108	0.094	NA	NA
	2	-9.01	-8.94	-9.09	1	0.133	0.157	0.113	NA	NA
	3	-9.16	-9.13	-9.20	1	0.095	0.103	0.088	NA	NA
Norethynodrel	1	-6.26	-6.19	-6.33	1	75.9	89.4	64.5	1.34E-03	-2.87
	2	-6.00	-5.92	-6.08	1	137.1	164.3	114.4	9.73E-04	-3.01
	3	-6.19	-6.10	-6.27	1	89.8	109.0	74.0	1.06E-03	-2.975
R1881	1	-5.34	-5.23	-5.46	1	NA	NA	NA	NA	NA
	2	-5.28	-5.20	-5.37	1	NA	NA	NA	NA	NA
	3	-5.51	-5.35	-5.67	1	NA	NA	NA	NA	NA
Test Chemical 11	1	-6.08	-5.99	-6.17	1	114.8	140.2	94.0	8.81E-04	-3.06
	2	-5.94	-5.87	-6.00	1	159.7	186.3	137.0	8.34E-04	-3.08
	3	-6.02	-5.97	-6.07	1	131.7	146.6	118.3	7.21E-04	-3.14
Test Chemical 12	1	-7.33	-7.22	-7.43	1	6.46	8.25	5.06	1.56E-02	-1.81
	2	-7.47	-7.43	-7.51	1	4.65	5.09	4.25	2.86E-02	-1.54
	3	-7.57	-7.35	-7.79	1	3.72	6.21	2.23	2.55E-02	-1.59
Test Chemical 15	1	-5.31	-5.23	-5.40	1	667.0	817.3	544.4	1.52E-04	-3.82
	2	-5.21	-5.11	-5.32	1	843.1	1075.3	661.0	1.58E-04	-3.80
	3	-5.31	-5.20	-5.42	1	673.3	864.7	524.2	1.41E-04	-3.85
Test Chemical 19	1	-3.84	-3.70	-3.98	1	19910	27232	14557	5.08E-06	-5.29
	2	-3.75	-3.61	-3.89	1	24249	33536	17534	5.50E-06	-5.26
	3	-3.83	-3.75	-3.90	1	20498	24491	17156	4.63E-06	-5.33
Test Chemical 21	1	NA	NA	NA	1	NA	NA	NA	NA	NA
	2	NA	NA	NA	1	NA	NA	NA	NA	NA
	3	NA	NA	NA	1	NA	NA	NA	NA	NA

Figure 3. Test Chemical Curve Fits









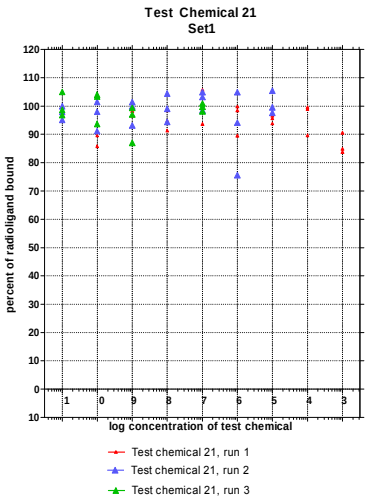
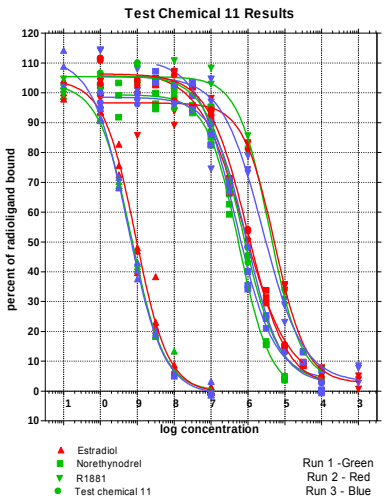
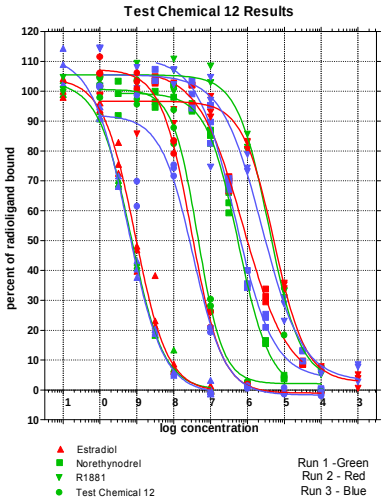
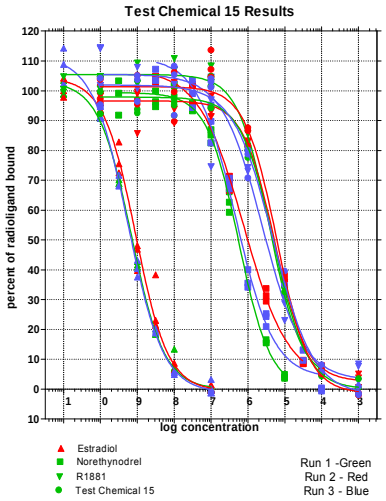
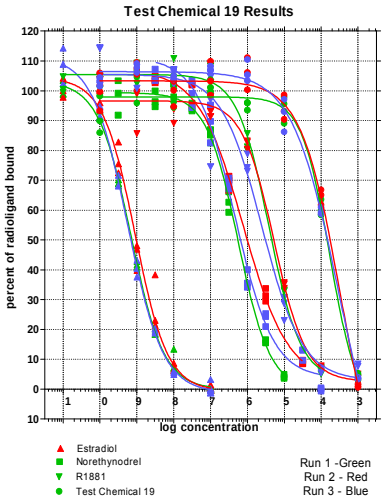


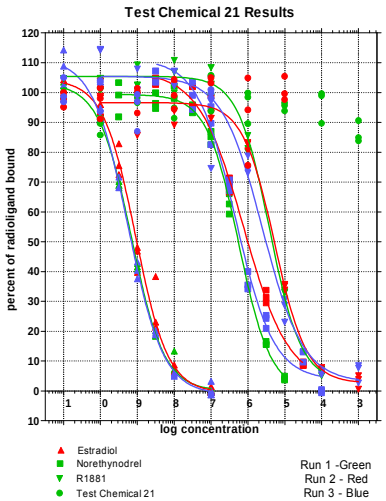
Figure 4. Curve Fits for Test Chemicals Plus Controls











4.0 Conclusion

This report presents data from estrogen receptor saturation and competitive binding assays using rat uterine cytosol. A new batch of cytosol was prepared in our laboratory for use in these assays and it contained 2.2 mg protein/mL. Historically, we have found cytosol preparation protein concentrations in this range. This batch of cytosol was tested in three independent saturation binding assays and was found to have an average Kd of 0.1596 nM. The results of the three saturation binding assays indicate that the concentration of protein used in the assay (50 μg) was appropriate and that the cytosol contained a single, high-affinity binding site.

A set of three test chemicals was tested in the competitive binding assay in three runs that also contained standard and control chemicals. Each of these assays met all performance criteria, except for Run 3, where the Top of the estradiol and norethynodrel curves were just outside the 90-110 range, with values of ca. 111. Since all other criteria were met, this near-miss was not considered to be sufficient reason to exclude that run. Test chemicals 11, 12, 15, and 19 were all found to be positive for estrogen receptor binding, while test chemical 21 was negative in this assay.

Appendix A

Solvent Concentration Calculations

Calculation of Maximum Solvent Concentration in Assay Tubes

Concentration of Ethanol in Assay Buffer

1. The 100 mM PMSF Stock Solution is prepared in 100 % ethanol.
2. The TEDG + PMSF buffer contains 1 mL of the PMSF stock per 100 mL buffer,
   therefore, the TEDG + PMSF buffer contains 1% ethanol.

Concentration of Ethanol in 50 nM [^{3}H]E2 solution.

1. 50 nM [^{3}H]E2 solution is prepared by diluting 5.4 μL [^{3}H]E2 stock (100% ethanol) to 1.0 mL with assay buffer.

=(5.4/1000 + 994.6/1000*0.01)*100
1.53%

Concentration of Ethanol in Master Mix

1. Master mix is prepared by combining 380 μL of assay buffer with 10 μL of 50 nM [^{3}H]E2 solution

=((380*0.01)+(10*0.0153))/390*100
1.01%

Total ethanol in assay tubes (total binding)

1. The total binding tubes contain 10 μL ethanol, 390 μL of master mix and 100 μL cytosol (in assay buffer) in a final volume of 500 μL

=(10/500 + (390* 0.0101/500)+ (100*0.01/500))*100
2.99%

2. Other tubes that have fairly high ethanol concentrations include P1 and NSB

The P1 dilution added to the tubes is 50% ethanol, so effectively 5 μL ethanol is added. As above
=(5/500 + (390* 0.0101/500)+ (100*0.01/500))*100
1.99%

The NSB dilution added to the tubes is 10% ethanol, so effectively 1 μL ethanol is added. As above:
=(1/500 + (390* 0.0101/500)+ (100*0.01/500))*100
1.19%

Other dilutions in each series approach a final ethanol concentration of 1%.

Appendix B

Control and Test Chemical Dilution Schemes

Estradiol and Control Solution Preparation

Date: _____

Assay Run ID _____

Balance Cal b ID _____

Ethanol supplier/lot # _____

Pipette	Serial Number
L20	
L200	
L1000	

Preparation of [^{3}H]17b-estradiol

[^{3}H]17b-estradiol

Lot #	3589221	
Label SA	110	Ci/mmol
Certification Date	06/28/07	
Label concentration	1	mCi/mL

1) Calculate the SA for the date of use.

Date of use	02/06/08
Days	223
% Remaining	0.966
New SA	106.2865

2) Calculate the concentration of the [^{3}H]17β-estradiol stock

SA (Ci/mmol)	106.2865
Stock concentration (mCi/mL)	1
Concentration (nM)	9408.532

3) For Competitive Assay

Want concentration in assay tube - 1 nM

Prepare stock at 50 nM

In 3 mL, need:

nmol/L = 0.15 nmol/ Y L

Y L = 0.15 nmol * L/nmol

Y µL = 0.15 nmol/ nM * 1E6

Y µL = 15.9

Dilute Y uL of stock to 3 mL

	µL stock
	µL buffer added
	mL total volume

Signature _____ Date _____

QC Initials/Date _____

Estradiol and Control Solution Preparation

Date: _____

Preparation of 17b-estradiol stock (non-radiolabeled)

Use amber glassware — **17b-estradiol**
Solvent: EtOH — Lot # 086K1611

Stock 1 - 1 mM
Weigh 6.74 - 6.88 mg 17β-estradiol into a 25 mL volumetric flask
Partially fill with ethanol. Mix until dissolved. Then q.s. with additonal ethanol

	mg estradiol
	mL final solution

Stock 2 - 50 µM
Dilute Stock 1 1:20 in ethanol (i.e., Dilute 0.5 mL of 1 mM stock to 10 mL)

	mL Stock 1
	mL final solution

Preparation of Negative Control (R1881) Stock — Methyltrienolone
Want stock concentration of 100 mM — Lot # 20070530
MW = 284.4 g/mol

Weight 27.02 - 29.86 mg R1881 into a 1 mL volumetric flask.
Partially fill with ethanol. Mix until dissolved. Then q.s. with additional ethanol

	mg R1881
	mL final solution

Preparation of Weak Positive Control (norethynodrel) Stock — Norethynodrel
Want stock concentration of 10 mM — Lot # G
MW = 298.4 g/mol

Weight 28.35 - 31.33 mg norethynodrel into a 10 mL volumetric flask.
Partially fill with ethanol. Mix until dissolved. Then q.s. with additional ethanol

	mg norethynodrel
	mL final solution

Preparation of Test Chemical Stock
Want stock concentration of 100 mM
The table calculates mass needed to prepare 1 mL at 100 mM.

TC Code	MW	mg needed	Actual mg	Final volume (mL)	Solvent
12	318.4	31.84			
19	298.33	29.83			
11	242.28	24.23			
21	276.5	27.65			
15	229.29	22.93			
		0			

Signature _____ Date _____

QC Initials/Date _____

Dilution of Standards, Controls and Test Chemicals

Assay Date: _____

Assay Run ID: _____

Pipet	Serial Number
L200	
L1000	

Non-radiolabeled 17β-estradiol

Tube #	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration (nM)	Final concentration in Assay (M)
NSB1	900	100	Stock (50 µM)	1	5000	1.00E-07
S2	900	100	NSB1	1	500	1.00E-08
S3	600	277	S2	0.877	158.0	1.00E-8.5
S4	900	100	S2	1	50.0	1.00E-09
S5	900	100	S3	1	15.8	1.00E-9.5
S6	900	100	S4	1	5	1.00E-10
S7	900	100	S6	1	0.5	1.00E-11

Norethynodrel

Tube #	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
P1	400	400	Stock (10 mM)	0.80	5000	1.00E-04
P2	800	150	Stock (10 mM)	0.95	1580	1.00E-4.5
P3	900	100	P2	1.0	158.0	1.00E-5.5
Intermed	900	100	P1	1.0	500.0	--
P4	900	100	Intermed	1.0	50.0	1.00E-06
P5	900	100	P3	1.0	15.8	1.00E-6.5
P6	900	100	P4	1.0	5	1.00E-07
P7	900	100	P5	1.0	1.58	1.00E-7.5
P8	900	100	P7	1.0	0.158	1.00E-8.5

R1881 and Test Chemicals

Tube #*	µL buffer	µL Sample	Sample	Total Volume (mL)	Soln concentration (µM)	Final concentration in Assay (M)
1	500	500	Stock (100 mM)	1.0	50000	1.00E-03
2	900	100	1	1.0	5000	1.00E-04
3	900	100	2	1.0	500.0	1.00E-05
4	900	100	3	1.0	50.0	1.00E-06
5	900	100	4	1.0	5.0	1.00E-07
6	900	100	5	1.0	0.5	1.00E-08
7	900	100	6	1.0	0.05	1.00E-09
8	900	100	7	1.0	0.005	1.00E-10

* For R1881, label the tubes N1 through N8; for test chemicals, label them nTC1 through nTC8 where n=test chemical code

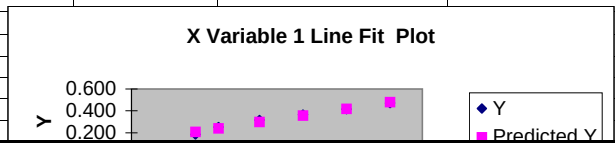
Signature _____ Date _____

QC Initials/Date _____

Appendix C

Protein Assay Results

	A	B	C	D	E	F	G	H	I	J	K	L
1	Standards:	0.89	0.74	0.59	0.44	0.3	0.22	Blk	Protein stock (mg/10 mL)	Protein stock ID		
2		1 092	0.917	0.938	0 885	0.810	0.742	0.546	14.8			
3		1.125	0.927	0.973	0.754	0.822	0.734	0.549				
4		0 819	1.026	0.805	0 927	0.744	0.700	0.527				
5	Samples:	1:5	1:10	0.59	0.3							
6		0 842	0.731	1.005	0.799							
7		0.799	0.745	0.836	0 828							
8		0 864	0.661	1.033	0.758							
9	mg Protein		µL Standard		mg Protein		A_{raw}	A_{adj}	Curve			
10	per µL		Used		Measured				Output	Variables	Regression results	
11	0.00089		100		0.0890		1 012	0.471	0.0863	m, b	0.242	-0.028
12	0.00074		100		0.0740		0 957	0.416	0.0729	se_m, se_b	0.017	0.006
13	0.00059		100		0.0590		0 905	0.365	0.0605	r^2, se_y	0.981	0.004
14	0.00044		100		0.0440		0 855	0.315	0.0484	F, df	202	4
15	0.00030		100		0.0300		0.792	0.251	0.0331	ss_{reg}, ss_{resid}	0.003	0.000
16	0.00022		100		0.0220		0.725	0.185	0.0169			
17		Blank	0.541		r^2=	0.981					Regression results are calculated using the function L NEST	
18					m=	0.242						
19					b=	-0.028						
20		A_{raw}	$A_{adj.}$	mg protein measured	µL diluted µSOMES	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)		mg protein/µL Prep.	average mg/µL	mg/mL	
21	1:5	0 842	0.301	0.045	100	80	400		2.26E-03	2.17E-03	2.2	
22	1:5	0.799	0.258	0.035	100	80	400		1.74E-03			
23	1:5	0 864	0.323	0.050	100	80	400		2.52E-03			
24	1:10	0.731	0.190	0.018	100	40	400		1.83E-03	1.38E-03	1 38	
25	1:10	0.745	0.204	0.022	100	40	400		2.17E-03			
26	1:10	0 661	0.120	0.001	100	40	400		1.37E-04			
27	0.59	1 005	0.464	0.085	100	1	1		8.46E-04	1.65E-03	1 65	
28	0.59	0 836	0.295	0.044	100	1	1		4.37E-04			
29	0.59	1 033	0.492	0.091	25	1	1		3.65E-03			
30	0.3	0.799	0.258	0.035	100	1	1		3.47E-04	5.86E-04	0 59	
31	0.3	0 828	0.287	0.042	100	1	1		4.18E-04			
32	0.3	0.758	0.217	0.025	25	1	1		9.93E-04			
33												
34												
35			SUMMARY OUTPUT									
36												
37			*Regression Statistics*									
38			Multiple R	0 990228334								
39			R Square	0 980552153								
40			Adjusted R Sq	0 975690192								
41			Standard Erro	0 016505403								



Appendix D

Saturation Assay Reports

Saturation Binding Report

Assay Date	1/28/2008
Assay ID	SAT1

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	• 98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol		
Rat Strain	Sprague Dawley		
Rat Age	94	days	
Ovariectomy Date	1/14/2008		
Uterus removal Date	1/22/2008		
Cytosol Prep Date	1/23/2008		
Cytosol Prep ID	1/23/2008		
Protein Content	2.20	mg/mL	
Protein Assay Date	1/23/2008		
RUC Storage Info	-70	°C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF	
Protein Concentration Used	50	µg/tube
Assay volume	0.5	mL
Incubation Time	19.03	h
Incubation Temp.	4	°C
Notes on problems:	None	

Results

Counter Data File	29JA2041.P00	
Excel Filename	Sat_#1_1_28_08.xls	
PRISM Filename	RTI Sat_#1_1_28_08.pzf	
Assay Date	1/28/2008	
Elapsed days since SA determination	214	
Adjusted SA	106.4	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	0.1508	nM
SE Kd	0.0164	
Estimated Bmax	71.03	fmol/100 µg
SE Bmax	2.26	

Discussion

		Enter Yes or No
1	Did specific binding curve reach a plateau?	Yes
2	Is the Scatchard plot linear?	Yes
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	Yes
4	Standard errors for Kd and Bmax acceptable?	
	ratio SE:parameter should be • 20%	
	SE:Kd 10.88	
	SE:Bmax 3.18	
5	Non-specific binding should be < 50% of total binding?	Yes

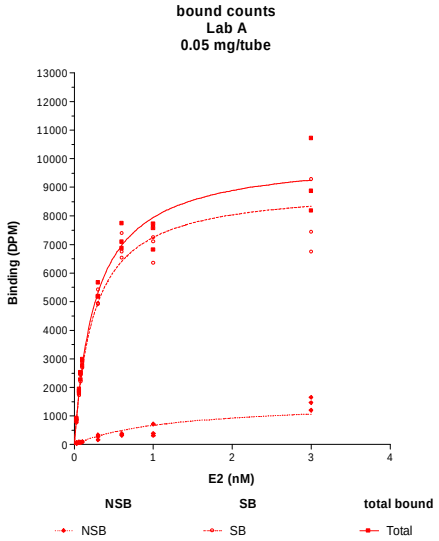
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

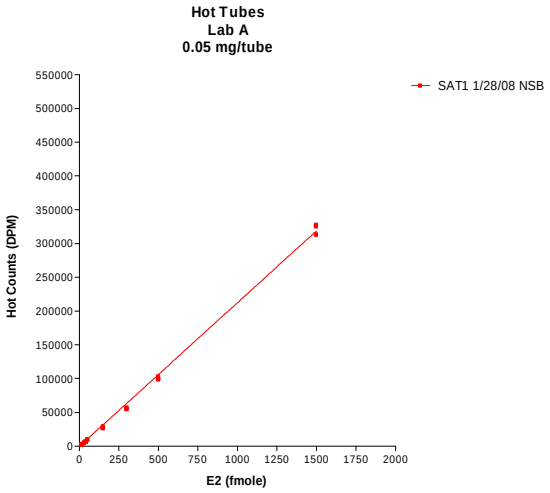
All acceptable? Yes

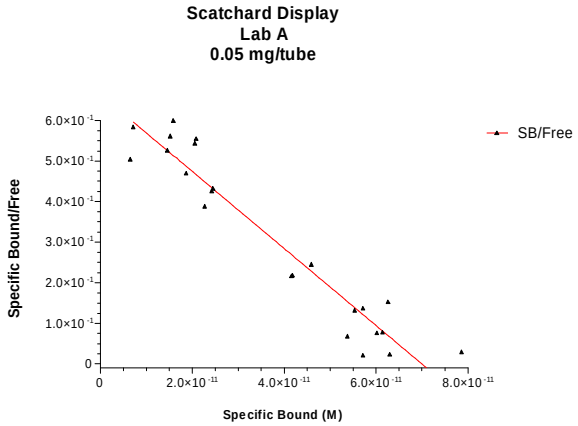
Conclusion

Note any reasons why confidence in the results should be high or low.

Run is acceptable.


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
Hot Counts (DPM)
E2 (fmole)
SAT1 1/28/08 NSB


Scatchard Display
Lab A
0.05 mg/tube
Specific Bound/Free
Specific Bound (M)
SB/Free

Raw file

File 29JA2041.P00

Via	Tim	DPM			tS	E
1	10	20.18	25.28	0	19.7	481.37
2	5	5461.61	2171 6	0	12.26	467.66
3	5	5922.35	2358	0	12.24	467.82
4	5	7152.93	2833.4	0	12.05	460.62
5	5	5154.38	2057	0	12.04	463.43
6	5	4552.91	1798.16	0	12.13	463.65
7	5	5052.35	2017.04	0	12.15	467.18
8	5	5165.74	2054.28	0	12.13	465.13
9	5	4589.03	1836.16	0	12.17	466.15
10	5	4732.88	1895 2	0	12.15	466.89
11	5	3790.39	1492.76	0	12.26	463.91
12	5	3450.34	1384.12	0	12.12	461.38
13	5	3466.85	1382.36	0	12.1	465.82
14	5	1992.81	789.16	0	12.27	467.14
15	5	1971.82	781 2	0	12.23	464.36
16	5	1850.49	729.36	0	12.13	464.51
17	5	1521.52	611.36	0	12.29	468.71
18	5	1671.55	663.84	0	12.38	467.25
19	5	1693.7	673.96	0	12.21	466.91
20	5	1301.65	506.72	0	12.34	464.31
21	5	1201.54	474.56	0	12.37	465 6
22	5	1251.05	489 6	0	12.53	463.36
23	5	549.69	217.96	0	12.31	466.42
24	5	623.83	243.92	0	12.85	465.64
25	5	600.58	237.52	0	12.26	462.86
26	5	0	0	0	0	463.53
27	5	802.49	320.56	0	11.91	466.49
28	5	1103.44	445.12	0	12.32	466.99
29	5	976.57	379.96	0	12.25	466.43
30	5	258.41	103.56	0	11.71	466.15
31	5	477.36	189.72	0	12.53	465.51
32	5	210.51	82.96	0	12.88	462.85
33	5	225.78	90.04	0	12.95	463.86
34	5	218.46	89.36	0	12.66	466.41
35	5	253.76	97 8	0	12.81	466.64
36	5	111 3	44.52	0	12.59	460.57
37	5	220.31	86.52	0	12.95	466.31
38	5	184.56	73	0	11.77	464.87
39	5	60.93	20.52	0	10.37	467.27
40	5	63.49	21.72	0	13.19	467.34
41	5	60.96	26.52	0	12.9	467.04
42	5	57 8	22.36	0	13.66	467.7
43	5	49.08	19.24	0	12.7	467.81
44	5	46.45	18.56	0	15.96	466.05
45	5	56.02	20 2	0	16.35	462.52
46	5	63.23	20.72	0	15.76	464.59
47	5	37.41	14.16	0	9.98	466.56
48	5	42.61	13.96	0	16.93	465.87
49	5	44.57	14.52	0	16.21	464.27
50	5	24.28	8.36	0	16.91	472.02
51	5	0	0	0	0	467.13
52	5	313545.03	146382.25	0	12.37	549.62
53	5	328033.53	149555.59	0	12.72	547.1
54	5	325540	148268.64	0	12.69	545.89
55	5	103263.75	47422.44	0	12.52	546.21
56	5	99191.12	45483.64	0	12.52	546.08
57	5	99133.96	45570.24	0	12.58	549.41
58	5	57518.49	26285.92	0	12.41	540.17
59	5	55779.04	25562.04	0	12.46	544.43
60	5	55349.27	25433.96	0	12.44	545.72
61	5	28963.71	13267.12	0	12.47	542.74
62	5	27117 9	12597.96	0	12.41	551.69
63	5	27336.45	12682.36	0	12.5	550.57
64	5	9799 2	4451 8	0	12.47	540.72
65	5	9813.85	4456.48	0	12.49	541.92
66	5	9440.55	4344.36	0	12.63	551.04
67	5	7196.45	3316 2	0	12.52	549.16
68	5	6907.08	3176.32	0	12.53	547.34
69	5	6832 5	3129	0	12.37	544.81
70	5	4948.54	2276.4	0	12.38	545.06
71	5	5140.76	2352.32	0	12.5	547.95
72	5	5151.63	2386.32	0	12.41	546.33
73	5	2400.55	1108.52	0	12.35	547.55
74	5	2347.57	1064.4	0	12.6	545.93
75	5	2295.55	1053.32	0	12.49	544 8
76	5	0	0	0	0	472.11
77	5	1636.35	642 6	0	12.17	463.17
78	5	1523 9	604 6	0	12.25	466.91
79	5	1559.76	625.04	0	12.19	465.67
80	5	36.19	13.16	0	11.97	465.87
81	5	34.11	13.32	0	17.1	466.18
82	5	38.92	14.52	0	18.77	462.54
83	5	0	0	0	0	461.19
84	5	273725.03	16344.32	0	7.06	109.73

File w/ backgrounds stripped out

Sample IDs

Type	Conc	Tube		DPM	Rounded DPM
H		1	1	549 69	550
H		1	2	623 83	624
H		1	3	600 58	601
H		2	1	1301 65	1302
H		2	2	1201 54	1202
H		2	3	1251 05	1251
H		3	1	1521 52	1522
H		3	2	1671 55	1672
H		3	3	1693.7	1694
H		4	1	1992 81	1993
H		4	2	1971 82	1972
H		4	3	1850.49	1850
H		5	1	3790 39	3790
H		5	2	3450 34	3450
H		5	3	3466 85	3467
H		6	1	5165.74	5166
H		6	2	4589 03	4589
H		6	3	4732 88	4733
H		7	1	5154 38	5154
H		7	2	4552 91	4553
H		7	3	5052 35	5052
H		8	1	5461 61	5462
H		8	2	5922 35	5922
H		8	3	7152 93	7153
HC		1	1	42 61	43
HC		1	2	44 57	45
HC		1	3	24 28	24
HC		2	1	56 02	56
HC		2	2	63 23	63
HC		2	3	37.41	37
HC		3	1	57 8	58
HC		3	2	49 08	49
HC		3	3	46.45	46
HC		4	1	60 93	61
HC		4	2	63.49	63
HC		4	3	60 96	61
HC		5	1	111 3	111
HC		5	2	220 31	220
HC		5	3	184 56	185
HC		6	1	225.78	226
HC		6	2	218.46	219
HC		6	3	253.76	254
HC		7	1	258.41	258
HC		7	2	477 36	477
HC		7	3	210 51	211
HC		8	1	802.49	803
HC		8	2	1103.44	1103
HC		8	3	976 57	977
Hot		1	1	2400 55	2401
Hot		1	2	2347 57	2348
Hot		1	3	2295 55	2296
Hot		2	1	4948 54	4949
Hot		2	2	5140.76	5141
Hot		2	3	5151 63	5152
Hot		3	1	7196.45	7196
Hot		3	2	6907 08	6907
Hot		3	3	6832 5	6833
Hot		4	1	9799 2	9799
Hot		4	2	9813 85	9814
Hot		4	3	9440 55	9441
Hot		5	1	28963.71	28960
Hot		5	2	27117 9	27120
Hot		5	3	27336.45	27340
Hot		6	1	57518.49	57520
Hot		6	2	55779 04	55780
Hot		6	3	55349 27	55350
Hot		7	1	103263.75	103300
Hot		7	2	99191.12	99190
Hot		7	3	99133 96	99130
Hot		8	1	313545 03	313500
Hot		8	2	328033 53	328000
Hot		8	3	325540	325500

Saturation Binding Report

Assay Date	1/30/2008
Assay ID	SAT2

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	• 98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol		
Rat Strain	Sprague Dawley		
Rat Age	94	days	
Ovariectomy Date	1/14/2008		
Uterus removal Date	1/22/2008		
Cytosol Prep Date	1/23/2008		
Cytosol Prep ID	1/23/2008		
Protein Content	2.20	mg/mL	
Protein Assay Date	1/23/2008		
RUC Storage Info	-70	°C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF	
Protein Concentration Used	50	µg/tube
Assay volume	0.5	mL
Incubation Time	19.25	h
Incubation Temp.	4	°C
Notes on problems:	None	

Results

Counter Data File	31JA2020.P00
Excel Filename	Sat_#2_1_30_08.xls
PRISM Filename	RTI Sat_#2_1_30_08.pzf

Assay Date	1/30/2008	
Elapsed days since SA determination	216	
Adjusted SA	106.4	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	0.1466	nM
SE Kd	0.0183	
Estimated Bmax	58.02	fmol/100 µg
SE Bmax	2.28	

Discussion

		Enter Yes or No
1	Did specific binding curve reach a plateau?	Yes
2	Is the Scatchard plot linear?	Yes
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	Yes
4	Standard errors for Kd and Bmax acceptable?	
	ratio SE:parameter should be • 20%	
	SE:Kd 12.48	
	SE:Bmax 3.93	
5	Non-specific binding should be < 50% of total binding?	Yes

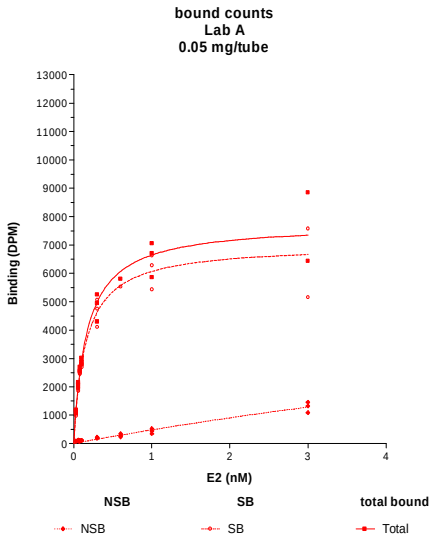
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

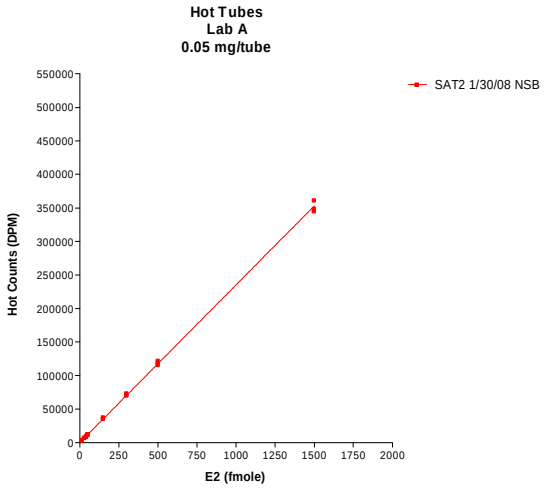
All acceptable? Yes

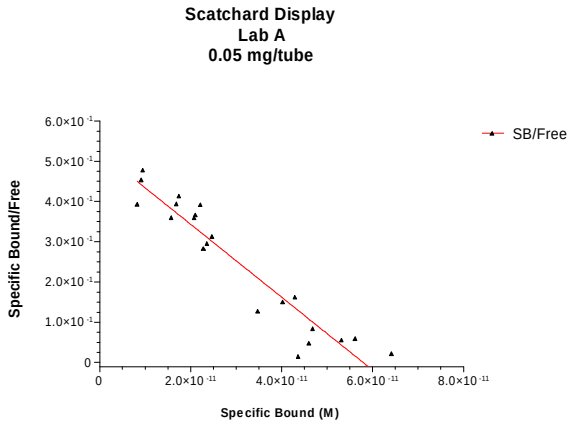
Conclusion

Note any reasons why confidence in the results should be high or low.

Run is acceptable.


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
Hot Counts (DPM)
E2 (fmole)
SAT2 1/30/08 NSB


Scatchard Display
Lab A
0.05 mg/tube
Specific Bound/Free
Specific Bound (M)
SB/Free

Raw file

	File	31JA2020.P00				
Via	Tim	DPM				tSIE
1	10	17 04	19.84	0	21.59	484.36
2	5	3759 84	1707.76	0	13.61	568.44
3	5	3391.15	1559.76	0	13.51	571.4
4	5	3457 94	1593.96	0	13.4	572.42
5	5	7260 24	3337.04	0	13.58	571.78
6	5	6971.47	3211.44	0	13.47	577.12
7	5	7190.48	3299.84	0	13.65	573.58
8	5	9280.15	4306.16	0	13.41	574.69
9	5	9523 29	4359 6	0	13.56	576.86
10	5	9309.78	4261.36	0	13.51	572.66
11	5	12596.46	5769.92	0	13.59	572.95
12	5	12323 28	5671.56	0	13.6	575.25
13	5	12083 82	5522 2	0	13.53	571.67
14	5	37668 09	17220.36	0	13.59	571.85
15	5	36021 66	16549.16	0	13.66	577 3
16	5	35778 54	16420.4	0	13.6	573.44
17	5	73799 69	33948.4	0	13.54	573.33
18	5	70801 67	32493.76	0	13.6	573.98
19	5	70818 69	32266.64	0	13.55	568.27
20	5	121873.77	55837.08	0	13.69	574.07
21	5	119145 53	54394.96	0	13.71	573.89
22	5	116233.5	53328.48	0	13.63	574.72
23	5	361536 09	165159 8	0	13.66	571.77
24	5	349058 03	159315.36	0	13.72	572.28
25	5	345425.41	157875 8	0	13.69	572.89
26	5	0	0	0	0	482.02
27	5	802.11	333.84	0	12.45	486.38
28	5	707 52	293 8	0	12.45	482.85
29	5	775 96	311.96	0	12.59	486.04
30	5	1443 86	596.56	0	12.57	485.78
31	5	1397 04	567.44	0	12.38	481.21
32	5	1312	530 8	0	12.45	480.44
33	5	1722 65	702.4	0	12.38	484.83
34	5	1698 09	692.76	0	12.43	484.41
35	5	1805.77	740.4	0	12.43	484.05
36	5	1869 03	768.76	0	12.35	483.01
37	5	2014 98	831.4	0	12.31	486.48
38	5	1928 82	785 6	0	12.44	483.11
39	5	2871 36	1182.96	0	12.35	480.81
40	5	3302 37	1348 2	0	12.43	481.39
41	5	3513 83	1451.88	0	12.39	484.18
42	5	3877.42	1581.96	0	12.46	482.41
43	5	2605 99	1069.44	0	12.58	485
44	5	2698.47	1104 8	0	12.46	484.99
45	5	3916.48	1605.56	0	12.34	485.67
46	5	4712.15	1931	0	12.32	481.85
47	5	4479 95	1829 2	0	12.41	484.37
48	5	4298.42	1761 2	0	12.39	482.23
49	5	3350 68	1374.36	0	12.46	485.99
50	5	5910.77	2398.64	0	12.39	480.93
51	5	0	0	0	0	477.45
52	5	57.1	22.6	0	15.27	481.99
53	5	61 24	27	0	11.02	483.57
54	5	57 21	21.56	0	15.34	485.24
55	5	86.14	34.16	0	17.56	482.06
56	5	72 54	30.6	0	15.57	482.47
57	5	58 63	26.36	0	11.99	483.66
58	5	75.3	31.84	0	14.12	485.43
59	5	51.49	22.44	0	13.81	483.34
60	5	66 36	28.4	0	13.41	482.32
61	5	68.3	27.6	0	13.15	481.81
62	5	71.5	28.4	0	12.84	485.31
63	5	73.46	29.6	0	11.95	484.55
64	5	145 03	60.96	0	12.91	482.24
65	5	117 03	47.96	0	12.6	482 6
66	5	138 23	56.8	0	12.79	482.61
67	5	231 07	92.36	0	12.94	482.64
68	5	155 89	63.28	0	13.17	482.42
69	5	182 05	73.88	0	12.98	480.51
70	5	232 34	98.8	0	12.89	481.55
71	5	291 24	118	0	12.57	480.73
72	5	350 34	142.76	0	13.2	482.27
73	5	883 85	361.16	0	12.72	482.24
74	5	968 88	397.84	0	12.6	485.71
75	5	727 97	294 2	0	12.51	482.03
76	5	275995.19	16415	0	7.06	109.54
77	5	15 36	4.36	0	22.18	482 9

File w/ backgrounds stripped out

Sample IDs				
Type	Conc	Tube	DPM	Rounded DPM
H	1	1	802.11	802
H	1	2	707.52	708
H	1	3	775.96	776
H	2	1	1443.86	1444
H	2	2	1397.04	1397
H	2	3	1312.00	1312
H	3	1	1722.65	1723
H	3	2	1698.09	1698
H	3	3	1805.77	1806
H	4	1	1869.03	1869
H	4	2	2014.98	2015
H	4	3	1928.82	1929
H	5	1	2871.36	2871
H	5	2	3302.37	3302
H	5	3	3513.83	3514
H	6	1	3877.42	3877
H	6	2	2605.99	2606
H	6	3	2698.47	2698
H	7	1	3916.48	3916
H	7	2	4712.15	4712
H	7	3	4479.95	4480
H	8	1	4298.42	4298
H	8	2	3350.68	3351
H	8	3	5910.77	5911
HC	1	1	57.10	57
HC	1	2	61.24	61
HC	1	3	57.21	57
HC	2	1	86.14	86
HC	2	2	72.54	73
HC	2	3	58.63	59
HC	3	1	75.30	75
HC	3	2	51.49	51
HC	3	3	66.36	66
HC	4	1	68.30	68
HC	4	2	71.50	72
HC	4	3	73.46	73
HC	5	1	145.03	145
HC	5	2	117.03	117
HC	5	3	138.23	138
HC	6	1	231.07	231
HC	6	2	155.89	156
HC	6	3	182.05	182
HC	7	1	232.34	232
HC	7	2	291.24	291
HC	7	3	350.34	350
HC	8	1	883.85	884
HC	8	2	968.88	969
HC	8	3	727.97	728
Hot	1	1	3759.84	3760
Hot	1	2	3391.15	3391
Hot	1	3	3457.94	3458
Hot	2	1	7260.24	7260
Hot	2	2	6971.47	6971
Hot	2	3	7190.48	7190
Hot	3	1	9280.15	9280
Hot	3	2	9523.29	9523
Hot	3	3	9309.78	9310
Hot	4	1	12596.46	12600
Hot	4	2	12323.28	12320
Hot	4	3	12083.82	12080
Hot	5	1	37668.09	37670
Hot	5	2	36021.66	36020
Hot	5	3	35778.54	35780
Hot	6	1	73799.69	73800
Hot	6	2	70801.67	70800
Hot	6	3	70818.69	70820
Hot	7	1	121873.77	121900
Hot	7	2	119145.53	119100
Hot	7	3	116233.50	116200
Hot	8	1	361536.09	361500
Hot	8	2	349058.03	349100
Hot	8	3	345425.41	345400

Saturation Binding Report

Assay Date	1/31/2008
Assay ID	SAT3

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol
Supplier	Perkin Elmer (Boston)
Catalog #	NET-517
Batch #	3589221
Specific Activity	110 Ci/mmol
SA date	6/28/2007
Concentration (mCi/mL)	1
Tested Concentrations (nM)	0.03, 0.06, 0.08, 0.1, 0.3, 0.6, 1, 3

Radioinert Ligand

Name	17β-estradiol
Supplier	Sigma (St. Louis)
Catalog #	E8875
Lot #	086K1611
CAS #	50-28-2
Purity	• 98%
Concentration in NSB tubes (nM)	3, 6, 8, 10, 30, 60, 100, 300

Estrogen Receptor

Type	Rat Uterine Cytosol		
Rat Strain	Sprague Dawley		
Rat Age	94	days	
Ovariectomy Date	1/14/2008		
Uterus removal Date	1/22/2008		
Cytosol Prep Date	1/23/2008		
Cytosol Prep ID	1/23/2008		
Protein Content	2.20	mg/mL	
Protein Assay Date	1/23/2008		
RUC Storage Info	-70	°C	(Ultra004)

Test Conditions

Buffer	TEDG + PMSF	
Protein Concentration Used	50	µg/tube
Assay volume	0.5	mL
Incubation Time	18.92	h
Incubation Temp.	4	°C
Notes on problems:	None	

Results

Counter Data File	01FE1939.P00
Excel Filename	Sat_#3_1_31_08.xls
PRISM Filename	RTI Sat_#3_1_31_08.pzf

Assay Date	1/31/2008	
Elapsed days since SA determination	217	
Adjusted SA	106.4	Ci/mmol

See attached graphs

1. Binding
2. Measured concentration of [^{3}H]estradiol
3. Scatchard plot

See attached raw data (dpm/tube)

Estimated Kd	0.1813	nM
SE Kd	0.0342	
Estimated Bmax	59.84	fmol/100 µg
SE Bmax	3.35	

Discussion

		Enter Yes or No	
1	Did specific binding curve reach a plateau?	Yes	
2	Is the Scatchard plot linear?	Yes	(but more scatter than other runs)
3	Is the Kd reasonable (generally 0.05 to 0.5 nM)?	Yes	
4	Standard errors for Kd and Bmax acceptable?		
	ratio SE:parameter should be • 20%		
	SE:Kd	18.86	
	SE:Bmax	5.60	
5	Non-specific binding should be < 50% of total binding?	Yes	

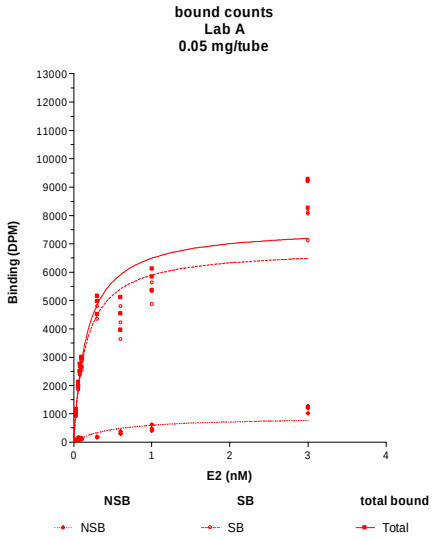
If any of the above are answered no, consider reducing protein prior to rerunning saturation assay

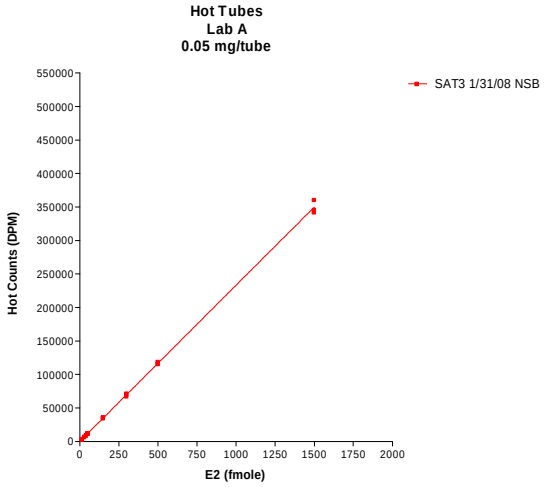
All acceptable? Yes

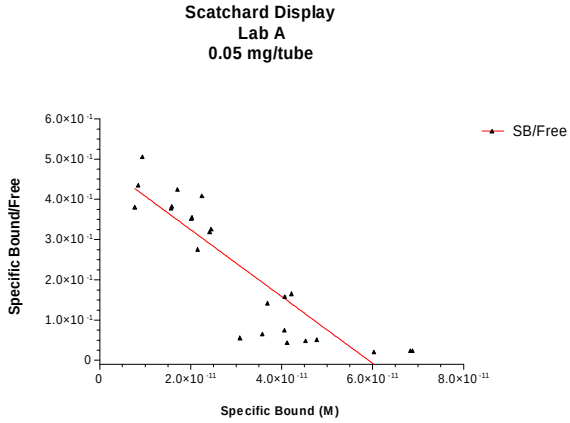
Conclusion

Note any reasons why confidence in the results should be high or low.

This run is acceptable, but there is higher variability than in the other runs, so parameters may be less reliable.


bound counts
Lab A
0.05 mg/tube
Binding (DPM)
E2 (nM)
NSB
SB
total bound
NSB
SB
Total


Hot Tubes
Lab A
0.05 mg/tube
SAT3 1/31/08 NSB
Hot Counts (DPM)
E2 (fmole)


Scatchard Display
Lab A
0.05 mg/tube
SB/Free
Specific Bound/Free
Specific Bound (M)

Raw file

File 01FE1939.P00

Via	Tim	DPM				tSIE
1	10	18.12	22.72	0	20.74	479.93
2	5	3510 08	1574.28	0	13.59	559.03
3	5	3276.14	1512.72	0	13.37	569.01
4	5	3335.18	1494.28	0	13.53	559.61
5	5	6991 63	3195.96	0	13.54	567.28
6	5	6815 05	3103 6	0	13.6	569.79
7	5	6861.1	3078.12	0	13.47	556.47
8	5	9519 25	4321.52	0	13.47	565.17
9	5	9146.49	4166.88	0	13.59	566.56
10	5	9162 61	4132.04	0	13.63	567.32
11	5	12204.43	5530.64	0	13.57	564.48
12	5	11767.41	5347.32	0	13.56	565.1
13	5	11785.49	5360.88	0	13.55	569.75
14	5	36254 64	16481.72	0	13.63	567.09
15	5	34933 93	15979.92	0	13.67	573.47
16	5	34861 26	15814.36	0	13.59	567.21
17	5	71613 38	32551 8	0	13.57	567.19
18	5	69980 25	31980.76	0	13.7	572.46
19	5	67454 23	30652.28	0	13.54	565 9
20	5	118817 63	54036.52	0	13.57	567.56
21	5	116306.18	53039.24	0	13.64	570 3
22	5	115943 52	52821.12	0	13.73	571.57
23	5	360715 53	163310.16	0	13.67	566.05
24	5	345662 97	156924.64	0	13.64	566.72
25	5	342544 25	156039.13	0	13.69	570 5
26	5	0	0	0	0	475.78
27	5	786 38	317.52	0	12.6	483.72
28	5	714.19	292.88	0	12.4	481.04
29	5	653	264.88	0	12.57	480.76
30	5	1424.47	591.48	0	12.22	480 6
31	5	1316.72	534.12	0	12.33	481.34
32	5	1327.44	535 8	0	12.42	477.39
33	5	1678.42	683.36	0	12.41	481.91
34	5	1669 99	684.12	0	12.51	481.09
35	5	1851 06	764.88	0	12.33	481.35
36	5	1983.47	807.28	0	12.52	478.52
37	5	2008 88	826.32	0	12.25	481.98
38	5	1774.72	725.72	0	12.33	482.19
39	5	3322.47	1362.88	0	12.44	484.69
40	5	3024.46	1230.72	0	12.43	481.76
41	5	3443 38	1407.32	0	12.35	480.68
42	5	3034.78	1239.28	0	12.37	479.35
43	5	2645 68	1087.52	0	12.31	481.53
44	5	3415 83	1402.88	0	12.35	479 9
45	5	3899.16	1570.68	0	12.46	477.99
46	5	3580 97	1456.88	0	12.3	480.41
47	5	4094 29	1667.68	0	12.34	479.45
48	5	6153.5	2514.68	0	12.44	482.08
49	5	5521 91	2245.72	0	12.44	480.16
50	5	6194 23	2511.48	0	12.44	477.73
51	5	0	0	0	0	477.63
52	5	42.47	16.76	0	10.38	484.61
53	5	42.4	16.72	0	12.83	484.03
54	5	55 29	22.68	0	15.3	483.68
55	5	113.71	40.16	0	14.49	483.51
56	5	61 24	25.28	0	12.4	480.7
57	5	60 62	28.52	0	11.63	486.54
58	5	73 91	28.92	0	13.83	480.08
59	5	61 36	26.16	0	10.86	481.68
60	5	108 02	41.12	0	15.11	480 8
61	5	80 86	30.48	0	12.65	478.77
62	5	59 83	24.16	0	15.79	484.02
63	5	94 08	37.68	0	11.91	481.44
64	5	123 03	47.52	0	13.35	480.49
65	5	115 31	43.32	0	13.27	481.51
66	5	118 08	47.96	0	12.49	480.87
67	5	195 81	77.52	0	12.17	483.68
68	5	202 35	79.32	0	13.09	481.37
69	5	252.6	100.92	0	12.6	481.54
70	5	267 98	108.52	0	12.5	480.71
71	5	417 24	178.52	0	11.91	480.11
72	5	318 94	129	0	12.94	481.18
73	5	849 03	345.52	0	12.35	480.82
74	5	797.3	323.56	0	12.54	480.58
75	5	679 37	275.28	0	12.51	484.09
76	5	277922.72	16178.76	0	7.03	108.56
77	5	0	0	0	56.79	481.17

File w/ backgrounds stripped out

Sample IDs

Type	Conc	Tube	DPM	Rounded DPM
H	1	1	786.38	786
H	1	2	714.19	714
H	1	3	653.00	653
H	2	1	1424.47	1424
H	2	2	1316.72	1317
H	2	3	1327.44	1327
H	3	1	1678.42	1678
H	3	2	1669.99	1670
H	3	3	1851.06	1851
H	4	1	1983.47	1983
H	4	2	2008.88	2009
H	4	3	1774.72	1775
H	5	1	3322.47	3322
H	5	2	3024.46	3024
H	5	3	3443.38	3443
H	6	1	3034.78	3035
H	6	2	2645.68	2646
H	6	3	3415.83	3416
H	7	1	3899.16	3899
H	7	2	3580.97	3581
H	7	3	4094.29	4094
H	8	1	6153.50	6154
H	8	2	5521.91	5522
H	8	3	6194.23	6194
HC	1	1	42.47	42
HC	1	2	42.40	42
HC	1	3	55.29	55
HC	2	1	113.71	114
HC	2	2	61.24	61
HC	2	3	60.62	61
HC	3	1	73.91	74
HC	3	2	61.36	61
HC	3	3	108.02	108
HC	4	1	80.86	81
HC	4	2	59.83	60
HC	4	3	94.08	94
HC	5	1	123.03	123
HC	5	2	115.31	115
HC	5	3	118.08	118
HC	6	1	195.81	196
HC	6	2	202.35	202
HC	6	3	252.60	253
HC	7	1	267.98	268
HC	7	2	417.24	417
HC	7	3	318.94	319
HC	8	1	849.03	849
HC	8	2	797.30	797
HC	8	3	679.37	679
Hot	1	1	3510.08	3510
Hot	1	2	3276.14	3276
Hot	1	3	3335.18	3335
Hot	2	1	6991.63	6992
Hot	2	2	6815.05	6815
Hot	2	3	6861.10	6861
Hot	3	1	9519.25	9519
Hot	3	2	9146.49	9146
Hot	3	3	9162.61	9163
Hot	4	1	12204.43	12200
Hot	4	2	11767.41	11770
Hot	4	3	11785.49	11790
Hot	5	1	36254.64	36250
Hot	5	2	34933.93	34930
Hot	5	3	34861.26	34860
Hot	6	1	71613.38	71610
Hot	6	2	69980.25	69980
Hot	6	3	67454.23	67450
Hot	7	1	118817.63	118800
Hot	7	2	116306.18	116300
Hot	7	3	115943.52	115900
Hot	8	1	360715.53	360700
Hot	8	2	345662.97	345700
Hot	8	3	342544.25	342500

Appendix E

Competitive Assay Reports

Competitive Binding Report

Test Chemicals Set 1

Tested Chemicals	11, 12, 15, 19, 21
Assay Dates	2/4, 2/6 and 2/7/2008

Radioactive Ligand

Name	[^{3}H]Estradiol; [2,4,6,7,16,17-^{3}H(N)]Estradiol	
Supplier	Perkin Elmer (Boston)	
Catalog #	NET-517	
Batch #	3589221	
Specific Ac ivity	110	Ci/mmol
SA date	6/28/2007	
Concentra ion (mCi/mL)	1	
Tested Concentrations (nM)	1	

Radioinert Ligand

Name	17β-estradiol
Supplier	Battelle Sequim
Lot #	086K1611
CAS #	50-28-2
Purity	≥98%
Concentration in standard curve tubes (nM)	0.01, 0.1, 0.316, 1, 3.16, 10, 100

Estrogen Receptor

Type	Rat Uterine Cytosol		
Rat Strain	Sprague Dawley		
Rat Age	94	days	
Ovariectomy Date	1/14/2008		
Uterus removal Date	1/22/2008		
Cytosol Prep Date	1/23/2008		
Cytosol Prep ID	1/23/2008		
Protein Content	2 20	mg/mL	
Protein Assay Date	1/23/2008		
RUC Storage Info	-70	°C	(Ultra004)

Positive Control

Name	Norethynodrel
Supplier	Battelle Sequim
Lot #	G
CAS #	68-23-5
Purity	100
Concentration in assay tubes (nM)	3.16, 31.6, 100, 316, 1000, 3160, 31600, 100000 (Runs 2 and 3) 0.316, 3.16, 10, 31.6, 100, 316, 3160, 10000 (Run 1)

Negative Control

Name	R1881
Supplier	Battelle Sequim
Lot #	20070530
CAS #	965-93-5
Purity	98.70%
Concentration in assay tubes (nM)	0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Runs 2 and 3) 0.01, 0.1, 1, 10, 100, 1000, 10000, 100000 (Run 1)

Test Conditions

Buffer	TEDG + PMSF		
Protein Concentration Used	50	µg/tube	
Assay volume	0.5	mL	
Solvent	Ethanol		
Max Solvent Concentration	2 99%	(present in total binding tubes)	

Incubation Time (h)	Run 1	Run 2	Run 3
	18.17	18.57	18.25

Incubation Temp.	4	°C
Kd for E2 (from saturation binding assays)	0.1596	nM

Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (xls)	S1R1_2_4_08	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

	Run 1	Run 2	Run 3
Assay Date	2/4/2008	2/6/2008	2/7/2008
Elapsed days since SA determination	221	223	224
Adjusted SA (Ci/mmol)	106.3	106 3	106 3

	Run 1	Run 2	Run 3
Solvent control response vs. negative control	1.11	0.82	0.81

standard curve- Run 1

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.5	0.2	1.0
-7.0	-0.4	-0.1	-0.1
-8.0	13.4	6.9	7.1
-8.5	18.4	20.1	18.4
-9.0	41.6	42.7	40.9
-9.5	68.9	70.2	68.9
-10.0	93.2	91.2	90.7
-11.0	103.5	98.8	99.0

standard curve- Run 2

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0.5	0.1	1.4
-7.0	-0.2	-0.4	-0.4
-8.0	8.7	6.1	6.5
-8.5	23.1	21.4	38.3
-9.0	46 9	48.1	39.7
-9.5	75 5	82 8	72.5
-10.0	93.6	102 8	96.0
-11.0	104 2	97 9	100.0

standard curve- Run 3

concentration (logM)	Y1-SC	Y2-SC	Y3-SC
-7.0	-0 5	-0.3	3.2
-7.0	-1 3	0.1	-1.3
-8.0	6.1	5.6	5 0
-8.5	19.4	20.3	18 9
-9.0	37.6	40.4	43 2
-9.5	71.6	68.2	68.1
-10.0	90.8	91.3	94.7
-11.0	108.9	114.3	102.4

weak positive- Run 1

concentration (logM)	y1-PC	y2-PC	y3-PC
-5.0	4.9	4.0	3.6
-5.5	15.6	16.6	15.5
-6.5	62.6	59.3	66.1
-7.0	86.9	82.5	85.1
-7.5	93.2	95.9	93.9
-8.0	102.2	102.1	98.0
-8.5	94.6	99.9	96.3
-9.5	103.3	91.8	99.1

weak positive- Run 2

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0			
-4.5	9.7	8 5	8.6
-5.5	33 8	29.4	31.3
-6.0			
-6.5	66 5	70 0	71.3
-7.0			
-7.5	102.1	101 9	95.1
-8.5	102.7	104 9	103.3

weak positive- Run 3

concentration (logM)	y1-PC	y2-PC	y3-PC
-4.0	0.0	-0.6	0.4
-4.5	13.0	9.6	13 2
-5.5	25.3	24.3	21 0
-6.0	40.0	34.2	35 5
-6.5	70.7	67.1	68 5
-7.0	89.7	82.6	95 2
-7.5	99.1	94.3	103 5
-8.5	107.2	103.5	120.1

negative- Run 1

concentration (logM)	Y1-NC	Y2-NC	Y3-NC
-4.0	6.0	7.3	5.1
-5.0	33.0	33.3	30.8
-6.0	85.7	80.1	81.2
-7.0	108.4	122.0	102.9
-8.0	106.9	99.9	110.8
-9.0	102.5	102.5	109.3
-10.0	100.6	103.9	104.8
-11.0	99.5	100.5	104.7

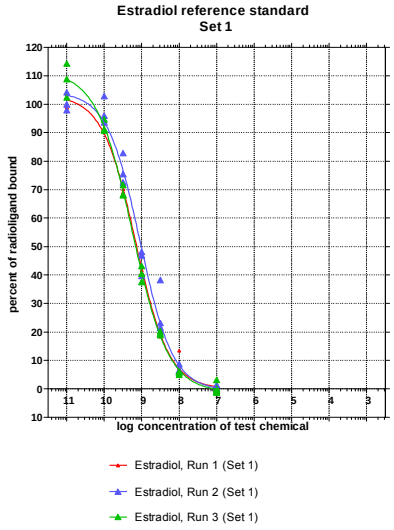
negative- Run 2

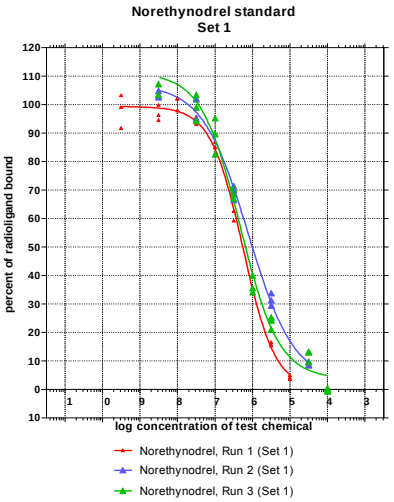
concentration (logM)	Y1-NC	Y2-NC	Y3-NC
-3.0	0.6	3 8	5.1
-4.0	6.7	7 8	6.9
-5.0	35.7	33 9	35.2
-6.0	83 2	80 5	82.8
-7.0	93 0	91.4	98.2
-8.0	93 8	89.1	102.8
-9.0	85.7	98 9	99.6
-10.0	98 2	103 9	98.8

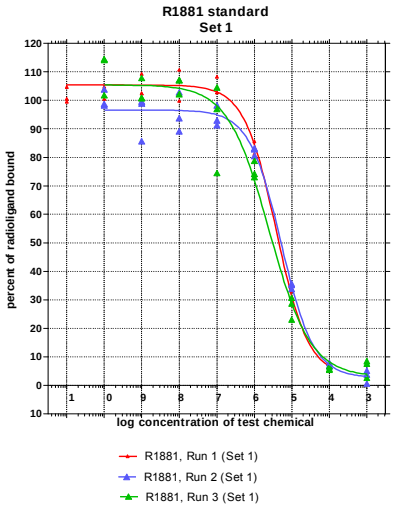
negative- Run 3

concentration (logM)	Y1-NC	Y2-NC	Y3-NC
-3.0	2.7	7.6	8 5
-4.0	5.9	5.4	6.7
-5.0	30.7	28.7	23 0
-6.0	74.3	78.8	73.1
-7.0	104.6	97.0	74.6
-8.0	102.2	107.2	107.1
-9.0	100.8	100.8	107 9
-10.0	101.8	114.2	114 5

Ligand	Run	Log(IC50)	Log(IC50) 95% CI		Method	Ki	Ki 95% CI		RBA	log(RBA)
Estradiol	1	-9.13	-9.10	-9.16	1	0.101	0.108	0.094	NA	NA
	2	-9.01	-8.94	-9.09	1	0.133	0.157	0.113	NA	NA
	3	-9.16	-9.13	-9.20	1	0.095	0.103	0.088	NA	NA
Norethynodrel	1	-6.26	-6.19	-6.33	1	75.9	89.4	64.5	1.34E-03	-2.87
	2	-6.00	-5.92	-6.08	1	137.1	164.3	114.4	9.73E-04	-3.01
	3	-6.19	-6.10	-6.27	1	89.8	109.0	74.0	1 059E-03	-2.975
R1881	1	-5.34	-5.23	-5.46	1					
	2	-5.28	-5.20	-5.37	1					
	3	-5.51	-5.35	-5.67	1					


Estradiol reference standard
Set 1
percent of radioligand bound
log concentration of test chemical
Estradiol, Run 1 (Set 1)
Estradiol, Run 2 (Set 1)
Estradiol, Run 3 (Set 1)


Norethynodrel standard
Set 1
percent of radioligand bound
log concentration of test chemical
Norethynodrel, Run 1 (Set 1)
Norethynodrel, Run 2 (Set 1)
Norethynodrel, Run 3 (Set 1)


R1881 standard
Set 1
percent of radioligand bound
log concentration of test chemical
R1881, Run 1 (Set 1)
R1881, Run 2 (Set 1)
R1881, Run 3 (Set 1)

Competitive Binding Report

Test Chemicals Set 1

Tested Chemicals 11, 12, 15, 19, 21
Assay Dates 2/4, 2/6 and 2/7/2008

Test Chemical

Name Test Chemical 11
Code TC11
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3
	18.17	18 57	18.25

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

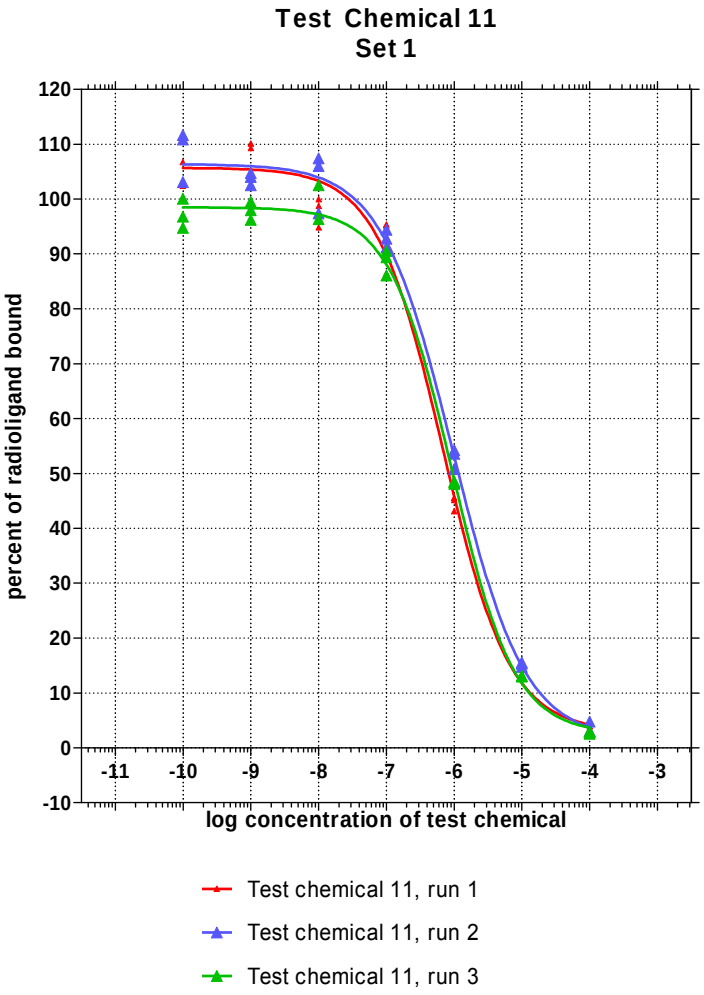
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (.xls)	S1R1_2_4_	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 11- Run 1				Test Chemical 11- Run 2				Test Chemical 11- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0				-3.0				-3.0			
-4.0	3.9	2.9	3.0	-4.0	4.8	3.0	2.9	-4.0	2.5	3.0	2 9
-5.0	14.3	12.9	13.1	-5.0	15.6	14.7	15.3	-5.0	13.0	13.1	13.1
-6.0	43.1	45.6	45.2	-6.0	50.8	54.3	53.6	-6.0	48.1	48.5	48.4
-7.0	86.5	95.2	95.4	-7.0	92.8	94.4	91.2	-7.0	89.4	90.5	86.0
-8.0	100.0	94.9	98.8	-8.0	97.4	106.0	107.4	-8.0	96.3	102.5	
-9.0	103.8	110.1	109.3	-9.0	102.5	104.0	104.7	-9.0	98.0	99.5	96.2
-10.0	102.4	106.7	111.6	-10.0	110.9	103.1	111.7	-10.0	100.1	96.8	94 8

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 11	1	-6.08	-5.99	-6.17	1	114.79	140.24	93.96	8.81E-04	-3 06
	2	-5.94	-5.87	-6.00	1	159.75	186.26	137.00	8.34E-04	-3 08
	3	-6.02	-5.97	-6.07	1	131.69	146.61	118.28	7.21E-04	-3.14


Test Chemical 11
Set 1
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
Test chemical 11, run 1
Test chemical 11, run 2
Test chemical 11, run 3

Competitive Binding Report

Test Chemicals Set 1

Tested Chemicals 11, 12, 15, 19, 21
Assay Dates 2/4, 2/6 and 2/7/2008

Test Chemical

Name Test Chemical 12
Code TC12
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3
	18.17	18 57	18.25

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

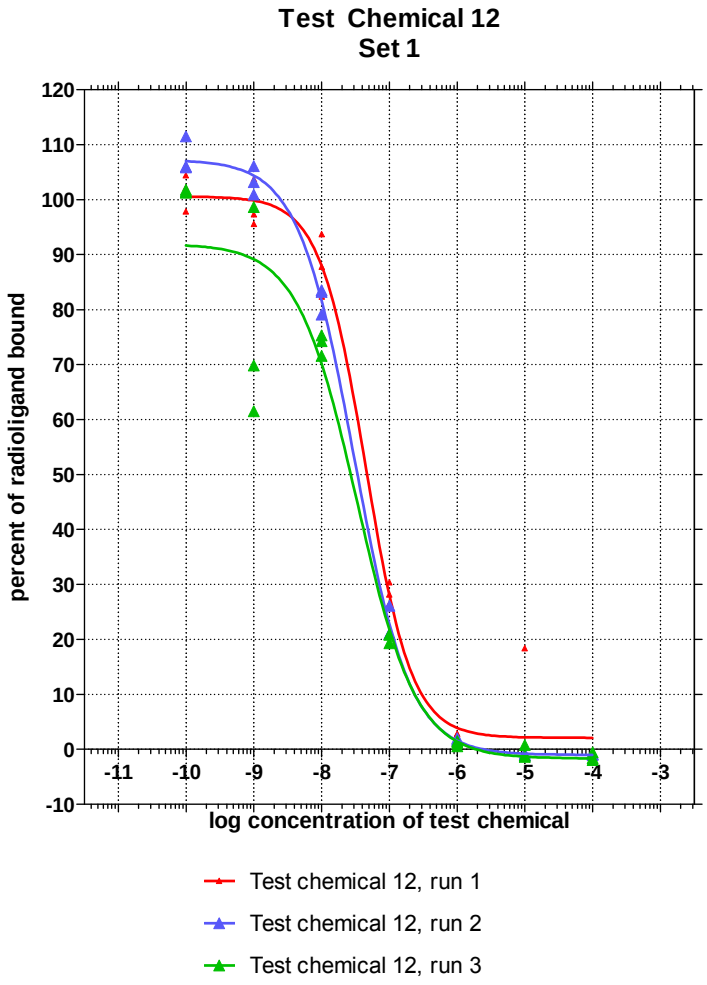
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (.xls)	S1R1_2_4_	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

Any precipitation of test chemicals? If yes, describe: Level 1 was cloudy for all runs

Test Chemical 12- Run 1				Test Chemical 12- Run 2				Test Chemical 12- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0				-3.0				-3.0			
-4.0		-0.5	-0.7	-4.0	-0.9	-1.0	-1.0	-4.0	-2.0	-1.6	-0.6
-5.0	-1.2	18.4	-1.1	-5.0	-0.8	-1.1	-1.1	-5.0	-1.2	-1.4	0.8
-6.0	2.7	2.9	1.5	-6.0	1.8	2.0	1.6	-6.0	1.2	1.5	0.5
-7.0	30.4	28.1	26.5	-7.0	26.1	21.0	21.0	-7.0	19.3	20.8	20.9
-8.0	93.7	87.8	82.4	-8.0	83.5	83.3	79.1	-8.0	71.6	74.3	75.3
-9.0	103.8	95.6	97.4	-9.0	106.1	100.9	103.2	-9.0	69.8	61.5	98.7
-10.0	104.5	97.9	102.2	-10.0	105.9	111.5	106.0	-10.0	101.8	101.3	101.4

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 12	1	-7.33	-7.22	-7.43	1	6.46	8.25	5.06	1.56E-02	-1 81
	2	-7.47	-7.43	-7.51	1	4.65	5.09	4.25	2.86E-02	-1 54
	3	-7.57	-7.35	-7.79	1	3.72	6.21	2.23	2.55E-02	-1 59


Test Chemical 12
Set 1
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
Test chemical 12, run 1
Test chemical 12, run 2
Test chemical 12, run 3

Competitive Binding Report

Test Chemicals Set 1

Tested Chemicals 11, 12, 15, 19, 21
Assay Dates 2/4, 2/6 and 2/7/2008

Test Chemical

Name Test Chemical 15
Code TC15
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Rep 1	Rep 2	Rep 3
	18.17	18 57	18.25

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

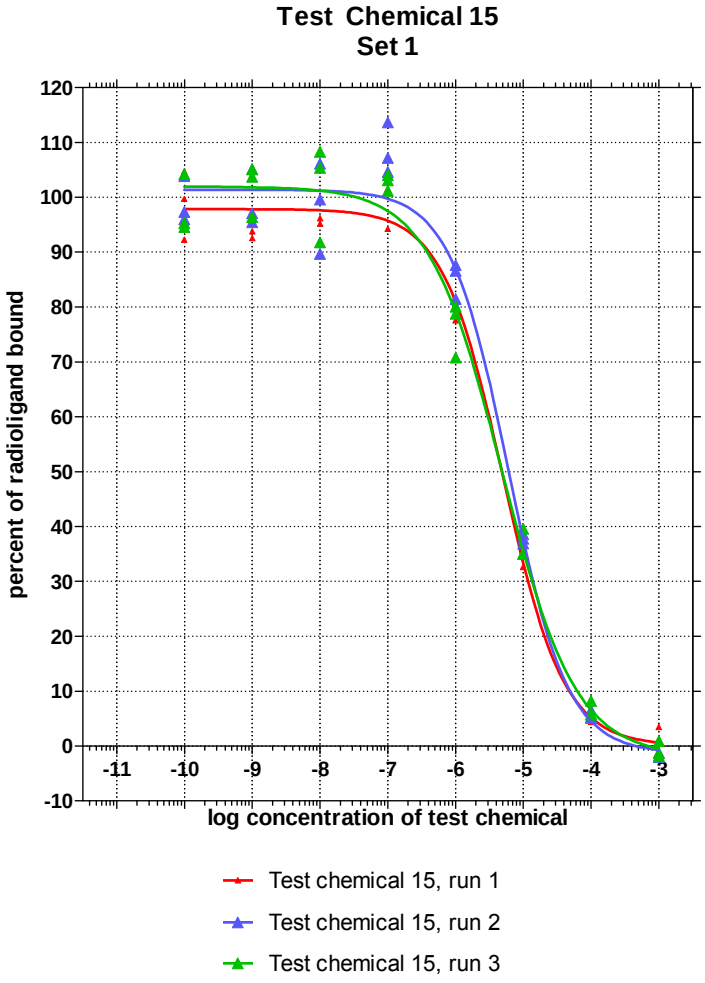
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (.xls)	S1R1_2_4_	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

Any precipitation of test chemicals? If yes, describe NA

Test Chemical 15- Run 1				Test Chemical 15- Run 2				Test Chemical 15- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0	0.4	3.5	-1.6	-3.0	-2.0	-1.2	-1.9	-3.0	1.0	-1.3	-1.9
-4.0	4.8	5.3	4.4	-4.0	6.5	5.2	5.1	-4.0	5.8	8.2	5.7
-5.0	34.5	34.5	32.6	-5.0	37.8	36.9	38.6	-5.0	39.6	35.0	35.0
-6.0	81.5	80.3	77.6	-6.0	81.5	86.6	87.6	-6.0	70.8	80.1	78.8
-7.0	104.9	94.3	101.6	-7.0	107.2	113.6	104.6	-7.0	101.2	104.1	103.1
-8.0	91.7	95.2	96.2	-8.0	89.7	99.6	106.2	-8.0	91.8	105.4	108.3
-9.0	93.8	92.6	103.3	-9.0	97.1	95.5	105.1	-9.0	103.7	105.1	96.4
-10.0	92.3	99.7	104.7	-10.0	97.3	96.0	103.8	-10.0	95.3	94.5	104.2

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 15	1	-5.31	-5.23	-5.40	1	667.05	817.29	544.43	1.52E-04	-3 82
	2	-5.21	-5.11	-5.32	1	843.10	1075.31	661.03	1.58E-04	-3 80
	3	-5.31	-5.20	-5.42	1	673.29	864.72	524.24	1.41E-04	-3 85


Test Chemical 15
Set 1
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
Test chemical 15, run 1
Test chemical 15, run 2
Test chemical 15, run 3

Competitive Binding Runort

Test Chemicals Set 1

Tested Chemicals 11, 12, 15, 19, 21
Assay Dates 2/4, 2/6 and 2/7/2008

Test Chemical

Name Test Chemical 19
Code TC19
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.1, 1, 10, 100, 1000, 10000, 100000, 1000000

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 μg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	18.17	18 57	18.25

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (.xls)	S1R1_2_4_	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

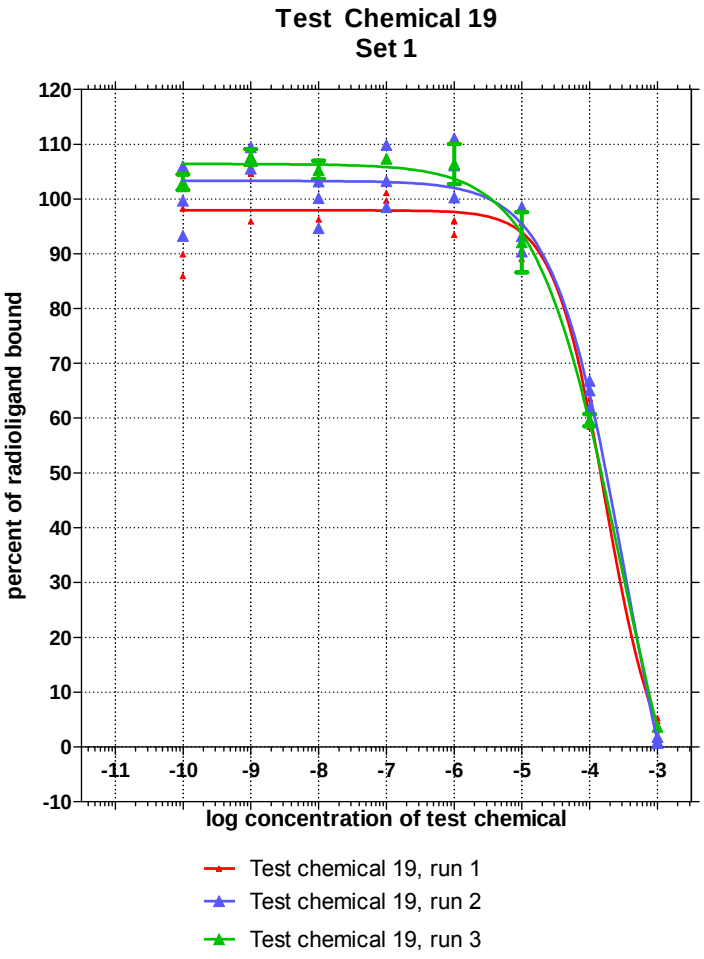
Any precipitation of test chemicals? If yes, describe NA

Test Chemical 19- Run 1			
concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0	3.1	4.7	5.3
-4.0	59.9	63.6	58.4
-5.0	97.9	95.6	89.1
-6.0	93.5	95.9	96.1
-7.0	99.7	103.6	101.1
-8.0	96.3	105.1	94.4
-9.0	95.9	106.8	104.6
-10.0	98.3	89.9	85.9

Test Chemical 19- Run 2			
concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0	1.8	1.0	0.7
-4.0	61.8	65.1	66.8
-5.0	98.6	90.4	93.2
-6.0	106.1	111.1	100.3
-7.0	109.9	98.5	103.3
-8.0	100.2	103.2	94.7
-9.0	107.5	105.5	109.5
-10.0	93.3	105.9	99.7

Test Chemical 19- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0	3.4	3.7	4.0
-4.0	60.9	58.8	59.3
-5.0	86.2	93.0	97.1
-6.0	103.3	105.3	110.5
-7.0	108.2	107.0	106.9
-8.0	105.0	103.9	107.2
-9.0	106.2	109.1	107.8
-10.0	103.2	101.6	104.4

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 19	1	-3.84	-3.70	-3.98	1	19910.07	27232.01	14556.76	5.08E-06	-5 29
	2	-3.75	-3.61	-3.89	1	24249.38	33536.50	17534.11	5.50E-06	-5 26
	3	-3.83	-3.75	-3.90	1	20498.29	24491.35	17156.30	4.63E-06	-5 33


Test Chemical 19
Set 1
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
Test chemical 19, run 1
Test chemical 19, run 2
Test chemical 19, run 3

Competitive Binding Runort

Test Chemicals Set 1

Tested Chemicals 11, 12, 15, 19, 21
Assay Dates 2/4, 2/6 and 2/7/2008

Test Chemical

Name Test Chemical 21
Code TC21
Supplier Battelle Sequim
Lot # N/A
CAS # N/A
Purity N/A
Concentration in assay tubes (nM) 0.01, 0.1, 1, 10, 100, 1000, 10000, 100000 (Run 2 and 3)
0.1, 1, 10, 100, 1000, 10000, 100000, 1000000 (Run 1)

Test Conditions

Buffer TEDG + PMSF
Protein Concentration Used 50 µg/tube
Assay volume 0.5 mL
Solvent Ethanol
Max Solvent Concentration 2.99% (present in total binding tubes)

Incubation Time (h)	Run 1	Run 2	Run 3
	18.17	18 57	18.25

Incubation Temp. 4 °C
Kd for E2 (from saturation binding assays) 0.1596 nM
Notes on problems: None

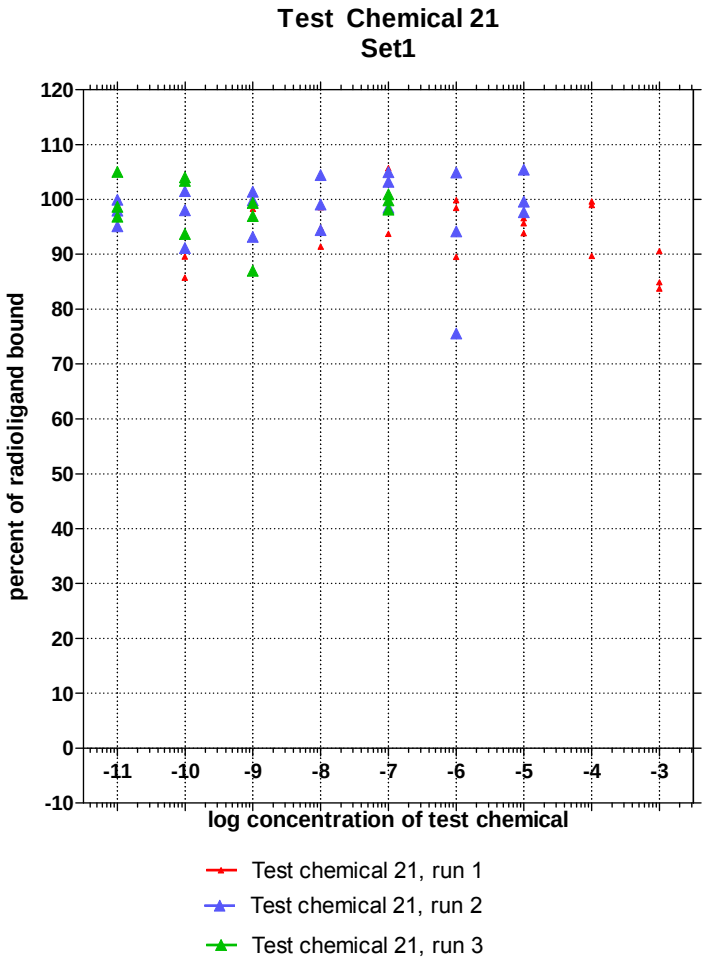
Results

Filenames	Run 1	Run 2	Run 3
Counter Data (.P00)	06FE1251	08FE1132	09FE1103
Excel (.xls)	S1R1_2_4_	S1R2_2_6_08	S1R3_2_7_08
Prism (.pzf)	RTI_Set 1		

Any precipitation of test chemicals? If yes, describe For Run-1, levels 1 and 2 were cloudy.

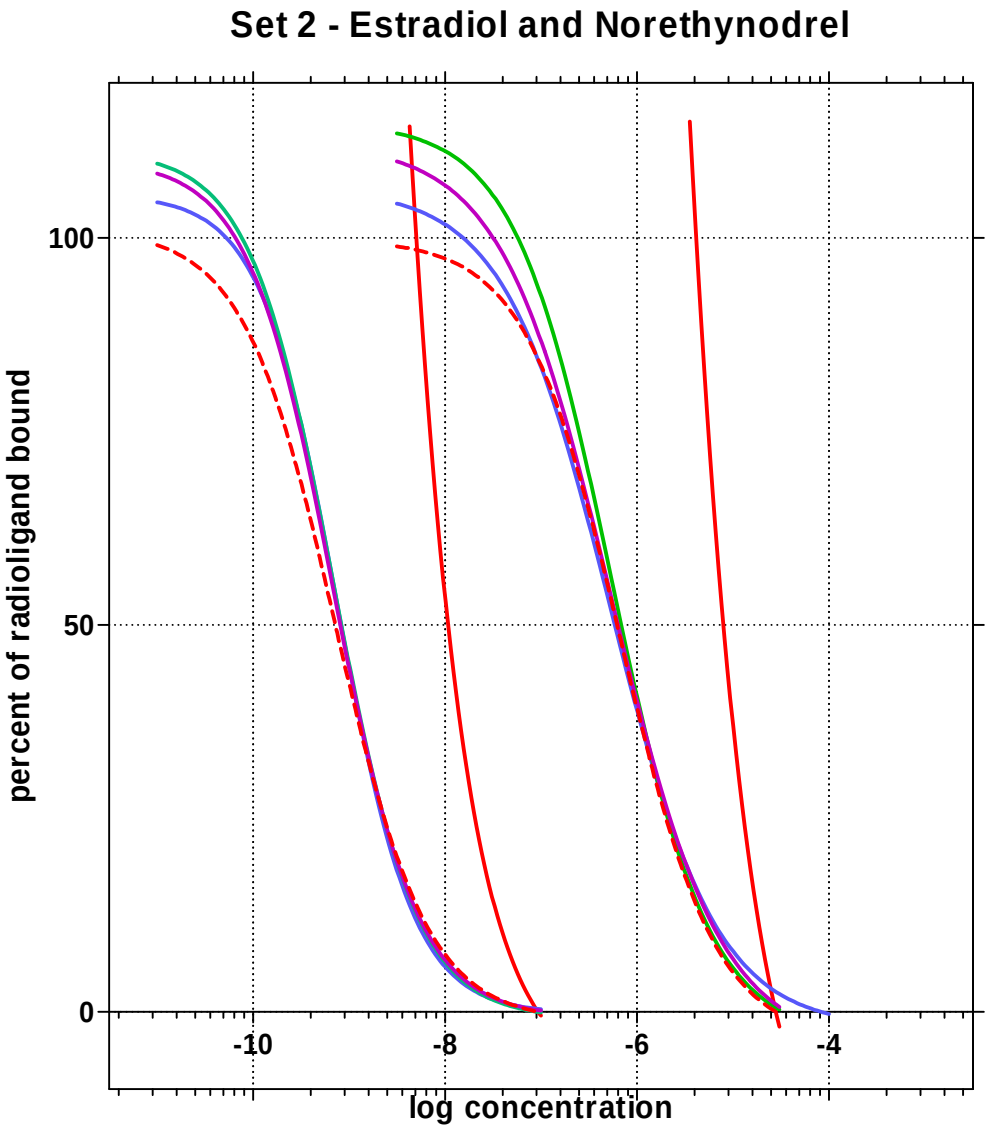
Test Chemical 21- Run 1				Test Chemical 21- Run 2				Test Chemical 21- Run 3			
concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U1	y2-U1	y3-U1
-3.0	90.6	84.9	83.8	-4.0				-4.0			
-4.0	99.0	89.7	99.6	-5.0	105.4	97.6	99.6	-5.0			
-5.0	95.7	93.8	96.6	-6.0	94.2	75.6	104.9	-6.0			
-6.0	89.6	98.4	99.8	-7.0	105.0	103.2	98.5	-7.0	101.0	98.1	99.8
-7.0	93.7	105.7	99.4	-8.0	94.4	99.1	104.4	-8.0			
-8.0	91.4	94.8	98.6	-9.0	93.2	99.8	101.4	-9.0	97.0	99.3	87.0
-9.0	98.3	96.8	96.6	-10.0	91.1	101.5	98.0	-10.0	104.0	93.7	103.4
-10.0	89.6	85.8	91.1	-11.0	98.0	95.1	100.0	-11.0	98.6	105.0	96 9

Ligand	Run	Log(IC50)	95% CI		Method	Ki	95% CI		RBA	log(RBA)
Test Chemical 21	1	NA	0.00	0.00	1	NA	NA	NA	NA	NA
	2	NA	0.00	0.00	1	NA	NA	NA	NA	NA
	3	NA	0.00	0.00	1	NA	NA	NA	NA	NA


Test Chemical 21
Set1
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
Test chemical 21, run 1
Test chemical 21, run 2
Test chemical 21, run 3

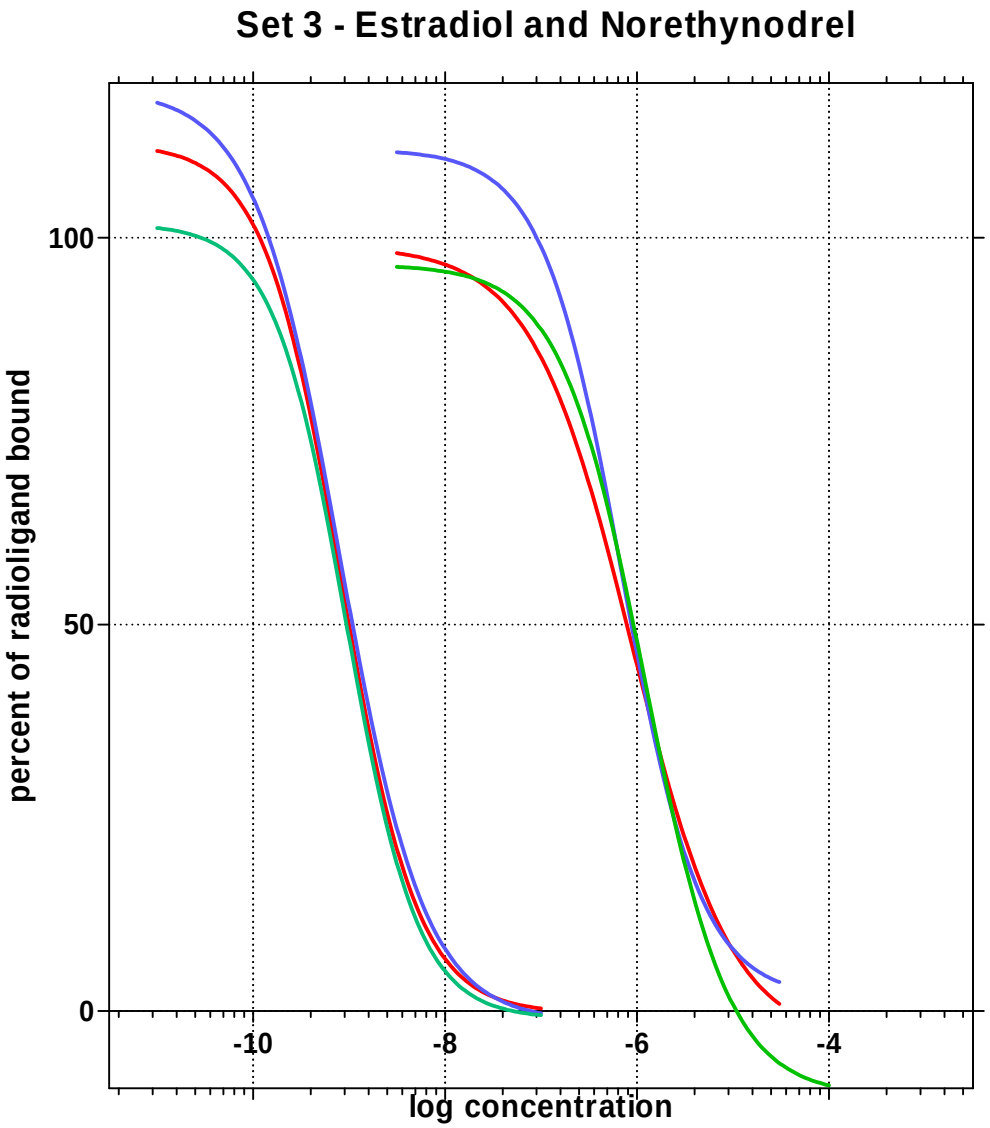
Appendix G

Standard Plots

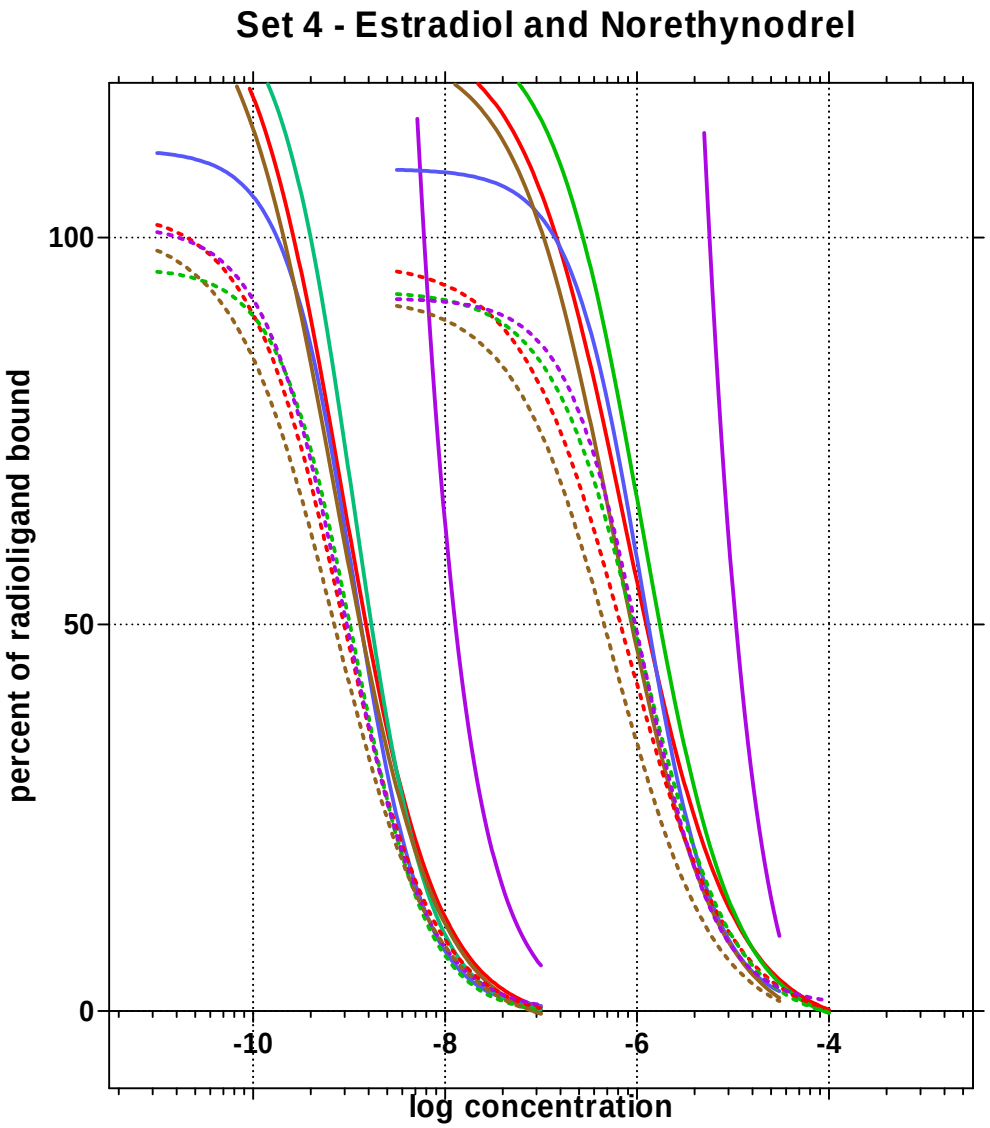


Run 1 - Red
Run 2 - Blue
Run 3 - Green
Run 4 - Purple

Dotted lines are the Run 1- normalized curves.

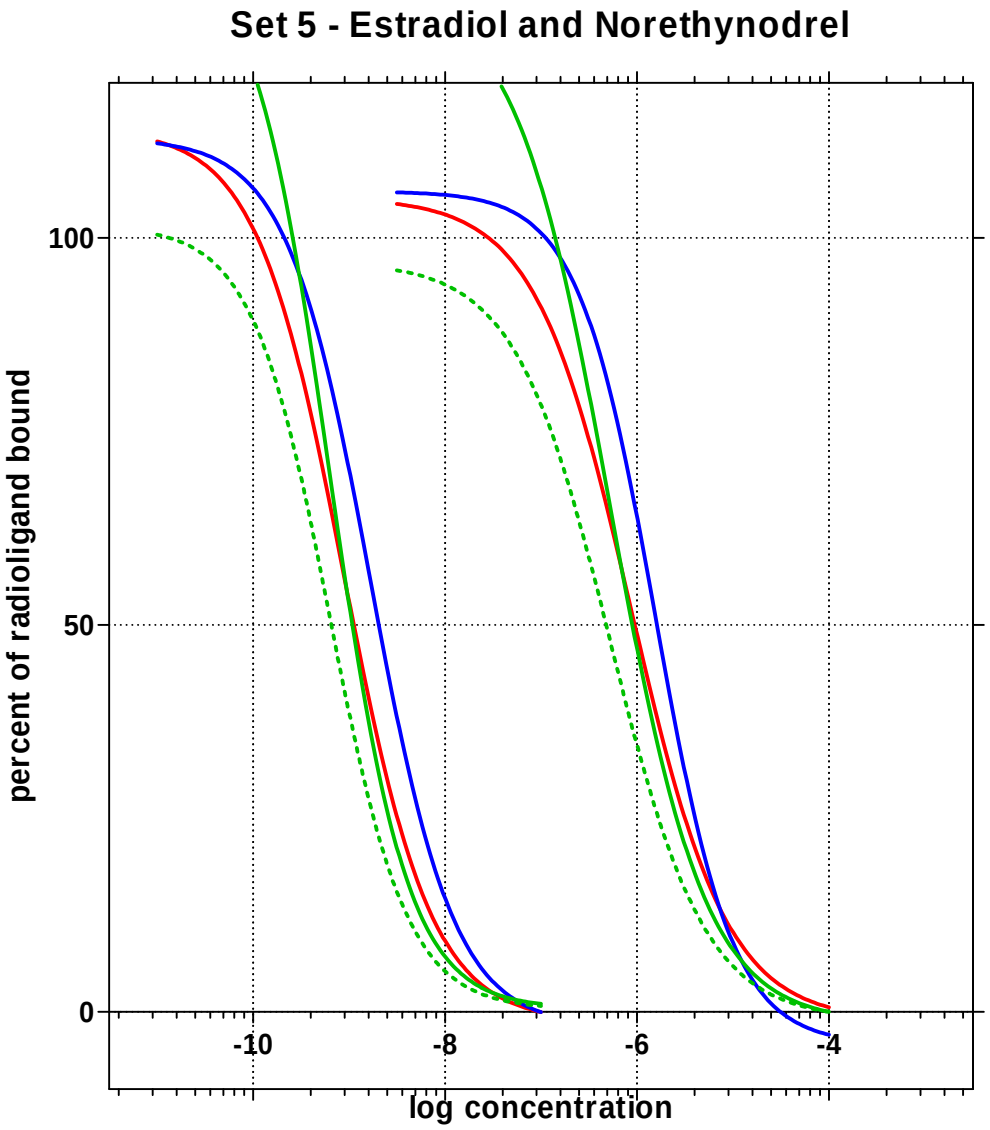


Run 1 - Red
Run 2 - Blue
Run 3 - Green



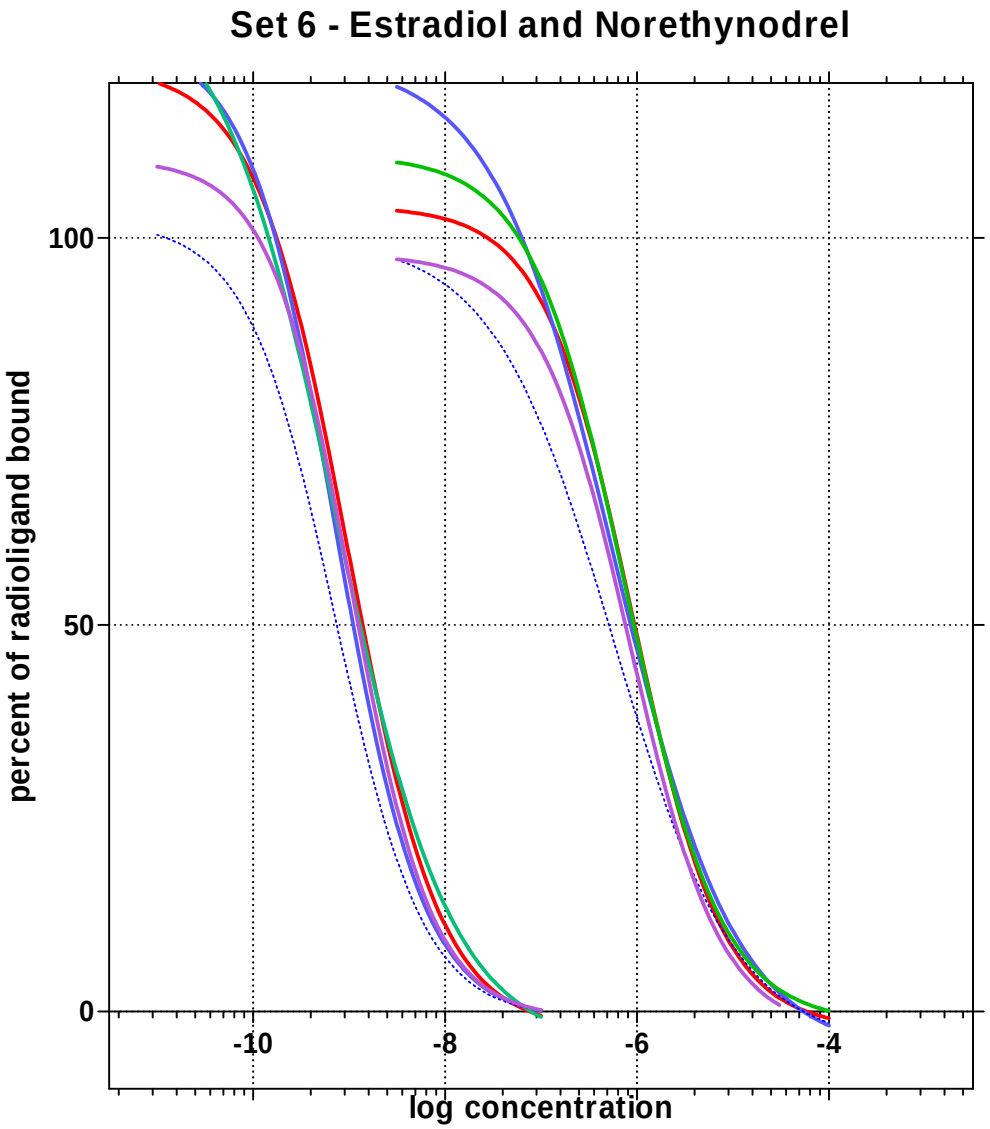
Run 1 - Red
Run 2 - Blue
Run 3 - Green
Run 4 - Purple
Run 5 - Brown

Dotted lines are the normalized curves. Runs are designated by color – 1-Red, 3-Green, 4-Purple, 5-Brown.



Run 1 - Red
Run 2 - Blue
Run 3 - Green

Dotted green lines are the Run 3 normalized curves



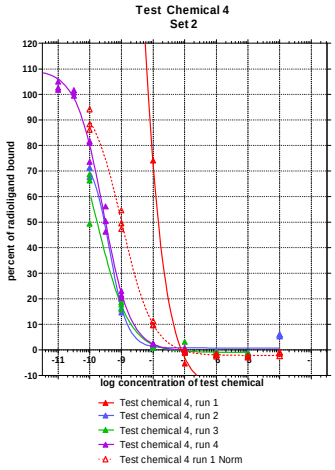
Run 1 -Red
Run 2 - Blue
Run 3 - Green
Run 4 - Purple

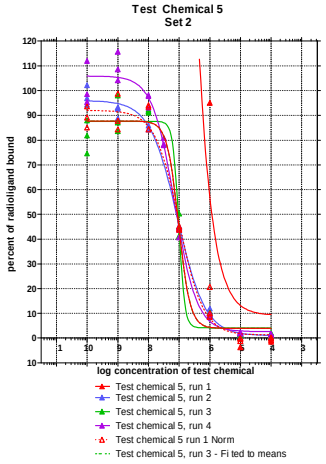
Dotted lines are the Run 2 Normalized curves.

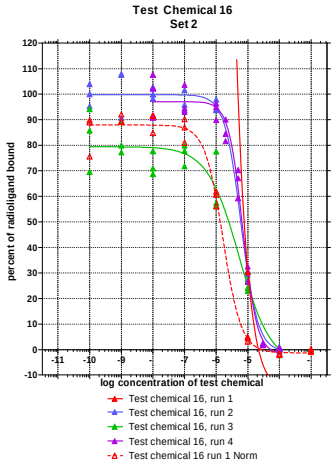
Appendix H

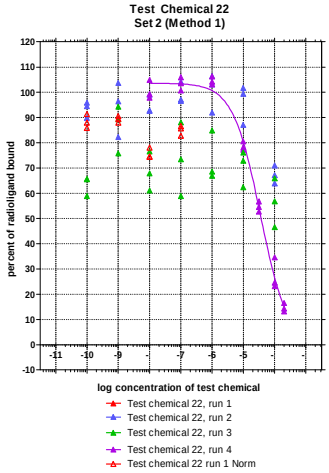
Test Chemical Plots

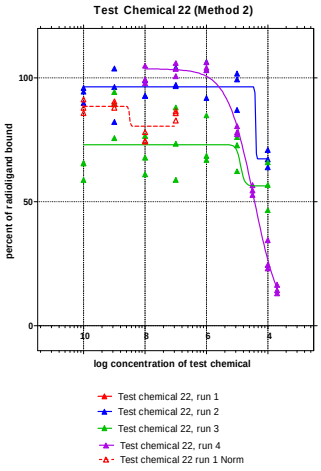
Set 2 Test Chemical Plots



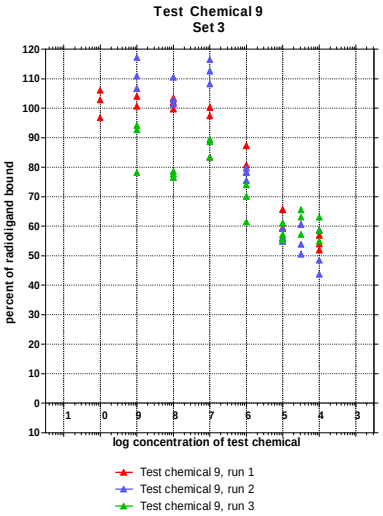


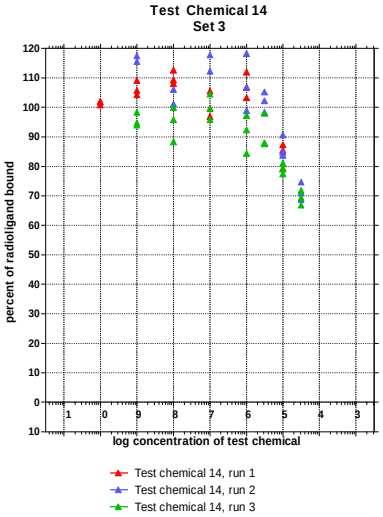


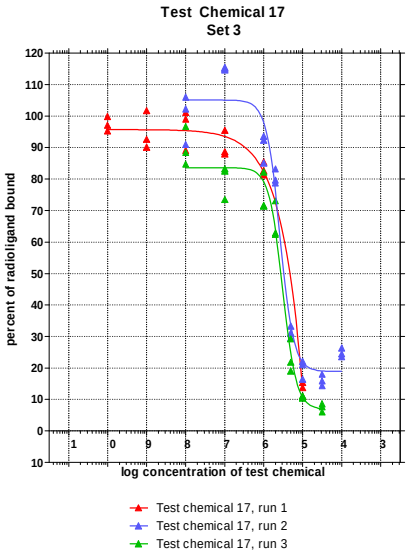


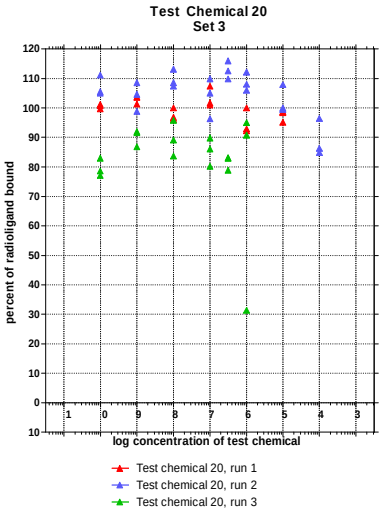


Set 3 Test Chemical Plots

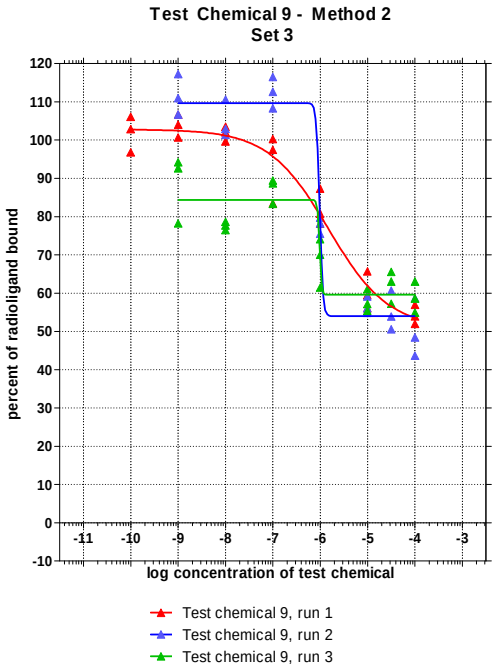


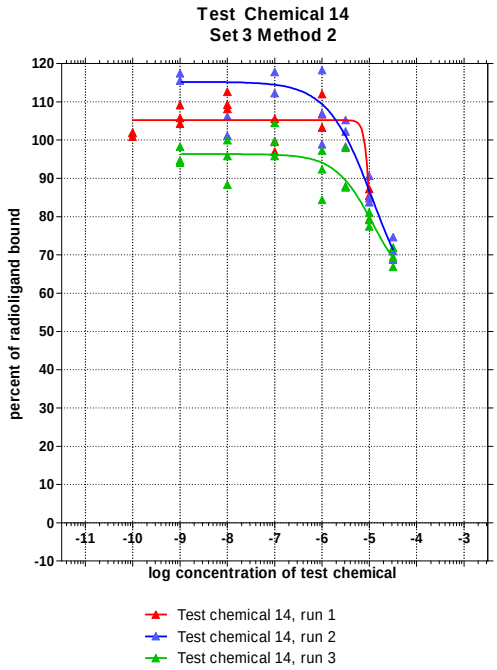


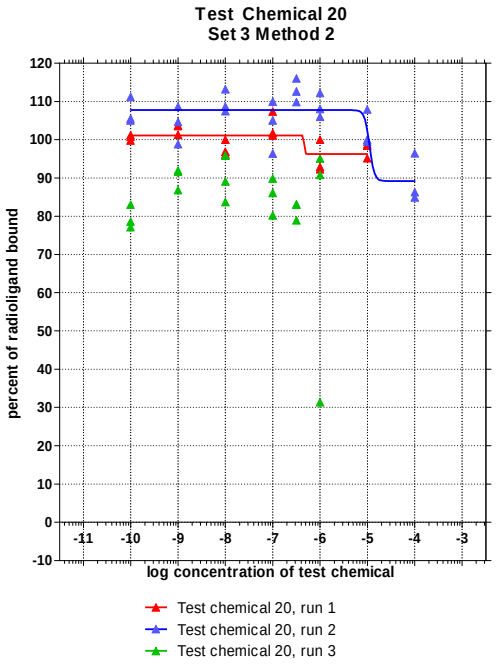




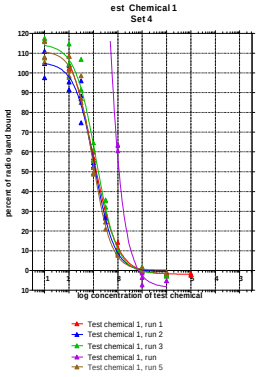
Set 3 Test Chemical Plots of Method 2 Fitting Results

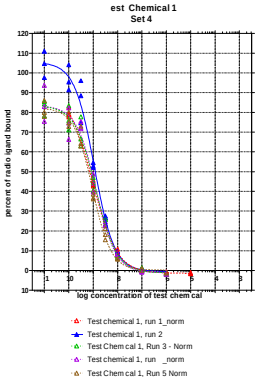


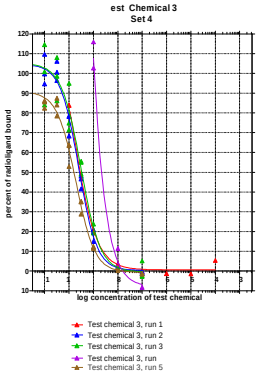


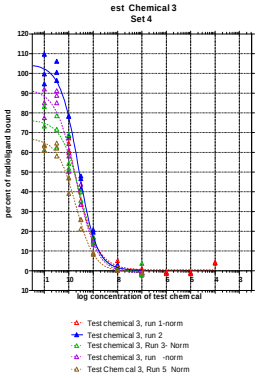


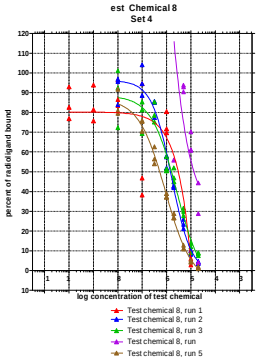
Set 4 Test Chemical Plots

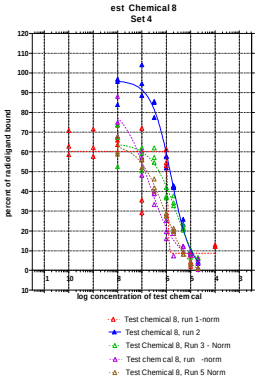


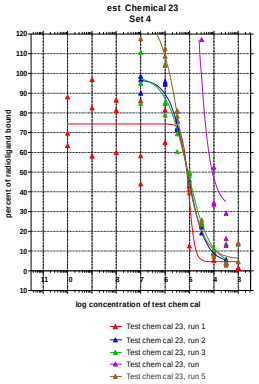


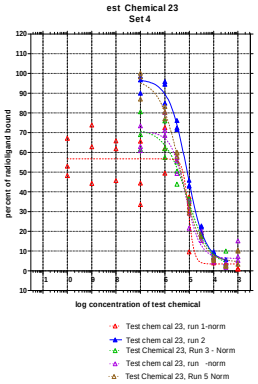




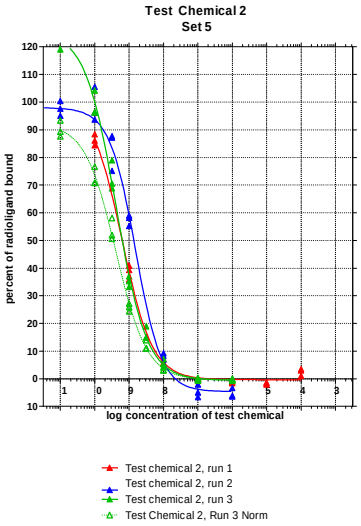


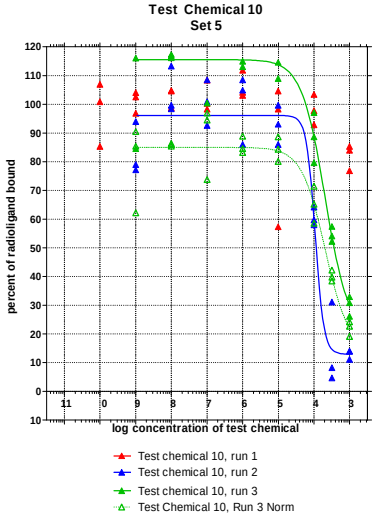


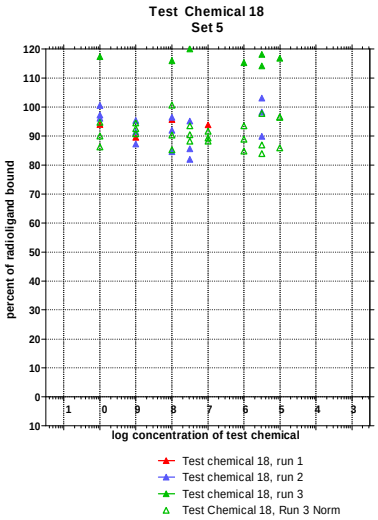




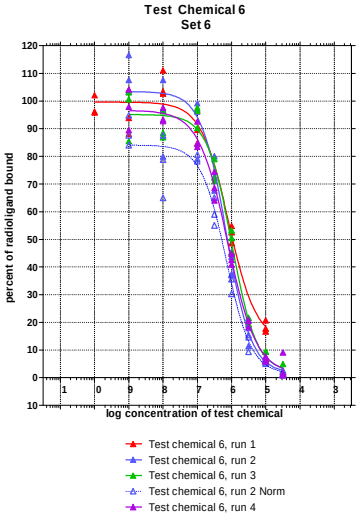
Set 5 Test Chemical Plots

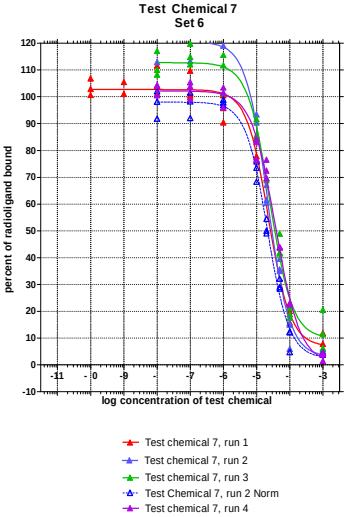


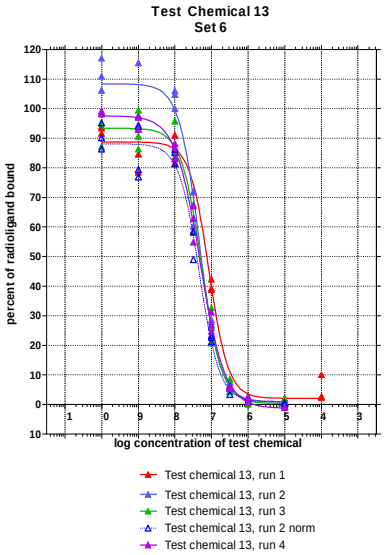




Set 6 Test Chemical Plots



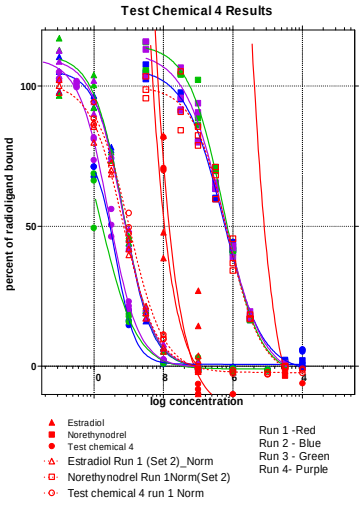


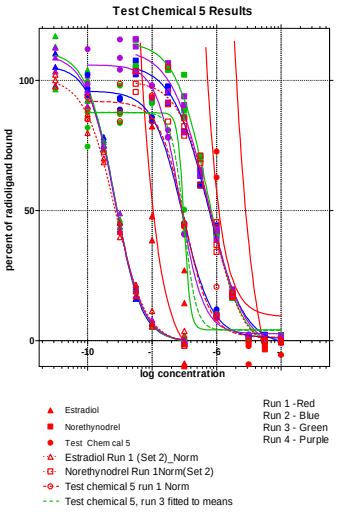


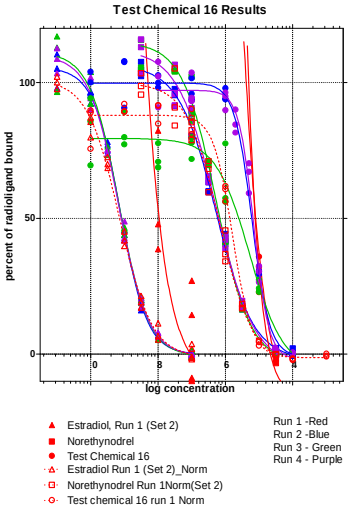
Appendix I

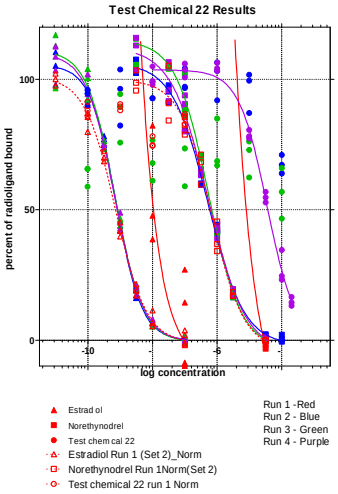
Plots of Standards and Test Chemicals

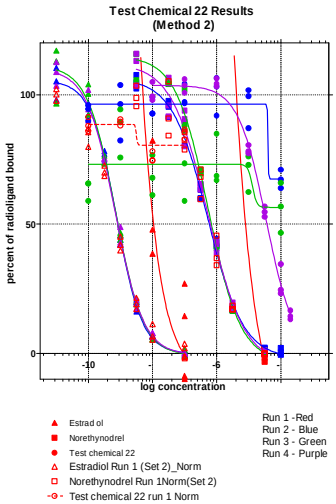
Set 2 Plots of All Standard and Test Chemical Runs



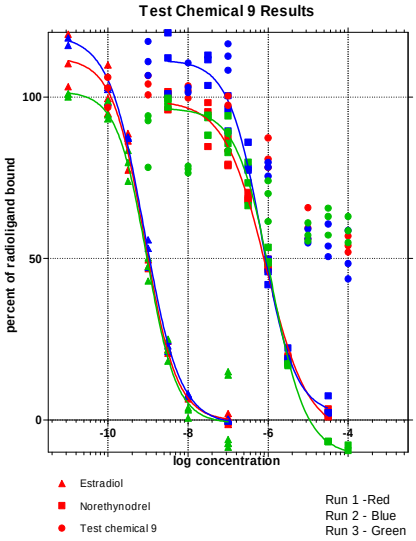


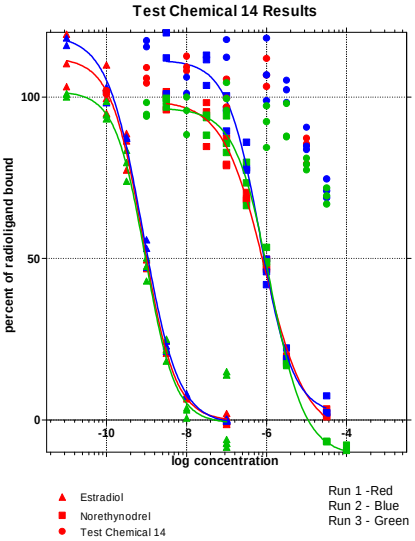


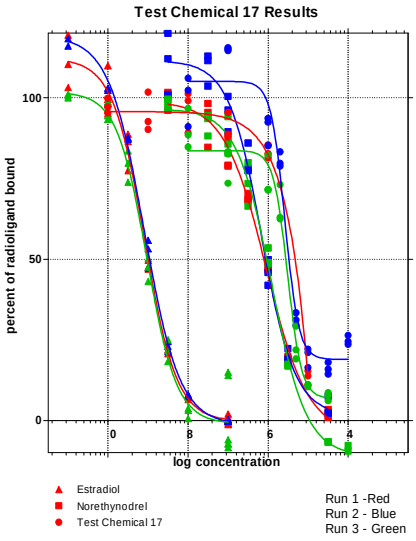


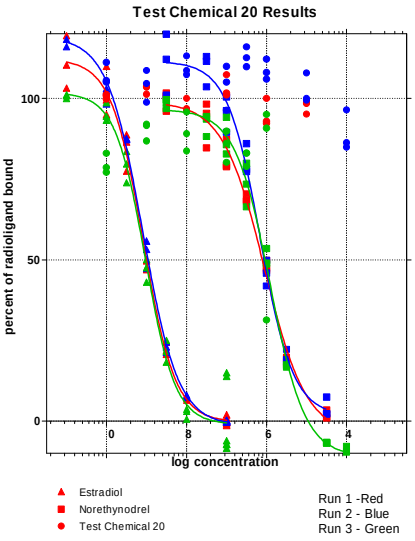


Set 3 Plots of All Standards and Test Chemical Runs









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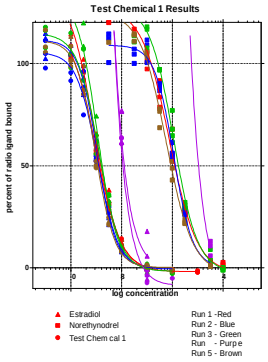
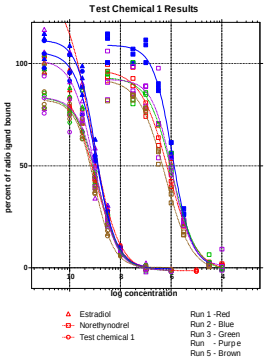
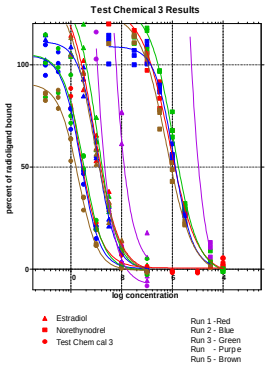
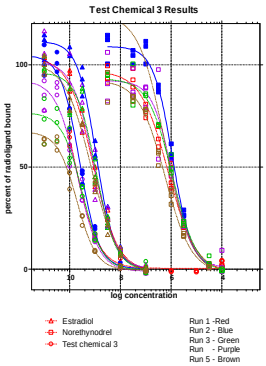
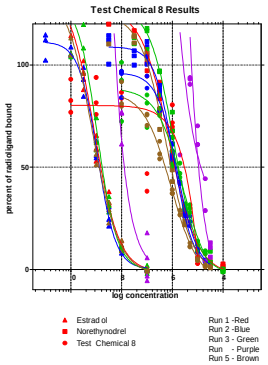
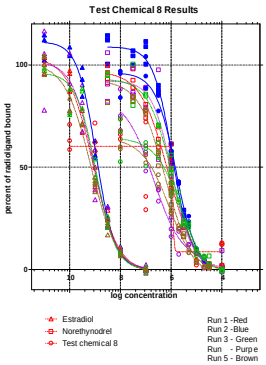


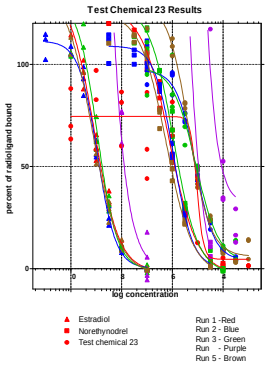


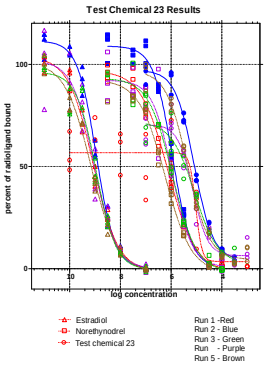




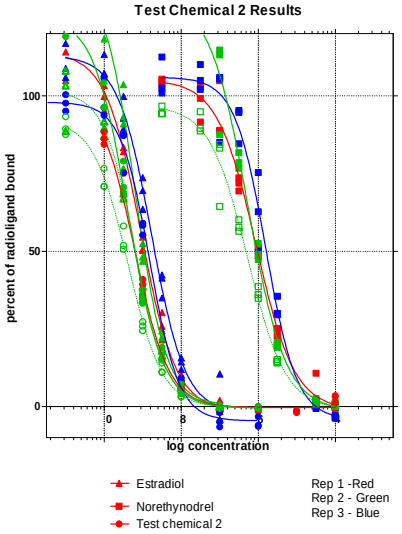


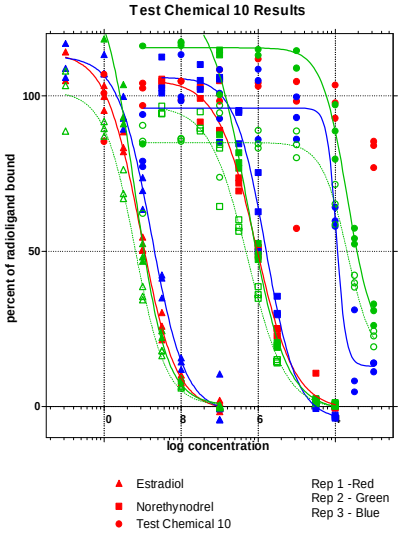


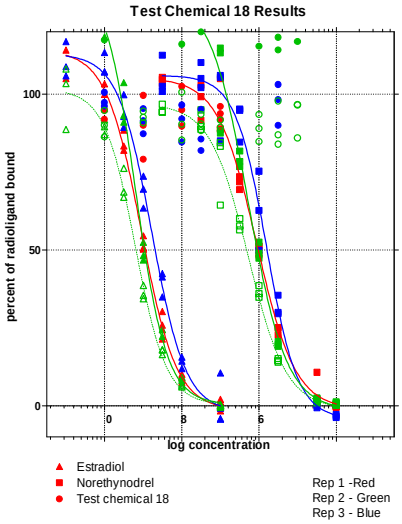




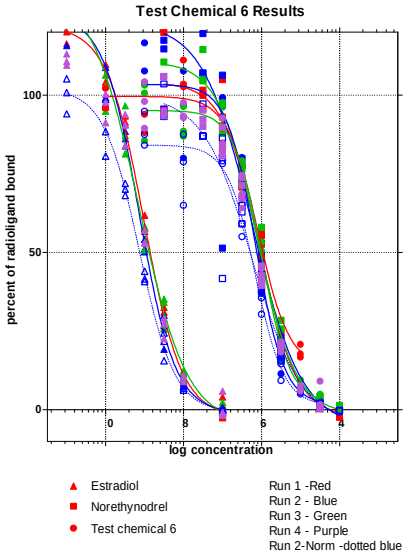
Set 5 Plots of All Standard and Test Chemical Runs

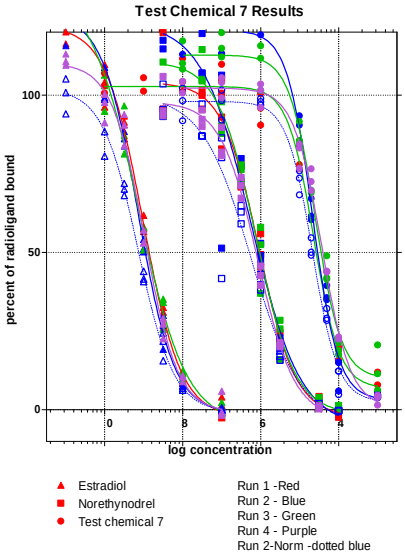


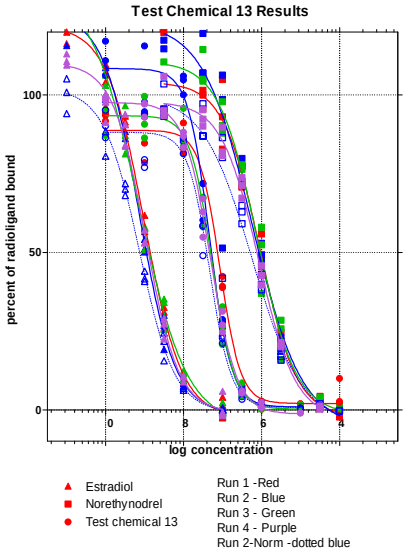




Set 6 Plots of All Standard and Test Chemical Runs







Appendix J

Protocol Deviations

The following protocol deviations are documented in the study records:
1. Planned Deviations

PLANNED PROTOCOL DEVIATION

DATE: 3/4/2008

TO: THE RECORD FOR 0210114.006.007

FROM: SHERRY BLACK

RE: 0210114.006.007 PLANNED PROTOCOL DEVIATIONS

Item 1: Water for assays

During the laboratory prequalification phase of this project (0210114.006.006) we interpreted ddH_2O to mean distilled, deionized water and proceeding to use the distilled, deionized water produced by the still in Lab 210 Hermann in the preparation of all ddH_2O-based solutions for the assay. Our results for that phase of the study showed a properly functioning assay and so the conclusion was made that the distilled, deionized water was appropriate for use. Based on that knowledge, we will continue to use distilled, deionized water in the preparation of all solutions requiring ddH_2O for the assays of the current (0210114.006.007.001) and all subsequent ER tasks.

Item 2: Test tubes

The protocol for the ER assay specifies 12 x 75 mm round-bottom siliconized borosilicate glass test tubes. These tubes are no longer readily available from the supplier. We had enough of the siliconized tubes to complete the Saturation binding assays that were conducted on Jan 28, 30 and 31, but not enough to conduct any further assays. As part of the Saturation assay set from Jan 28, a few extra samples were run using 12 x 75 mm round-bottom borocilicate glass test tubes that had be silanized according to our SOP (DPK-EQP-002.002). The results of that test (while limited) indicated similar results would be obtained regardless of whether siliconized or silanized tubes were used. Silanized tubes were used for all competitive binding assays of the first set of 5 test chemicals and satisfactory results were obtained. The client authorized our use of silanized tubes for all future assays. See attached email.

Item 3: R1881

The results for the negative control (R1881) generated for Set 1 were not as expected. This chemical should not show any binding, but definite and reproducible binding was demonstrated. The reason for this is not clear, but may be chemical related (impurity). When presented with the data from Set 1, the client instructed us to discontinue use of R1881 in all future assays. See attached email.

2. Unplanned Deviation

TO6, Task 7 Protocol Deviation 5[a]

ITEM 1

ORIGINAL DOCUMENT SPECIFICATIONS:

Assay Protocol, Page 38, Section 9.3.2 Individual Tubes

Incubate assay tubes at 4 °C for 16 to 20 hours. Assay tubes should be placed on a rotator during the incubation period.

DEVIATION:

In Assay S4R5, the tubes were not rotated because the rotator was not turned on.

REASON/IMPACT OF CHANGE:
The assay results appear to be unaffected by this error.

a Note that protocol deviations were numbered consecutively throughout Task Order 6. Deviations 1-4 are documented in the Task 6 files.