Appendix 8

Final report from the second interlaboratory validation study

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

Lab Z

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.

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FINAL REPORT

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

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Contract No.: 6-340-0210114
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CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

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1. SUMMARY

The purpose of this study was to perform chemical testing of twenty-three (23) unknown, coded test chemicals on the basis of application of the "Protocol for the In Vitro Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol" (the Protocol). The Protocol was developed as part of the Endocrine Disruptor Screening Program (EDSP) of the U.S. Environmental Protection Agency. This Report provides data and results for 23 chemicals tested under the Protocol, designated Chemical Codes 1 through 23 inclusive.

Test chemicals are characterized as "Positive," "Negative" or "Equivocal" based on the ability to bind to rat uterine estrogen receptor. The calculated log IC_{50} and Hill slope of the displacement curve (if any) were used as quantitative criteria to characterize each test chemical. Test Chemicals 2, 4, 5, 6, 7, 11, 12, and 15 were determined to be Positive for binding to rat uterine estrogen receptor under the conditions of the Protocol. Test Chemicals 9, 18, 20, 21 and 22 were determined to be Negative for binding to rat uterine estrogen receptor under the conditions of the Protocol. Test Chemicals 1, 3, 8, 10, 13, 14, 16, 17, 19 and 23 were determined to be Equivocal for binding to rat uterine estrogen receptor, based on a qualitative evaluation of binding parameters (upper and lower binding plateaus levels and Hill slope)

In conclusion, this Draft Report provides results for 23 chemicals tested under the experimental conditions of the Protocol, and comments on suitability of the Protocol as sole guidance for determining the estrogen binding affinity of unknown chemicals.

2. INTRODUCTION

The purpose of this study was to perform chemical testing of twenty-three (23) unknown, coded test chemicals on the basis of application of the "Protocol for the In Vitro Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol" (the Protocol). The Protocol was developed as part of the Endocrine Disruptor Screening Program (EDSP) of the U.S. Environmental Protection Agency.

All methods and procedures were conducted in the spirit of the EPA Good Laboratory Practices Regulations. This is a non-GLP study. The Sponsor provided the "Quality Assurance Project Plan (QAPP) for Task Order 7, June 2007," which provided specific Quality Assurance parameters for the study.

3. MATERIALS AND METHODS

3.1 Reference Chemical and Weak Positive and Negative Control Articles:

Reference Chemical

Name:	17β-estradiol
Lot No.:	26K1611
Physical Description:	White to off-white powder

Weak Positive Control:

Name:	Norethynodrel
Lot No.:	G
Physical Description:	White or nearly white crystalline powder

Radiotracer:

Name:	[^{3}H]-Estradiol, specific activity 110 Ci/mmole
Lot No.:	3589221/1 (runs TTN001 – 003)
	3589791/1 (runs TTN004 – 007; RN001 – 008)
	3589481/1 (runs TTN008 – 019; RN009 – 013)
Physical Description:	Solution in ethanol

Test Chemicals

Twenty three chemicals were tested in this study. The test chemicals were supplied as coded chemicals of unknown identity by the Sponsor, with designated code numbers 1 through 23 inclusive. All study personnel were blinded to test chemical identity. Molecular weights for each test chemical were provided to allow preparation of serial dilutions at specific molar concentrations. All chemicals were tested using ethanol as solvent, with the exception of test chemicals 1, 5, 10, 18, 22 and 23, which were tested using dimethylsuloxide (DMSO) as the solvent. It had no effect on the study since DMSO did not interfere with the estrogen binding assay. Storage conditions to maintain stability were provided by the Sponsor and were documented in the raw data.

Identification, purity, stability and any other characteristics that define the reference chemical and weak positive and negative controls, and test chemicals were the sole responsibility of the Sponsor and were not included in the TRL study report.

3.2 Test System

Rat uterine cytosol is the specified test system in the Protocol, and was specified by the Sponsor. Ovariectomized Sprague-Dawley female rats, 85 – 100 days old, were obtained from Taconic Farms, Inc. The uteri were collected at UIC-TRL. Uterine cytosol was prepared according to the Protocol, section 6.0.

3.3 Experimental Design

The estrogen receptor competitive binding assays were conducted per "Protocol for the *in vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol" (Endocrine Disruptor Screening Program, U.S. Environmental Protection Agency, April 2007), with modifications and adjustments per RTI and EPA. The negative control compound R1881 was eliminated from the Protocol per EPA directive.

Data for each assay were collected on one of two liquid scintillation counters, a Packard TRI-CARB 3100TR or a Beckman-Coulter LS6500. Data were reduced using Microsoft Excel 2000 and Prism v5.0 software. Excel and Prism file templates containing data entry and data reduction formulae were included in the Protocol provided by the Sponsor.

4. RESULTS

4.1 Rat Uterine Cytosol Preparation and Saturation Binding Assay

Five batches of rat uterine cytosol were prepared over the course of the study. The saturation binding parameters used to characterize the cytosol are summarized in Table 1. Excel and Prism files of saturation binding data and analysis are presented in Appendices A to E.

Protein concentrations ranged from 1.2 to 3.2 mg/ml. The estimated saturation binding constants (K_d) ranged from 0.8 to 1.5 nM. The estimated maximum receptors bound (B_{max}) ranged from 41.2 to 146.0 fmole/100 µg protein. Each of the saturation assays resulted in observation of maximum specific binding and a linear Scatchard plot.

4.2 Rat Uterine Cytosol Estrogen Receptor Competition Binding Assay

Results of competition binding assays are summarized in Table 2. Raw data files (Excel and Prism) are presented in Appendices F through X. Raw data files are identified by a filename consisting of the performing lab initials (TRL), a sequential run number associated with each technician (e.g., TTN001), the assay start date as yy/mm/dd, and the Chemical Code numbers run in the assay. The final audited data files were appended with "Rev#." Complete data file names thus appear as, for example, "Rev1-TRL run TTN001 080312 Chem Codes 12, 19.xls" for the Excel file and "Rev1-TRL run TTN001 080312 Chem Codes 12, 19.pxf" for the Prism file.

Summary results are based on assay runs that met Protocol standard control (estradiol) and weak positive (norethynodrel) criteria for top and bottom plateau levels, Hill slope and within run standard deviation, or that were within 5% of the parameter value for meeting these criteria. In cases where no assay runs met criteria for that chemical, all available data from unacceptable runs was used to provide the tentative chemical characterization presented in the summary descriptions.

Test Chemical 1 was determined to be Equivocal for estrogen receptor binding in three competition assays, TTN015, TTN018 and TTN019 (Table 3; Fig. 1). Chemical 1 displaced 100% of the [^{3}H]-estradiol tracer over the concentration range 100 µM to 100 nM. Data from higher concentrations were truncated from analysis avoid a U-shaped binding curve. A sigmoidal binding curve was not observed. A top plateau was not observed, and an IC_{50} or Hill slope could not be determined. Test Chemical 1 dissolved in DMSO (75% DMSO in working assay buffer in dilution 1 and 50% or less in

subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 8.92%, 2.12% and 9.77%. Test Chemical 1 was characterized as Equivocal because some concentrations of test chemical appeared to compete the radiotracer, but a characteristic binding curve was not observed.

Test Chemical 2 was determined to be Positive for estrogen receptor binding in two competition assays, RN008 and RN009 (Table 3; Fig. 2). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The mean log IC_{50} was –9.1 nM and the mean relative binding affinity (RBA) was 1.13 compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 2 was –1.39. Test Chemical 2 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 53% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 7.3% and 12.7%. Test Chemical 2 was characterized as Positive because it yielded a competition binding curve with upper and lower binding plateaus near 100% and 0%, respectively, a Hill slope near -1 and a value for log IC_{50}.

Test Chemical 3 was determined to be Equivocal for estrogen receptor binding in three competition assays, RN009, TTN015 and TTN018 (Table 3; Fig. 3). The three assays yielded similar 100% competition of [^{3}H]-estradiol tracer over the concentration range 100 μM to 100nM, with varying upward slopes at lower concentrations to 100 pM. Data from the 1 mM concentration was truncated from analysis to avoid a U-shaped binding curve. A top plateau was not observed, and an IC_{50} or Hill slope could be determined in only two of the four assays. Test Chemical 3 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 50% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 1.5%, 1.2% and 2.7%. Test Chemical 3 was characterized as Equivocal because some concentrations of test chemical appeared to compete the radiotracer, but a characteristic binding curved was not observed in all assay runs.

Test Chemical 4 was tentatively determined to be Positive for estrogen receptor binding in three competition assays, TTN010, TTN011 and TTN013 (Table 3; Fig. 4). The three assays yielded similar competition binding curves in terms of slope and lower binding plateau. The mean log IC_{50} was –8.6 nM and the mean RBA was 2.7 compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 4 was –1.05. Test Chemical 4 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 45% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 6.4%, 13.8% and 2.5%. Test Chemical 4 was tentatively characterized as Positive because the bottom binding plateaus and Hill slopes were consistent and within criteria ranges, but with the caveat that the assay runs did not meet criteria parameters for the control and weak positive binding curves.

Test Chemical 5 was determined to be Positive for estrogen receptor binding in three competition assays, TTN007, TTN008 and TTN009 (Table 3; Fig. 5). The three assays yielded similar competition binding curves in terms of slope, lower plateaus and logIC_{50}s. The mean log IC_{50} was –6.4 nM and the mean RBA was 4.02 x 10^{-3} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 5 was –0.98. Test Chemical 5 dissolved in DMSO (50% DMSO in working assay buffer in dilution 1 and 45% or less in subsequent dilutions) but appeared to precipitate in the assay mixture at the 1 mM to 10 μM concentrations. The within-run standard deviations for the three assays were 3.6%, 11.6% and 2.9%. Test Chemical 5 was characterized as Positive because it showed a competition binding curve with clearly defined upper and lower plateaus near 100% and 0%, respectively, and a Hill slope close to –1.0.

Test Chemical 6 was determined to be Positive for estrogen receptor binding in three competition assays, RN004, RN005 and RN006 (Table 3; Fig. 6). The three assays yielded similar competition binding curves in terms of slope, upper plateaus and logIC_{50}s. Data from the 1 mM concentration in three assays, and from the 1 mM and 100 mM concentrations in two assays, was truncated from analysis to avoid U-shaped binding curves. Therefore, the lower binding plateau was not clearly defined. The mean log IC_{50} was –5.8 nM and the mean RBA was 1.3 x 10^{-3} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 6 was –1.35. Test Chemical 6 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 53% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 2.7%, 22.5% and 6.7%. Test Chemical 6 was characterized as Positive because it yielded a competition binding curve with upper and lower binding plateaus near 100% and 0%, respectively, a Hill slope near -1 and a value for log IC_{50}.

Test Chemical 7 was determined to be Positive for estrogen receptor binding in three competition assays, RN004, RN005 and RN006 (Table 3; Fig. 7). The three assays yielded similar binding curves in terms of slope, upper plateaus and logIC_{50}s. The mean log IC_{50} was –3.7 nM and the mean RBA was 8.6 x 10^{-6} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 7 was –0.95. Test Chemical 7 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 8.3%, 5.7% and 3.8%. Test Chemical 7 was characterized as Positive because at least one data point showed greater than 50% radioligand displacement and it was possible to obtain a value for log IC_{50} from an unconstrained curve with a Hill slope near -1.

Test Chemical 8 was determined to be Equivocal for estrogen receptor binding in two competition assays, RN008 and TTN019 (Table 3; Fig. 8). The assays yielded divergent results. Data from the 1 mM concentration was truncated from analysis in both assays to avoid U-shaped binding curves. In run RN008, Test Chemical 8 competed the radiotracer at 1 mM (70%) and 100 mM (30%) concentrations, although a lower plateau was not observed. In run TTN019 all concentrations of test chemical competed the

radiotracer greater than 60%. Chemical 8 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 26.6% and 4.5%. Test Chemical 8 was characterized as Equivocal because two assays gave divergent results, and a clearly defined competition binding curve was not seen in either.

Test Chemical 9 was tentatively determined to be Negative for estrogen receptor binding in three competition assays, TTN010, TTN011 and TTN012 (Table 3; Fig. 9). No displacement of the [^{3}H]-estradiol radiotracer exceeding 50% was observed at any of the tested concentrations (final assay concentrations 100 pM – 1 mM). A logIC_{50} and RBA could not be determined for Test Chemical 9. Test Chemical 9 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 50% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 5.8%, 9.6% and 17.6%. Test Chemical 9 was tentatively characterized as Negative because it did not yield a competition binding curve, and did not compete the radiotracer greater than 50% at even the highest concentration, but with the caveat that the assay runs did not meet criteria parameters for the control and weak positive binding curves.

Test Chemical 10 was determined to be Equivocal for estrogen receptor binding in two competition assays, RN008 and RN009 (Table 3; Fig. 10). The assays yielded similar competition binding in terms of the upper plateau and an ability to compete the radiotracer greater than 50% at the highest concentration of 1 mM. A lower binding plateau was not apparent. The Hill slopes of the two curves for Test Chemical 10 varied; one was indicated an excessively shallow curve at –2.0 and the other was –0.7. Test Chemical 10 dissolved in DMSO (50% DMSO in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) but appeared to precipitate in the assay mixture at the 1 mM and 100 μM concentrations (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 6.5% and 7.3%. Test Chemical 10 was characterized as Equivocal because the Hill slope was variable and deemed excessively shallow in one case and the maximum competition at 1 mM was inconsistent.

Test Chemical 11 was determined to be Positive for estrogen receptor binding in three competition assays, TTN002, TTN006 and RN008 (Table 3; Fig. 11). The three assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The mean log IC_{50} was –5.5 nM and the mean RBA was 3.9×10^{-4} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 11 was –0.95. Test Chemical 11 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 3.8%, 7.8% and 14.5%. Test Chemical 11 was characterized as Positive because it showed a competition binding curve with clearly defined upper and lower plateaus near 100% and 0%, respectively, and a Hill slope close to –1.0.

Test Chemical 12 was determined to be Positive for estrogen receptor binding in two competition assays, TTN001 and TTN006 (Table 3; Fig. 12). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The mean log IC_{50} was –6.9 nM and the mean RBA was 1.1 x 10^{-2} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 12 was –0.98. Test Chemical 12 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions), but appeared to precipitate in the assay mixture at the 1 mM to 10 μM concentrations. The within-run standard deviations for the three assays were 3.2% and 2.9%. Test Chemical 12 was characterized as Positive because it showed a competition binding curve with clearly defined upper and lower plateaus near 100% and 0%, respectively, and a Hill slope close to –1.0.

Test Chemical 13 was determined to be Equivocal for estrogen receptor binding in two competition assays, RN005 and RN006 (Table 3; Fig. 13). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. Data from the 1 mM concentration was truncated from analysis to avoid U-shaped binding curves. The lower binding plateau was clearly defined over two concentration levels. The mean log IC_{50} was –5.8 nM and the mean RBA was 1.4 x 10^{-3} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 13 was –2.1. Test Chemical 13 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 53% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 6.2% and 6.3%. Test Chemical 13 was characterized as Equivocal because the Hill slope was deemed excessively steep at –2.1.

Test Chemical 14 was tentatively determined to be Equivocal for estrogen receptor binding in three competition assays, TTN010, TTN011 and TTN014 (Table 3; Fig. 14). Two assay runs (TTN010, TTN014) showed displacement of the [^{3}H]-estradiol radiotracer exceeding 25% at 10 μM. Data from the two highest concentrations (1 mM and 100 μM) were truncated from analysis in all assay runs avoid a U-shaped binding curve. A sigmoidal binding curve was not observed. A logIC_{50} and RBA could not be determined for Test Chemical 14. Test Chemical 14 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 50% or less in subsequent dilutions) but appeared to precipitate in the assay mixture at the 1 mM to 10 μM concentrations (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 10.2%, 19.0% and 23.9%. Test Chemical 14 was tentatively characterized as Equivocal because it did not meet all criteria for a determination of Negative, but with the caveat that the assay runs did not meet criteria parameters for the control and weak positive binding curves.

Test Chemical 15 was determined to be Positive for estrogen receptor binding in two competition assays, TTN002 and TTN006 (Table 3; Fig. 15). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The mean log IC_{50} was –5.3 nM and the mean RBA was 1.8 x 10^{-4} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 15 was –0.99. Test Chemical 15 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1

and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 3.7% and 4.5%. Test Chemical 15 was characterized as Positive because it showed a competition binding curve with clearly defined upper and lower plateaus near 100% and 0%, respectively, and a Hill slope close to –1.0.

Test Chemical 16 was determined to be Equivocal for estrogen receptor binding in three competition assays, TTN007, TTN008 and TTN009 (Table 3; Fig. 16). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The lower binding plateau was clearly defined over two concentration levels. The mean log IC_{50} was –5.1 nM and the mean RBA was 1.8×10^{-4} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 16 was –3.2. Test Chemical 16 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 45% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 5.6%, 2.8% and 3.3%. Test Chemical 13 was characterized as Equivocal because the Hill slope was deemed excessively steep at –3.2.

Test Chemical 17 was determined to be Equivocal for estrogen receptor binding in two competition assays, TTN008 and TTN009 (Table 3; Fig. 17). The assays yielded similar competition binding curves in terms of slope, upper and lower plateaus and logIC_{50}s. The lower binding plateau was clearly defined in one of the assays. The mean log IC_{50} was –5.4 nM and the mean RBA was 3.5×10^{-4} compared to 17β-estradiol. The mean Hill slope of the competition binding curve for Test Chemical 17 was –1.5. Test Chemical 17 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 50% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 3.4% and 5.1%. Test Chemical 17 was characterized as Equivocal because the Hill slope was deemed excessively steep at –2 in one of the assays.

Test Chemical 18 was determined to be Negative for estrogen receptor binding in three competition assays, RN004, RN005 and RN006 (Table 3; Fig. 18). No displacement of the [^{3}H]-estradiol radiotracer was observed at any of the tested concentrations in two of the assays (final assay concentrations 100 pM – 1 mM). In the third assay, approximately 65% displacement was seen at the highest concentration of 1 mM. A logIC_{50} and RBA could not be determined for Test Chemical 18. Test Chemical 18 dissolved in DMSO (100% in all dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 3.0%, 11.4% and 3.9%. Test Chemical 18 was characterized as Negative because it did not yield a competition binding curve, and showed the ability to compete the radiotracer only at the highest concentration and in one assay run only.

Test Chemical 19 was determined to be Equivocal for estrogen receptor binding in two competition assays, TTN001 and TTN007 (Table 3; Fig. 19). The assays yielded similar curves in terms of slope and upper plateaus. In both assays, greater than 50%

displacement of the radiotracer was seen at the highest concentration only (1mM). A $logIC_{50}$ and RBA could be determined in one assay run only. Test Chemical 19 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the two assays were 20.3% and 2.5%. Test Chemical 19 was characterized as Equivocal because it competed with the radiotracer only at the maximum concentration and the one calculated Hill slope was excessively steep at –2.3.

Test Chemical 20 was determined to be Negative for estrogen receptor binding in two competition assays, TTN015 and TTN018 (Table 3; Fig. 20). Displacement of the [^{3}H]-estradiol radiotracer was observed at concentrations from 10 μM to 100 pM. Data from the 1 mM and 100 μM concentrations were truncated from analysis avoid a U-shaped binding curve. A sigmoidal binding curve was not observed. A reasonable $logIC_{50}$ and RBA could not be determined for Test Chemical 20. The mean calculated $logIC_{50}$ was 12.22 and the mean calculated Hill slope was –16. Upper and lower bounds of the competition curve were approximately 65% and 25%, respectively. Test Chemical 20 dissolved in ethanol (75% EtOH in working assay buffer in dilution 1 and 45% or less in subsequent dilutions) but appeared to precipitate in the assay mixture at the 1 mM to 10 μM concentrations. The within-run standard deviations for the two assays were 10.4% and 14.1%. Test Chemical 20 was characterized as Negative because it did not yield a competition binding curve that met acceptable criteria, i.e. upper and lower bounds near 100% and 0%, respectively, and a Hill slope of approximately –1.0.

Test Chemical 21 was determined to be Negative for estrogen receptor binding in one competition assay, TTN006 (Table 3; Fig. 21). No displacement of the estradiol radiotracer was observed at any of the tested concentrations (final assay concentrations 100 pM – 1 mM). A $logIC_{50}$ and RBA could not be determined for Test Chemical 21. Test Chemical 21 dissolved in ethanol (50% EtOH in working assay buffer in dilution 1 and 5% or less in subsequent dilutions), however the diluted test chemical in assay buffer formed a cloudy solution at the 1 mM and 100 μM concentrations. The within-run standard deviation was 4.5%. Test Chemical 21 was characterized as Negative because it did not yield a competition binding curve, and was unable to compete with the radiotracer at any concentration up to 1 mM.

Test Chemical 22 was determined to be Negative for estrogen receptor binding in three competition assays, TTN007, TTN008 and TTN009 (Table 3; Fig. 22). No displacement of the [^{3}H]-estradiol radiotracer was observed at any of the tested concentrations in two of the assays (final assay concentrations 100 pM – 1 mM). In the third assay, approximately 60% displacement was seen at the highest concentration of 1 mM. A $logIC_{50}$ and RBA could not be determined for Test Chemical 22. Test Chemical 22 dissolved in DMSO (50% DMSO in working assay buffer in dilution 1 and 5% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 4.7%, 7.0% and 10.1%. Test Chemical 22 was characterized as Negative because it did not yield a competition binding curve, and showed the ability to compete the radiotracer only at the highest concentration and in one assay run only.

Test Chemical 23 was determined to be Equivocal for estrogen receptor binding in three competition assays, TTN015, TTN018 and TTN019 (Table 3; Fig. 23) . Displacement of the [^{3}H]-estradiol radiotracer was observed at concentrations from 10 μM to 100 pM, and was highly variable. A sigmoidal binding curve was not observed. Upper and lower curve plateaus, logIC_{50} and RBA could not be determined for Test Chemical 23. Test Chemical 23 dissolved in DMSO (75% in dilution 1 and 45% or less in subsequent dilutions) and was soluble in the assay mixture at all concentrations tested (final assay concentrations 100 pM – 1 mM). The within-run standard deviations for the three assays were 10.1%, 7.0% and 9.8%. Test Chemical 23 was characterized as Equivocal competitive displacement of the radioligand was observed, but highly variable over the range of concentrations tested.

Control criteria were evaluated for 17β-estradiol and the weak positive norethynodrel competition curves, and included the top and bottom plateau levels, hill slope and within-run standard deviation. Control criteria values are summarized in Table 4, and Summary Plots are presented in Figures 24 and 25. Of 32 total assay runs, 14 (44%) met acceptable performance criteria, or were within 5% of meeting criteria values. Four of the assays (12%) met all performance criteria. This analysis excludes the P1 norethynodrel standard, which was consistently truncated from analysis to avoid a U-shaped competition binding curve. The most common reason for an assay to not meet acceptable performance criteria was an elevated upper binding plateau levels.

5. DISCUSSION

5.1 Saturation Binding of Rat Uterine Cytosol

Rat uterine cytosol was prepared at TRL from ovariectomized rats. Protein concentrations and saturation binding parameters (K_d and B_{max}) were consistent between batches although they did not fall into the ranges described in the Protocol. Each batch of cytosol assayed showed a plateau of maximum specific binding and a linear Scatchard plot indicative of one-site binding.

5.2 Estrogen Binding Potential of the Test Chemicals

Test Chemicals 2, 4, 5, 6, 7, 11, 12, and 15 were determined to be Positive for binding to rat uterine estrogen receptor under the conditions of the Protocol. For each chemical, the log IC_{50}s and Hill slopes for the competition curves were consistent and typically within a narrow range. The relative binding affinities showed a broader between-run range of values, as they are calculated relative to the estradiol log IC_{50} determined in each assay. Test chemical 4 was ascribed a tentative determination because no assay runs for this chemical met control chemical performance criteria.

Test Chemicals 9, 18, 20, 21, and 22 were determined to be Negative for binding to rat uterine estrogen receptor under the conditions of the Protocol. These chemicals did not yield competition binding curves, and each test concentration yielded values of

approximately 100% or greater radioligand bound. Between-run variability was more pronounced for Negative chemicals, possibly due to solubility issues. Test chemicals 9 and 14 were ascribed tentative determinations because no assay runs for these chemicals met control chemical performance criteria.

Test Chemicals 1, 3, 8, 10, 13, 14, 16, 17, 19 and 23 were Equivocal for binding to rat uterine estrogen receptor under the conditions of the Protocol. Equivocal determinations were qualitative in nature, based on evaluation of binding parameters (percent binding levels and Hill slope) and fell into three general categories. In the first category, Test Chemicals were determined to be Equivocal because although displacement of the radioligand was observed over several concentrations, a characteristic binding curve was not obtained and a log IC_{50} could not be calculated. Test Chemicals 1, 3 and 23 fell into this category. In the second category, one or two of the highest concentrations (1 mM and 100 μM) could displace at least 50% of the radioligand, but a characteristic binding curve was not obtained and a log IC_{50} could not be calculated. Test Chemicals 8, 10, 14 and 19 fell into this category. In the third category, the Test Chemicals yielded sigmoidal competition binding curves with clearly defined upper and lower binding plateaus, but the Hill slopes deviated from a value of –1 significantly enough to cast doubt on true estrogen-like binding. Hill slopes were –2 or greater, indicating excessively steep slopes for the binding curves. Test Chemicals 13, 16 and 17 fell into this category.

Of 32 total assay runs, 44% met performance criteria or were within 5% of meeting criteria values. In the assays that failed to meet performance criteria, the most common reason was an elevated upper binding plateau level. The upper plateaus in these assays ranged from 130% to 200% or greater radioligand bound. This phenomenon appeared to be related to low DPM counts in the ethanol control tubes, since calculation of the % bound values for each chemical concentration X is based on the ratio of X DPM counts to the average ethanol DPM counts (after subtraction of nonspecific DPM). Conversely, one could posit enhanced binding of the radioligand in the presence of low concentrations of unlabled ligand as a cause of the elevated upper plateaus.

5.3 Comments on the Protocol and Inter-Laboratory Validation

The specific causes of some assays not meeting performance criteria remain elusive. At the Sponsor's (RTI / EPA) request, the following discussion comments on technical issues that arose during implementation of the Protocol.

Rat uterine cytosol preparation. The Protocol gives sufficient direction on uterine dissection and tissue / buffer ratios for the preparation of the cytosolic fraction. Two clarifications are warranted. The first is regarding the age range of the rats. It is not clear if the 85-100 day age range refers to the age at sacrifice or the age at ovariectomy. The second is regarding uteri collection from large numbers of animals. At the TRL, we dissected uteri and placed them immediately in ice-cold buffer. Out of concern for receptor degradation during the collection period, we limited the number of animals to 30 – 35, so that uteri were in buffer approximately 45 minutes prior to homogenization. This approach resulted in functional cytosol for the assay, but also small batches that necessitated multiple saturation assays to characterize each batch. The Sponsor later

clarified that uteri could be dissected and snap-frozen in liquid nitrogen, so that larger numbers of animals, 50 – 100, could be handled in one session, yielding larger batches of cytosol. TRL used this approach for the last batch of 60 animals, with the frozen uteri homogenized and further prepared immediately after the dissection session, and it worked quite satisfactorily. For scientists not accustomed to implementing large-scale biochemical assays, this minor but critical point is not obvious. It would be useful to include this point about snap-freezing uteri in the protocol, in addition to some guidance on approximate quantities, e.g. that X number of rats are expected to yield Y milliliters of uterine cytosol, sufficient for Z test chemicals assayed in triplicate.

Characterization of rat uterine cytosol. Guidance from the Protocol and Sponsor regarding saturation assay results and interpretation was not consistent. Section 7.0 of the Protocol states that there are no specific performance criteria regarding the outcome of saturation assays, and refers to general considerations in section 8.6. However, section 8.6 describes specific value ranges for Kd and Bmax, accompanied by the statement that several saturation assays may be required to attain these value ranges, thereby implying that these are genuine target values. Feedback from the Sponsor similarly implied that saturation assays should be yielding values in these ranges. TRL lowered the cytosolic protein concentration for a series of saturation assays, which brought Kd and Bmax values closer to the Protocol values. Subsequent competition assays utilized these lowered protein concentrations (40 μg / assay tube) until the Sponsor directed TRL to perform all assays at a standardized 50 μg / assay tube. Guidance from the Sponsor was on the one hand to approach the typical values stated in the Protocol, and on the other to utilize 50 μg protein per assay tube. It would be helpful to the performing laboratories for the Protocol to articulate a priority for utilizing a set protein concentration, or for attaining historic Kd and Bmax value ranges, or some other unambiguous metric.

Ligand depletion. Section 8.1.5 of the Protocol states that 15% ligand depletion is a case for adjustment of the assay, specifically cytosolic protein concentrations. The Excel spreadsheet template accompanying the Protocol flags ligand depletion above 10% as indicating a problem with the assay. Feedback from the Sponsor (conference call 4-16-08) indicated that up to 20% ligand depletion is acceptable in saturation assays and not a concern in competition assays. This should be clarified.

Data Handling. The Excel and Prism templates were well laid out and greatly facilitated data reduction. It would be very useful to have a more clear "roadmap" to the sequence of data input and transformation, for example an MS word document that took the user step-by-step from DPM entry, through the formulae used to transform DPM into percent bound, to the cut-and-paste steps used to transfer data to Prism. The main utility would be to accelerate learning for new users, but also it would serve to clarify why data is truncated or flagged.

Test chemical solubility. Solubility appears to play a major role in assay outcomes, specifically variability and reproducibility, even with the control chemicals estradiol and noerthynodrel. The most common and significant cause of assays not meeting acceptance criteria was a low DPM yield for the ethanol control tubes relative to control chemical tubes, which resulted in elevated upper binding plateau levels. Therefore it

appears that solvent itself, in addition to test chemical solubility, is an important factor. If this is the case, it seems necessary that the Protocol include solubility testing of the test chemicals as a distinct and standardized operation that is performed separate from the competition assay, and similarly a test of solvent compatibility of ethanol and DMSO with the cytosolic fraction.

Noerthynodrel weak positive control. The TRL experienced a months-long delay in implementation of the competition assays because the "P1" or 100 μM concentration of norethynodrel gave an elevated % binding value and thus a U-shaped competition curve. Solubility issues and adjustments to how norethynodrel was prepared, diluted and handled were investigated in depth. The elevated % binding value was consistent over time and in the hands of four technicians. During the course of running the competition assays, select assays did show an acceptable % binding value between 1% and –5%, seemingly at random given that in back to back assays run by the same technician with the same reagents, one would and one would not yield acceptable P1 values. Interestingly, in two competition assays that were discarded because all sample DPM counts were very low (500 DPM or less), the P1 DPMs were remained high (over 1500 DPM), suggesting the occurrence of some phenomena other than ligand binding.

Hydroxyapatite. Not all hydroxyapatite is the same. For the CAS number listed in the Protocol, (1306-06-5), multiple forms are available. TRL first purchased "Fast Flow" hydroxyapatite from Fluka (catalog no. 55497), a granular form which produced a stable, easily washable pellet but was expensive and difficult to keep in suspension and to distribute into the assay tubes because it would clog the repeating pipetter. We also tried hydroxyapatite from Riedel-de Haen (catalog no. 04238), a finer granule form which was significantly less expensive and much easier to keep in suspension and distribute to the assay tubes, but which formed a pellet that was very difficult to resuspend during the washing phases of the assay, even with prolonged vortexing. We also used hydroxyapatite slurry obtained from BioRad (catalog no 130-0150), which performed similarly to the Fluka Fast Flow. It would be helpful, if possible, to specify a particular vendor and catalog number of hydroxyapatite, as well as other chemicals and reagents, in the Protocol.

Radiotracer stability. The Protocol and the shipping materials accompanying the [^{3}H]-estradiol radiotracer did not specify handling requirements, only storage at –20^{o}C. Radiotracer degradation appears to have played a major role in several competition assays not meeting performance criteria, as switching to a previously unopened stock solution significantly improved assays results.

R1881. The R1881 "negative" control chemical yielded competition binding curves and measurable log IC50s in the five competition assays in which this control was used. R1881 was poorly soluble in absolute ethanol and formed a precipitate in the assay tubes. It was eliminated from subsequent competition assays at the Sponsor's request.

Performance Criteria. The Protocol stipulates strict performance criteria of control chemicals for "acceptable runs, " specifically with-in run variation less than 5% (estradiol) or 5.7% (norethynodrel), bottom binding plateau between –5.0 and 1.0%, top

binding plateau between 90 and 110% and Hill slope between –1.1 and –0.7. Under strict adherence to these criteria, and ignoring the P1 noethynodrel standard, which consistently gave a U-shaped binding curve, 12% of 32 assays run were acceptable runs. Under slightly relaxed criteria (i.e. expanding acceptable ranges 5%), 44% of the assays gave acceptable runs. The performance criteria stipulated in the Protocol are typical for instrumental analytical methods (e.g., chromatographic methods) and immunologic analytical methods (e.g. enzyme immunoassay). However these criteria may be too narrow for this assay, which although biochemical is sourced from live animals, is non-automated and entails multiple steps, all of which add to variability.

Classification Criteria. The Protocol guidance on classification of chemicals as positive, negative or equivocal is not completely clear as written. The main problem is that all the classification descriptions appear to be predicated on the test chemical producing a recognizable competition binding curve, and that judgment is based primarily on the percent radioligand displacement. What quantitative value is ascribed to a Hill slope that is "unusually steep" or "unusually shallow"? Values less than –1.5, or –2.0, or greater than –0.5? Chemicals that are negative most commonly did not produce any binding curve, e.g. a straight line at 100%, not "an acceptable binding curve…which do[es] not displace more than 25% of the radioligand…" Thus this description of the negative classification is confusing because it does not include an option of absence of any binding curve. Several chemicals yielded a U-shaped binding curve (prior to data truncation during analysis). This is not addressed in classification criteria.

Inter-laboratory collaboration. The TRL recognizes the two major goals of the study as to provide data on the estrogenicity of the 23 blind-coded test chemicals and to demonstrate the utility of the Protocol as stand-alone directions for successfully assaying the test chemicals. These two goals at times worked at cross-purposes to each other. The Protocol as written contains inconsistencies and ambiguities that were eventually clarified by the Sponsors. However there were troubleshooting issues – notably the high DPM counts in the P1 norethynodrel standards, the low DPM counts in the ethanol controls, and inconsistency in meeting performance criteria – that were not resolved by conference call between lab and Sponsor. TRL advocated for site visits between TRL technicians and Study Director and the technical staff of the Sponsor laboratory to resolve these issues, an approach that was deemed by the Sponsor to be contrary to demonstration of the Protocol as a stand-alone approach. However this impeded the other goal of generating useable data on a very aggressive deadline. We comment here to suggest that separating these two goals based on their collaborative requirements may have facilitated delivery of both.

6. CONCLUSION

Twenty-three blind-coded Test Chemicals were assayed for estrogenicity according to the "Protocol for the In Vitro Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol" (the Protocol). Test Chemicals 2, 4, 5, 6, 7, 11, 12, and 15 were determined to be Positive for binding to rat uterine estrogen receptor under the conditions of the Protocol. Test Chemicals 9, 18, 20, 21 and 22 were determined to be Negative for binding to rat

uterine estrogen receptor under the conditions of the Protocol. Test Chemicals 1, 3, 8, 10, 13, 14, 16, 17, 19 and 23 were Equivocal for binding to rat uterine estrogen receptor under the conditions of the Protocol. The Protocol has potential to function as a stand-alone directions for testing unknown chemicals for estrogenicity, but requires some revision and accommodations for assay performance troubleshooting.

7. PERSONNEL

Study Director:	Daniel Tessier, Ph.D., D.A.B.T.
Principal Investigator	Alexander V. Lyubimov, M.D., Ph.D., D.A.B.T.
Receptor Binding Analysts	Thuly Nguyen, B.S.
	Rita Nisman, B.S.

8. ARCHIVES

Upon completion of the study and submission of the final report, all raw data, documentation and other materials necessary to reconstruct the study will be stored for five years in the UIC/TRL archives maintained by Quality Assurance, University of Illinois at Chicago (UIC), Department of Pharmacology, Chicago, IL 60612. The Sponsor will then be contacted regarding further archival arrangements.

Table 1

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Saturation Binding Assay Results by Cytosol Batch

Cytosol Batch ID	Protein Concentration (mg/ml)	Est. Kd (nM)	Est. Bmax (fmole/100 µg protein)
RUC2-18-08	1.16	1.4	146.0
RUC2-21-08	3.16	1.1	85.2
RUC2-22-08	2.69	1.5	107.9
RUC3-3-08	2.75	1.0	41.2
RUC6-6-08	2.52	0.8	113.2

Table 2

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR
BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Competition Binding Assay Results by Chemical Code *

Chemical Codes	**1**	**2**	**3**	**4**	**5**
Bottom Plateau Level	-2.67 ± 0.99	-1.33 ± 1.60	-2.91 ± 1.50	-6.89 ± 5.24	1.13 ± 1.26
Top Plateau Level	29.17 ± 25.79	100.97 ± 31.24	87.02 ± 21.71	133.71 ± 46.94	100.33 ± 9.37
Log IC_{50} (nM)	N/A	-9.06 ± 0.49	-9.43 ± 0.51	-8.61 ± 0.31	-6.45 ± 0.05
Hill Slope	N/A	-1.39 ± 5.14	-1.10 ± 0.13	-1.05 ± 0.21	-0.98 ± 0.05
SD within run	6.94 ± 4.19	11.35 ± 1.91	1.79 ± 0.80	7.58 ± 5.77	6.05 ± 0.52
RBA**	N/A	$1.13 \pm 4.05 \times 10^{-2}$	5.73 ± 3.28	$2.67 \pm 6.96 \times 10^{-1}$	$4.02 \times 10^{-3} \pm 3.73 \times 10^{-4}$
Conclusion	EQUIVOCAL	POSITIVE	EQUIVOCAL	POSITIVE	POSITIVE

N/A = Not Applicable
*Values are averages of repeated assays run in triplicate ± SD.
**Relative Binding Affinity; 17β-estradiol = 1.0.

Table 2 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR
BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Competition Binding Assay Results by Chemical Code *

Chemical Codes	6	7	8	9	10
Bottom Plateau Level	5.12 ± 9.70	-382673.22 ± 662792.43	10.61 ± 22.64	56.24 ± 2.07	-89.74 ± 137.69
Top Plateau Level	98.28 ± 6.90	97.95 ± 2.39	67.97 ± 45.09	159.30 ± 50.51	109.60 ± 9.48
Log IC_{50} (nM)	-5.83 ± 0.08	-3.68 ± 0.11	-4.67 ± N/A	N/A	-3.41 ± 0.52
Hill Slope	-1.35 ± 0.63	-0.95 ± 0.40	-1.30 ± N/A	N/A	-1.34 ± 0.89
SD within run	10.63 ± 10.47	5.93 ± 2.26	15.54 ± 15.64	11.02 ± 6.02	6.90 ± 0.57
RBA**	$1.25 \times 10^{-3} \pm 4.03 \times 10^{-4}$	$8.63 \times 10^{-6} \pm 1.61 \times 10^{-6}$	1.04×10^{-4} ± N/A	N/A	$1.18 \times 10^{-5} \pm 1.32 \times 10^{-5}$
Conclusion	POSITIVE	POSITIVE	EQUIVOCAL	NEGATIVE	EQUIVOCAL

N/A = Not Applicable
*Values are averages of repeated assays ± SD.
**Relative Binding Affinity; 17β-estradiol = 1.0.

Table 2 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR
BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Competition Binding Assay Results by Chemical Code *

Chemical Codes	11	12	13	14	15
Bottom Plateau Level	-1.68 ± 1.20	-1.41 ± 0.97	1.73 ± 1.02	46.66 ± 40.92	-0.21 ± 2.72
Top Plateau Level	97.28 ± 5.19	107.80 ± 5.80	95.13 ± 6.55	125.97 ± 10.63	102.10 ± 2.26
Log IC_{50} (nM)	-5.48 ± 0.23	-6.89 ± 0.11	-5.77 ± 0.16	-6.60 ± N/A	-5.28 ± 0.20
Hill Slope	-0.95 ± 0.09	-0.98 ± 0.10	-2.06 ± 0.01	-0.33 ± N/A	-0.99 ± 0.09
SD within run	8.70 ± 5.41	3.06 ± 0.23	6.25 ± 0.07	21.80 ± 13.27	4.10 ± 0.57
RBA**	$3.93 \times 10^{-4} \pm 1.04 \times 10^{-4}$	$1.13 \times 10^{-2} \pm 5.92 \times 10^{-3}$	$1.38 \times 10^{-3} \pm 5.66 \times 10^{-4}$	$1.50 \times 10^{-2} \pm$ N/A	$1.84 \times 10^{-4} \pm 4.57 \times 10^{-5}$
Conclusion	POSITIVE	POSITIVE	EQUIVOCAL	EQUIVOCAL	POSITIVE

N/A = Not Applicable
*Values are averages of repeated assays ± SD.
**Relative Binding Affinity; 17β-estradiol = 1.0.

Table 2 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR
BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Competition Binding Assay Results by Chemical Code *

Chemical Codes	16	17	18	19	20
Bottom Plateau Level	-1.41 ± 1.18	4.86 ± 12.30	74.69 ± 2.47	-115.59 ± 175.38	28.24 ± 8.13
Top Plateau Level	103.29 ± 7.39	107.50 ± 0.42	102.49 ± 4.69	101.45 ± 9.13	64.06 ± 6.01
Log IC_{50} (nM)	-5.09 ± 0.04	-5.38 ± 0.00	-3.04 ± N/A	N/A	12.22 ± 2.61
Hill Slope	-3.22 ± 2.15	-1.54 ± 0.65	N/A	N/A	-16.02 ± 3.97
SD within run	3.88 ± 1.46	4.25 ± 1.20	6.10 ± 4.61	11.38 ± 12.61	12.22 ± 2.61
RBA**	$1.76 \times 10^{-4} \pm 2.99 \times 10^{-5}$	$3.51 \times 10^{-4} \pm 2.12 \times 10^{-5}$	$2.34 \times 10^{-6} \pm$ N/A	N/A	$1.66 \times 10^{-2} \pm 5.40 \times 10^{-4}$
Conclusion	EQUIVOCAL	EQUIVOCAL	NEGATIVE	EQUIVOCAL	NEGATIVE

N/A = Not Applicable
*Values are averages of repeated assays ± SD.
**Relative Binding Affinity; 17β-estradiol = 1.0.

Table 2 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR
BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary of Competition Binding Assay Results by Chemical Code *

Chemical Codes	21	22	23
Bottom Plateau Level	97.44 ± N/A	63.55 ± 25.24	N/A
Top Plateau Level	121.17 ± N/A	103.73 ± 6.60	N/A
Log IC_{50} (nM)	N/A	-3.27 ± N/A	N/A
Hill Slope	N/A	N/A	N/A
SD within run	4.70 ± N/A	7.42 ± 3.79	9.98 ± 2.46
RBA**	N/A	N/A	N/A
Conclusion	NEGATIVE	NEGATIVE	EQUIVOCAL

N/A = Not Applicable
*Values are averages of repeated assays ± SD.
**Relative Binding Affinity; 17β-estradiol = 1.0.

Table 3

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 1

Run No.	TTN015	TTN018	TTN019
Bottom Plateau Level	-2.011	-3.808	-2.196
Top Plateau Level	2.78×10^{14}	10.93	47.4
Log IC_{50}	-9.938	N/A	N/A
Hill Slope	-0.5993	N/A	N/A
SD within run	8.92	2.12	9.77
RBA*	1.524×10^{1}	N/A	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 2

Run No.	RN008	RN009
Bottom Plateau Level	-0.1944	-2.46
Top Plateau Level	95.73	106.2
Log IC_{50}	-8.716	-8.496
Hill Slope	-1.23	-1.092
SD within run	7.3	12.7
RBA*	1.159	1.102

*Relative Binding Affinity, 17β-estradiol = 1.0

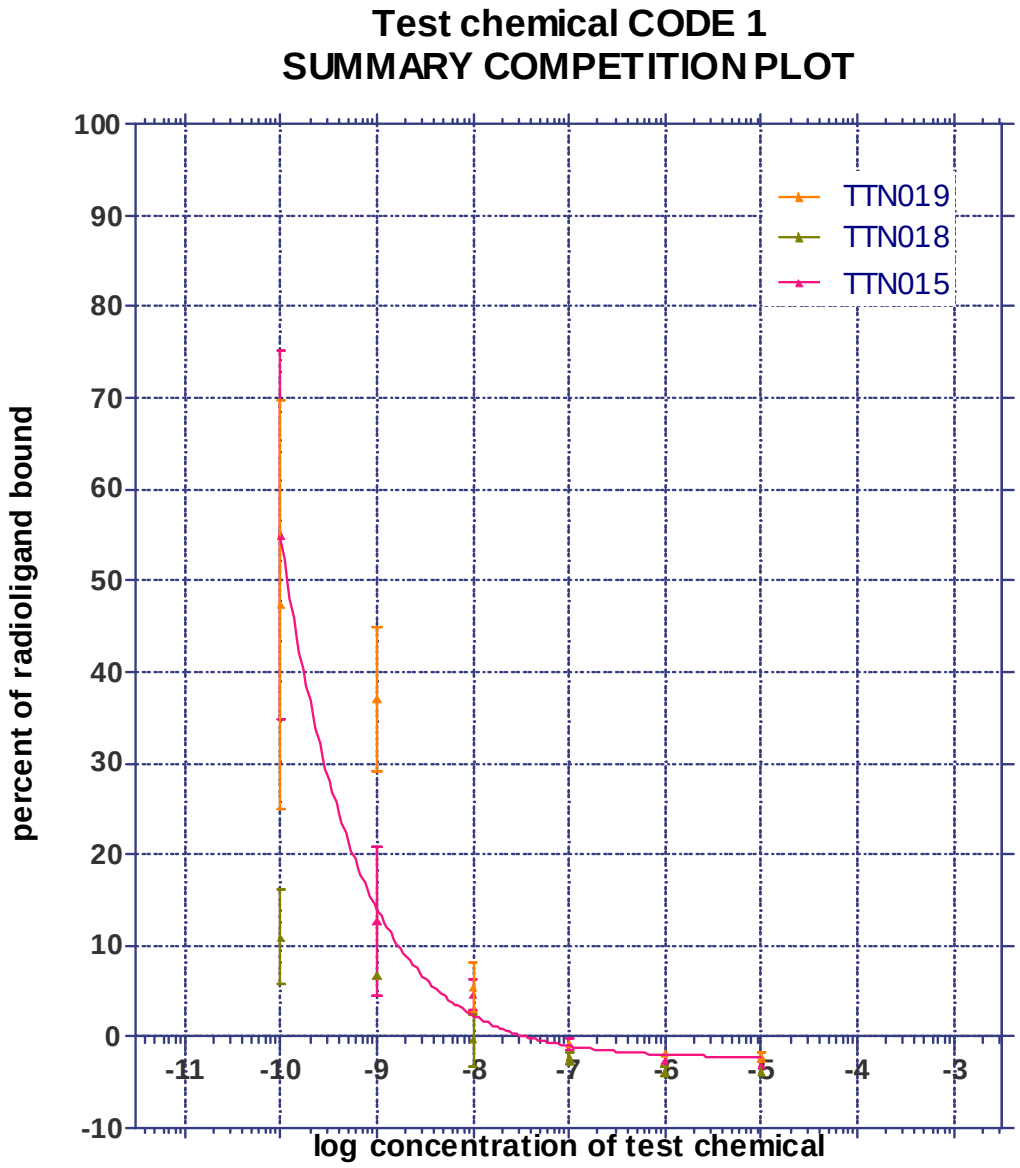

Test chemical CODE 1
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN019
TTN018
TTN015
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 1.

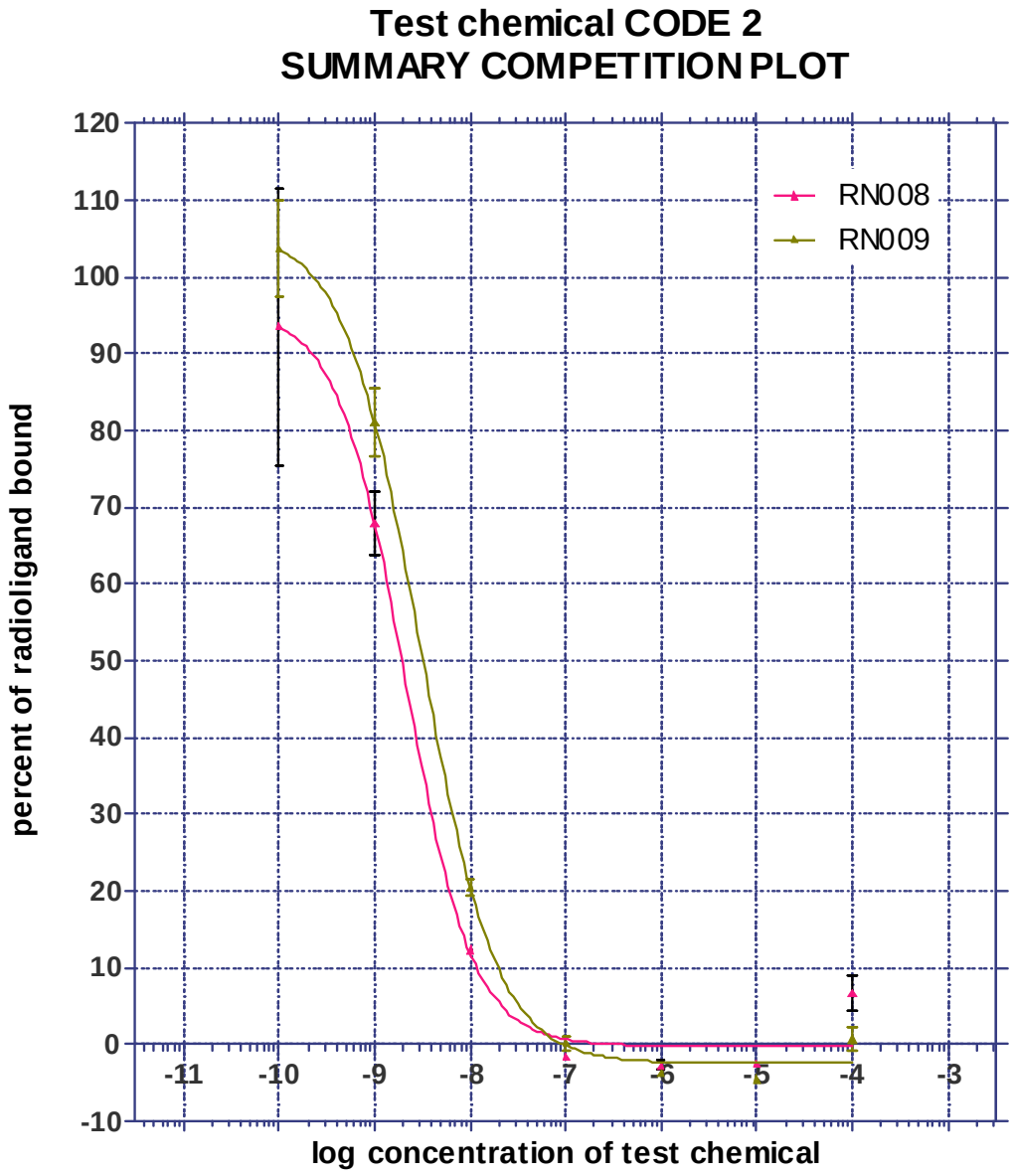

Test chemical CODE 2
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN008
RN009
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 2.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 3

Run No.	TTN015	TTN018	RN009
Bottom Plateau Level	-1.187	-3.935	-3.601
Top Plateau Level	70.44	79.02	111.6
Log IC_{50}	-9.459	-9.934	-8.906
Hill Slope	-1.246	-1.039	-1.002
SD within run	1.47	1.21	2.7
RBA*	5.058	9.290	2.831

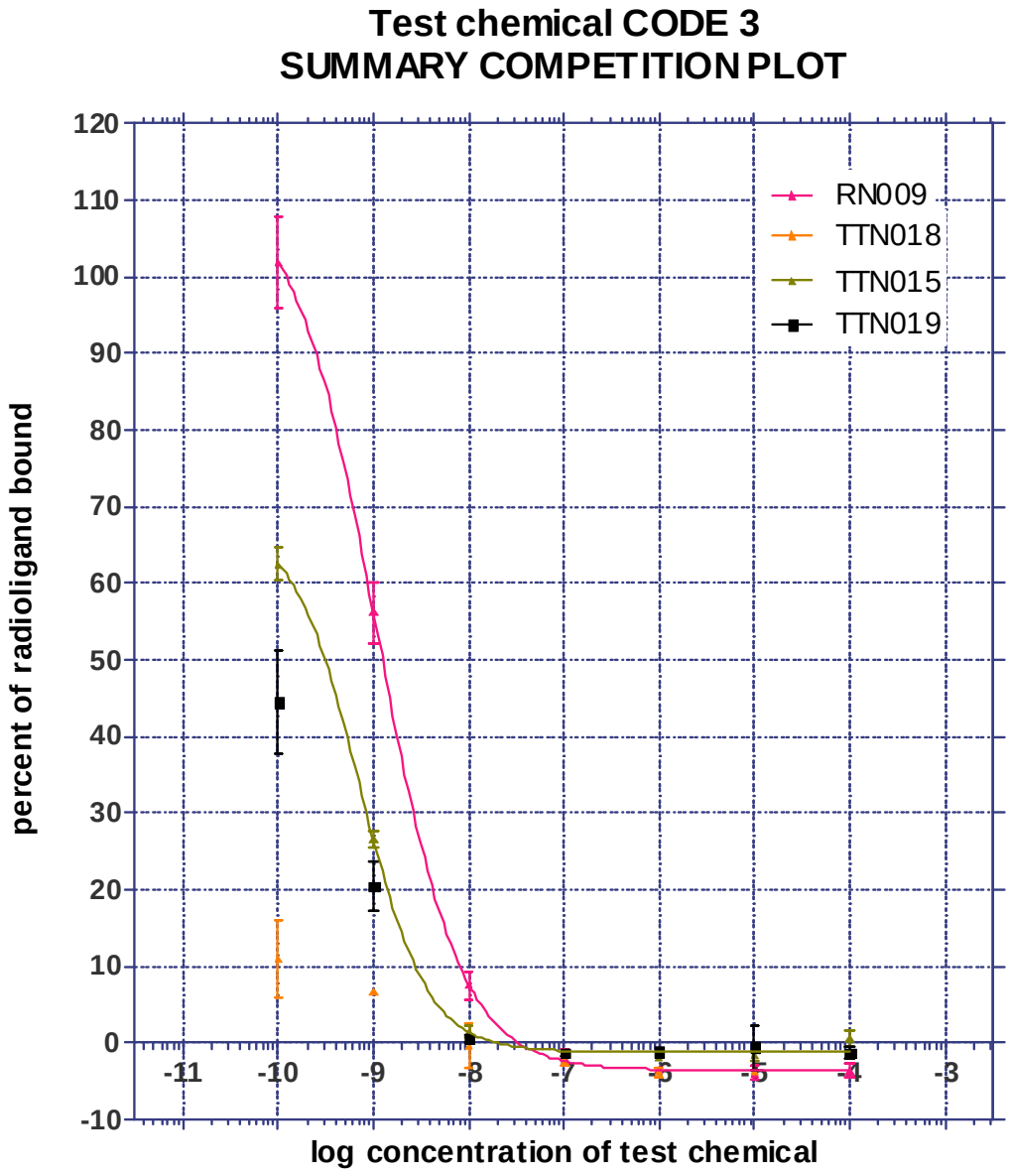
N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 4

Run No.	TTN010	TTN011	TTN013
Bottom Plateau Level	-5.069	-12.79	-2.803
Top Plateau Level	86.24	134.8	180.1
Log IC_{50}	-8.551	-8.338	-8.593
Hill Slope	-1.28	-0.8706	-1.003
SD within run	13.85	6.37	2.51
RBA*	2.600	2.009	3.396

*Relative Binding Affinity, 17β-estradiol = 1.0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SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN009
TTN018
TTN015
TTN019
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 3.

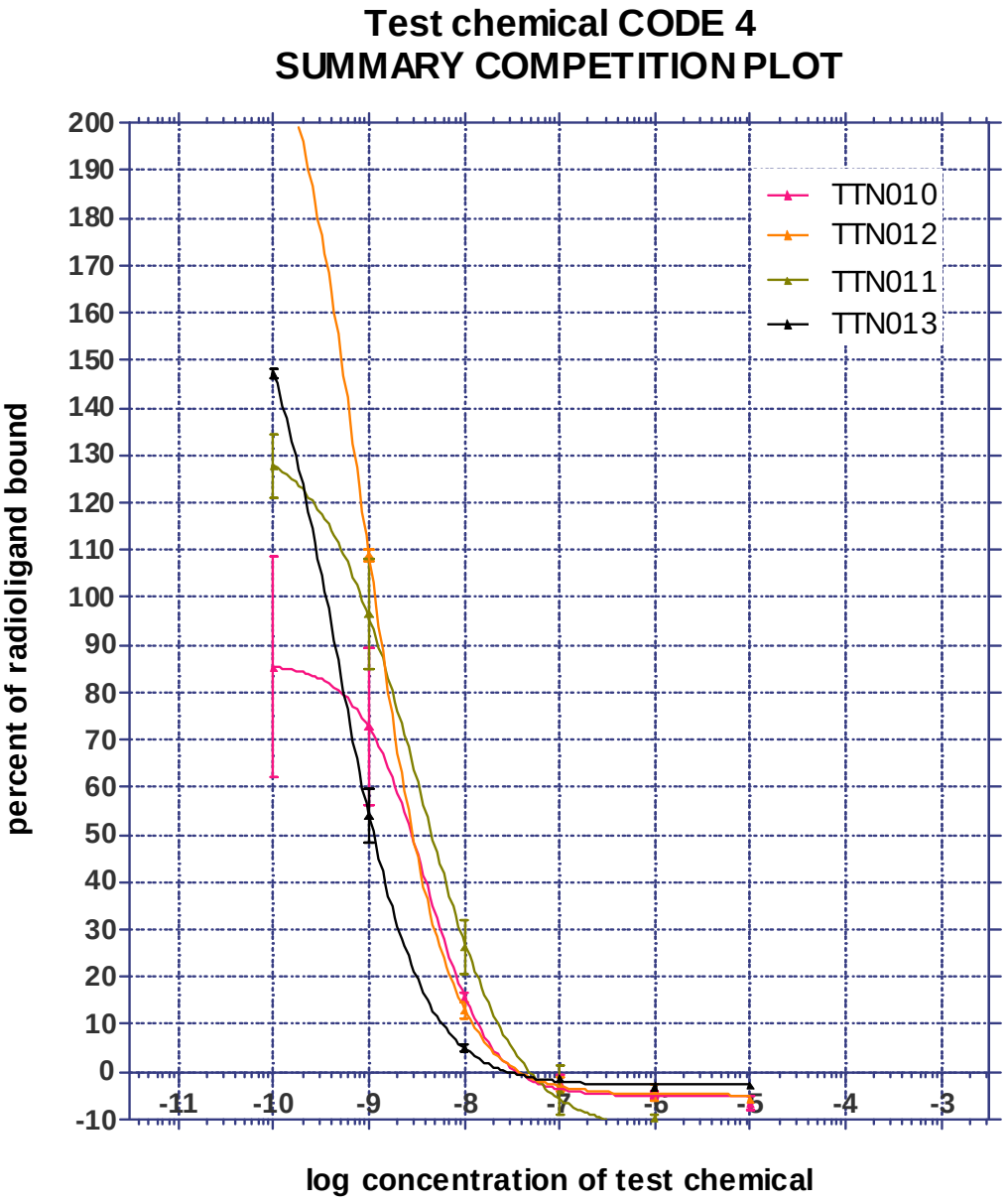

Test chemical CODE 4
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN010
TTN012
TTN011
TTN013
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 4.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 5

Run No.	TTN007	TTN008	TTN009
Bottom Plateau Level	1.557	2.121	-0.2946
Top Plateau Level	89.78	103.5	107.7
Log IC_{50}	-6.447	-6.508	-6.4
Hill Slope	-0.9321	-1.03	-0.9702
SD within run	3.64	11.6	2.9
RBA*	3.715×10^{-3}	4.436×10^{-3}	3.908×10^{-3}

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 6

Run No.	RN004	RN005	RN006
Bottom Plateau Level	0.4869	16.27	-1.399
Top Plateau Level	95.65	93.08	106.1
Log IC_{50}	-5.897	-5.853	-5.741
Hill Slope	-1.104	-2.063	-0.8889
SD within run	2.7	22.5	6.7
RBA*	8.810×10^{-4}	1.679×10^{-3}	1.183×10^{-3}

*Relative Binding Affinity, 17β-estradiol = 1.0

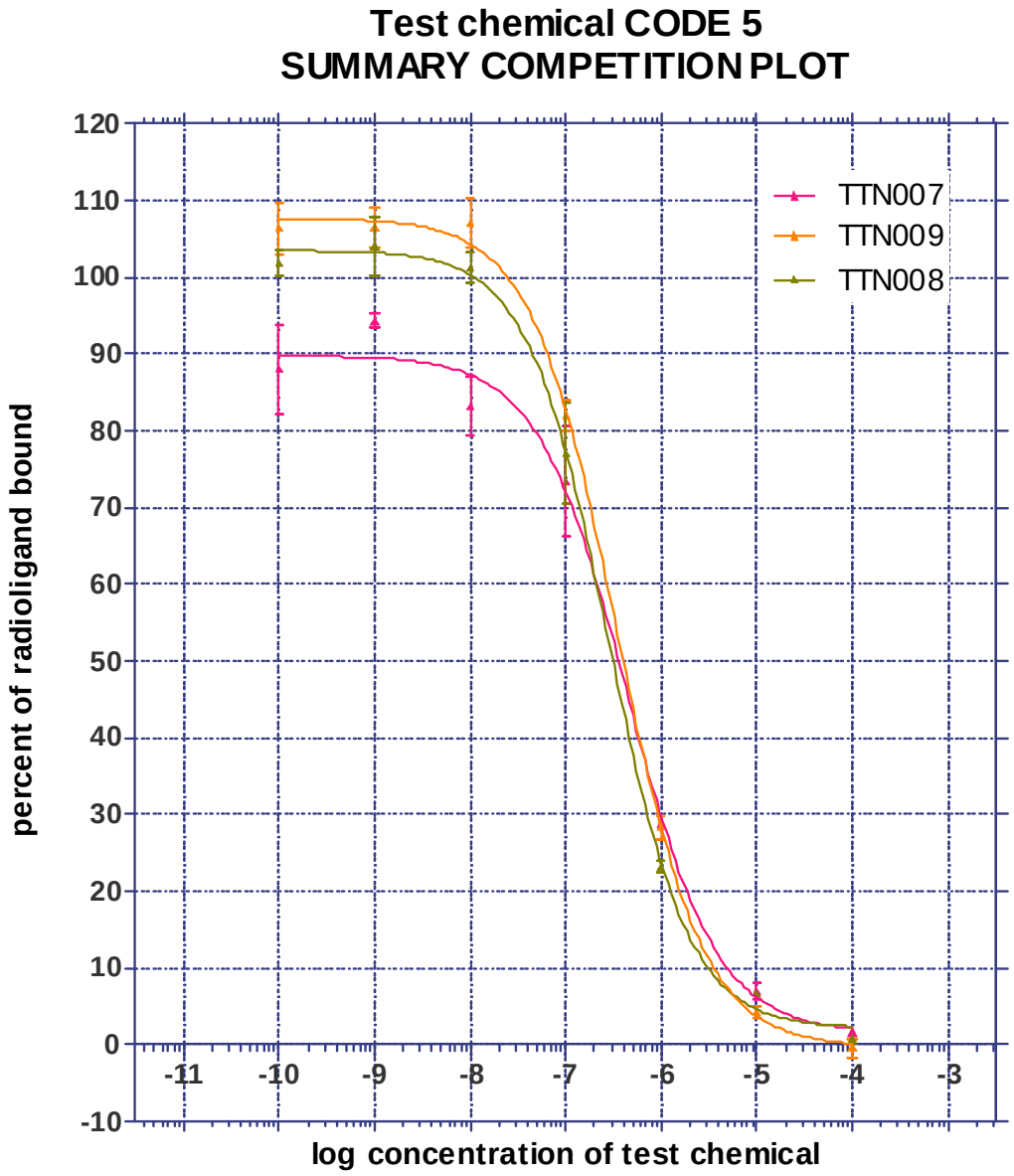

Test chemical CODE 5
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN007
TTN009
TTN008
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 5.

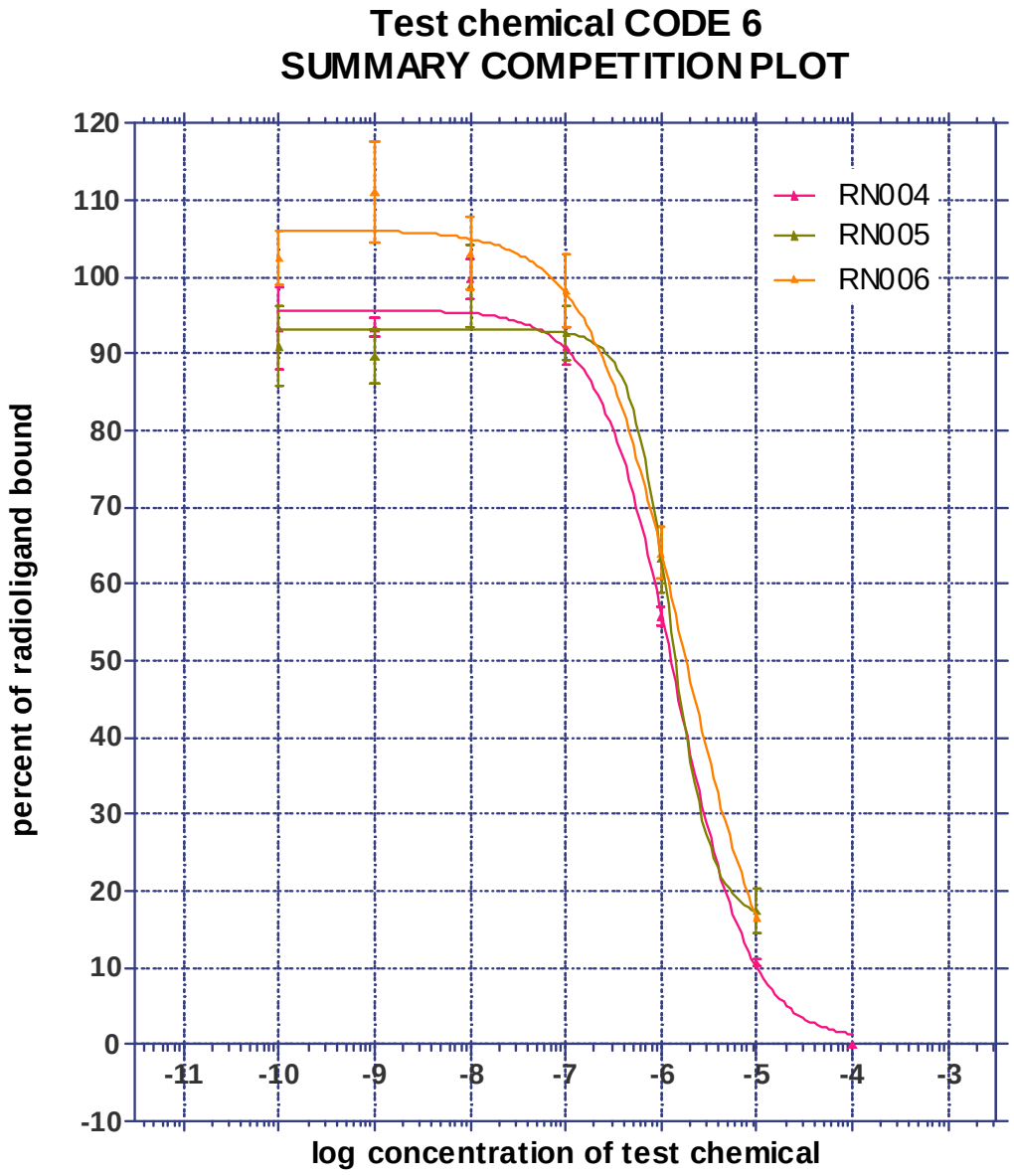

Test chemical CODE 6
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN004
RN005
RN006
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 6.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 7

Run No.	RN004	RN005	RN006
Bottom Plateau Level	-7.978	-1148000	-11.68
Top Plateau Level	95.97	97.29	100.6
Log IC_{50}	-3.796	-3.568	-3.677
Hill Slope	-1.311	-0.5173	-1.012
SD within run	8.3	5.7	3.8
RBA*	6.982×10^{-6}	8.710×10^{-6}	1.021×10^{-5}

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 8

Run No.	RN008	TTN019
Bottom Plateau Level	26.62	-5.401
Top Plateau Level	99.85	36.09
Log IC_{50}	-4.671	N/A
Hill Slope	-1.299	N/A
SD within run	26.6	4.48
RBA*	1.045×10^{-4}	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

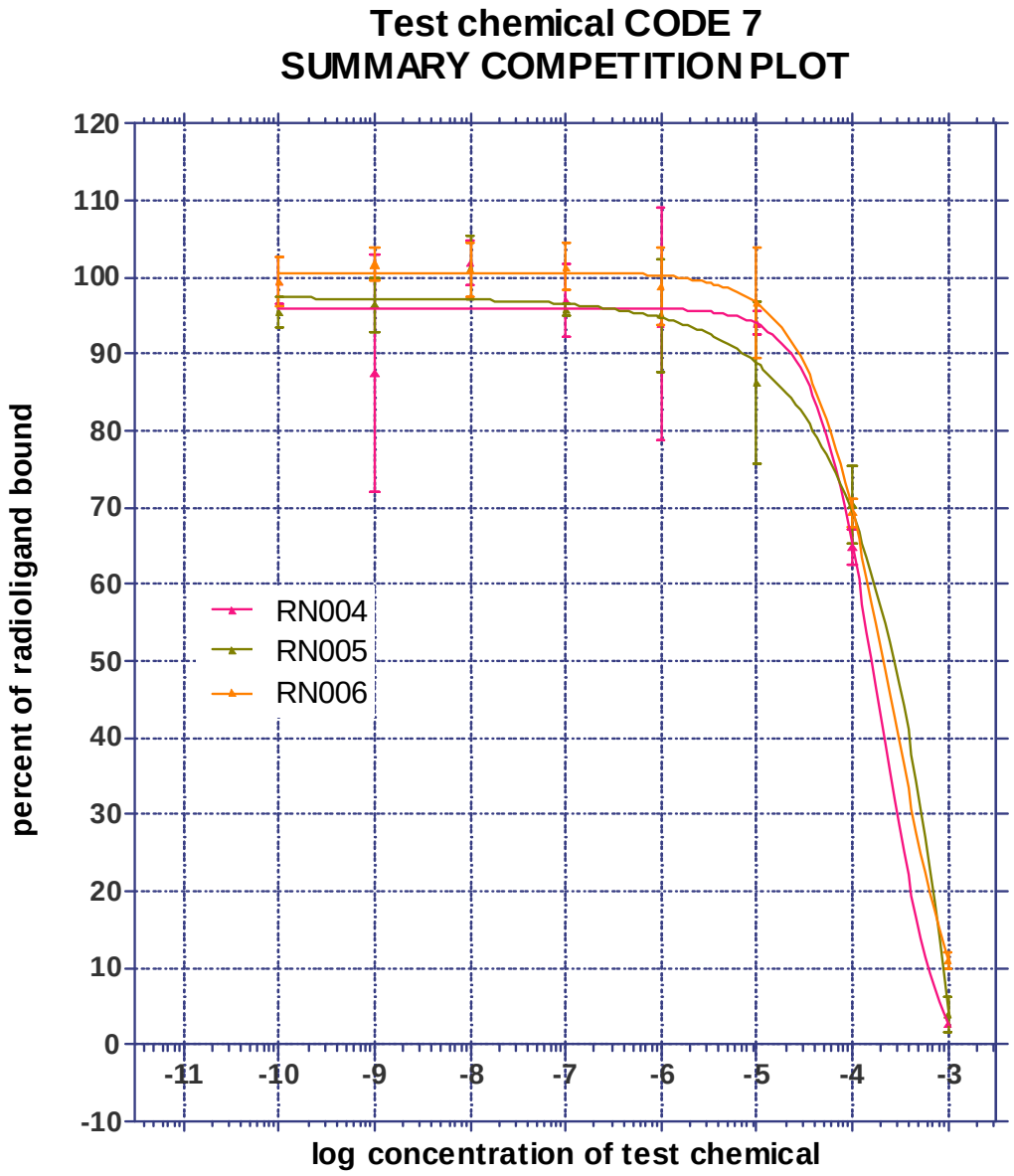

Test chemical CODE 7
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN004
RN005
RN006
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 7.

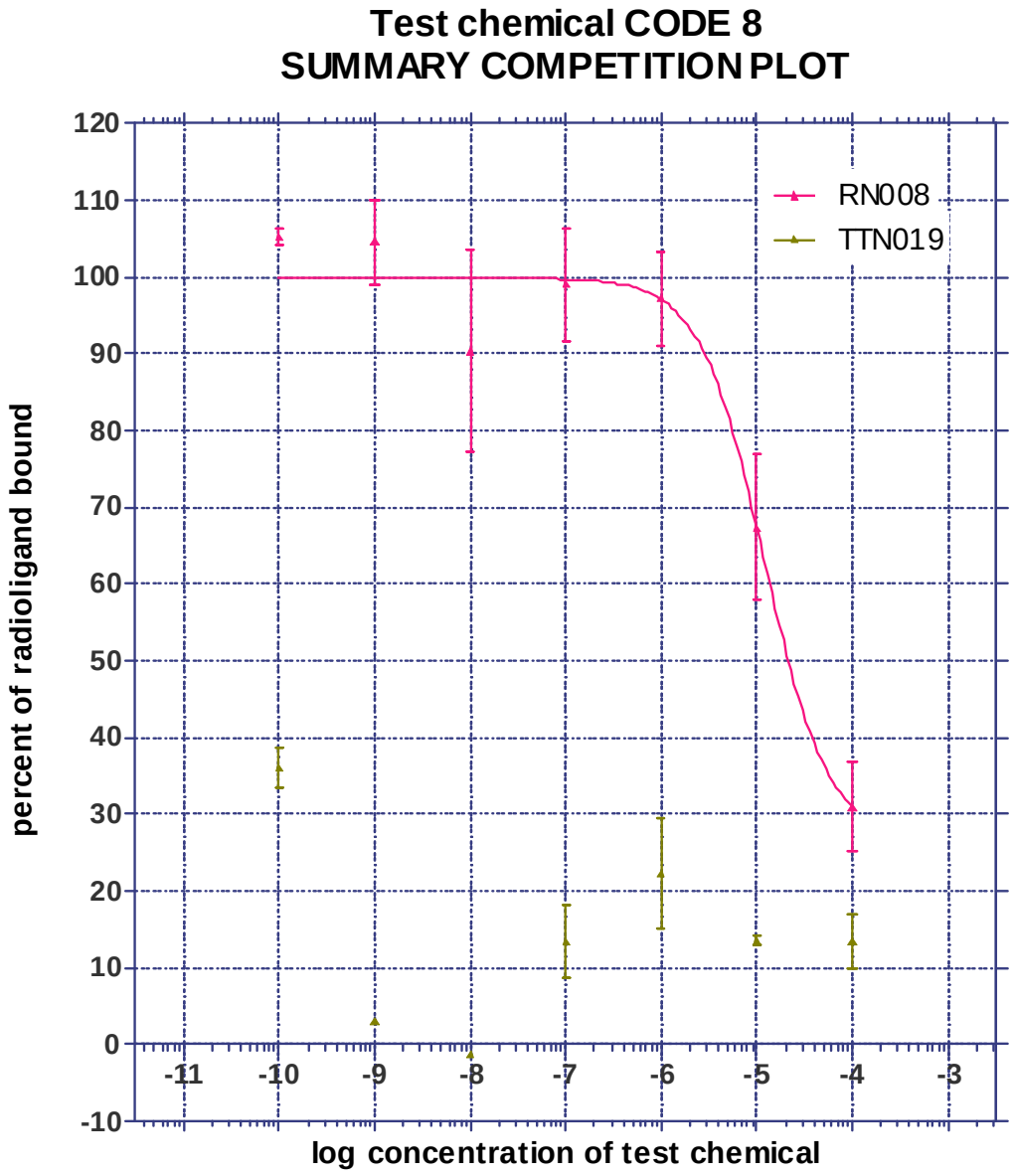

Test chemical CODE 8
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN008
TTN019
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 8.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 9

Run No.	TTN010	TTN011	TTN012
Bottom Plateau Level	55	58.63	5.09
Top Plateau Level	128.7	131.6	217.6
Log IC_{50}	N/A	N/A	N/A
Hill Slope	N/A	N/A	N/A
SD within run	5.84	9.59	17.62
RBA*	N/A	N/A	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 10

Run No.	RN008	RN009
Bottom Plateau Level	-187.1	7.623
Top Plateau Level	102.9	116.3
Log IC_{50}	-3.044	-3.779
Hill Slope	-0.7143	-1.971
SD within run	6.5	7.3
RBA*	2.466×10^{-6}	2.113×10^{-5}

*Relative Binding Affinity, 17β-estradiol = 1.0

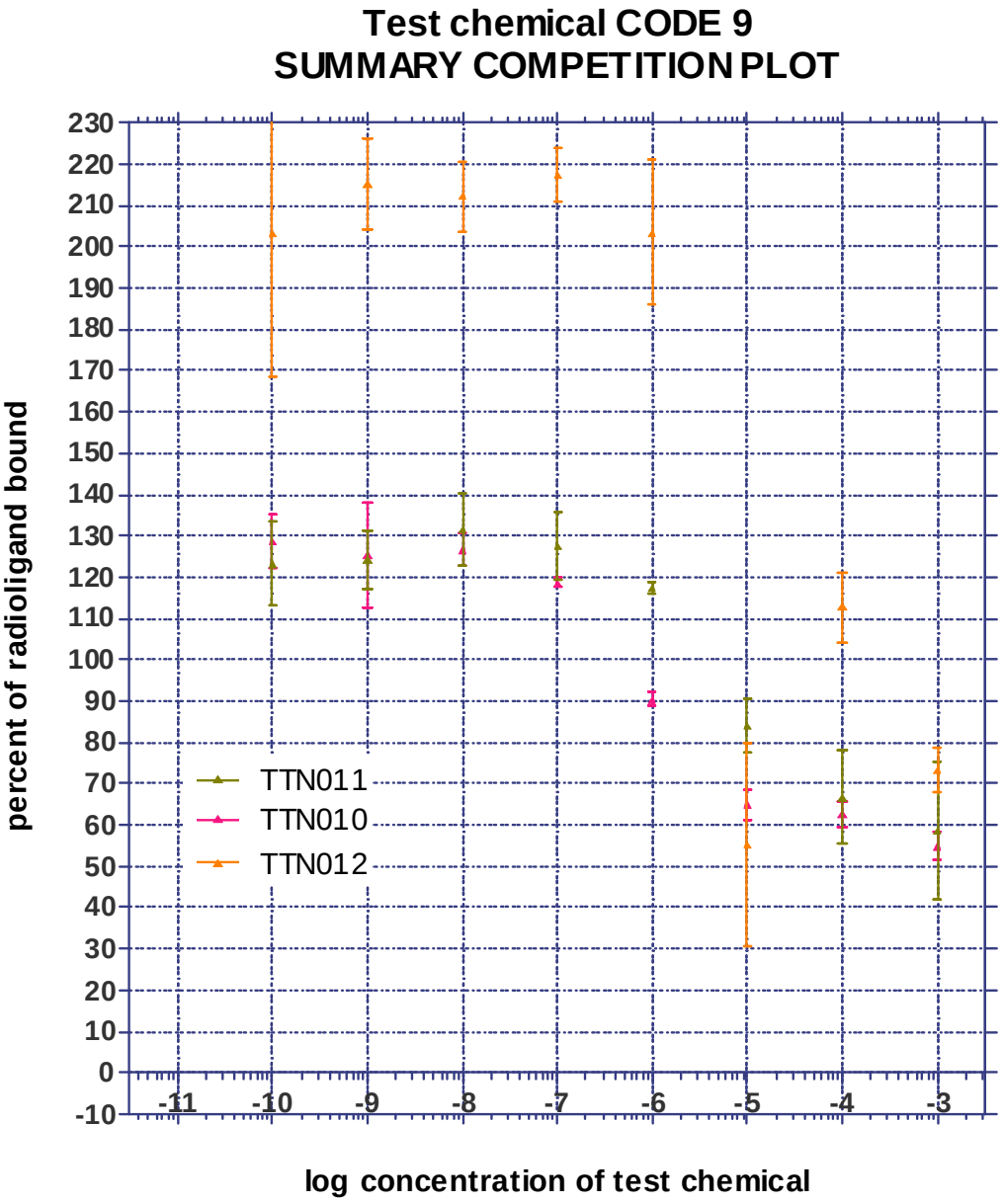

Test chemical CODE 9
SUMMARY COMPETITION PLOT
percent of radioligand bound
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
TTN011
TTN010
TTN012
log concentration of test chemical

Figure 9.

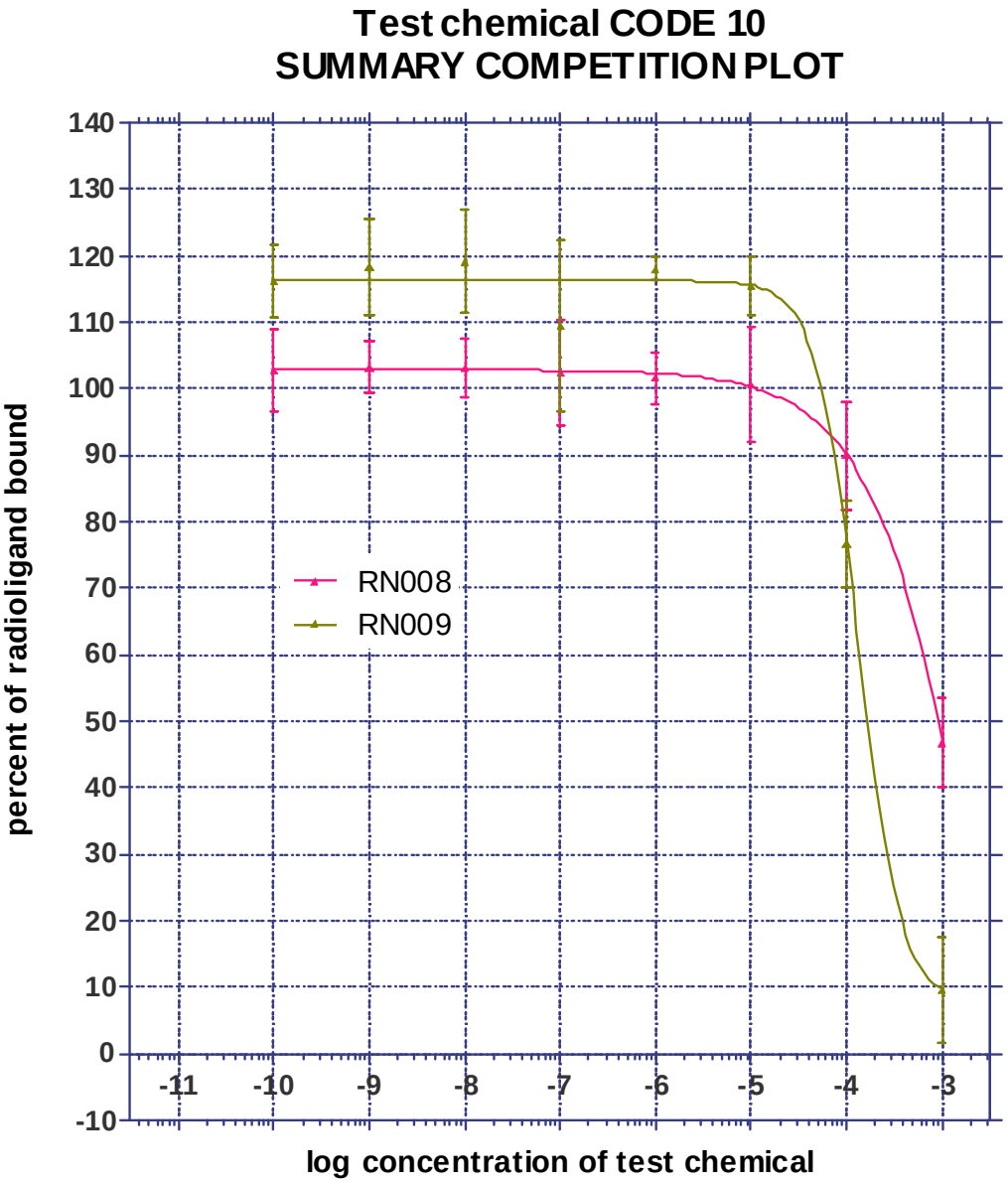

Test chemical CODE 10
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
RN008
RN009
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 10.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 11

Run No.	TTN002	TTN006	RN008
Bottom Plateau Level	-1.626	-0.5095	-.2.902
Top Plateau Level	102.3	97.61	91.93
Log IC_{50}	-5.57	-5.661	-5.223
Hill Slope	-0.8412	-1.003	-1.074
SD within run	3.79	7.8	14.5
RBA*	3.006×10^{-4}	5.047×10^{-4}	3.724×10^{-4}

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 12

Run No.	TTN001	TTN006
Bottom Plateau Level	-0.7165	-2.095
Top Plateau Level	111.9	103.7
Log IC_{50}	-6.973	-6.812
Hill Slope	-1.048	-0.905
SD within run	3.22	2.89
RBA*	1.552×10^{-2}	7.145×10^{-3}

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

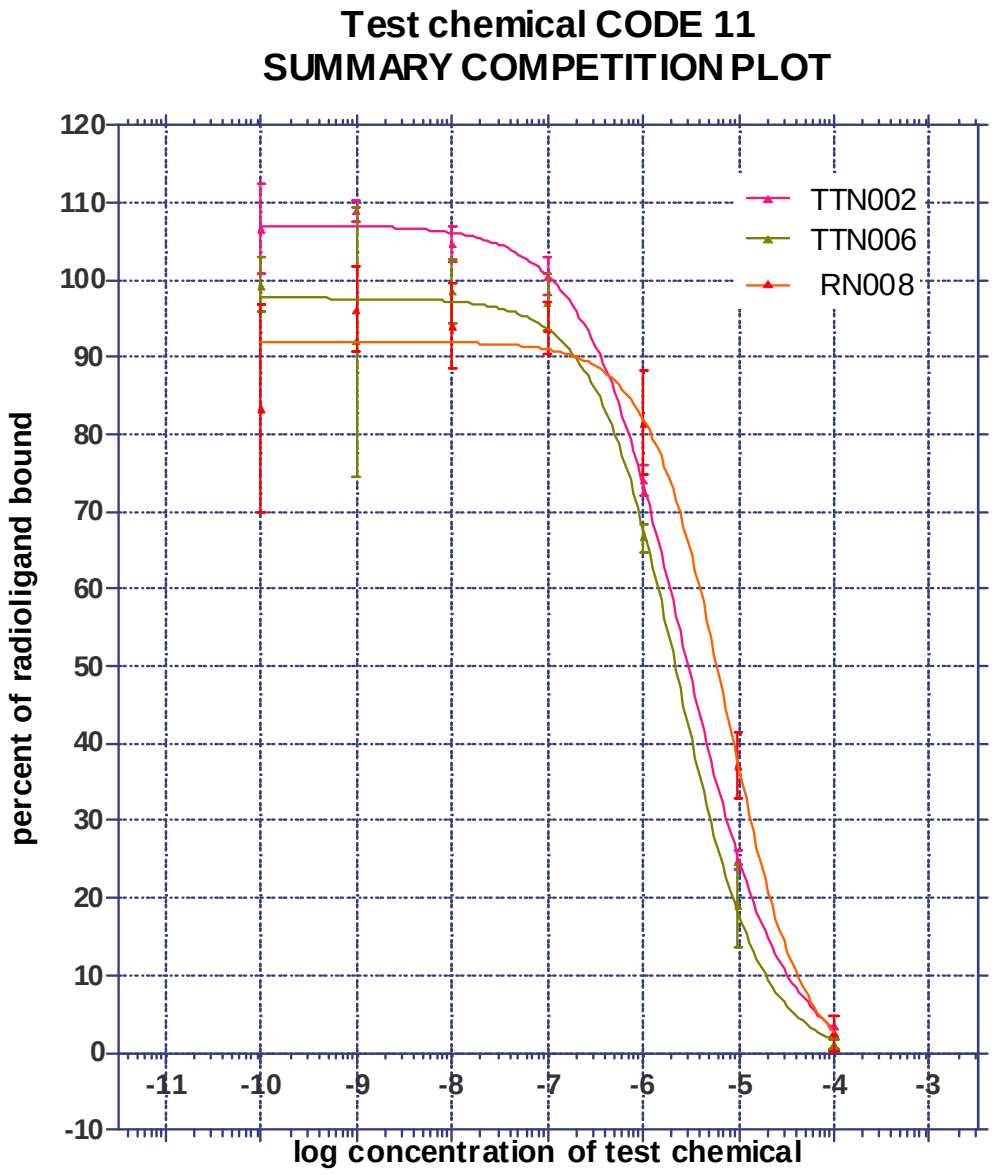

Test chemical CODE 11
SUMMARY COMPETITION PLOT
TTN002
TTN006
RN008
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 11.

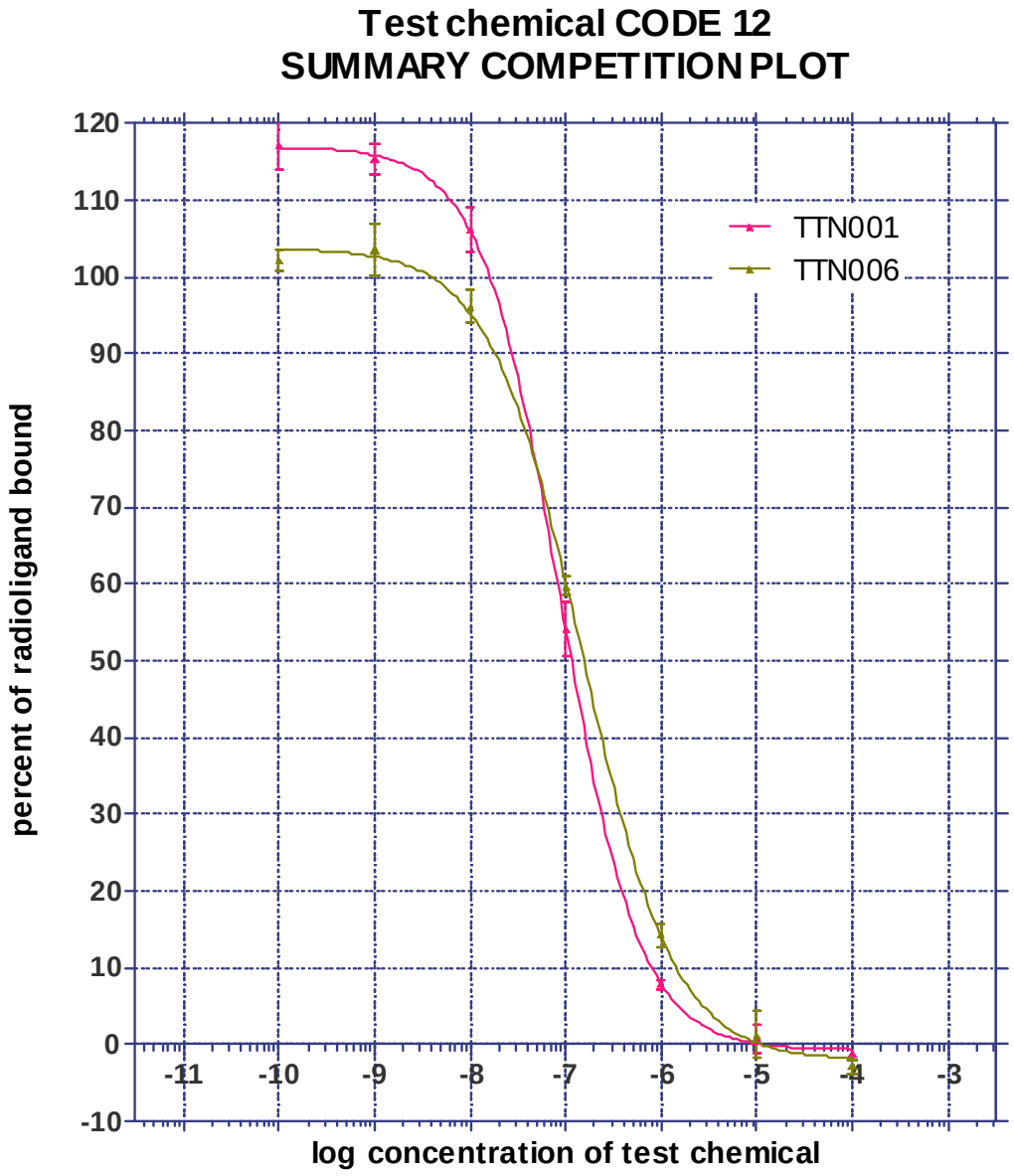

Test chemical CODE 12
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN001
TTN006
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 12.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 13

Run No.	RN005	RN006
Bottom Plateau Level	2.451	1.009
Top Plateau Level	90.49	99.76
Log IC_{50}	-5.878	-5.658
Hill Slope	-2.063	-2.048
SD within run	6.2	6.3
RBA*	1.778×10^{-3}	9.772×10^{-4}

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 14

Run No.	TTN010	TTN011	TTN014
Bottom Plateau Level	70.77	69.8	-0.5798
Top Plateau Level	134.6	129.2	114.1
Log IC_{50}	N/A	N/A	-6.596
Hill Slope	N/A	N/A	-0.3343
SD within run	10.15	19.01	36.25
RBA*	N/A	N/A	1.503×10^{-2}

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

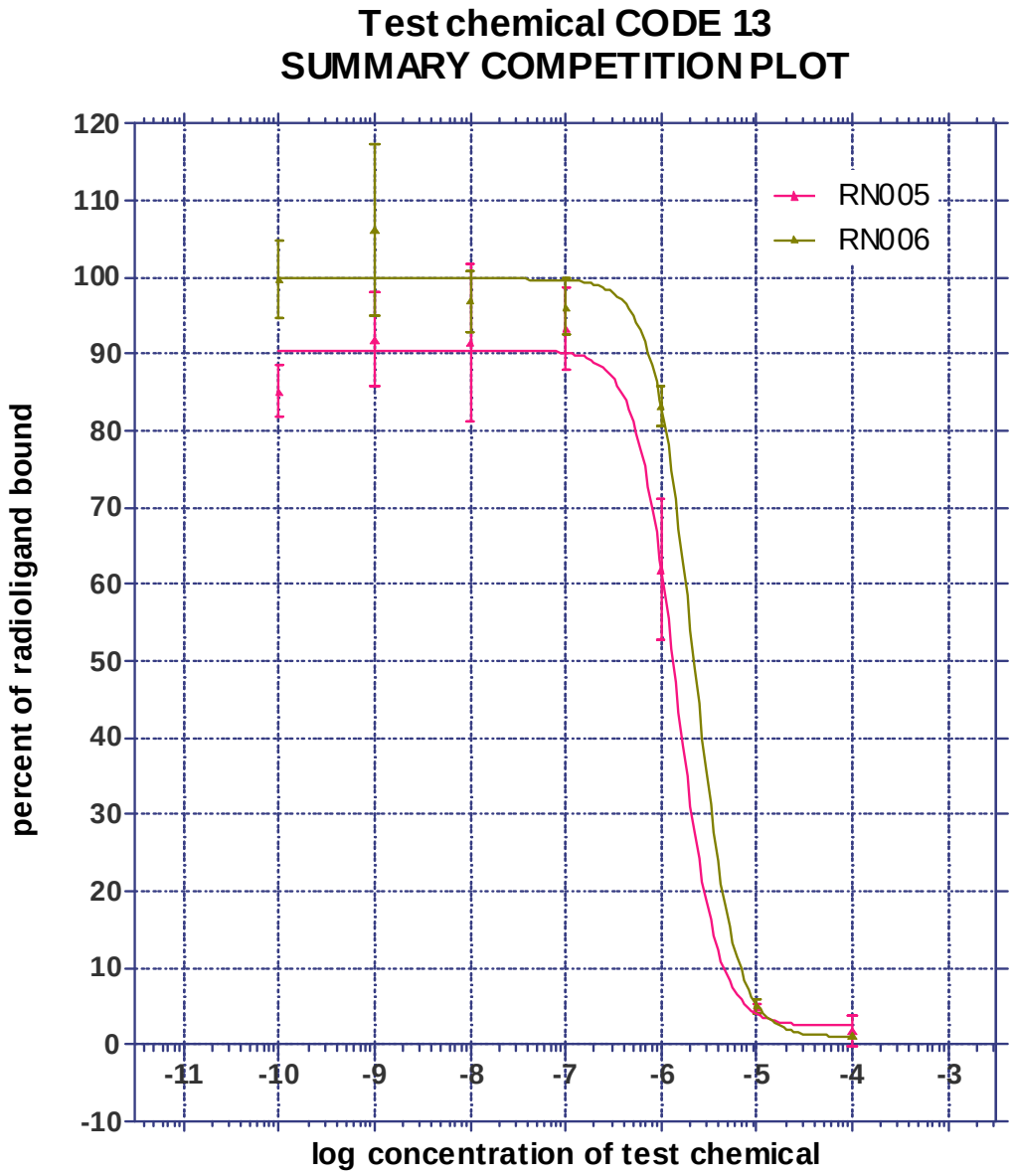

Test chemical CODE 13
SUMMARY COMPETITION PLOT
RN005
RN006
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 13.

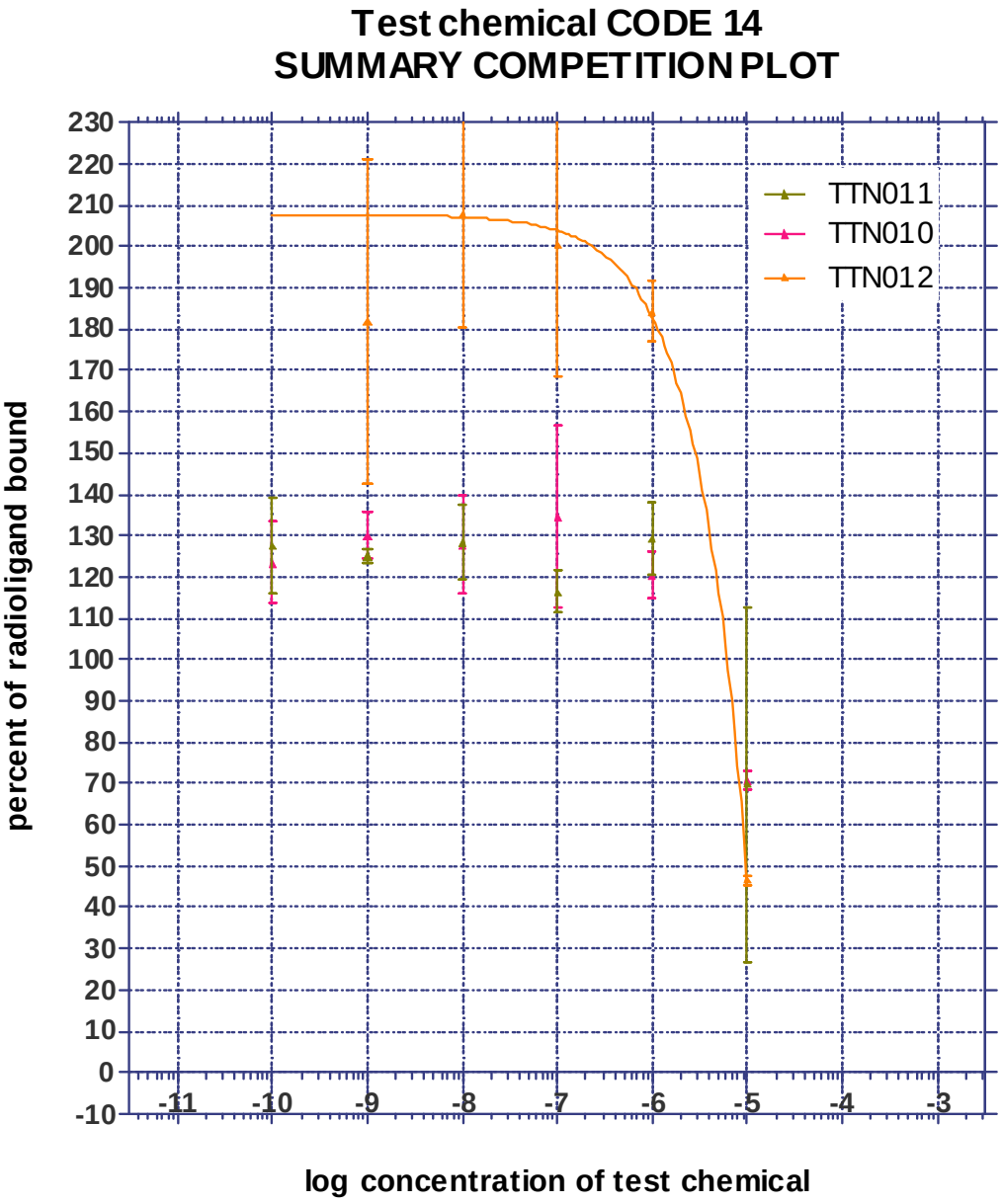

Test chemical CODE 14
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN011
TTN010
TTN012
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 14.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 15

Run No.	TTN002	TTN006
Bottom Plateau Level	-2.127	1.713
Top Plateau Level	103.7	100.5
Log IC_{50}	-5.427	-5.139
Hill Slope	-0.9262	-1.05
SD within run	3.7	4.5
RBA*	2.163×10^{-4}	1.517×10^{-4}

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 16

Run No.	TTN007	TTN008	TTN009
Bottom Plateau Level	-0.2026	-1.483	-2.558
Top Plateau Level	94.76	107.3	107.8
Log IC_{50}	-5.041	-5.111	-5.121
Hill Slope	-5.665	-1.649	-2.348
SD within run	5.55	2.8	303
RBA*	1.459×10^{-4}	1.778×10^{-4}	2.056×10^{-4}

*Relative Binding Affinity, 17β-estradiol = 1.0

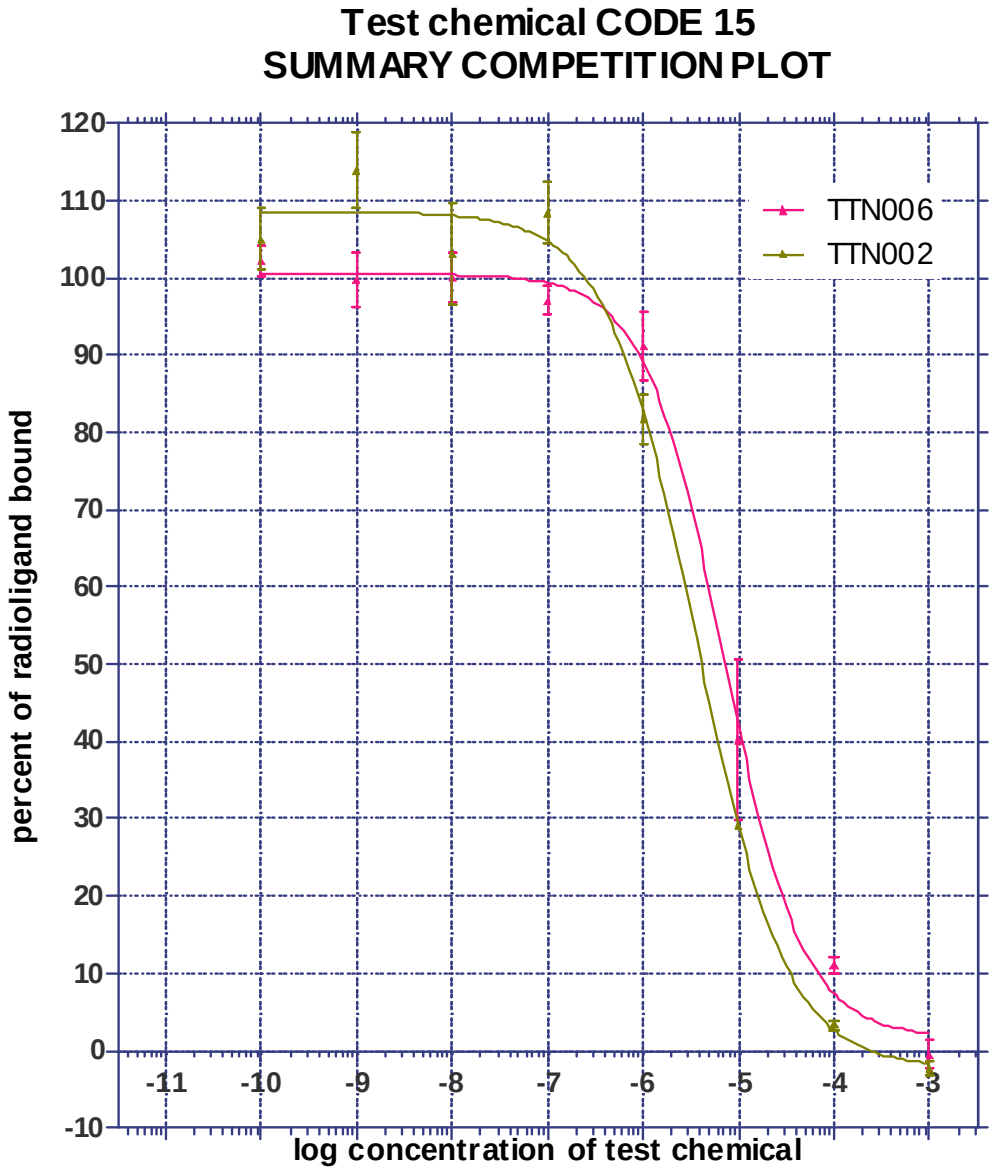

Test chemical CODE 15
SUMMARY COMPETITION PLOT
TTN006
TTN002
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 15.

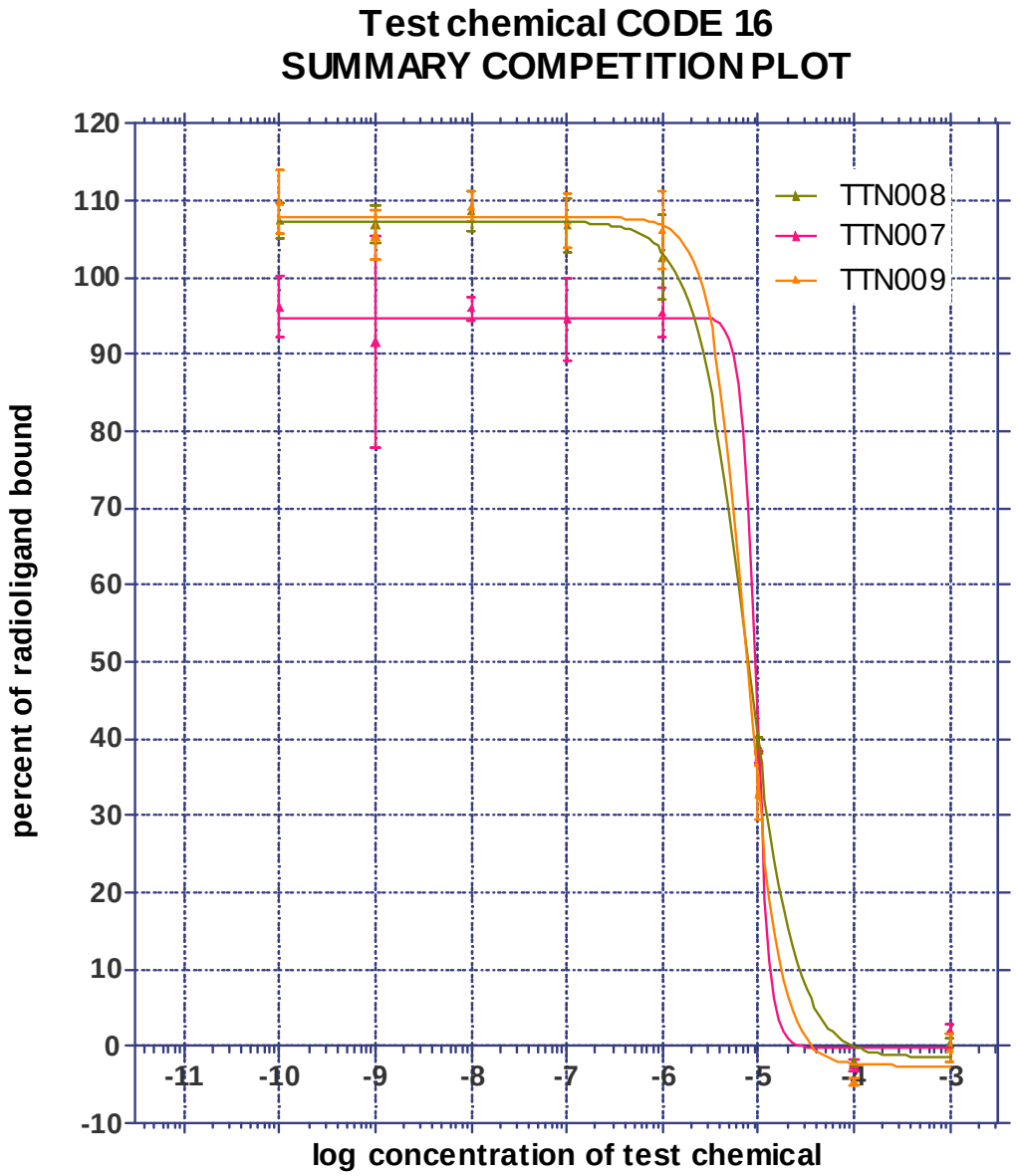

Test chemical CODE 16
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN008
TTN007
TTN009
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 16.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 17

Run No.	TTN008	TTN009
Bottom Plateau Level	-3.839	13.56
Top Plateau Level	107.8	107.2
Log IC_{50}	-5.381	-5.378
Hill Slope	-1.08	-2.001
SD within run	3.4	5.1
RBA*	3.311×10^{-4}	3.715×10^{-4}

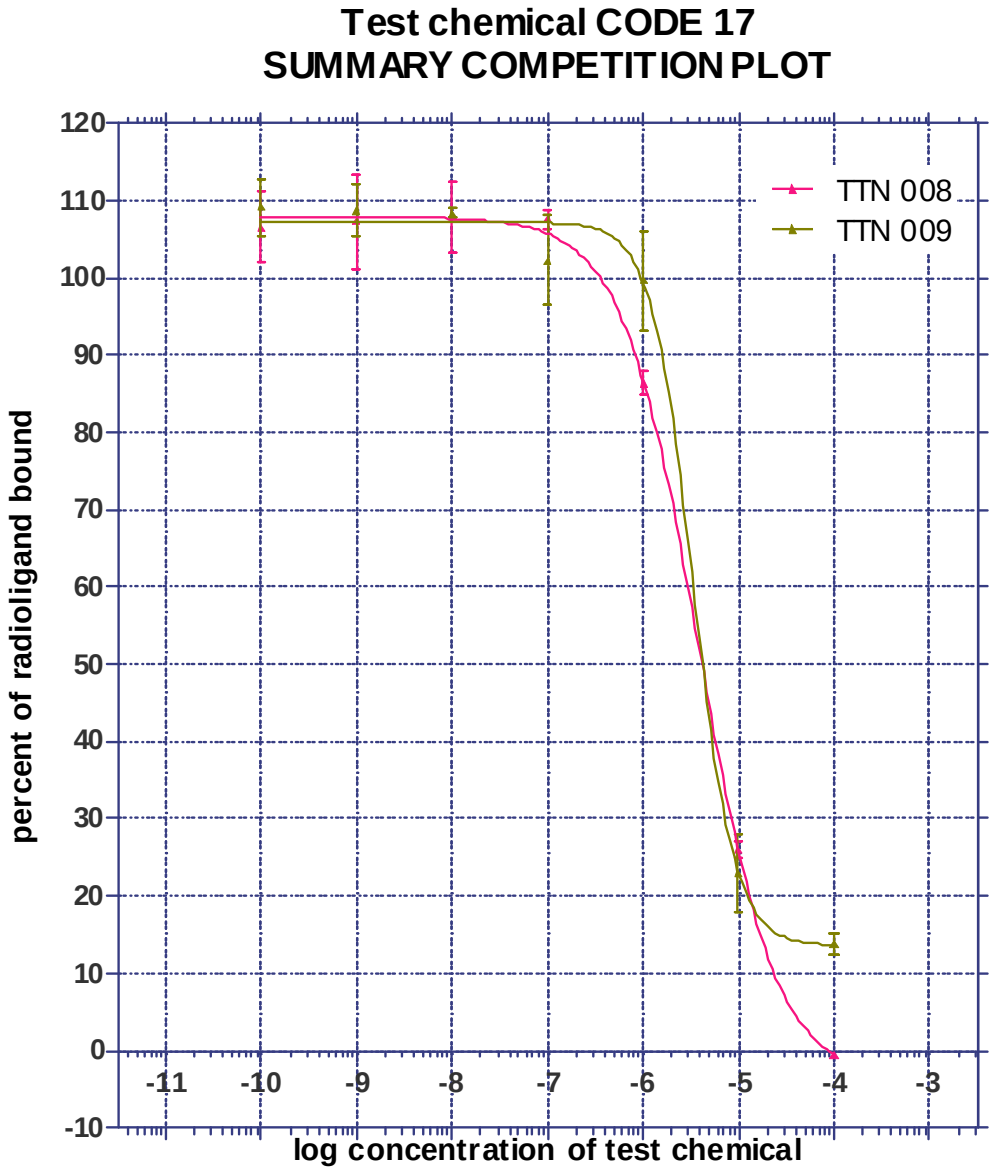
*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 18

Run No.	RN004	RN005	RN006
Bottom Plateau Level	72.94	76.43	$\sim -8.477 \times 10^{-11}$
Top Plateau Level	99.17	105.8	~ 95.42
Log IC_{50}	N/A	N/A	-3.038
Hill Slope	N/A	N/A	~ -3.400
SD within run	3	11.4	3.9
RBA*	N/A	N/A	2.344×10^{-6}

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0


Test chemical CODE 17
SUMMARY COMPETITION PLOT
TTN 008
TTN 009
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
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0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 17.

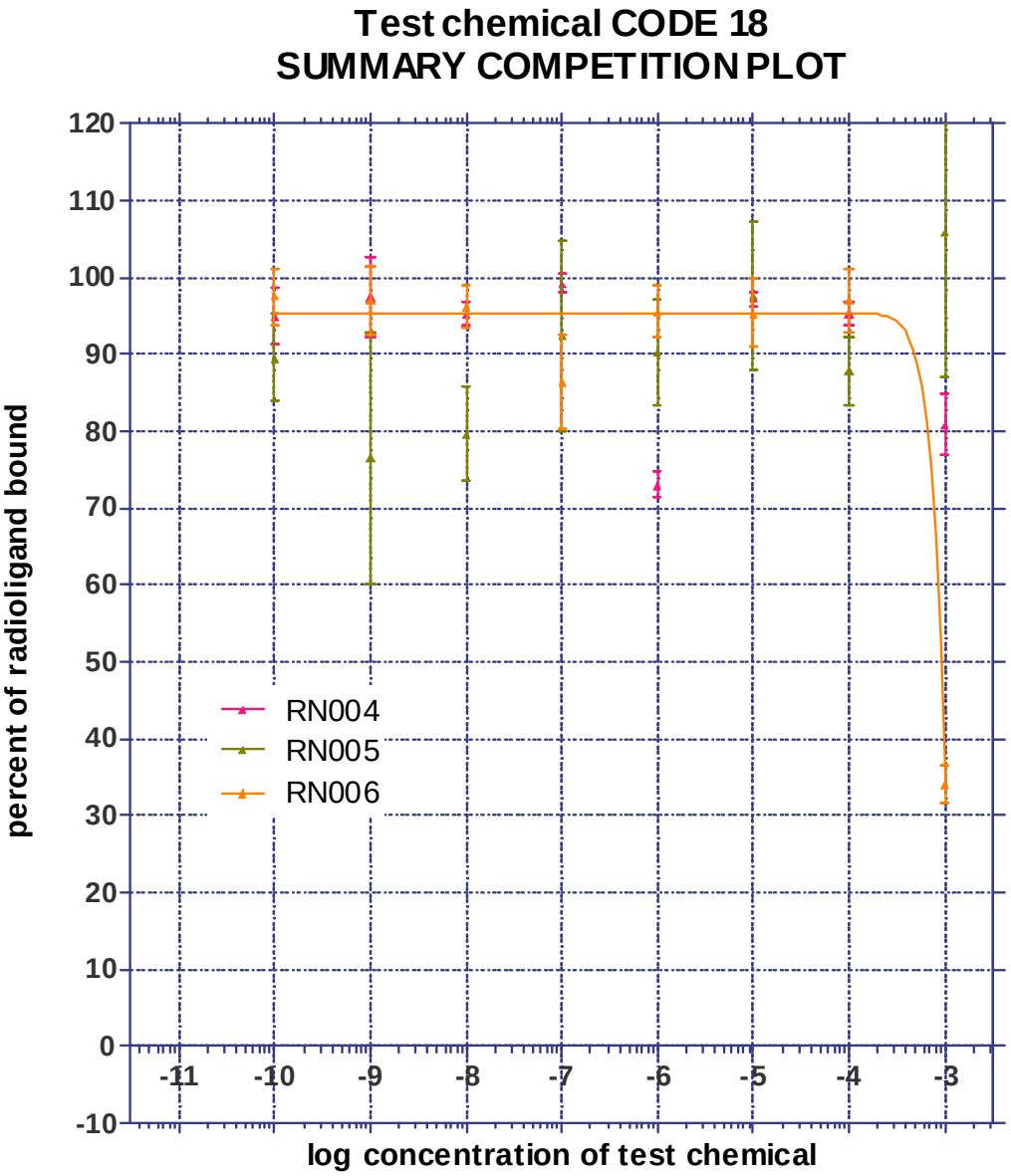

Test chemical CODE 18
SUMMARY COMPETITION PLOT
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical
RN004
RN005
RN006

Figure 18.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 19

Run No.	TTN001	TTN007
Bottom Plateau Level	-239.6	8.426
Top Plateau Level	107.9	94.99
Log IC_{50}	N/A	-3.563
Hill Slope	N/A	-2.308
SD within run	20.3	2.46
RBA*	N/A	4.853×10^{-6}

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 20

Run No.	TTN001	TTN007
Bottom Plateau Level	22.49	33.99
Top Plateau Level	68.31	59.81
Log IC_{50}	-6.985	-7.176
Hill Slope	-1.568	-30.48
SD within run	10.37	14.06
RBA*	1.698×10^{-2}	1.622×10^{-2}

*Relative Binding Affinity, 17β-estradiol = 1.0

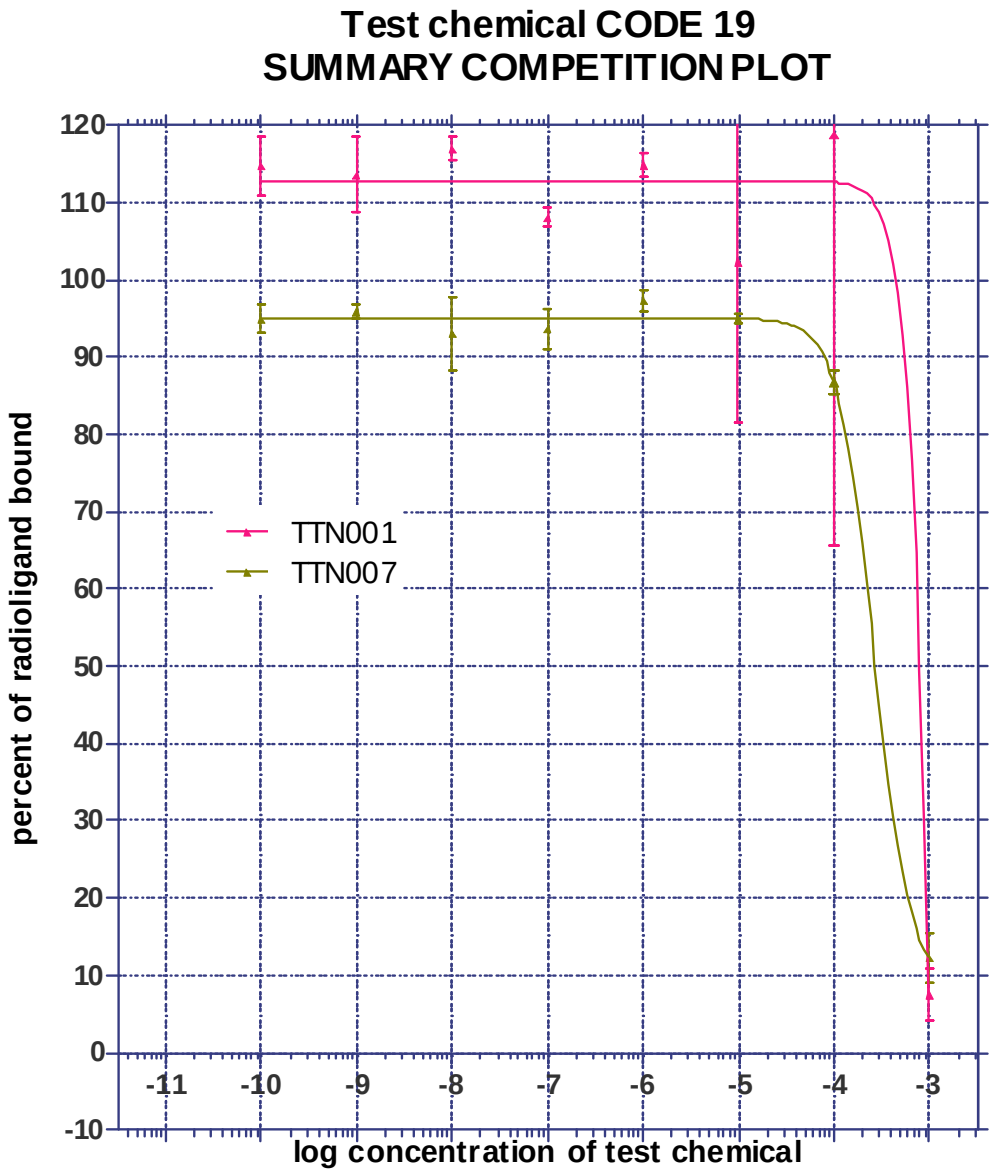

Test chemical CODE 19
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN001
TTN007
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 19.

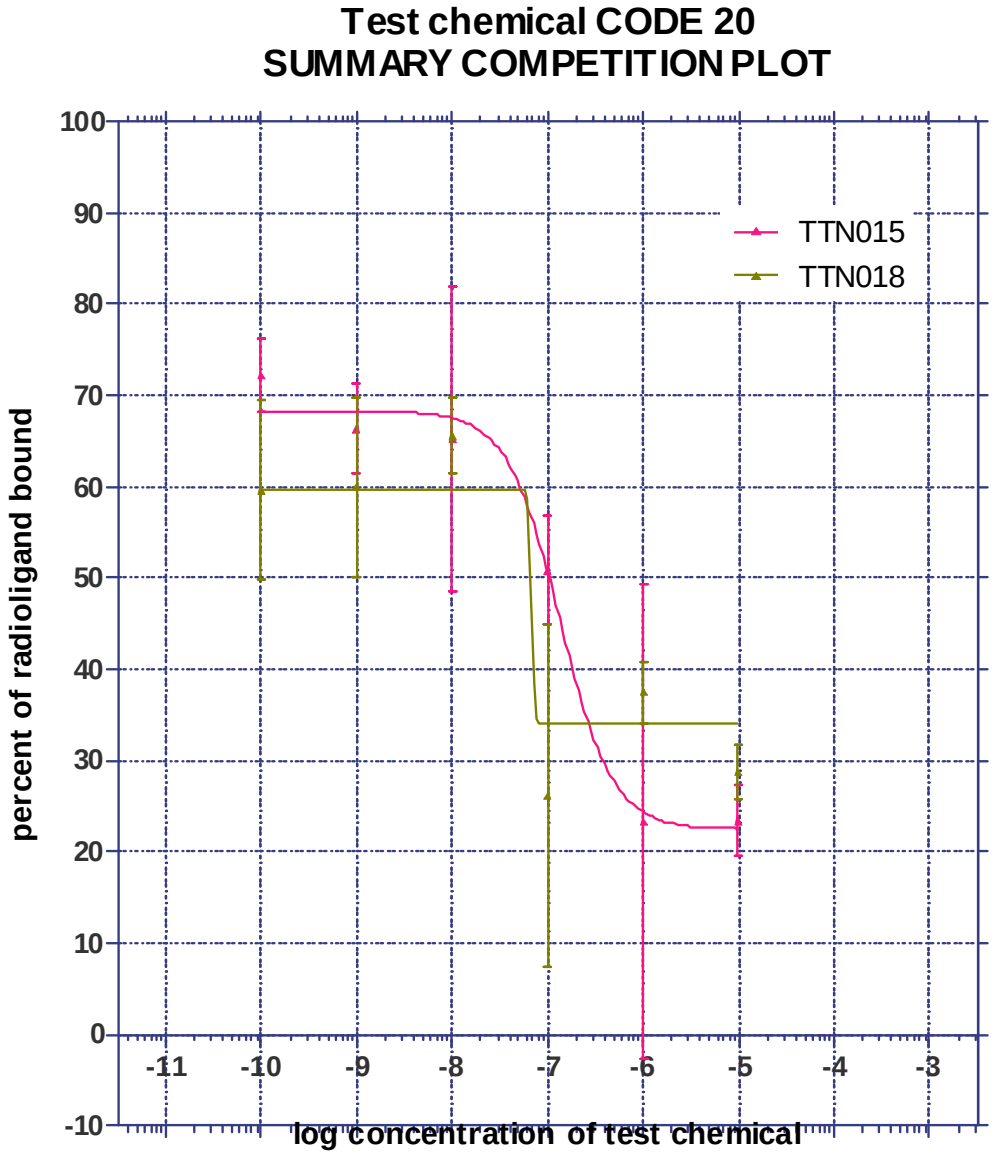

Test chemical CODE 20
SUMMARY COMPETITION PLOT
TTN015
TTN018
percent of radioligand bound
log concentration of test chemical
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 20.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code
Chemical Code No. 21

Run No.	TTN006
Bottom Plateau Level	97.44
Top Plateau Level	102.9
Log IC_{50}	N/A
Hill Slope	N/A
SD within run	4.7
RBA*	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

Chemical Code No. 22

Run No.	TTN007	TTN008	TTN009
Bottom Plateau Level	72.54	35.05	83.07
Top Plateau Level	96.48	105.3	109.4
Log IC_{50}	N/A	-3.267	N/A
Hill Slope	N/A	-1.627	N/A
SD within run	4.74	7	10.1
RBA*	N/A	2.547×10^{-6}	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

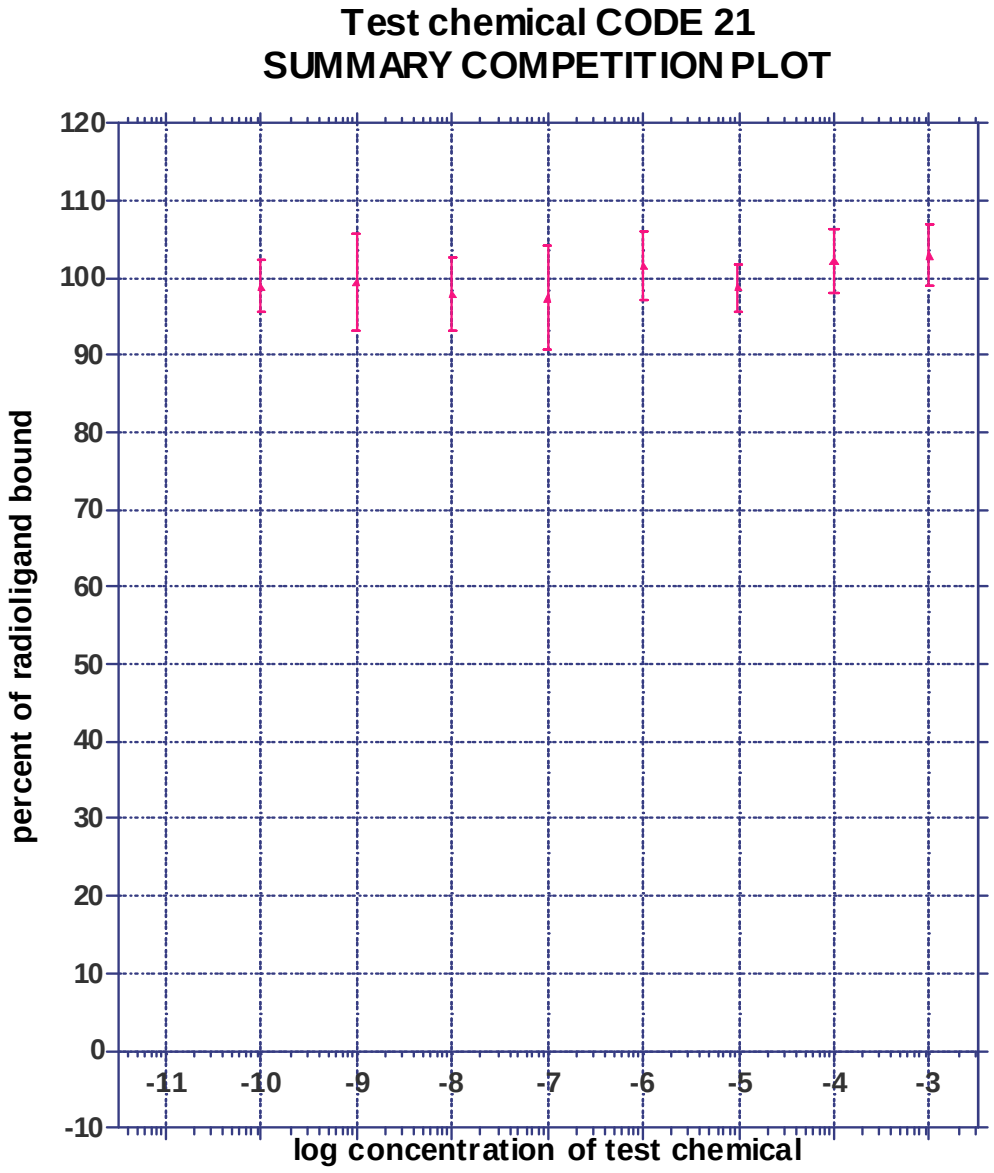

Test chemical CODE 21
SUMMARY COMPETITION PLOT
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Figure 21.

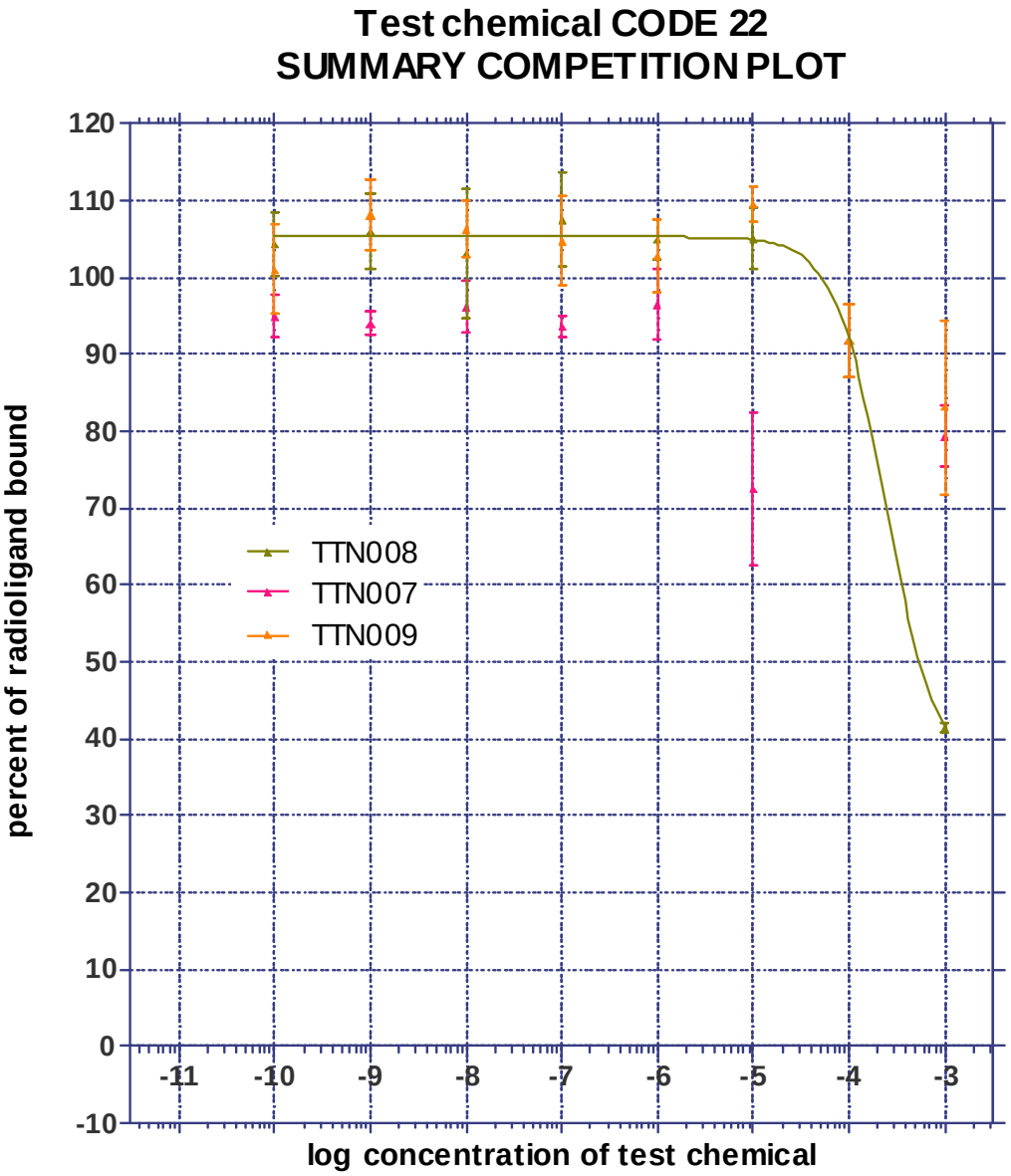

Test chemical CODE 22
SUMMARY COMPETITION PLOT
percent of radioligand bound
log concentration of test chemical
TTN008
TTN007
TTN009
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 22.

Table 3 (contd.)

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Individual Competition Binding Assay Results by Chemical Code

Chemical Code No. 23

Run No.	TTN015	TTN018	TTN019
Bottom Plateau Level	43.3	N/A	N/A
Top Plateau Level	68.43	N/A	N/A
Log IC_{50}	N/A	N/A	N/A
Hill Slope	N/A	N/A	N/A
SD within run	10.1	6.98	9.83
RBA**	N/A	N/A	N/A

N/A = Not Applicable

*Relative Binding Affinity, 17β-estradiol = 1.0

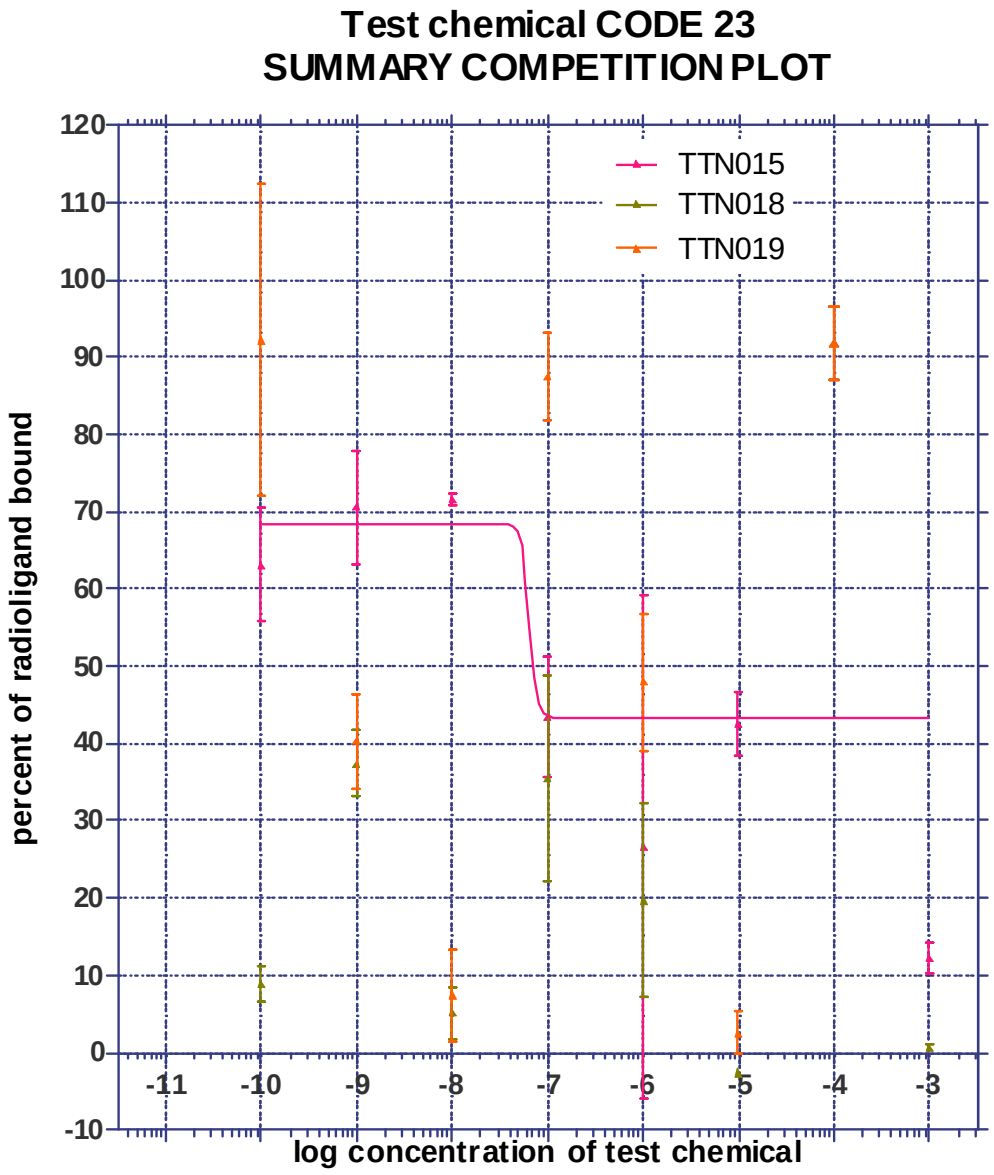

Test chemical CODE 23
SUMMARY COMPETITION PLOT
TTN015
TTN018
TTN019
percent of radioligand bound
log concentration of test chemical
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 23.

Table 4

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Summary Results for Standard 17β-Estradiol and Weak Positive Control Norethynodrel

Parameter	17β-Estradiol	Norethynodrel
Bottom Plateau Level	-1.2 ± 1.4*	-0.7 ± 3.9
Top Plateau Level	105.8 ± 9.1	104.2 ± 6.2
Log IC_{50}	-8.3 ± 0.2	-5.6 ± 0.2
Hill Slope	-1.02 ± 0.1	-1.1 ± 0.2
SD within run	2.8 ± 0.8	3.8 ± 1.6
RBA**	1.0	$1.4 \times 10^{-3} \pm 5.8 \times 10^{-4}$

*Values are averages of 14 assays run in triplicate ± SD. Criteria-satisfying assays were TTN001, -002, -006, -007, -008, -009, -015, -018, -019, RN004, -005, -006, -008, -009.
**Relative Binding Affinity, 17β-Estradiol = 1.0

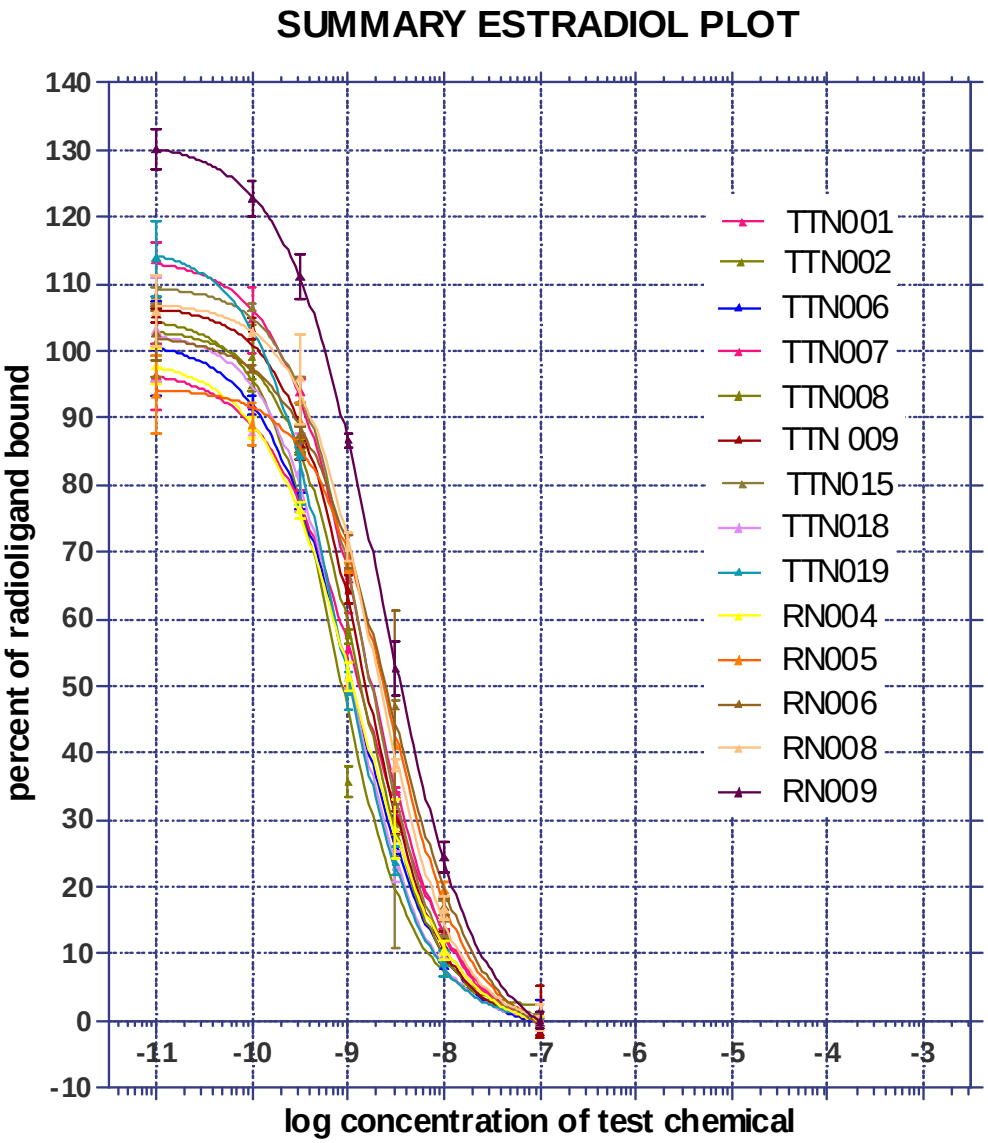

SUMMARY ESTRADIOL PLOT
percent of radioligand bound
log concentration of test chemical
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
TTN001
TTN002
TTN006
TTN007
TTN008
TTN 009
TTN015
TTN018
TTN019
RN004
RN005
RN006
RN008
RN009

Figure 24.

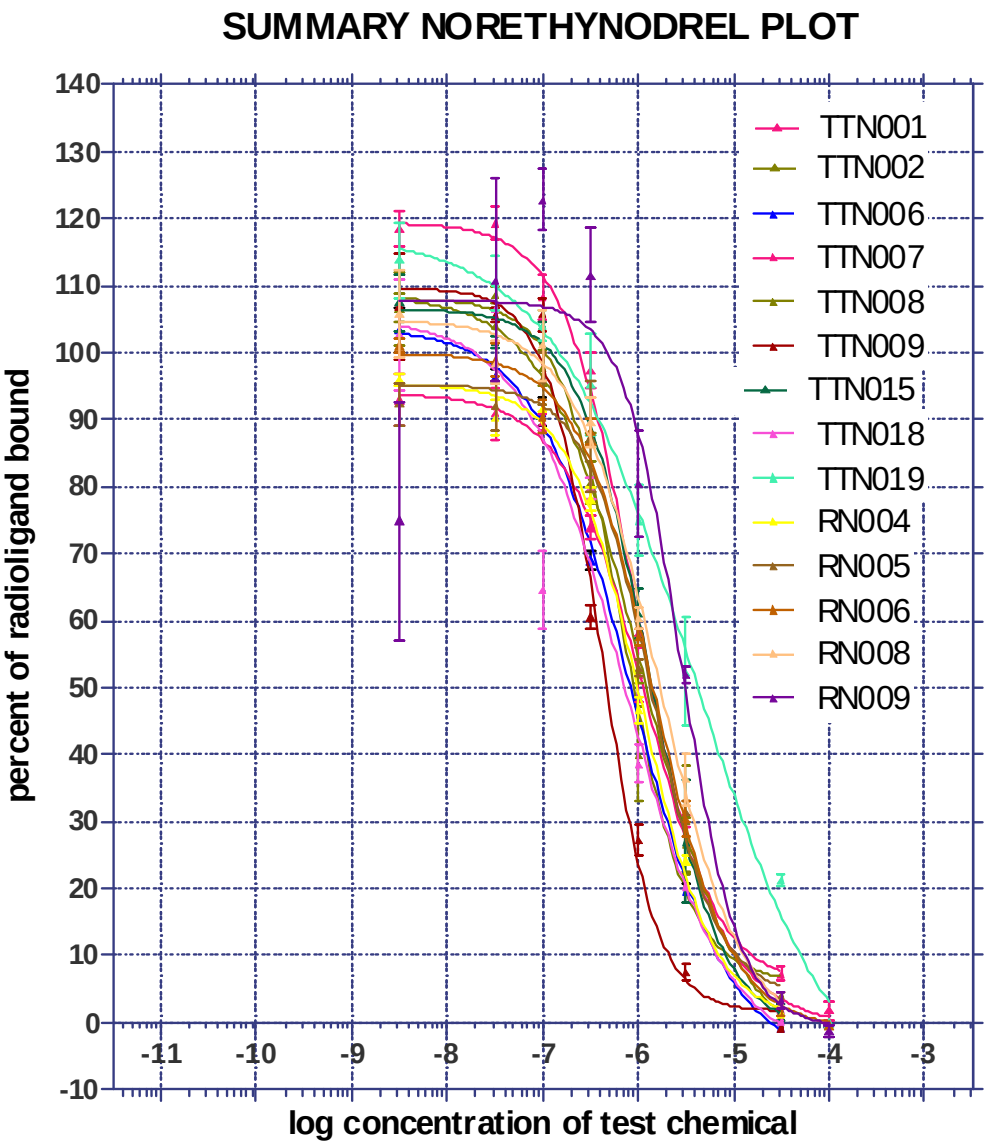

SUMMARY NORETHYNODREL PLOT
percent of radioligand bound
log concentration of test chemical
TTN001
TTN002
TTN006
TTN007
TTN008
TTN009
TTN015
TTN018
TTN019
RN004
RN005
RN006
RN008
RN009
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3

Figure 25.

APPENDIX A

Excel and Prism Files of Saturation Binding Data and Analysis for Run Identifier
TRL RUC2-18-08

Laboratory Code B

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B	
Run identification:	TTN sat. binding	
Assay start date:	3/4/2008	
Tracer lot number:	3589221/1	
Specific activity on day of assay:	105.85	Ci/mmole
Cytosol lot or vial number:	2/18/2008	
protein (cytosol) per tube:	40	ug
protein (cytosol) per tube:	0.04	mg
KD	1.40E+00	nM
Bmax	146	fmole/100 ug
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	
total volume in tubes	500	uL

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot E2 Initial Concentration (nM)*	*Hot E2 Volume (uL)*	*Hot E2 Final Concentration (nM)*	*Cold E2 Initial Concentration (nM)*	*Cold E2 Volume (uL)*	*Cold E2 Final Concentration (nM)*	*Buffer Volume (uL)*	*Receptor Volume (uL)*
1	1	H	0.3	50	0.03	—	—	—	350	100
2	2	H	0.3	50	0.03	—	—	—	350	100
3	3	H	0.3	50	0.03	—	—	—	350	100
4	1	H	0.6	50	0.06	—	—	—	350	100
5	2	H	0.6	50	0.06	—	—	—	350	100
6	3	H	0.6	50	0.06	—	—	—	350	100
7	1	H	0.8	50	0.08	—	—	—	350	100
8	2	H	0.8	50	0.08	—	—	—	350	100
9	3	H	0.8	50	0.08	—	—	—	350	100
10	1	H	1.0	50	0.10	—	—	—	350	100
11	2	H	1.0	50	0.10	—	—	—	350	100
12	3	H	1.0	50	0.10	—	—	—	350	100
13	1	H	3.0	50	0.30	—	—	—	350	100
14	2	H	3.0	50	0.30	—	—	—	350	100
15	3	H	3.0	50	0.30	—	—	—	350	100
16	1	H	6.0	50	0.60	—	—	—	350	100
17	2	H	6.0	50	0.60	—	—	—	350	100
18	3	H	6.0	50	0.60	—	—	—	350	100
19	1	H	10.0	50	1.00	—	—	—	350	100
20	2	H	10.0	50	1.00	—	—	—	350	100
21	3	H	10.0	50	1.00	—	—	—	350	100
22	1	H	30.0	50	3.00	—	—	—	350	100
23	2	H	30.0	50	3.00	—	—	—	350	100
24	3	H	30.0	50	3.00	—	—	—	350	100
25	1	HC	0.3	50	0.03	30	50	3	300	100
26	2	HC	0.3	50	0.03	30	50	3	300	100

raw dpm	*dpm for 1.5 mL*	*Use this value?*	*Notes to explain why "Use this value" is set to "FALSE"*	*Ten Percent Rule*
240	360	TRUE		9.6%
244	366	TRUE		9.8%
252	378	TRUE		10.1%
502	753	TRUE		9.1%
489	733.5	TRUE		8.8%
507	760.5	TRUE		9.2%
240	#VALUE!	NO	Pipetting error	#VALUE!
642	963	TRUE		8.8%
669	1003.5	TRUE		9.2%
858	1287	TRUE		9.4%
828	1242	TRUE		9.1%
797	1195.5	TRUE		8.8%
2075	3112.5	TRUE		7.9%
2189	3283.5	TRUE		8.4%
1784	2676	TRUE		6.8%
3415	5122.5	TRUE		6.4%
3639	5458.5	TRUE		6.8%
3507	5260.5	TRUE		6.6%
4979	7468.5	TRUE		5.6%
5062	7593	TRUE		5.7%
4568	6852	TRUE		5.1%
4143	6214.5	TRUE		1.4%
7803	11704.5	TRUE		2.6%
7613	11419.5	TRUE		2.6%
60	90	TRUE		
73	109.5	TRUE		

Output to Curve Fitting Software

Saturation X values	*Bound y values*	*NSB y values*
0.03	360.0	95.0
0.03	366.0	95.0
0.03	378.0	95.0
0.06	753.0	130.5
0.06	733.5	130.5
0.06	760.5	130.5
0.08	#VALUE!	142.5
0.08	963.0	142.5
0.08	1003.5	142.5
0.1	1287.0	144.0
0.1	1242.0	144.0
0.1	1195.5	144.0
0.3	3112.5	237.5
0.3	3283.5	237.5
0.3	2676.0	237.5
0.6	5122.5	330.0
0.6	5458.5	330.0
0.6	5260.5	330.0
1	7468.5	438.5
1	7593.0	438.5
1	6852.0	438.5
3	6214.5	1003.0
3	11704.5	1003.0
3	11419.5	1003.0

27	3	HC	0.3	50	0.03	30	50	3	300	100
28	1	HC	0.6	50	0.06	60	50	6	300	100
29	2	HC	0.6	50	0.06	60	50	6	300	100
30	3	HC	0.6	50	0.06	60	50	6	300	100
31	1	HC	0.8	50	0.08	80	50	8	300	100
32	2	HC	0.8	50	0.08	80	50	8	300	100
33	3	HC	0.8	50	0.08	80	50	8	300	100
34	1	HC	1.0	50	0.10	100	50	10	300	100
35	2	HC	1.0	50	0.10	100	50	10	300	100
36	3	HC	1.0	50	0.10	100	50	10	300	100
37	1	HC	3.0	50	0.30	300	50	30	300	100
38	2	HC	3.0	50	0.30	300	50	30	300	100
39	3	HC	3.0	50	0.30	300	50	30	300	100
40	1	HC	6.0	50	0.60	600	50	60	300	100
41	2	HC	6.0	50	0.60	600	50	60	300	100
42	3	HC	6.0	50	0.60	600	50	60	300	100
43	1	HC	10.0	50	1.00	1000	50	100	300	100
44	2	HC	10.0	50	1.00	1000	50	100	300	100
45	3	HC	10.0	50	1.00	1000	50	100	300	100
46	1	HC	30.0	50	3.00	3000	50	300	300	100
47	2	HC	30.0	50	3.00	3000	50	300	300	100
48	3	HC	30.0	50	3.00	3000	50	300	300	100
49	1	Hot	0.3	50	0.03	—	—	—	—	—
50	2	Hot	0.3	50	0.03	—	—	—	—	—
51	3	Hot	0.3	50	0.03	—	—	—	—	—
52	1	Hot	0.6	50	0.06	—	—	—	—	—
53	2	Hot	0.6	50	0.06	—	—	—	—	—
54	3	Hot	0.6	50	0.06	—	—	—	—	—
55	1	Hot	0.8	50	0.08	—	—	—	—	—
56	2	Hot	0.8	50	0.08	—	—	—	—	—
57	3	Hot	0.8	50	0.08	—	—	—	—	—
58	1	Hot	1.0	50	0.10	—	—	—	—	—
59	2	Hot	1.0	50	0.10	—	—	—	—	—
60	3	Hot	1.0	50	0.10	—	—	—	—	—
61	1	Hot	3.0	50	0.30	—	—	—	—	—
62	2	Hot	3.0	50	0.30	—	—	—	—	—
63	3	Hot	3.0	50	0.30	—	—	—	—	—
64	1	Hot	6.0	50	0.60	—	—	—	—	—
65	2	Hot	6.0	50	0.60	—	—	—	—	—
66	3	Hot	6.0	50	0.60	—	—	—	—	—
67	1	Hot	10.0	50	1.00	—	—	—	—	—
68	2	Hot	10.0	50	1.00	—	—	—	—	—
69	3	Hot	10.0	50	1.00	—	—	—	—	—
70	1	Hot	30.0	50	3.00	—	—	—	—	—
71	2	Hot	30.0	50	3.00	—	—	—	—	—
72	3	Hot	30.0	50	3.00	—	—	—	—	—

57	85.5	TRUE
83	124.5	TRUE
92	138	TRUE
86	129	TRUE
98	147	TRUE
90	135	TRUE
97	145.5	TRUE
103	154.5	TRUE
97	145.5	TRUE
88	132	TRUE
158	237	TRUE
158	237	TRUE
159	238.5	TRUE
232	348	TRUE
224	336	TRUE
204	306	TRUE
317	475.5	TRUE
289	433.5	TRUE
271	406.5	TRUE
669	1003.5	TRUE
629	943.5	TRUE
708	1062	TRUE
2502	3753	TRUE
2445	3667.5	TRUE
2556	3834	TRUE
5528	8292	TRUE
5437	8155.5	TRUE
5617	8425.5	TRUE
7350	11025	TRUE
7253	10879.5	TRUE
7185	10777.5	TRUE
8881	13321.5	TRUE
9308	13962	TRUE
9074	13611	TRUE
25818	38727	TRUE
26315	39472.5	TRUE
26443	39664.5	TRUE
52360	78540	TRUE
53617	80425.5	TRUE
54303	81454.5	TRUE
87592	131388	TRUE
89372	134058	TRUE
89158	133737	TRUE
262349	393523.5	TRUE
358592	537888	TRUE
273624	410436	TRUE

Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
specific bound/molar	bound/free %	*average total added molar*	*specific bound/molar*	*total added dpm*	*NSB dpm*	*spcific bound/dpm*	*total bound dpm*
2.25535E-12	0.0781	3.19E-11	2.2554E-12	3753.00	90.00	265.00	360.00
2.30641E-12	0.0800	3.19E-11	2.3064E-12	3667.50	109.50	271.00	366.00
2.40854E-12	0.0839	3.19E-11	2.4085E-12	3834.00	85.50	283.00	378.00
5.29795E-12	0.0826	7.06E-11	5.2979E-12	8292.00	124.50	622.50	753.00
5.13199E-12	0.0798	7.06E-11	5.1320E-12	8155.50	138.00	603.00	733.50
5.36178E-12	0.0837	7.06E-11	5.3618E-12	8425.50	129.00	630.00	760.50
#VALUE!	#VALUE!	9.27E-11	#VALUE!	11025.00	147.00	#VALUE!	#VALUE!
6.98307E-12	0.0826	9.27E-11	6.9831E-12	10879.50	135.00	820.50	963.00
7.32776E-12	0.0871	9.27E-11	7.3278E-12	10777.50	145.50	861.00	1003.50
9.72779E-12	0.0926	1.16E-10	9.7278E-12	13321.50	154.50	1143.00	1287.00
9.34481E-12	0.0886	1.16E-10	9.3448E-12	13962.00	145.50	1098.00	1242.00
8.94906E-12	0.0846	1.16E-10	8.9491E-12	13611.00	132.00	1051.50	1195.50
2.44684E-11	0.0795	3.34E-10	2.4468E-11	38727.00	237.00	2875.00	3112.50
2.59238E-11	0.0846	3.34E-10	2.5924E-11	39472.50	237.00	3046.00	3283.50
2.07535E-11	0.0666	3.34E-10	2.0753E-11	39664.50	238.50	2438.50	2676.00
4.07878E-11	0.0639	6.82E-10	4.0788E-11	78540.00	348.00	4792.50	5122.50
4.36474E-11	0.0687	6.82E-10	4.3647E-11	80425.50	336.00	5128.50	5458.50
4.19623E-11	0.0658	6.82E-10	4.1962E-11	81454.50	306.00	4930.50	5260.50
5.98306E-11	0.0560	1.13E-09	5.9831E-11	131388.00	475.50	7030.00	7468.50
6.08902E-11	0.0570	1.13E-09	6.0890E-11	134058.00	433.50	7154.50	7593.00
5.45837E-11	0.0508	1.13E-09	5.4584E-11	133737.00	406.50	6413.50	6852.00
4.43538E-11	0.0118	3.81E-09	4.4354E-11	393523.50	1003.50	5211.50	6214.50
9.10778E-11	0.0246	3.81E-09	9.1078E-11	537888.00	943.50	10701.50	11704.50
8.86523E-11	0.0239	3.81E-09	8.8652E-11	410436.00	1062.00	10416.50	11419.50

	3/5/2008
One site binding (hyperbola)	
Best-fit values	
BMAX	1.168E-10
KD	1.396E-09
Std. Error	
BMAX	1.149E-11
KD	1.739E-10
95% Confidence Intervals	
BMAX	9.289e-011 to 1.407e-010
KD	1.034e-009 to 1.757e-009
Goodness of Fit	
Degrees of Freedom	21
R^2 (unweighted)	0.8849
Weighted Sum of Squares ($1/Y^2$)	0.4174
Absolute Sum of Squares	1.937E-21
Sy.x	0.141
Data	
Number of X values	23
Number of Y replicates	
Total number of values	
Number of missing values	

Bmax molar	1.17E-10	KD molar	1.40E-09
mole to molar conversion value	0.0005	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	2.35E+17	**kd nM =**	1.40E+00
Bmax molar to Bmax moles	5.84E-14		
= DPM/((DPM/mmole)*1000)	5.84E-14		
=Bmax DPM	1.37E+04		
assay date	3/4/2008		
Bmax(dpm)	13723.81105		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	105.85		
DPM/mmole	2.35E+14		
DPM/pmole	2.35E+05		
1/(DPM/mmole)	4.26E-15		
1/(DPM/pmole)	4.26E-06		
SA(dpm/pmole)	2.35E+05		
protein/tube (ug)	40		
protein./tube(mg)	0.04		
bmax pmole	0.058400		
bmax pmole/mg	1.46		
Bmax fmole/mg	1460		
Bmax (fmole/100 ug)	146		
Bmax(fmole/100 ug)/Bmax molar	1.25E+12		

Table format: XY		X	A	B	C	D	E	F	G
		X Title	03-05-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G
		X	Y	Y	Y	Y	Y	Y	Y
1	Title	0.030000	360.000	95.0000					
2	Title	0.030000	366.000	95.0000					
3	Title	0.030000	378.000	95.0000					
4	Title	0.060000	753.000	130.5000					
5	Title	0.060000	733.500	130.5000					
6	Title	0.060000	760.500	130.5000					
7	Title	0.080000		142.5000					
8	Title	0.080000	963.000	142.5000					
9	Title	0.080000	1003.500	142.5000					
10	Title	0.100000	1287.000	144.0000					
11	Title	0.100000	1242.000	144.0000					
12	Title	0.100000	1195.500	144.0000					
13	Title	0.300000	3112.500	237.5000					
14	Title	0.300000	3283.500	237.5000					
15	Title	0.300000	2676.000	237.5000					
16	Title	0.600000	5122.500	330.0000					
17	Title	0.600000	5458.500	330.0000					
18	Title	0.600000	5260.500	330.0000					
19	Title	1.000000	7468.500	438.5000					
20	Title	1.000000	7593.000	438.5000					
21	Title	1.000000	6852.000	438.5000					
22	Title	3.000000	6214.500	1003.0000					
23	Title	3.000000	11704.500	1003.0000					
24	Title	3.000000	11419.500	1003.0000					

	H	I	J	K	L
	Data Set-H	Data Set-I	Data Set-J	Data Set-K	Data Set-L
	Y	Y	Y	Y	Y
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Table format: XY		X	A	B	C	D	E	F
		X Title	03-05-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	15.000000	3753.000					
2	Title	15.000000	3667.500					
3	Title	15.000000	3834.000					
4	Title	30.000000	8292.000					
5	Title	30.000000	8155.500					
6	Title	30.000000	8425.500					
7	Title	40.000000	11025.000					
8	Title	40.000000	10879.500					
9	Title	40.000000	10777.500					
10	Title	50.000000	13321.500					
11	Title	50.000000	13962.000					
12	Title	50.000000	13611.000					
13	Title	150.000000	38727.000					
14	Title	150.000000	39472.500					
15	Title	150.000000	39664.500					
16	Title	300.000000	78540.000					
17	Title	300.000000	80425.500					
18	Title	300.000000	81454.500					
19	Title	500.000000	131388.000					
20	Title	500.000000	134058.000					
21	Title	500.000000	133737.000					
22	Title	1500.000000	393523.500					
23	Title	1500.000000	537888.000					
24	Title	1500.000000	410436.000					

Table format: XY		X	A	B	C	D	E	F
		X Title	03-05-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	90.00					
2	Title	0.030000	109.50					
3	Title	0.030000	85.50					
4	Title	0.060000	124.50					
5	Title	0.060000	138.00					
6	Title	0.060000	129.00					
7	Title	0.080000	147.00					
8	Title	0.080000	135.00					
9	Title	0.080000	145.50					
10	Title	0.100000	154.50					
11	Title	0.100000	145.50					
12	Title	0.100000	132.00					
13	Title	0.300000	237.00					
14	Title	0.300000	237.00					
15	Title	0.300000	238.50					
16	Title	0.600000	348.00					
17	Title	0.600000	336.00					
18	Title	0.600000	306.00					
19	Title	1.000000	475.50					
20	Title	1.000000	433.50					
21	Title	1.000000	406.50					
22	Title	3.000000	1003.50					
23	Title	3.000000	943.50					
24	Title	3.000000	1062.00					

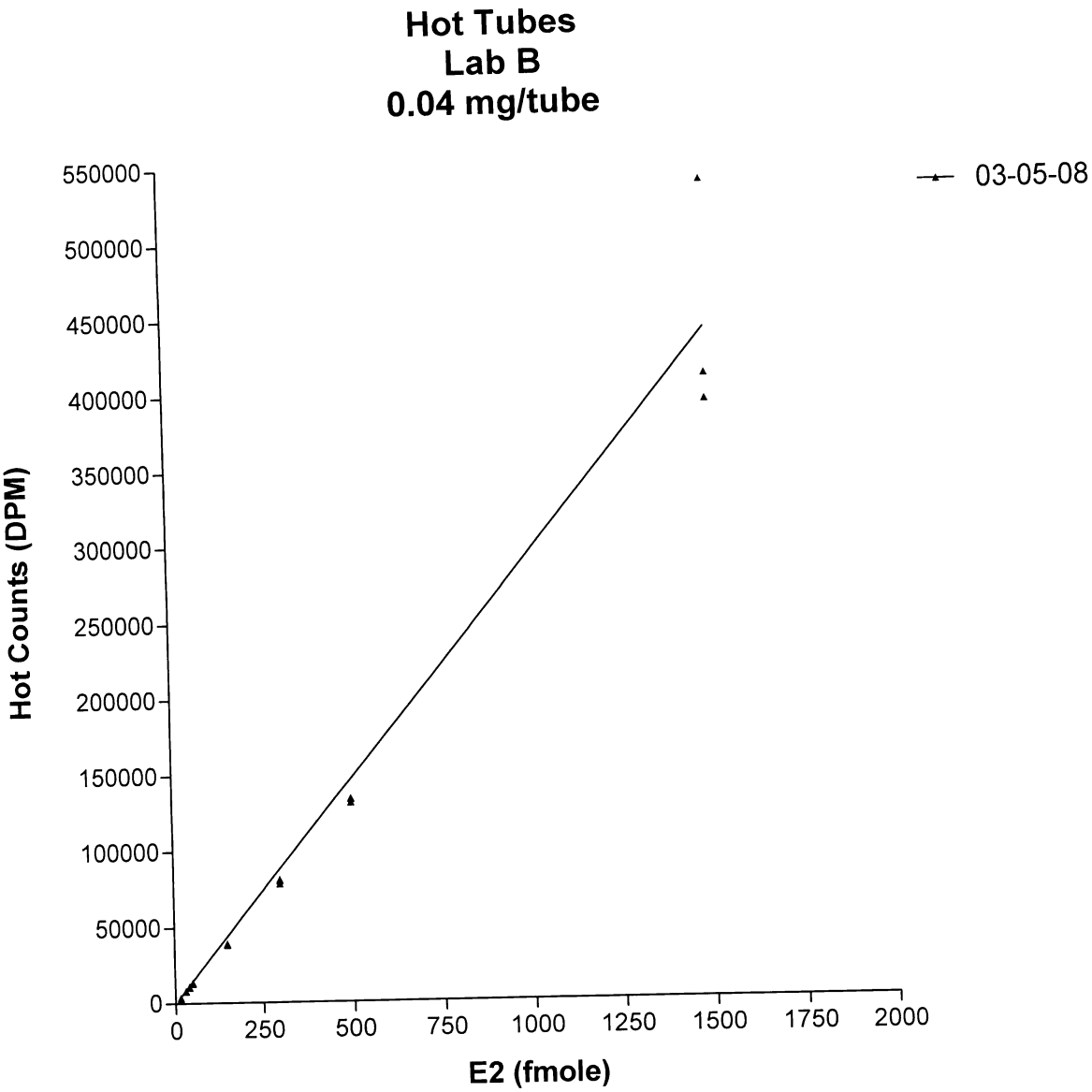
Table format: XY		X	A	B	C	D	E	F
		X Title	03-05-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	265.00000					
2	Title	0.030000	271.00000					
3	Title	0.030000	283.00000					
4	Title	0.060000	622.50000					
5	Title	0.060000	603.00000					
6	Title	0.060000	630.00000					
7	Title	0.080000						
8	Title	0.080000	820.50000					
9	Title	0.080000	861.00000					
10	Title	0.100000	1143.00000					
11	Title	0.100000	1098.00000					
12	Title	0.100000	1051.50000					
13	Title	0.300000	2875.00000					
14	Title	0.300000	3046.00000					
15	Title	0.300000	2438.50000					
16	Title	0.600000	4792.50000					
17	Title	0.600000	5128.50000					
18	Title	0.600000	4930.50000					
19	Title	1.000000	7030.00000					
20	Title	1.000000	7154.50000					
21	Title	1.000000	6413.50000					
22	Title	3.000000	5211.50000					
23	Title	3.000000	0701.50000					
24	Title	3.000000	0416.50000					

Table format: XY		X	A	B	C	D	E	F
		X Title	03-05-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	360.00000					
2	Title	0.030000	366.00000					
3	Title	0.030000	378.00000					
4	Title	0.060000	753.00000					
5	Title	0.060000	733.50000					
6	Title	0.060000	760.50000					
7	Title	0.080000						
8	Title	0.080000	963.00000					
9	Title	0.080000	1003.50000					
10	Title	0.100000	1287.00000					
11	Title	0.100000	1242.00000					
12	Title	0.100000	1195.50000					
13	Title	0.300000	3112.50000					
14	Title	0.300000	3283.50000					
15	Title	0.300000	2676.00000					
16	Title	0.600000	5122.50000					
17	Title	0.600000	5458.50000					
18	Title	0.600000	5260.50000					
19	Title	1.000000	7468.50000					
20	Title	1.000000	7593.00000					
21	Title	1.000000	6852.00000					
22	Title	3.000000	6214.50000					
23	Title	3.000000	1704.50000					
24	Title	3.000000	1419.50000					

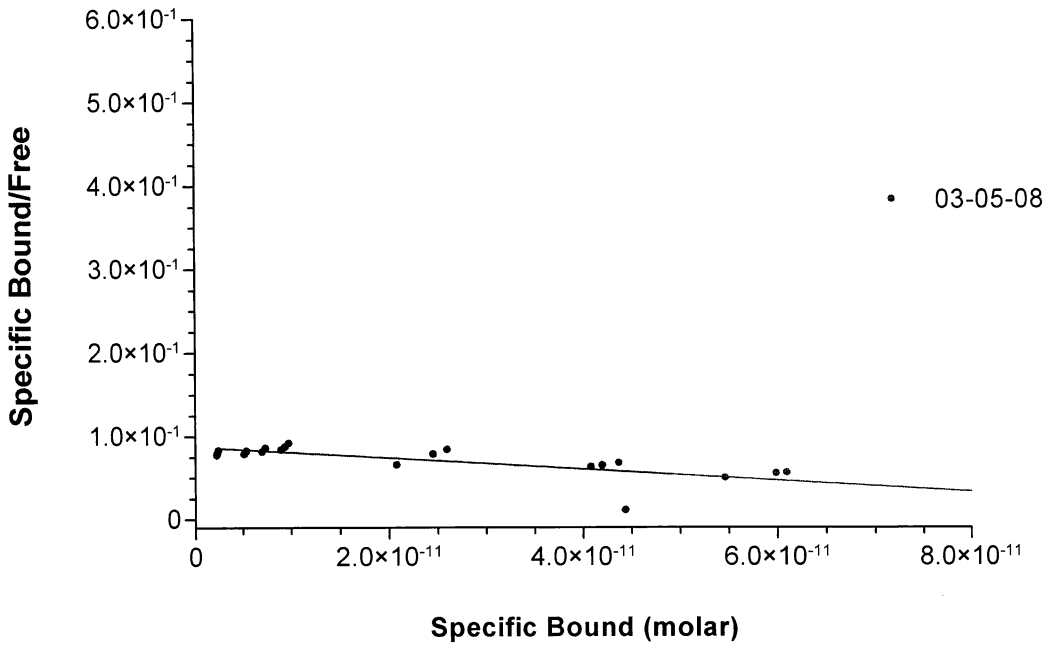
Table format: XY		X	A
		specific bound molar	03-05-08
		X	Y
1	Title	2.255350e-012	0.07810
2	Title	2.306410e-012	0.08000
3	Title	2.408540e-012	0.08390
4	Title	5.297950e-012	0.08260
5	Title	5.131990e-012	0.07980
6	Title	5.361780e-012	0.08370
7	Title		
8	Title	6.983070e-012	0.08260
9	Title	7.327760e-012	0.08710
10	Title	9.727790e-012	0.09260
11	Title	9.344810e-012	0.08860
12	Title	8.949060e-012	0.08460
13	Title	2.446840e-011	0.07950
14	Title	2.592380e-011	0.08460
15	Title	2.075350e-011	0.06660
16	Title	4.078780e-011	0.06390
17	Title	4.364740e-011	0.06870
18	Title	4.196230e-011	0.06580
19	Title	5.983060e-011	0.05600
20	Title	6.089020e-011	0.05700
21	Title	5.458370e-011	0.05080
22	Title	4.435380e-011	0.01180
23	Title	9.107780e-011	0.02460
24	Title	8.865230e-011	0.02390

Table format: XY		X	A
		mean total added molar	03-05-08
		X	Y
1	Title	3.19000e-011	2.25540e-012
2	Title	3.19000e-011	2.30640e-012
3	Title	3.19000e-011	2.40850e-012
4	Title	7.06000e-011	5.29790e-012
5	Title	7.06000e-011	5.13200e-012
6	Title	7.06000e-011	5.36180e-012
7	Title	9.27000e-011	
8	Title	9.27000e-011	6.98310e-012
9	Title	9.27000e-011	7.32780e-012
10	Title	1.16000e-010	9.72780e-012
11	Title	1.16000e-010	9.34480e-012
12	Title	1.16000e-010	8.94910e-012
13	Title	3.34000e-010	2.44680e-011
14	Title	3.34000e-010	2.59240e-011
15	Title	3.34000e-010	2.07530e-011
16	Title	6.82000e-010	4.07880e-011
17	Title	6.82000e-010	4.36470e-011
18	Title	6.82000e-010	4.19620e-011
19	Title	1.13000e-009	5.98310e-011
20	Title	1.13000e-009	6.08900e-011
21	Title	1.13000e-009	5.45840e-011
22	Title	3.81000e-009	4.43540e-011
23	Title	3.81000e-009	9.10780e-011
24	Title	3.81000e-009	8.86520e-011

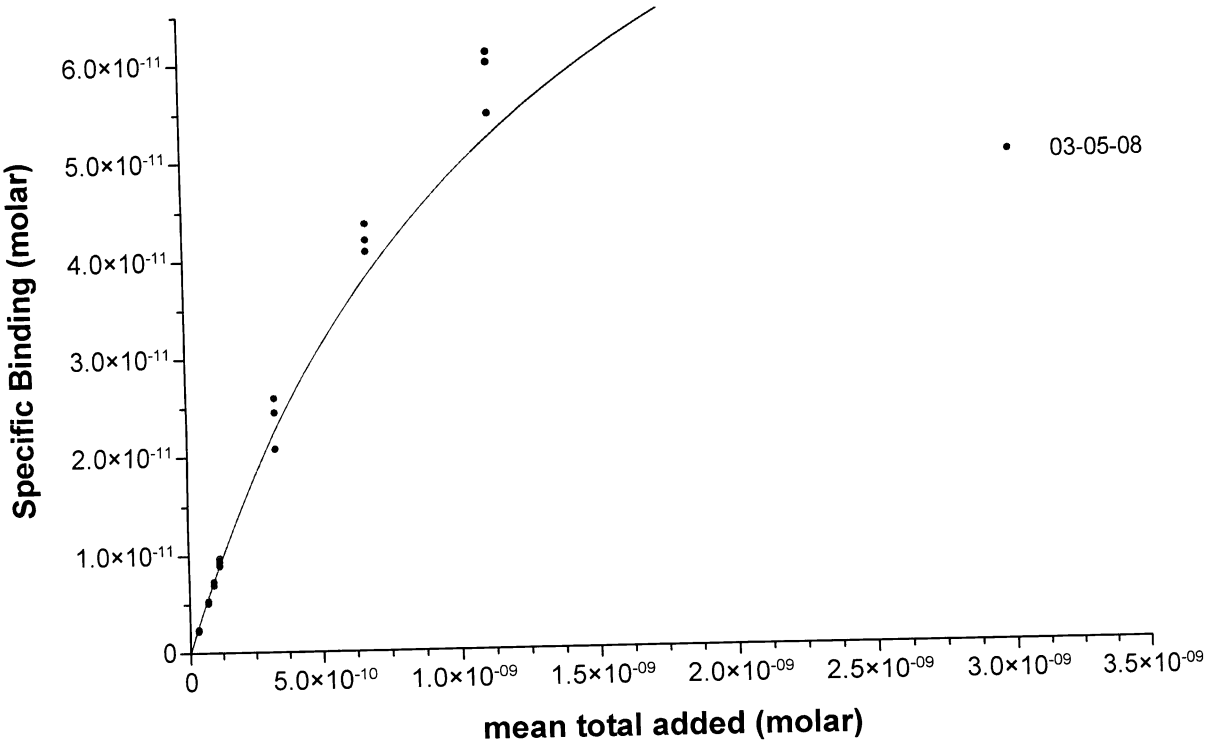
	Nonlin fit Table of results	A 03-05-08
		Y
1	One site binding (hyperbola)	
2	Best-fit values	
3	BMAX	1.168e-010
4	KD	1.396e-009
5	Std. Error	
6	BMAX	1.149e-011
7	KD	1.739e-010
8	95% Confidence Intervals	
9	BMAX	9.289e-011 to 1.407e-010
10	KD	1.034e-009 to 1.757e-009
11	Goodness of Fit	
12	Degrees of Freedom	21
13	R^2 (unweighted)	0.8849
14	Weighted Sum of Squares ($1/Y^2$)	0.4174
15	Absolute Sum of Squares	1.937e-021
16	Sy.x	0.1410
17	Number of points	
18	Analyzed	23

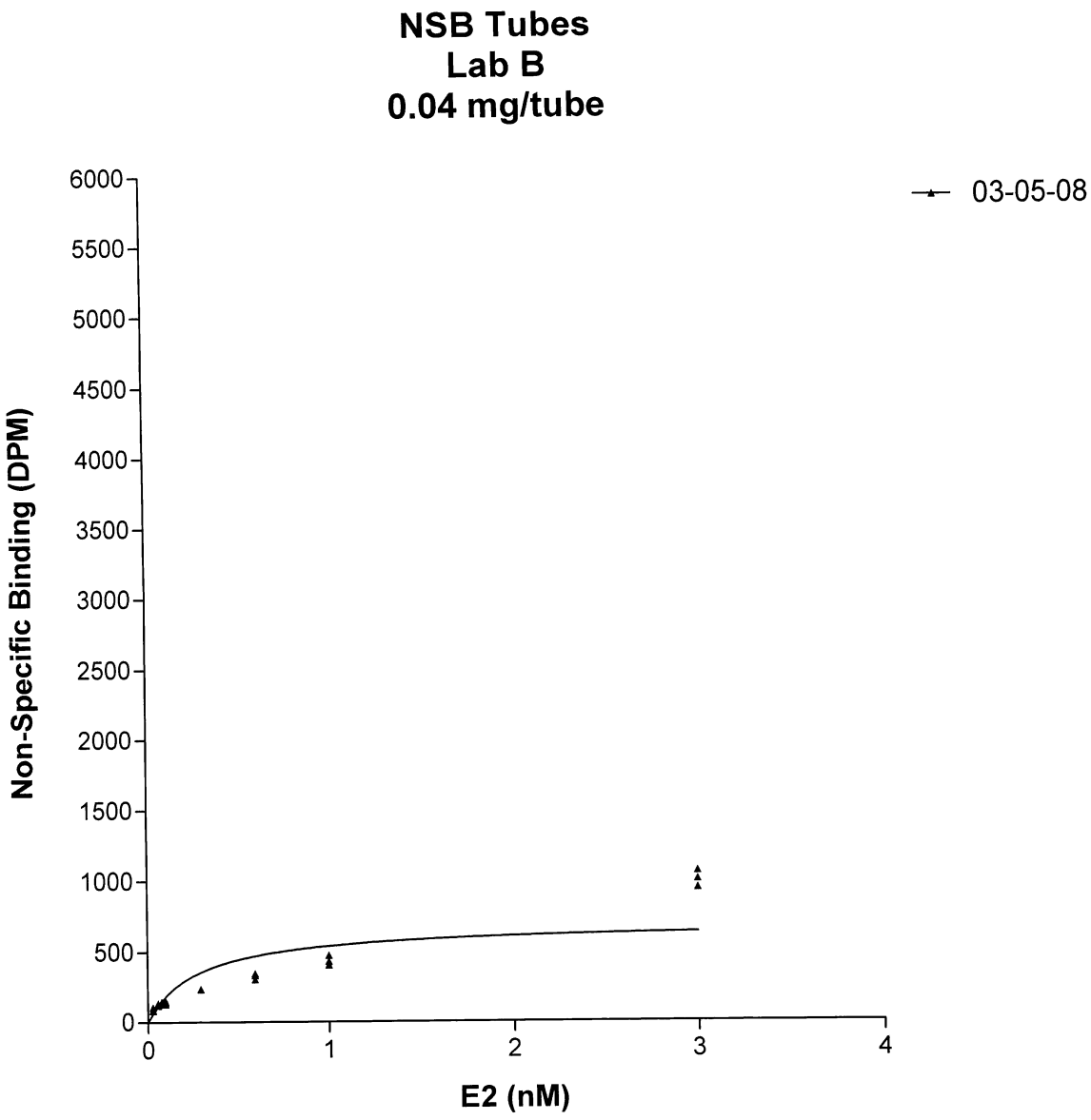

Hot Tubes
Lab B
0.04 mg/tube
03-05-08
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
1250
1500
1750
2000
E2 (fmole)

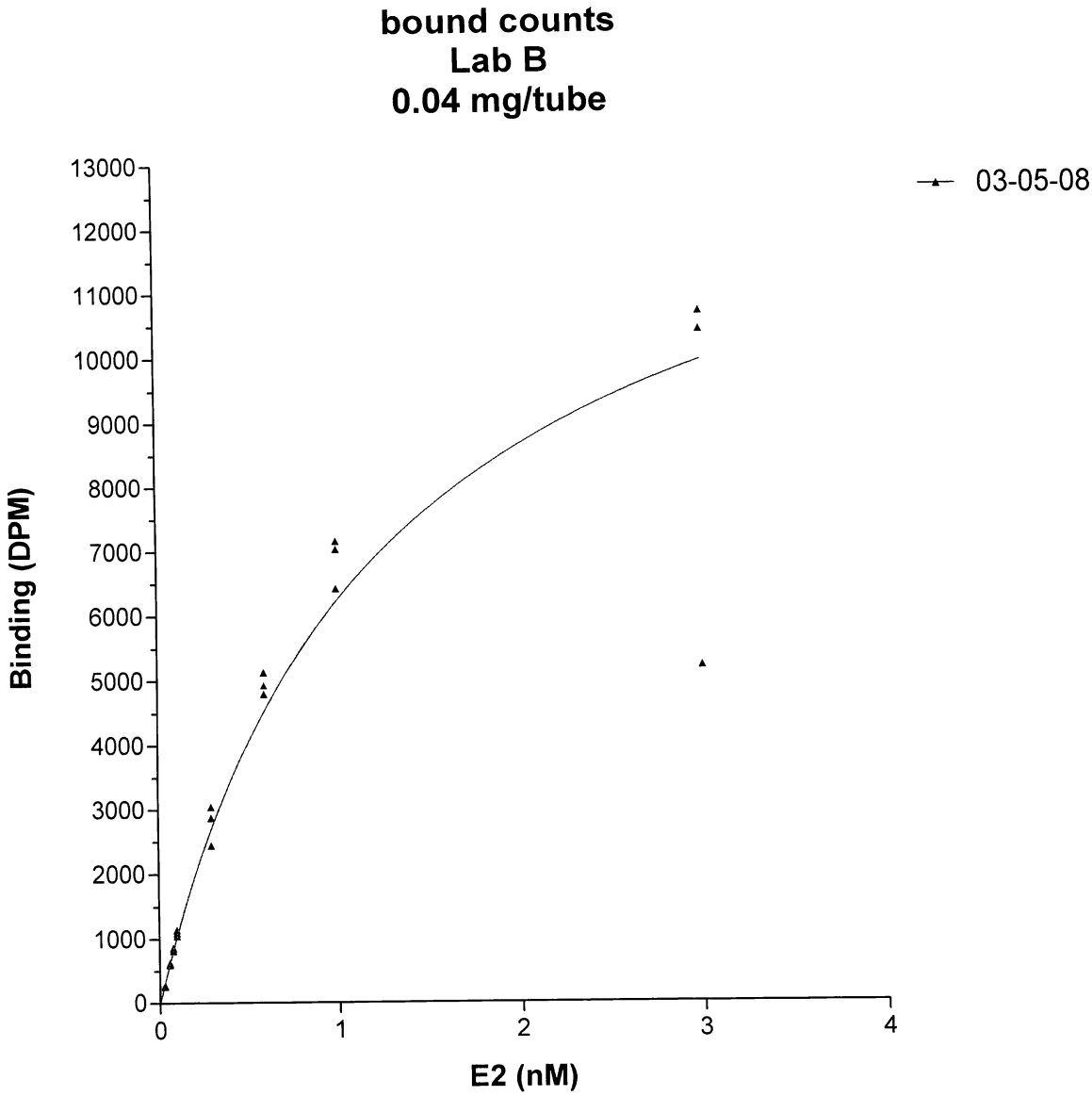
Scatchard Display
Lab B
0.04 mg/tube



Lab B
0.04 mg/tube




NSB Tubes
Lab B
0.04 mg/tube
03-05-08
Non-Specific Binding (DPM)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
E2 (nM)


bound counts
Lab B
0.04 mg/tube
03-05-08
Binding (DPM)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
E2 (nM)

APPENDIX B

Excel and Prism Files of Saturation Binding Data and Analysis for Run Identifier
TRL RUC2-21-08

Laboratory Code B/TRL

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B
Run identification:	TTN sat. binding
Assay start date:	3/31/2008
Tracer lot number:	3589221/1
Specific activity on day of assay:	105.42 Ci/mmole
Cytosol lot or vial number:	RUC-02-21-08
protein (cytosol) per tube:	40 ug
protein (cytosol) per tube:	0.04 mg
KD	1.10E+00 nM
Bmax	85.2375 fmole/100 ug
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5
total volume in tubes	500 uL

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot E2 Initial Concentration (nM)	Hot E2 Volume (uL)	Hot E2 Final Concentration (nM)	Cold E2 Initial Concentration (nM)	Cold E2 Volume (uL)	Cold E2 Final Concentration (nM)	Buffer Volume (uL)	Receptor Volume (uL)	raw dpm	dpm for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Output to Curve Fitting Software: Saturation X values	Bound y values	NSB y values
1	1	H	0.3	50	0.03	—	—	—	350	100	197	295.5	TRUE		6.7%	0.03	295.5	45.5
2	2	H	0.3	50	0.03	—	—	—	350	100	175	262.5	TRUE		6.0%	0.03	262.5	45.5
3	3	H	0.3	50	0.03	—	—	—	350	100	166	249	TRUE		5.7%	0.03	249.0	45.5
4	1	H	0.6	50	0.06	—	—	—	350	100	421	631.5	TRUE		7.2%	0.06	631.5	71.0
5	2	H	0.6	50	0.06	—	—	—	350	100	417	625.5	TRUE		7.1%	0.06	625.5	71.0
6	3	H	0.6	50	0.06	—	—	—	350	100	401	601.5	TRUE		6.8%	0.06	601.5	71.0
7	1	H	0.8	50	0.08	—	—	—	350	100	430	645	TRUE		5.5%	0.08	645.0	86.0
8	2	H	0.8	50	0.08	—	—	—	350	100	520	780	TRUE		6.7%	0.08	780.0	86.0
9	3	H	0.8	50	0.08	—	—	—	350	100	531	796.5	TRUE		6.8%	0.08	796.5	86.0
10	1	H	1.0	50	0.10	—	—	—	350	100	563	844.5	TRUE		5.6%	0.1	844.5	87.0
11	2	H	1.0	50	0.10	—	—	—	350	100	654	981	TRUE		6.5%	0.1	981.0	87.0
12	3	H	1.0	50	0.10	—	—	—	350	100	702	1053	TRUE		6.9%	0.1	1053.0	87.0
13	1	H	3.0	50	0.30	—	—	—	350	100	1631	2446.5	TRUE		5.6%	0.3	2446.5	162.0
14	2	H	3.0	50	0.30	—	—	—	350	100	1628	2442	TRUE		5.6%	0.3	2442.0	162.0
15	3	H	3.0	50	0.30	—	—	—	350	100	1554	2331	TRUE		5.3%	0.3	2331.0	162.0
16	1	H	6.0	50	0.60	—	—	—	350	100	2368	3552	TRUE		4.0%	0.6	3552.0	332.5
17	2	H	6.0	50	0.60	—	—	—	350	100	2490	3735	TRUE		4.2%	0.6	3735.0	332.5
18	3	H	6.0	50	0.60	—	—	—	350	100	2168	3252	TRUE		3.6%	0.6	3252.0	332.5
19	1	H	10.0	50	1.00	—	—	—	350	100	3135	4702.5	TRUE		3.2%	1	4702.5	596.0
20	2	H	10.0	50	1.00	—	—	—	350	100	3322	4983	TRUE		3.4%	1	4983.0	596.0
21	3	H	10.0	50	1.00	—	—	—	350	100	3117	4675.5	TRUE		3.2%	1	4675.5	596.0
22	1	H	30.0	50	3.00	—	—	—	350	100	5114	7671	TRUE		1.8%	3	7671.0	1543.5
23	2	H	30.0	50	3.00	—	—	—	350	100	4917	7375.5	TRUE		1.7%	3	7375.5	1543.5
24	3	H	30.0	50	3.00	—	—	—	350	100	5140	7710	TRUE		1.8%	3	7710.0	1543.5
25	1	HC	0.3	50	0.03	30	50	3	300	100	26	39	TRUE					
26	2	HC	0.3	50	0.03	30	50	3	300	100	29	43.5	TRUE					

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
48	27	3	HC	0.3	50	0.03	30	50	3	300	100			36	54	TRUE					
49	28	1	HC	0.6	50	0.06	60	50	6	300	100			42	63	TRUE					
50	29	2	HC	0.6	50	0.06	60	50	6	300	100			48	72	TRUE					
51	30	3	HC	0.6	50	0.06	60	50	6	300	100			52	78	TRUE					
52	31	1	HC	0.8	50	0.08	80	50	8	300	100			50	75	TRUE					
53	32	2	HC	0.8	50	0.08	80	50	8	300	100			59	88.5	TRUE					
54	33	3	HC	0.8	50	0.08	80	50	8	300	100			63	94.5	TRUE					
55	34	1	HC	1.0	50	0.10	100	50	10	300	100			56	84	TRUE					
56	35	2	HC	1.0	50	0.10	100	50	10	300	100			59	88.5	TRUE					
57	36	3	HC	1.0	50	0.10	100	50	10	300	100			59	88.5	TRUE					
58	37	1	HC	3.0	50	0.30	300	50	30	300	100			104	156	TRUE					
59	38	2	HC	3.0	50	0.30	300	50	30	300	100			110	165	TRUE					
60	39	3	HC	3.0	50	0.30	300	50	30	300	100			110	165	TRUE					
61	40	1	HC	6.0	50	0.60	600	50	60	300	100			199	298.5	TRUE					
62	41	2	HC	6.0	50	0.60	600	50	60	300	100			237	355.5	TRUE					
63	42	3	HC	6.0	50	0.60	600	50	60	300	100			229	343.5	TRUE					
64	43	1	HC	10.0	50	1.00	1000	50	100	300	100			370	555	TRUE					
65	44	2	HC	10.0	50	1.00	1000	50	100	300	100			431	646.5	TRUE					
66	45	3	HC	10.0	50	1.00	1000	50	100	300	100			391	586.5	TRUE					
67	46	1	HC	30.0	50	3.00	3000	50	300	300	100			976	1464	TRUE					
68	47	2	HC	30.0	50	3.00	3000	50	300	300	100			1043	1564.5	TRUE					
69	48	3	HC	30.0	50	3.00	3000	50	300	300	100			1068	1602	TRUE					
70	49	1	Hot	0.3	50	0.03	—	—	—	—	—			2920	4380	TRUE					
71	50	2	Hot	0.3	50	0.03	—	—	—	—	—			2979	4468.5	TRUE					
72	51	3	Hot	0.3	50	0.03	—	—	—	—	—			2873	4309.5	TRUE					
73	52	1	Hot	0.6	50	0.06	—	—	—	—	—			5824	8736	TRUE					
74	53	2	Hot	0.6	50	0.06	—	—	—	—	—			5855	8782.5	TRUE					
75	54	3	Hot	0.6	50	0.06	—	—	—	—	—			5930	8895	TRUE					
76	55	1	Hot	0.8	50	0.08	—	—	—	—	—			7641	11461.5	TRUE					
77	56	2	Hot	0.8	50	0.08	—	—	—	—	—			7996	11994	TRUE					
78	57	3	Hot	0.8	50	0.08	—	—	—	—	—			7759	11638.5	TRUE					
79	58	1	Hot	1.0	50	0.10	—	—	—	—	—			10229	15343.5	TRUE					
80	59	2	Hot	1.0	50	0.10	—	—	—	—	—			10217	15325.5	TRUE					
81	60	3	Hot	1.0	50	0.10	—	—	—	—	—			9907	14860.5	TRUE					
82	61	1	Hot	3.0	50	0.30	—	—	—	—	—			29681	44521.5	TRUE					
83	62	2	Hot	3.0	50	0.30	—	—	—	—	—			29555	44332.5	TRUE					
84	63	3	Hot	3.0	50	0.30	—	—	—	—	—			28631	42946.5	TRUE					
85	64	1	Hot	6.0	50	0.60	—	—	—	—	—			57737	86605.5	TRUE					
86	65	2	Hot	6.0	50	0.60	—	—	—	—	—			60472	90708	TRUE					
87	66	3	Hot	6.0	50	0.60	—	—	—	—	—			60272	90408	TRUE					
88	67	1	Hot	10.0	50	1.00	—	—	—	—	—			101613	152419.5	TRUE					
89	68	2	Hot	10.0	50	1.00	—	—	—	—	—			99868	149802	TRUE					
90	69	3	Hot	10.0	50	1.00	—	—	—	—	—			93248	139872	TRUE					
91	70	1	Hot	30.0	50	3.00	—	—	—	—	—			288426	432639	TRUE					
92	71	2	Hot	30.0	50	3.00	—	—	—	—	—			290867	436300.5	TRUE					
93	72	3	Hot	30.0	50	3.00	—	—	—	—	—			275224	412836	TRUE					

	DL	DM	DN	DO	DP	DQ	DR
21		*4/1/2008*					
22	One site binding (hyperbola)		Bmax molar	6.82E-11		KD molar	1.10E-09
23	Best-fit values		mole to molar conversion value	0.0005		molar to nM converison	1.00E+09
24	BMAX	6.82E-11	DPM/mole = (DPM/mmole)*1000	2.34E+17		**kd nM =**	1.10E+00
25	KD	1.10E-09	Bmax molar to Bmax moles	3.4095E-14			
26	Std. Error		= DPM/((DPM/mmole)*1000)	3.4095E-14			
27	BMAX	4.31E-12	=Bmax DPM	7.98E+03			
28	KD	9.15E-11					
29	95% Confidence Intervals						
30	BMAX	5.926e-011 to 7.713e-011	assay date	3/31/2008			
31	KD	9.118e-010 to 1.291e-009	Bmax(dpm)	7979.041611			
32	Goodness of Fit		DPM/Ci (definition)	2.22E+12			
33	Degrees of Freedom	22	Ci/mmole	105.42			
34	R^2 (unweighted)	0.9945	DPM/mmole	2.34E+14			
35	Weighted Sum of Squares ($1/Y^2$)	0.2263	DPM/pmole	2.34E+05			
36	Absolute Sum of Squares	3.67E-23	1/(DPM/mmole)	4.27E-15			
37	Sy.x	0.1014	1/(DPM/pmole)	4.27E-06			
38	Data		SA(dpm/pmole)	2.34E+05			
39	Number of X values	24	protein/tube (ug)	40			
40	Number of Y replicates		protein./tube(mg)	0.04			
41	Total number of values		bmax pmole	0.034095			
42	Number of missing values		bmax pmole/mg	0.852375			
43			Bmax fmole/mg	852.375			
44			**Bmax (fmole/100 ug)**	85.2375			
45			Bmax(fmole/100 ug)/Bmax molar	1.25E+12			

	CF	CG	CH	CI	CJ	CK	CL	CM
20	Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
21	specific bound/molar	bound/free %	*average total added molar*	*specific bound/molar*	*total added dpm*	*NSB dpm*	*spcific bound/dpm*	*total bound dpm*
22	2.13653E-12	0.0611	3.75E-11	2.1365E-12	4380.00	39.00	250.00	295.50
23	1.85451E-12	0.0526	3.75E-11	1.8545E-12	4468.50	43.50	217.00	262.50
24	1.73914E-12	0.0492	3.75E-11	1.7391E-12	4309.50	54.00	203.50	249.00
25	4.79011E-12	0.0686	7.52E-11	4.7901E-12	8736.00	63.00	560.50	631.50
26	4.73883E-12	0.0678	7.52E-11	4.7388E-12	8782.50	72.00	554.50	625.50
27	4.53373E-12	0.0647	7.52E-11	4.5337E-12	8895.00	78.00	530.50	601.50
28	4.77729E-12	0.0506	1.00E-10	4.7773E-12	11461.50	75.00	559.00	645.00
29	5.93102E-12	0.0636	1.00E-10	5.9310E-12	11994.00	88.50	694.00	780.00
30	6.07203E-12	0.0652	1.00E-10	6.0720E-12	11638.50	94.50	710.50	796.50
31	6.4737E-12	0.0529	1.30E-10	6.4737E-12	15343.50	84.00	757.50	844.50
32	7.64025E-12	0.0630	1.30E-10	7.6402E-12	15325.50	88.50	894.00	981.00
33	8.25557E-12	0.0684	1.30E-10	8.2556E-12	14860.50	88.50	966.00	1053.00
34	1.95237E-11	0.0551	3.75E-10	1.9524E-11	44521.50	156.00	2284.50	2446.50
35	1.94852E-11	0.0550	3.75E-10	1.9485E-11	44332.50	165.00	2280.00	2442.00
36	1.85366E-11	0.0521	3.75E-10	1.8537E-11	42946.50	165.00	2169.00	2331.00
37	2.75143E-11	0.0376	7.63E-10	2.7514E-11	86605.50	298.50	3219.50	3552.00
38	2.90782E-11	0.0398	7.63E-10	2.9078E-11	90708.00	355.50	3402.50	3735.00
39	2.49505E-11	0.0340	7.63E-10	2.4950E-11	90408.00	343.50	2919.50	3252.00
40	3.50947E-11	0.0288	1.26E-09	3.5095E-11	152419.50	555.00	4106.50	4702.50
41	3.74919E-11	0.0308	1.26E-09	3.7492E-11	149802.00	646.50	4387.00	4983.00
42	3.4864E-11	0.0286	1.26E-09	3.4864E-11	139872.00	586.50	4079.50	4675.50
43	5.23665E-11	0.0146	3.65E-09	5.2366E-11	432639.00	1464.00	6127.50	7671.00
44	4.98411E-11	0.0139	3.65E-09	4.9841E-11	436300.50	1564.50	5832.00	7375.50
45	5.26998E-11	0.0147	3.65E-09	5.2700E-11	412836.00	1602.00	6166.50	7710.00

Table format: XY		X	A	B	C	D	E	F	G
		X Title	04-01-08	04-01-08	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G
		X	Y	Y	Y	Y	Y	Y	Y
1	Title	0.030000	295.500	45.5000					
2	Title	0.030000	262.500	45.5000					
3	Title	0.030000	249.000	45.5000					
4	Title	0.060000	631.500	71.0000					
5	Title	0.060000	625.500	71.0000					
6	Title	0.060000	601.500	71.0000					
7	Title	0.080000	645.000	86.0000					
8	Title	0.080000	780.000	86.0000					
9	Title	0.080000	796.500	86.0000					
10	Title	0.100000	844.500	87.0000					
11	Title	0.100000	981.000	87.0000					
12	Title	0.100000	1053.000	87.0000					
13	Title	0.300000	2446.500	162.0000					
14	Title	0.300000	2442.000	162.0000					
15	Title	0.300000	2331.000	162.0000					
16	Title	0.600000	3552.000	332.5000					
17	Title	0.600000	3735.000	332.5000					
18	Title	0.600000	3252.000	332.5000					
19	Title	1.000000	4702.500	596.0000					
20	Title	1.000000	4983.000	596.0000					
21	Title	1.000000	4675.500	596.0000					
22	Title	3.000000	7671.000	1543.5000					
23	Title	3.000000	7375.500	1543.5000					
24	Title	3.000000	7710.000	1543.5000					

	H	I	J	K	L
	Data Set-H	Data Set-I	Data Set-J	Data Set-K	Data Set-L
	Y	Y	Y	Y	Y
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Table format: XY		X	A	B	C	D	E	F
		X Title	04-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	15.000000	4380.000					
2	Title	15.000000	4468.500					
3	Title	15.000000	4309.500					
4	Title	30.000000	8736.000					
5	Title	30.000000	8782.500					
6	Title	30.000000	8895.000					
7	Title	40.000000	11461.500					
8	Title	40.000000	11994.000					
9	Title	40.000000	11638.500					
10	Title	50.000000	15343.500					
11	Title	50.000000	15325.500					
12	Title	50.000000	14860.500					
13	Title	150.000000	44521.500					
14	Title	150.000000	44332.500					
15	Title	150.000000	42946.500					
16	Title	300.000000	86605.500					
17	Title	300.000000	90708.000					
18	Title	300.000000	90408.000					
19	Title	500.000000	152419.500					
20	Title	500.000000	149802.000					
21	Title	500.000000	139872.000					
22	Title	1500.000000	432639.000					
23	Title	1500.000000	436300.500					
24	Title	1500.000000	412836.000					

Table format: XY		X	A	B	C	D	E	F
		X Title	04-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	39.00					
2	Title	0.030000	43.50					
3	Title	0.030000	54.00					
4	Title	0.060000	63.00					
5	Title	0.060000	72.00					
6	Title	0.060000	78.00					
7	Title	0.080000	75.00					
8	Title	0.080000	88.50					
9	Title	0.080000	94.50					
10	Title	0.100000	84.00					
11	Title	0.100000	88.50					
12	Title	0.100000	88.50					
13	Title	0.300000	156.00					
14	Title	0.300000	165.00					
15	Title	0.300000	165.00					
16	Title	0.600000	298.50					
17	Title	0.600000	355.50					
18	Title	0.600000	343.50					
19	Title	1.000000	555.00					
20	Title	1.000000	646.50					
21	Title	1.000000	586.50					
22	Title	3.000000	1464.00					
23	Title	3.000000	1564.50					
24	Title	3.000000	1602.00					

Table format: XY		X	A	B	C	D	E	F
		X Title	04-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	250.00000					
2	Title	0.030000	217.00000					
3	Title	0.030000	203.50000					
4	Title	0.060000	560.50000					
5	Title	0.060000	554.50000					
6	Title	0.060000	530.50000					
7	Title	0.080000	559.00000					
8	Title	0.080000	694.00000					
9	Title	0.080000	710.50000					
10	Title	0.100000	757.50000					
11	Title	0.100000	894.00000					
12	Title	0.100000	966.00000					
13	Title	0.300000	2284.50000					
14	Title	0.300000	2280.00000					
15	Title	0.300000	2169.00000					
16	Title	0.600000	3219.50000					
17	Title	0.600000	3402.50000					
18	Title	0.600000	2919.50000					
19	Title	1.000000	4106.50000					
20	Title	1.000000	4387.00000					
21	Title	1.000000	4079.50000					
22	Title	3.000000	6127.50000					
23	Title	3.000000	5832.00000					
24	Title	3.000000	6166.50000					

Table format: XY		X	A	B	C	D	E
		X Title	04-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E
		X	Y	Y	Y	Y	Y
1	Title	0.030000	295.50000				
2	Title	0.030000	262.50000				
3	Title	0.030000	249.00000				
4	Title	0.060000	631.50000				
5	Title	0.060000	625.50000				
6	Title	0.060000	601.50000				
7	Title	0.080000	645.00000				
8	Title	0.080000	780.00000				
9	Title	0.080000	796.50000				
10	Title	0.100000	844.50000				
11	Title	0.100000	981.00000				
12	Title	0.100000	1053.00000				
13	Title	0.300000	2446.50000				
14	Title	0.300000	2442.00000				
15	Title	0.300000	2331.00000				
16	Title	0.600000	3552.00000				
17	Title	0.600000	3735.00000				
18	Title	0.600000	3252.00000				
19	Title	1.000000	4702.50000				
20	Title	1.000000	4983.00000				
21	Title	1.000000	4675.50000				
22	Title	3.000000	7671.00000				
23	Title	3.000000	7375.50000				
24	Title	3.000000	7710.00000				

Table format: XY		X	A
		specific bound molar	04-01-08
		X	Y
1	Title	2.136530e-012	0.06110
2	Title	1.854510e-012	0.05260
3	Title	1.739140e-012	0.04920
4	Title	4.790110e-012	0.06860
5	Title	4.738830e-012	0.06780
6	Title	4.533730e-012	0.06470
7	Title	4.777290e-012	0.05060
8	Title	5.931020e-012	0.06360
9	Title	6.072030e-012	0.06520
10	Title	6.473700e-012	0.05290
11	Title	7.640250e-012	0.06300
12	Title	8.255570e-012	0.06840
13	Title	1.952370e-011	0.05510
14	Title	1.948520e-011	0.05500
15	Title	1.853660e-011	0.05210
16	Title	2.751430e-011	0.03760
17	Title	2.907820e-011	0.03980
18	Title	2.495050e-011	0.03400
19	Title	3.509470e-011	0.02880
20	Title	3.749190e-011	0.03080
21	Title	3.486400e-011	0.02860
22	Title	5.236650e-011	0.01460
23	Title	4.984110e-011	0.01390
24	Title	5.269980e-011	0.01470

Table format: XY		X	A
		mean total added molar	04-01-08
		X	Y
1	Title	3.75000e-011	2.13650e-012
2	Title	3.75000e-011	1.85450e-012
3	Title	3.75000e-011	1.73910e-012
4	Title	7.52000e-011	4.79010e-012
5	Title	7.52000e-011	4.73880e-012
6	Title	7.52000e-011	4.53370e-012
7	Title	1.00000e-010	4.77730e-012
8	Title	1.00000e-010	5.93100e-012
9	Title	1.00000e-010	6.07200e-012
10	Title	1.30000e-010	6.47370e-012
11	Title	1.30000e-010	7.64020e-012
12	Title	1.30000e-010	8.25560e-012
13	Title	3.75000e-010	1.95240e-011
14	Title	3.75000e-010	1.94850e-011
15	Title	3.75000e-010	1.85370e-011
16	Title	7.63000e-010	2.75140e-011
17	Title	7.63000e-010	2.90780e-011
18	Title	7.63000e-010	2.49500e-011
19	Title	1.26000e-009	3.50950e-011
20	Title	1.26000e-009	3.74920e-011
21	Title	1.26000e-009	3.48640e-011
22	Title	3.65000e-009	5.23660e-011
23	Title	3.65000e-009	4.98410e-011
24	Title	3.65000e-009	5.27000e-011

	Nonlin fit Table of results	A 04-01-08
		Y
1	One site binding (hyperbola)	
2	Best-fit values	
3	BMAX	6.819e-011
4	KD	1.102e-009
5	Std. Error	
6	BMAX	4.309e-012
7	KD	9.151e-011
8	95% Confidence Intervals	
9	BMAX	5.926e-011 to 7.713e-011
10	KD	9.118e-010 to 1.291e-009
11	Goodness of Fit	
12	Degrees of Freedom	22
13	R^2 (unweighted)	0.9945
14	Weighted Sum of Squares ($1/Y^2$)	0.2263
15	Absolute Sum of Squares	3.671e-023
16	Sy.x	0.1014
17	Number of points	
18	Analyzed	24

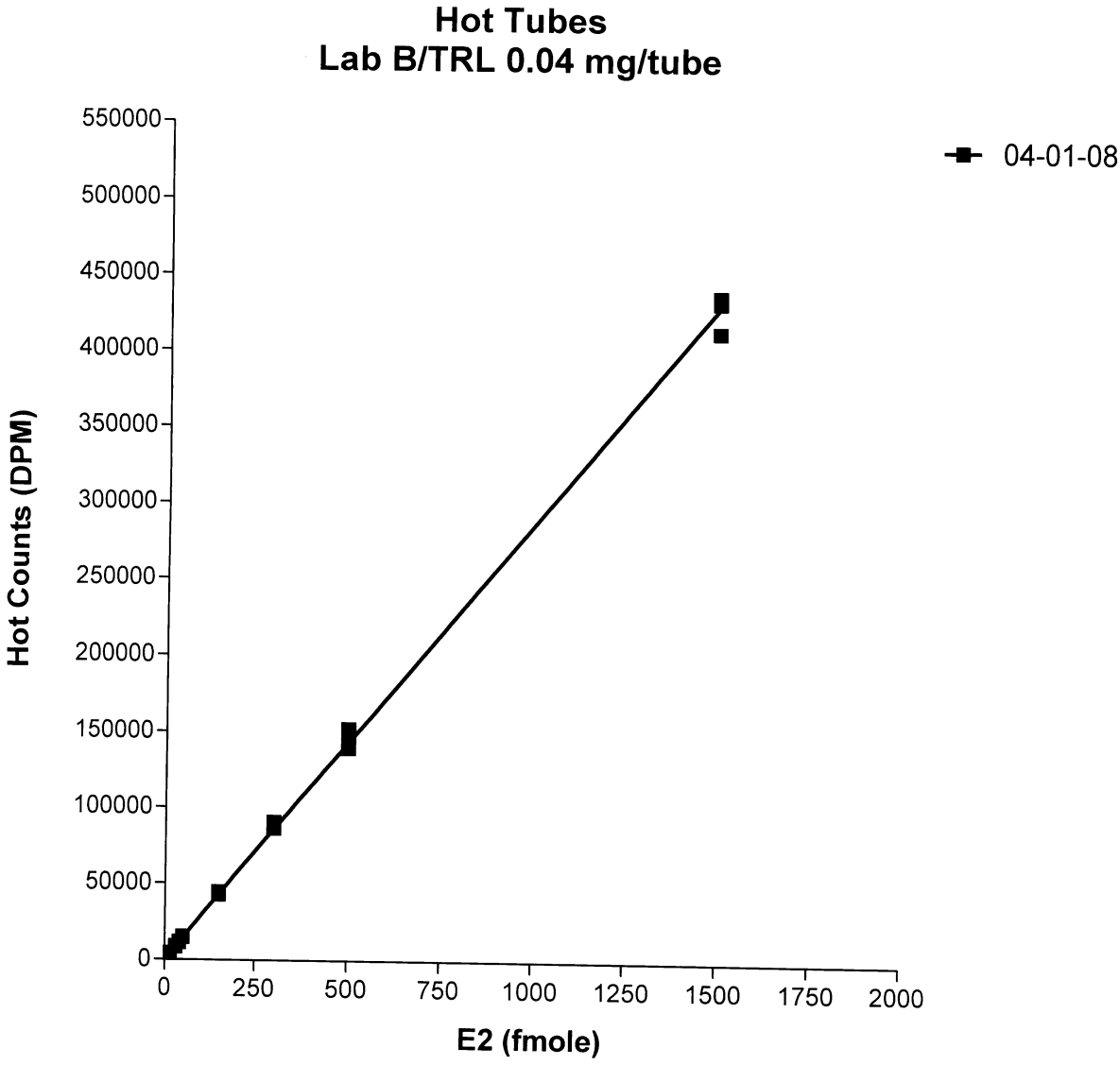
	X Seq.	A
	mean total added molar	04-01-08
	X	Y
1	0.000000	0.000
2	0.000000	1.483e-012
3	0.000000	2.904e-012
4	0.000000	4.265e-012
5	0.000000	5.570e-012
6	0.000000	6.823e-012
7	0.000000	8.027e-012
8	0.000000	9.185e-012
9	0.000000	1.030e-011
10	0.000000	1.137e-011
11	0.000000	1.241e-011
12	0.000000	1.340e-011
13	0.000000	1.436e-011
14	0.000000	1.529e-011
15	0.000000	1.619e-011
16	0.000000	1.706e-011
17	0.000000	1.790e-011
18	0.000000	1.871e-011
19	0.000000	1.949e-011
20	0.000000	2.025e-011
21	0.000000	2.099e-011
22	0.000000	2.171e-011
23	0.000000	2.240e-011
24	0.000000	2.308e-011
25	0.000000	2.373e-011
26	0.000000	2.436e-011
27	0.000000	2.498e-011
28	0.000000	2.558e-011
29	0.000000	2.617e-011
30	0.000000	2.673e-011
31	0.000000	2.729e-011
32	0.000000	2.783e-011
33	0.000000	2.835e-011
34	0.000000	2.886e-011
35	0.000000	2.936e-011
36	0.000000	2.985e-011
37	0.000000	3.032e-011
38	0.000000	3.078e-011
39	0.000000	3.123e-011
40	0.000000	3.167e-011
41	0.000000	3.210e-011
42	0.000000	3.252e-011
43	0.000000	3.293e-011
44	0.000000	3.333e-011

	X Seq.	A
	mean total added molar	04-01-08
	X	Y
45	0.000000	3.372e-011
46	0.000000	3.411e-011
47	0.000000	3.448e-011
48	0.000000	3.485e-011
49	0.000000	3.521e-011
50	0.000000	3.556e-011
51	0.000000	3.590e-011
52	0.000000	3.624e-011
53	0.000000	3.657e-011
54	0.000000	3.689e-011
55	0.000000	3.721e-011
56	0.000000	3.752e-011
57	0.000000	3.782e-011
58	0.000000	3.812e-011
59	0.000000	3.841e-011
60	0.000000	3.870e-011
61	0.000000	3.898e-011
62	0.000000	3.925e-011
63	0.000000	3.952e-011
64	0.000000	3.979e-011
65	0.000000	4.005e-011
66	0.000000	4.031e-011
67	0.000000	4.056e-011
68	0.000000	4.080e-011
69	0.000000	4.105e-011
70	0.000000	4.129e-011
71	0.000000	4.152e-011
72	0.000000	4.175e-011
73	0.000000	4.198e-011
74	0.000000	4.220e-011
75	0.000000	4.242e-011
76	0.000000	4.263e-011
77	0.000000	4.284e-011
78	0.000000	4.305e-011
79	0.000000	4.325e-011
80	0.000000	4.346e-011
81	0.000000	4.365e-011
82	0.000000	4.385e-011
83	0.000000	4.404e-011
84	0.000000	4.423e-011
85	0.000000	4.441e-011
86	0.000000	4.460e-011
87	0.000000	4.478e-011
88	0.000000	4.496e-011

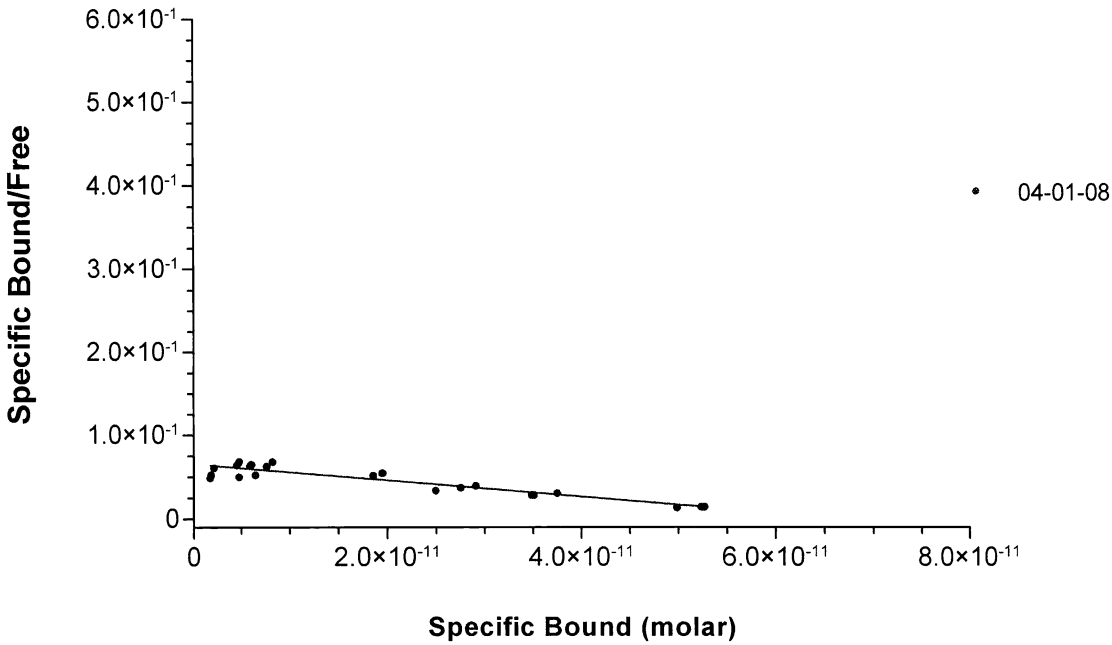
	X Seq.	A
	mean total added molar	04-01-08
	X	Y
89	0.000000	4.513e-011
90	0.000000	4.530e-011
91	0.000000	4.547e-011
92	0.000000	4.564e-011
93	0.000000	4.580e-011
94	0.000000	4.597e-011
95	0.000000	4.613e-011
96	0.000000	4.628e-011
97	0.000000	4.644e-011
98	0.000000	4.659e-011
99	0.000000	4.674e-011
100	0.000000	4.689e-011
101	0.000000	4.704e-011
102	0.000000	4.718e-011
103	0.000000	4.733e-011
104	0.000000	4.747e-011
105	0.000000	4.761e-011
106	0.000000	4.774e-011
107	0.000000	4.788e-011
108	0.000000	4.801e-011
109	0.000000	4.815e-011
110	0.000000	4.828e-011
111	0.000000	4.840e-011
112	0.000000	4.853e-011
113	0.000000	4.866e-011
114	0.000000	4.878e-011
115	0.000000	4.890e-011
116	0.000000	4.902e-011
117	0.000000	4.914e-011
118	0.000000	4.926e-011
119	0.000000	4.938e-011
120	0.000000	4.949e-011
121	0.000000	4.960e-011
122	0.000000	4.972e-011
123	0.000000	4.983e-011
124	0.000000	4.994e-011
125	0.000000	5.004e-011
126	0.000000	5.015e-011
127	0.000000	5.026e-011
128	0.000000	5.036e-011
129	0.000000	5.046e-011
130	0.000000	5.056e-011
131	0.000000	5.067e-011
132	0.000000	5.077e-011

	X Seq.	A
	mean total added molar	04-01-08
	X	Y
133	0.000000	5.086e-011
134	0.000000	5.096e-011
135	0.000000	5.106e-011
136	0.000000	5.115e-011
137	0.000000	5.125e-011
138	0.000000	5.134e-011
139	0.000000	5.143e-011
140	0.000000	5.152e-011
141	0.000000	5.161e-011
142	0.000000	5.170e-011
143	0.000000	5.179e-011
144	0.000000	5.188e-011
145	0.000000	5.196e-011
146	0.000000	5.205e-011
147	0.000000	5.213e-011
148	0.000000	5.222e-011
149	0.000000	5.230e-011
150	0.000000	5.238e-011

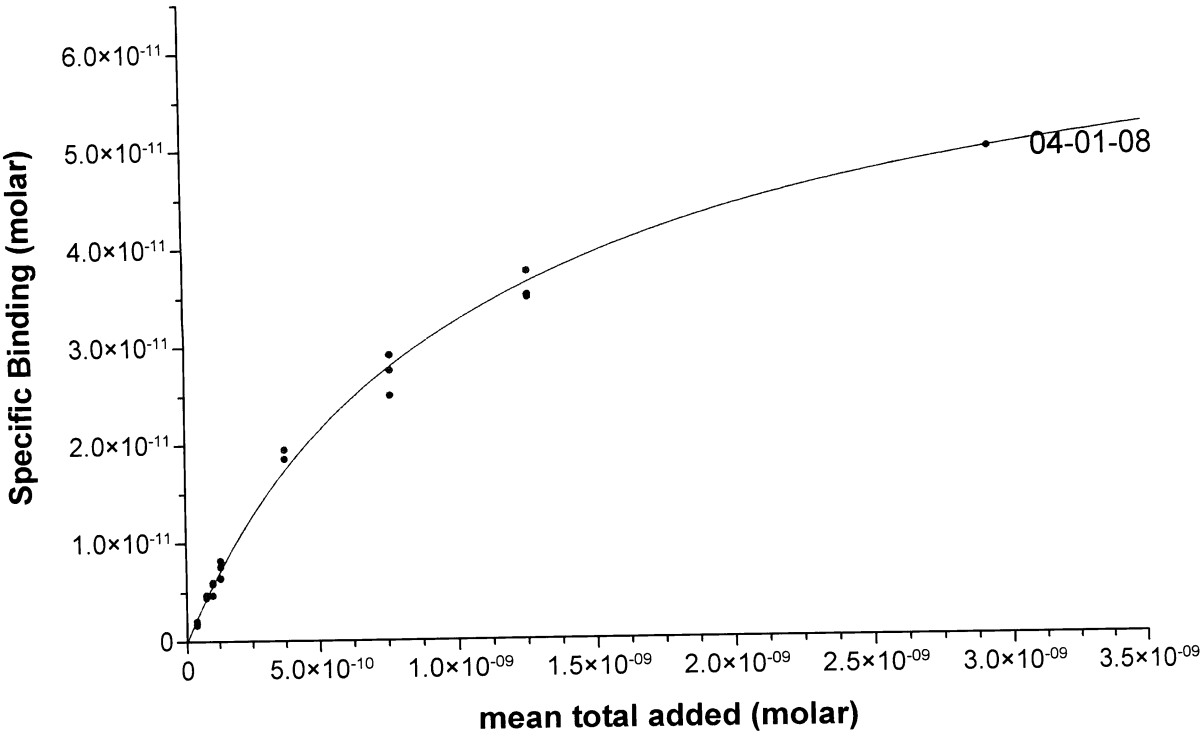
	X	A	B
	X Title	BMAX	KD
	X	Y	Y
1		6.819208e-011	1.101613e-009

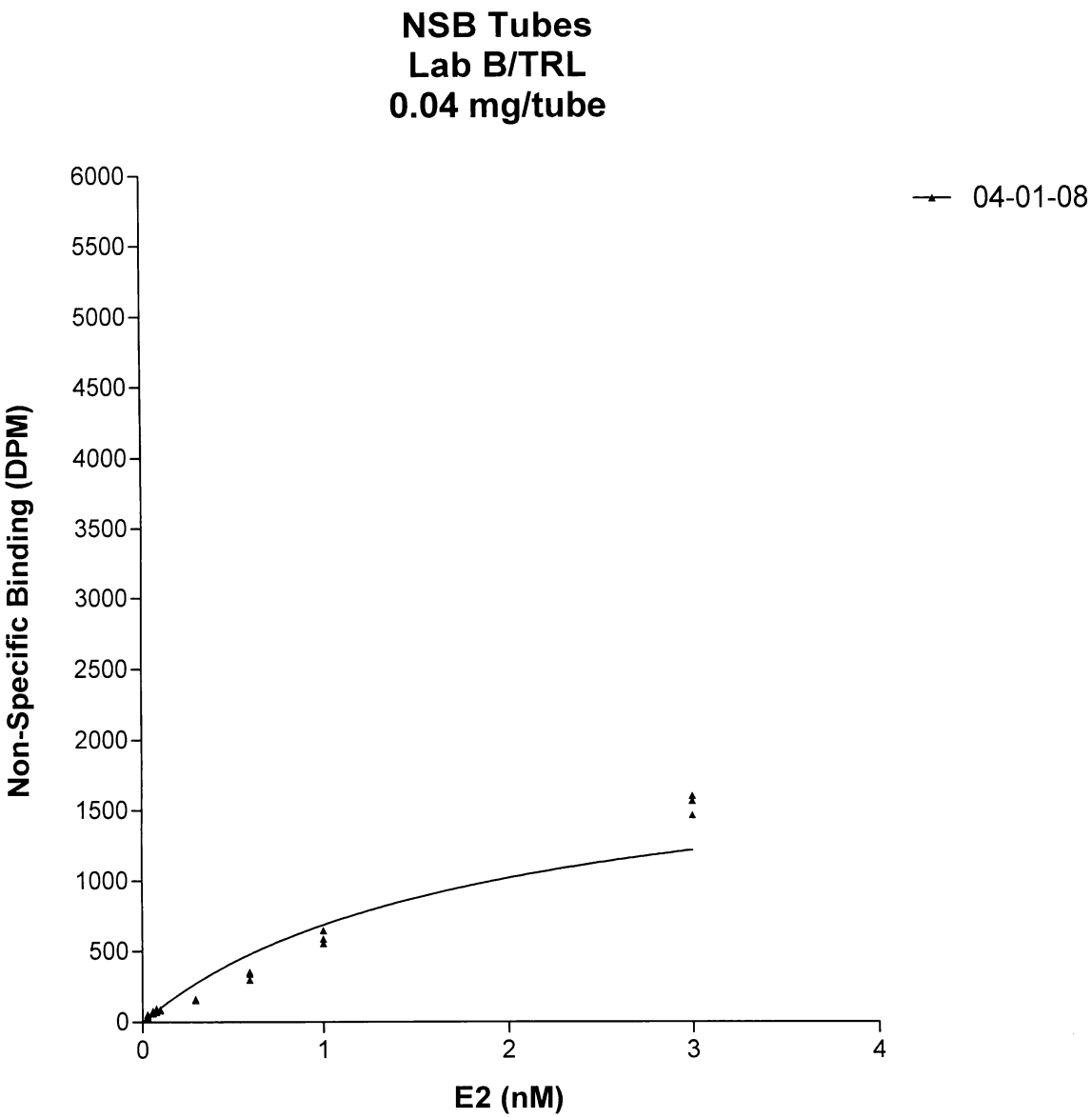

Hot Tubes
Lab B/TRL 0.04 mg/tube
04-01-08
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
1250
1500
1750
2000
E2 (fmole)

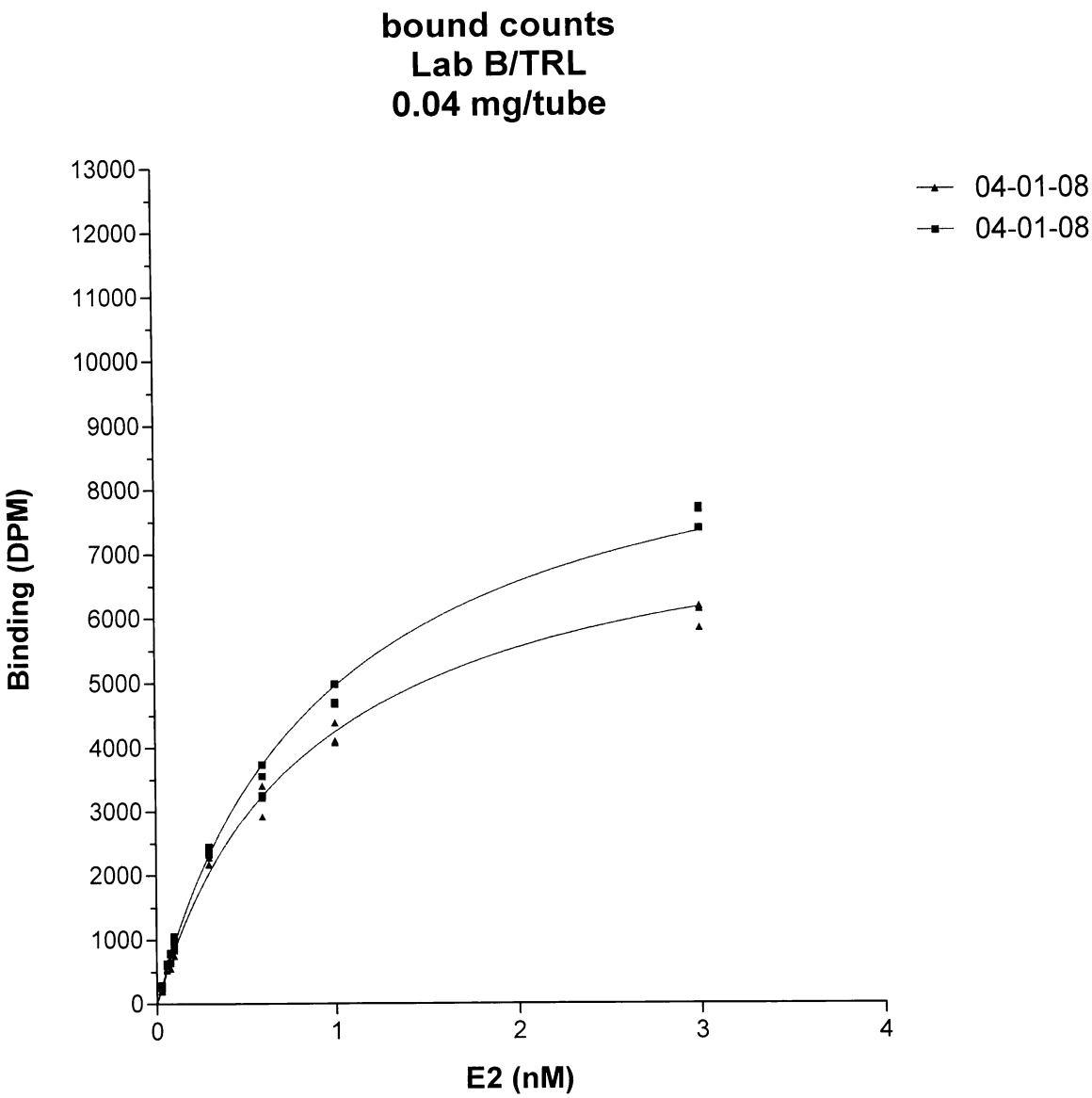
Scatchard Display
Lab B/TRL
0.04 mg/tube



Lab B/TRL
0.04 mg/tube




NSB Tubes
Lab B/TRL
0.04 mg/tube
04-01-08
Non-Specific Binding (DPM)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
E2 (nM)


bound counts
Lab B/TRL
0.04 mg/tube
04-01-08
04-01-08
Binding (DPM)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
E2 (nM)

APPENDIX C

Excel and Prism Files of Saturation Binding Data and Analysis for Run Identifier
TRL RUC2-22-08

Laboratory Code B/TRL

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B	
Run identification:	TTN sat. binding	
Assay start date:	3/24/2008	
Tracer lot number:	3589221/1	
Specific activity on day of assay:	105.53	Ci/mmole
Cytosol lot or vial number:	RUC-02-22-08	
protein (cytosol) per tube:	40	ug
protein (cytosol) per tube:	0.04	mg
KD	1.51E+00	nM
Bmax	107.9125	fmole/100 ug
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	
total volume in tubes	500	uL

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot E2 Initial Concentration (nM)*	*Hot E2 Volume (uL)*	*Hot E2 Final Concentration (nM)*	*Cold E2 Initial Concentration (nM)*	*Cold E2 Volume (uL)*	*Cold E2 Final Concentration (nM)*	*Buffer Volume (uL)*	*Receptor Volume (uL)*	*raw dpm*	*dpm for 1.5 mL*	*Use this value?*	*Notes to explain why "Use this value" is set to "FALSE"*	*Ten Percent Rule*
1	1	H	0.3	50	0.03	—	—	—	350	100	191	286.5	TRUE		6.5%
2	2	H	0.3	50	0.03	—	—	—	350	100	156	234	TRUE		5.3%
3	3	H	0.3	50	0.03	—	—	—	350	100	188	282	TRUE		6.4%
4	1	H	0.6	50	0.06	—	—	—	350	100	333	499.5	TRUE		5.6%
5	2	H	0.6	50	0.06	—	—	—	350	100	355	532.5	TRUE		6.0%
6	3	H	0.6	50	0.06	—	—	—	350	100	367	550.5	TRUE		6.2%
7	1	H	0.8	50	0.08	—	—	—	350	100	529	793.5	TRUE		6.7%
8	2	H	0.8	50	0.08	—	—	—	350	100	474	711	TRUE		6.0%
9	3	H	0.8	50	0.08	—	—	—	350	100	474	711	TRUE		6.0%
10	1	H	1.0	50	0.10	—	—	—	350	100	637	955.5	TRUE		6.5%
11	2	H	1.0	50	0.10	—	—	—	350	100	669	1003.5	TRUE		6.8%
12	3	H	1.0	50	0.10	—	—	—	350	100	657	985.5	TRUE		6.7%
13	1	H	3.0	50	0.30	—	—	—	350	100	1488	2232	TRUE		5.2%
14	2	H	3.0	50	0.30	—	—	—	350	100	1597	2395.5	TRUE		5.5%
15	3	H	3.0	50	0.30	—	—	—	350	100	1513	2269.5	TRUE		5.2%
16	1	H	6.0	50	0.60	—	—	—	350	100	2577	3865.5	TRUE		4.5%
17	2	H	6.0	50	0.60	—	—	—	350	100	2673	4009.5	TRUE		4.7%
18	3	H	6.0	50	0.60	—	—	—	350	100	2581	3871.5	TRUE		4.5%
19	1	H	10.0	50	1.00	—	—	—	350	100	3610	5415	TRUE		3.7%
20	2	H	10.0	50	1.00	—	—	—	350	100	3289	4933.5	TRUE		3.4%
21	3	H	10.0	50	1.00	—	—	—	350	100	3603	5404.5	TRUE		3.7%
22	1	H	30.0	50	3.00	—	—	—	350	100	5567	8350.5	TRUE		2.0%
23	2	H	30.0	50	3.00	—	—	—	350	100	5590	8385	TRUE		2.0%
24	3	H	30.0	50	3.00	—	—	—	350	100	5625	8437.5	TRUE		2.0%
25	1	HC	0.3	50	0.03	30	50	3	300	100	40	60	TRUE		
26	2	HC	0.3	50	0.03	30	50	3	300	100	38	57	TRUE		

Output to Curve Fitting Software

Saturation X values	*Bound y values*	*NSB y values*
0.03	286.5	57.5
0.03	234.0	57.5
0.03	282.0	57.5
0.06	499.5	80.5
0.06	532.5	80.5
0.06	550.5	80.5
0.08	793.5	84.0
0.08	711.0	84.0
0.08	711.0	84.0
0.1	955.5	107.5
0.1	1003.5	107.5
0.1	985.5	107.5
0.3	2232.0	181.5
0.3	2395.5	181.5
0.3	2269.5	181.5
0.6	3865.5	384.0
0.6	4009.5	384.0
0.6	3871.5	384.0
1	5415.0	654.0
1	4933.5	654.0
1	5404.5	654.0
3	8350.5	1635.0
3	8385.0	1635.0
3	8437.5	1635.0

27	3	HC	0.3	50	0.03	30	50	3	300	100
28	1	HC	0.6	50	0.06	60	50	6	300	100
29	2	HC	0.6	50	0.06	60	50	6	300	100
30	3	HC	0.6	50	0.06	60	50	6	300	100
31	1	HC	0.8	50	0.08	80	50	8	300	100
32	2	HC	0.8	50	0.08	80	50	8	300	100
33	3	HC	0.8	50	0.08	80	50	8	300	100
34	1	HC	1.0	50	0.10	100	50	10	300	100
35	2	HC	1.0	50	0.10	100	50	10	300	100
36	3	HC	1.0	50	0.10	100	50	10	300	100
37	1	HC	3.0	50	0.30	300	50	30	300	100
38	2	HC	3.0	50	0.30	300	50	30	300	100
39	3	HC	3.0	50	0.30	300	50	30	300	100
40	1	HC	6.0	50	0.60	600	50	60	300	100
41	2	HC	6.0	50	0.60	600	50	60	300	100
42	3	HC	6.0	50	0.60	600	50	60	300	100
43	1	HC	10.0	50	1.00	1000	50	100	300	100
44	2	HC	10.0	50	1.00	1000	50	100	300	100
45	3	HC	10.0	50	1.00	1000	50	100	300	100
46	1	HC	30.0	50	3.00	3000	50	300	300	100
47	2	HC	30.0	50	3.00	3000	50	300	300	100
48	3	HC	30.0	50	3.00	3000	50	300	300	100
49	1	Hot	0.3	50	0.03	—	—	—	—	—
50	2	Hot	0.3	50	0.03	—	—	—	—	—
51	3	Hot	0.3	50	0.03	—	—	—	—	—
52	1	Hot	0.6	50	0.06	—	—	—	—	—
53	2	Hot	0.6	50	0.06	—	—	—	—	—
54	3	Hot	0.6	50	0.06	—	—	—	—	—
55	1	Hot	0.8	50	0.08	—	—	—	—	—
56	2	Hot	0.8	50	0.08	—	—	—	—	—
57	3	Hot	0.8	50	0.08	—	—	—	—	—
58	1	Hot	1.0	50	0.10	—	—	—	—	—
59	2	Hot	1.0	50	0.10	—	—	—	—	—
60	3	Hot	1.0	50	0.10	—	—	—	—	—
61	1	Hot	3.0	50	0.30	—	—	—	—	—
62	2	Hot	3.0	50	0.30	—	—	—	—	—
63	3	Hot	3.0	50	0.30	—	—	—	—	—
64	1	Hot	6.0	50	0.60	—	—	—	—	—
65	2	Hot	6.0	50	0.60	—	—	—	—	—
66	3	Hot	6.0	50	0.60	—	—	—	—	—
67	1	Hot	10.0	50	1.00	—	—	—	—	—
68	2	Hot	10.0	50	1.00	—	—	—	—	—
69	3	Hot	10.0	50	1.00	—	—	—	—	—
70	1	Hot	30.0	50	3.00	—	—	—	—	—
71	2	Hot	30.0	50	3.00	—	—	—	—	—
72	3	Hot	30.0	50	3.00	—	—	—	—	—

37	55.5	TRUE
58	87	TRUE
53	79.5	TRUE
50	75	TRUE
60	90	TRUE
53	79.5	TRUE
55	82.5	TRUE
76	114	TRUE
79	118.5	TRUE
60	90	TRUE
90	135	TRUE
130	195	TRUE
143	214.5	TRUE
268	402	TRUE
238	357	TRUE
262	393	TRUE
473	709.5	TRUE
455	682.5	TRUE
380	570	TRUE
1116	1674	TRUE
1130	1695	TRUE
1024	1536	TRUE
2975	4462.5	TRUE
2984	4476	TRUE
2914	4371	TRUE
5913	8869.5	TRUE
5935	8902.5	TRUE
5923	8884.5	TRUE
7805	11707.5	TRUE
7965	11947.5	TRUE
7887	11830.5	TRUE
9655	14482.5	TRUE
9937	14905.5	TRUE
9958	14937	TRUE
28714	43071	TRUE
28896	43344	TRUE
28976	43464	TRUE
57009	85513.5	TRUE
58035	87052.5	TRUE
57267	85900.5	TRUE
98213	147319.5	TRUE
97038	145557	TRUE
98981	148471.5	TRUE
277135	415702.5	TRUE
289799	434698.5	TRUE
281373	422059.5	TRUE

Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
specific bound/molar	bound/free %	*average total added molar*	*specific bound/molar*	*total added dpm*	*NSB dpm*	*spcific bound/dpm*	*total bound dpm*
1.95496E-12	0.0552	3.79E-11	1.9550E-12	4462.50	60.00	229.00	286.50
1.50677E-12	0.0420	3.79E-11	1.5068E-12	4476.00	57.00	176.50	234.00
1.91655E-12	0.0540	3.79E-11	1.9165E-12	4371.00	55.50	224.50	282.00
3.57698E-12	0.0500	7.59E-11	3.5770E-12	8869.50	87.00	419.00	499.50
3.8587E-12	0.0541	7.59E-11	3.8587E-12	8902.50	79.50	452.00	532.50
4.01237E-12	0.0564	7.59E-11	4.0124E-12	8884.50	75.00	470.00	550.50
6.05697E-12	0.0643	1.01E-10	6.0570E-12	11707.50	90.00	709.50	793.50
5.35267E-12	0.0564	1.01E-10	5.3527E-12	11947.50	79.50	627.00	711.00
5.35267E-12	0.0564	1.01E-10	5.3527E-12	11830.50	82.50	627.00	711.00
7.23933E-12	0.0614	1.26E-10	7.2393E-12	14482.50	114.00	848.00	955.50
7.64911E-12	0.0651	1.26E-10	7.6491E-12	14905.50	118.50	896.00	1003.50
7.49544E-12	0.0637	1.26E-10	7.4954E-12	14937.00	90.00	878.00	985.50
1.7505E-11	0.0499	3.70E-10	1.7505E-11	43071.00	135.00	2050.50	2232.00
1.89008E-11	0.0541	3.70E-10	1.8901E-11	43344.00	195.00	2214.00	2395.50
1.78252E-11	0.0509	3.70E-10	1.7825E-11	43464.00	214.50	2088.00	2269.50
2.97214E-11	0.0423	7.36E-10	2.9721E-11	85513.50	402.00	3481.50	3865.50
3.09507E-11	0.0441	7.36E-10	3.0951E-11	87052.50	357.00	3625.50	4009.50
2.97726E-11	0.0424	7.36E-10	2.9773E-11	85900.50	393.00	3487.50	3871.50
4.06444E-11	0.0336	1.26E-09	4.0644E-11	147319.50	709.50	4761.00	5415.00
3.65339E-11	0.0301	1.26E-09	3.6534E-11	145557.00	682.50	4279.50	4933.50
4.05548E-11	0.0335	1.26E-09	4.0555E-11	148471.50	570.00	4750.50	5404.50
5.73299E-11	0.0162	3.62E-09	5.7330E-11	415702.50	1674.00	6715.50	8350.50
5.76244E-11	0.0162	3.62E-09	5.7624E-11	434698.50	1695.00	6750.00	8385.00
5.80726E-11	0.0164	3.62E-09	5.8073E-11	422059.50	1536.00	6802.50	8437.50

	3/25/2008
One site binding (hyperbola)	
Best-fit values	
BMAX	8.633E-11
KD	1.509E-09
Std. Error	
BMAX	6.262E-12
KD	1.376E-10
95% Confidence Intervals	
BMAX	7.334e-011 to 9.931e-011
KD	1.224e-009 to 1.795e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9928
Weighted Sum of Squares ($1/Y^2$)	0.2269
Absolute Sum of Squares	6.21E-23
Sy.x	0.1016
Data	
Number of X values	24
Number of Y replicates	
Total number of values	
Number of missing values	

Bmax molar	8.63E-11	KD molar	1.51E-09
mole to molar conversion value	0.0005	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	2.34E+17	**kd nM =**	1.51E+00
Bmax molar to Bmax moles	4.3165E-14		
= DPM/((DPM/mmole)*1000)	4.3165E-14		
=Bmax DPM	1.01E+04		
assay date	3/24/2008		
Bmax(dpm)	10112.50912		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	105.53		
DPM/mmole	2.34E+14		
DPM/pmole	2.34E+05		
1/(DPM/mmole)	4.27E-15		
1/(DPM/pmole)	4.27E-06		
SA(dpm/pmole)	2.34E+05		
protein/tube (ug)	40		
protein./tube(mg)	0.04		
bmax pmole	0.043165		
bmax pmole/mg	1.079125		
Bmax fmole/mg	1079.125		
Bmax (fmole/100 ug)	107.9125		
Bmax(fmole/100 ug)/Bmax molar	1.25E+12		

Laboratory Code B

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B	
Run identification:	TTN sat. binding	
Assay start date:	5/1/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.97	Ci/mmole
Cytosol lot or vial number:	RUC-03-03-08	
protein (cytosol) per tube:	50	ug
protein (cytosol) per tube:	0.05	mg
KD	9.80E-01	nM
Bmax	41.16	fmole/100 ug
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	
total volume in tubes	500	uL

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot E2 Initial Concentration (nM)*	*Hot E2 Volume (uL)*	*Hot E2 Final Concentration (nM)*	*Cold E2 Initial Concentration (nM)*	*Cold E2 Volume (uL)*	*Cold E2 Final Concentration (nM)*	*Buffer Volume (uL)*	*Receptor Volume (uL)*	*raw dpm*	*dpm for 1.5 mL*	*Use this value?*	*Notes to explain why "Use this value" is set to "FALSE"*	*Ten Percent Rule*	*Saturation X values*	*Bound y values*	*NSB y values*
1	1	H	0.3	50	0.03	—	—	—	350	100	63.67	95.505	TRUE		8.8%	0.03	95.5	56.5
2	2	H	0.3	50	0.03	—	—	—	350	100	61.87	92.805	TRUE		8.5%	0.03	92.8	56.5
3	3	H	0.3	50	0.03	—	—	—	350	100	64.47	96.705	TRUE		8.9%	0.03	96.7	56.5
4	1	H	0.6	50	0.06	—	—	—	350	100	89.8	134.7	TRUE		6.5%	0.06	134.7	67.2
5	2	H	0.6	50	0.06	—	—	—	350	100	100.13	150.195	TRUE		7.2%	0.06	150.2	67.2
6	3	H	0.6	50	0.06	—	—	—	350	100	101	151.5	TRUE		7.3%	0.06	151.5	67.2
7	1	H	0.8	50	0.08	—	—	—	350	100	127.4	191.1	TRUE		6.8%	0.08	191.1	64.8
8	2	H	0.8	50	0.08	—	—	—	350	100	129.67	194.505	TRUE		6.9%	0.08	194.5	64.8
9	3	H	0.8	50	0.08	—	—	—	350	100	120.54	180.81	TRUE		6.4%	0.08	180.8	64.8
10	1	H	1.0	50	0.10	—	—	—	350	100	152.14	228.21	TRUE		6.6%	0.1	228.2	72.5
11	2	H	1.0	50	0.10	—	—	—	350	100	131.2	196.8	TRUE		5.7%	0.1	196.8	72.5
12	3	H	1.0	50	0.10	—	—	—	350	100	159.54	239.31	TRUE		6.9%	0.1	239.3	72.5
13	1	H	3.0	50	0.30	—	—	—	350	100	368.01	552.015	TRUE		4.8%	0.3	552.0	93.6
14	2	H	3.0	50	0.30	—	—	—	350	100	416.54	624.81	TRUE		5.4%	0.3	624.8	93.6
15	3	H	3.0	50	0.30	—	—	—	350	100	351.54	527.31	TRUE		4.6%	0.3	527.3	93.6
16	1	H	6.0	50	0.60	—	—	—	350	100	683.82	1025.73	TRUE		4.4%	0.6	1025.7	131.3
17	2	H	6.0	50	0.60	—	—	—	350	100	707.89	1061.835	TRUE		4.5%	0.6	1061.8	131.3
18	3	H	6.0	50	0.60	—	—	—	350	100	663.55	995.325	TRUE		4.2%	0.6	995.3	131.3
19	1	H	10.0	50	1.00	—	—	—	350	100	806.89	1210.335	TRUE		3.0%	1	1210.3	182.1
20	2	H	10.0	50	1.00	—	—	—	350	100	1020.97	1531.455	TRUE		3.8%	1	1531.5	182.1
21	3	H	10.0	50	1.00	—	—	—	350	100	990.83	1486.245	TRUE		3.7%	1	1486.2	182.1
22	1	H	30.0	50	3.00	—	—	—	350	100	1879.47	2819.205	TRUE		2.3%	3	2819.2	415.8
23	2	H	30.0	50	3.00	—	—	—	350	100	1997.28	2995.92	TRUE		2.4%	3	2995.9	415.8
24	3	H	30.0	50	3.00	—	—	—	350	100	1906.94	2860.41	TRUE		2.3%	3	2860.4	415.8
25	1	HC	0.3	50	0.03	30	50	3	300	100	40.8	61.2	TRUE					
26	2	HC	0.3	50	0.03	30	50	3	300	100	34.2	51.3	TRUE					

Output to Curve Fitting Software

27	3	HC	0.3	50	0.03	30	50	3	300	100	37.94	56.91	TRUE
28	1	HC	0.6	50	0.06	60	50	6	300	100	40.74	61.11	TRUE
29	2	HC	0.6	50	0.06	60	50	6	300	100	47.2	70.8	TRUE
30	3	HC	0.6	50	0.06	60	50	6	300	100	46.4	69.6	TRUE
31	1	HC	0.8	50	0.08	80	50	8	300	100	38.34	57.51	TRUE
32	2	HC	0.8	50	0.08	80	50	8	300	100	46.34	69.51	TRUE
33	3	HC	0.8	50	0.08	80	50	8	300	100	44.94	67.41	TRUE
34	1	HC	1.0	50	0.10	100	50	10	300	100	47.67	71.505	TRUE
35	2	HC	1.0	50	0.10	100	50	10	300	100	48.27	72.405	TRUE
36	3	HC	1.0	50	0.10	100	50	10	300	100	49	73.5	TRUE
37	1	HC	3.0	50	0.30	300	50	30	300	100	58	87	TRUE
38	2	HC	3.0	50	0.30	300	50	30	300	100	62.14	93.21	TRUE
39	3	HC	3.0	50	0.30	300	50	30	300	100	67.14	100.71	TRUE
40	1	HC	6.0	50	0.60	600	50	60	300	100	77.81	116.715	TRUE
41	2	HC	6.0	50	0.60	600	50	60	300	100	93.47	140.205	TRUE
42	3	HC	6.0	50	0.60	600	50	60	300	100	91.27	136.905	TRUE
43	1	HC	10.0	50	1.00	1000	50	100	300	100	116.61	174.915	TRUE
44	2	HC	10.0	50	1.00	1000	50	100	300	100	124.61	186.915	TRUE
45	3	HC	10.0	50	1.00	1000	50	100	300	100	123.01	184.515	TRUE
46	1	HC	30.0	50	3.00	3000	50	300	300	100	308.49	462.735	TRUE
47	2	HC	30.0	50	3.00	3000	50	300	300	100	275.75	413.625	TRUE
48	3	HC	30.0	50	3.00	3000	50	300	300	100	247.29	370.935	TRUE
49	1	Hot	0.3	50	0.03	—	—	—	—	—	726.99	1090.485	TRUE
50	2	Hot	0.3	50	0.03	—	—	—	—	—	710.53	1065.795	TRUE
51	3	Hot	0.3	50	0.03	—	—	—	—	—	736.13	1104.195	TRUE
52	1	Hot	0.6	50	0.06	—	—	—	—	—	1401.65	2102.475	TRUE
53	2	Hot	0.6	50	0.06	—	—	—	—	—	1363.65	2045.475	TRUE
54	3	Hot	0.6	50	0.06	—	—	—	—	—	1398.06	2097.09	TRUE
55	1	Hot	0.8	50	0.08	—	—	—	—	—	1933.98	2900.97	TRUE
56	2	Hot	0.8	50	0.08	—	—	—	—	—	1721.89	2582.835	TRUE
57	3	Hot	0.8	50	0.08	—	—	—	—	—	1967.92	2951.88	TRUE
58	1	Hot	1.0	50	0.10	—	—	—	—	—	2402.83	3604.245	TRUE
59	2	Hot	1.0	50	0.10	—	—	—	—	—	2072.07	3108.105	TRUE
60	3	Hot	1.0	50	0.10	—	—	—	—	—	2471.45	3707.175	TRUE
61	1	Hot	3.0	50	0.30	—	—	—	—	—	7641.11	11461.665	TRUE
62	2	Hot	3.0	50	0.30	—	—	—	—	—	7618.92	11428.38	TRUE
63	3	Hot	3.0	50	0.30	—	—	—	—	—	7821.22	11731.83	TRUE
64	1	Hot	6.0	50	0.60	—	—	—	—	—	15685.53	23528.295	TRUE
65	2	Hot	6.0	50	0.60	—	—	—	—	—	15496.81	23245.215	TRUE
66	3	Hot	6.0	50	0.60	—	—	—	—	—	15754.53	23631.795	TRUE
67	1	Hot	10.0	50	1.00	—	—	—	—	—	26133.44	39200.16	TRUE
68	2	Hot	10.0	50	1.00	—	—	—	—	—	26691.42	40037.13	TRUE
69	3	Hot	10.0	50	1.00	—	—	—	—	—	27582.83	41374.245	TRUE
70	1	Hot	30.0	50	3.00	—	—	—	—	—	83644.08	125466.12	TRUE
71	2	Hot	30.0	50	3.00	—	—	—	—	—	83912.91	125869.365	TRUE
72	3	Hot	30.0	50	3.00	—	—	—	—	—	81828.68	122743.02	TRUE

Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
specific bound/molar	bound/free %	*average total added molar*	*specific bound/molar*	*total added dpm*	*NSB dpm*	*spcific bound/dpm*	*total bound dpm*
3.22708E-13	0.0394	8.98E-12	3.2271E-13	1090.49	61.20	39.04	95.51
3.00386E-13	0.0366	8.98E-12	3.0039E-13	1065.80	51.30	36.34	92.81
3.32628E-13	0.0406	8.98E-12	3.3263E-13	1104.20	56.91	40.24	96.71
5.5828E-13	0.0347	1.72E-11	5.5828E-13	2102.48	61.11	67.53	134.70
6.86379E-13	0.0430	1.72E-11	6.8638E-13	2045.48	70.80	83.03	150.20
6.97168E-13	0.0437	1.72E-11	6.9717E-13	2097.09	69.60	84.33	151.50
1.04406E-12	0.0482	2.32E-11	1.0441E-12	2900.97	57.51	126.29	191.10
1.07221E-12	0.0496	2.32E-11	1.0722E-12	2582.84	69.51	129.70	194.51
9.58988E-13	0.0441	2.32E-11	9.5899E-13	2951.88	67.41	116.00	180.81
1.28752E-12	0.0480	2.87E-11	1.2875E-12	3604.25	71.51	155.74	228.21
1.02785E-12	0.0379	2.87E-11	1.0279E-12	3108.11	72.41	124.33	196.80
1.37929E-12	0.0516	2.87E-11	1.3793E-12	3707.18	73.50	166.84	239.31
3.78945E-12	0.0417	9.54E-11	3.7894E-12	11461.67	87.00	458.38	552.02
4.39126E-12	0.0487	9.54E-11	4.3913E-12	11428.38	93.21	531.17	624.81
3.58521E-12	0.0394	9.54E-11	3.5852E-12	11731.83	100.71	433.67	527.31
7.39458E-12	0.0399	1.94E-10	7.3946E-12	23528.30	116.72	894.46	1025.73
7.69307E-12	0.0415	1.94E-10	7.6931E-12	23245.22	140.21	930.56	1061.84
7.14322E-12	0.0384	1.94E-10	7.1432E-12	23631.80	136.91	864.05	995.33
8.50044E-12	0.0264	3.32E-10	8.5004E-12	39200.16	174.92	1028.22	1210.34
1.11552E-11	0.0349	3.32E-10	1.1155E-11	40037.13	186.92	1349.34	1531.46
1.07814E-11	0.0337	3.32E-10	1.0781E-11	41374.25	184.52	1304.13	1486.25
1.98696E-11	0.0197	1.03E-09	1.9870E-11	125466.12	462.74	2403.44	2819.21
2.13305E-11	0.0212	1.03E-09	2.1330E-11	125869.37	413.63	2580.16	2995.92
2.02102E-11	0.0201	1.03E-09	2.0210E-11	122743.02	370.94	2444.65	2860.41

	5/1/2008
One site binding (hyperbola)	
Best-fit values	
BMAX	4.12E-11
KD	9.80E-10
Std. Error	
BMAX	5.40E-12
KD	1.46E-10
95% Confidence Intervals	
BMAX	2.996e-011 to 5.235e-011
KD	6.763e-010 to 1.283e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9916
Weighted Sum of Squares ($1/Y^2$)	0.3135
Absolute Sum of Squares	8.64E-24
Sy.x	0.1194
Data	
Number of X values	24
Number of Y replicates	
Total number of values	
Number of missing values	

Bmax molar	4.12E-11	KD molar	9.80E-10
mole to molar conversion value	0.0005	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	2.42E+17	**kd nM =**	9.80E-01
Bmax molar to Bmax moles	2.058E-14		
= DPM/((DPM/mmole)*1000)	2.058E-14		
=Bmax DPM	4.98E+03		
assay date	5/1/2008		
Bmax(dpm)	4978.7485		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	108.97		
DPM/mmole	2.42E+14		
DPM/pmole	2.42E+05		
1/(DPM/mmole)	4.13E-15		
1/(DPM/pmole)	4.13E-06		
SA(dpm/pmole)	2.42E+05		
protein/tube (ug)	50		
protein./tube(mg)	0.05		
bmax pmole	0.020580		
bmax pmole/mg	0.4116		
Bmax fmole/mg	411.6		
Bmax (fmole/100 ug)	41.16		
Bmax(fmole/100 ug)/Bmax molar	1.00E+12		

Table format: XY		X	A	B	C	D	E	F	G
		X Title	05-01-08	05-01-08	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G
		X	Y	Y	Y	Y	Y	Y	Y
1	Title	0.030000	95.500	56.5000					
2	Title	0.030000	92.800	56.5000					
3	Title	0.030000	96.700	56.5000					
4	Title	0.060000	134.700	67.2000					
5	Title	0.060000	150.200	67.2000					
6	Title	0.060000	151.500	67.2000					
7	Title	0.080000	191.100	64.8000					
8	Title	0.080000	194.500	64.8000					
9	Title	0.080000	180.800	64.8000					
10	Title	0.100000	228.200	72.5000					
11	Title	0.100000	196.800	72.5000					
12	Title	0.100000	239.300	72.5000					
13	Title	0.300000	552.000	93.6000					
14	Title	0.300000	624.800	93.6000					
15	Title	0.300000	527.300	93.6000					
16	Title	0.600000	1025.700	131.3000					
17	Title	0.600000	1061.800	131.3000					
18	Title	0.600000	995.300	131.3000					
19	Title	1.000000	1210.300	182.1000					
20	Title	1.000000	1531.500	182.1000					
21	Title	1.000000	1486.200	182.1000					
22	Title	3.000000	2819.200	415.8000					
23	Title	3.000000	2995.900	415.8000					
24	Title	3.000000	2860.400	415.8000					

	H	I	J	K	L
	Data Set-H	Data Set-I	Data Set-J	Data Set-K	Data Set-L
	Y	Y	Y	Y	Y
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	15.000000	1090.490					
2	Title	15.000000	1065.800					
3	Title	15.000000	1104.200					
4	Title	30.000000	2102.480					
5	Title	30.000000	2045.480					
6	Title	30.000000	2097.090					
7	Title	40.000000	2900.970					
8	Title	40.000000	2582.840					
9	Title	40.000000	2951.880					
10	Title	50.000000	3604.250					
11	Title	50.000000	3108.110					
12	Title	50.000000	3707.180					
13	Title	150.000000	11461.670					
14	Title	150.000000	11428.380					
15	Title	150.000000	11731.830					
16	Title	300.000000	23528.300					
17	Title	300.000000	23245.220					
18	Title	300.000000	23631.800					
19	Title	500.000000	39200.160					
20	Title	500.000000	40037.130					
21	Title	500.000000	41374.250					
22	Title	1500.000000	125466.100					
23	Title	1500.000000	125869.400					
24	Title	1500.000000	122743.000					

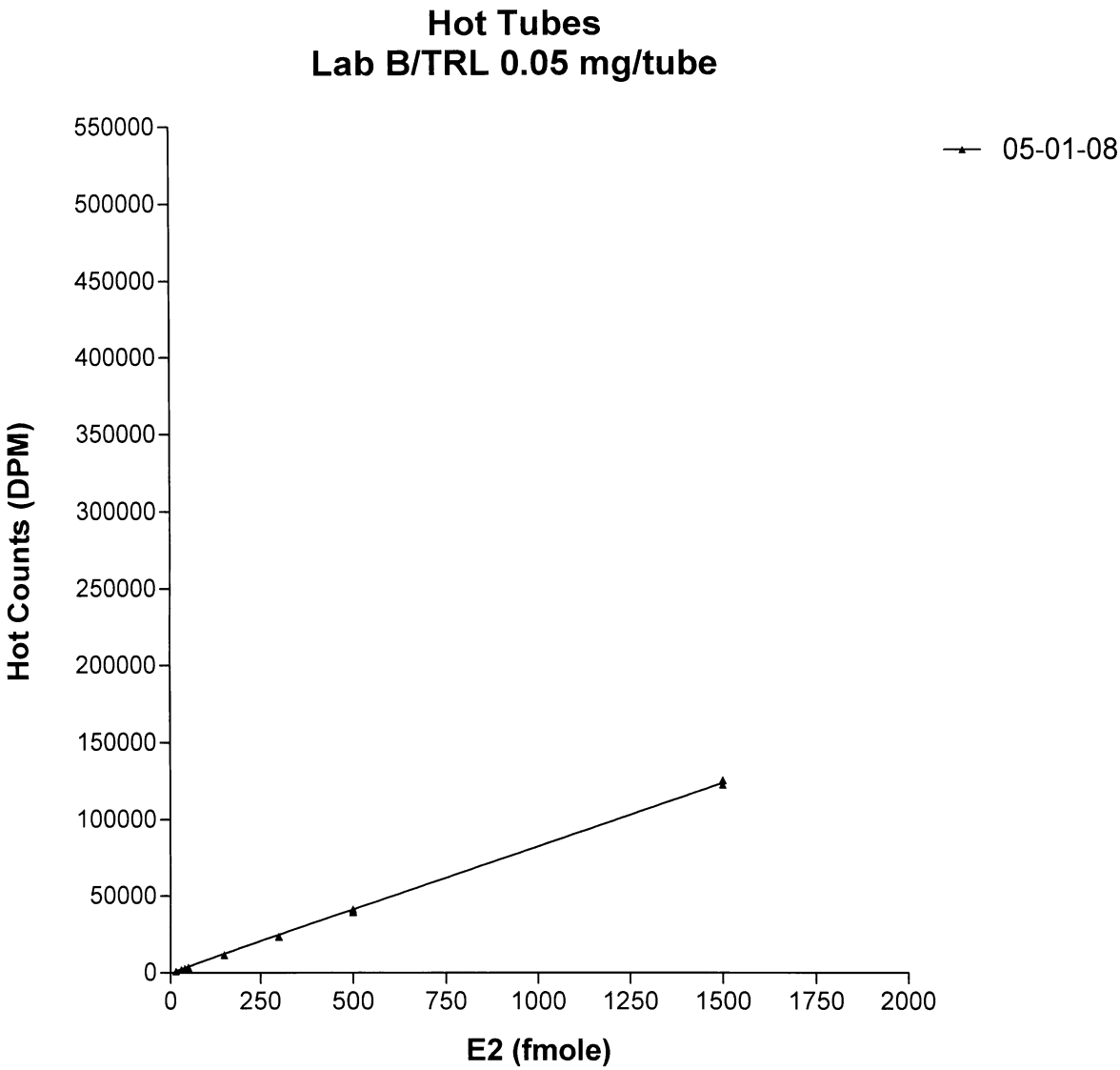
Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	61.20					
2	Title	0.030000	51.30					
3	Title	0.030000	56.91					
4	Title	0.060000	61.11					
5	Title	0.060000	70.80					
6	Title	0.060000	69.60					
7	Title	0.080000	57.51					
8	Title	0.080000	69.51					
9	Title	0.080000	67.41					
10	Title	0.100000	71.51					
11	Title	0.100000	72.41					
12	Title	0.100000	73.50					
13	Title	0.300000	87.00					
14	Title	0.300000	93.21					
15	Title	0.300000	100.71					
16	Title	0.600000	116.72					
17	Title	0.600000	140.21					
18	Title	0.600000	136.91					
19	Title	1.000000	174.92					
20	Title	1.000000	186.92					
21	Title	1.000000	184.52					
22	Title	3.000000	462.74					
23	Title	3.000000	413.63					
24	Title	3.000000	370.94					

Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	39.04000					
2	Title	0.030000	36.34000					
3	Title	0.030000	40.24000					
4	Title	0.060000	67.53000					
5	Title	0.060000	83.03000					
6	Title	0.060000	84.33000					
7	Title	0.080000	126.29000					
8	Title	0.080000	129.70000					
9	Title	0.080000	116.00000					
10	Title	0.100000	155.74000					
11	Title	0.100000	124.33000					
12	Title	0.100000	166.84000					
13	Title	0.300000	458.38000					
14	Title	0.300000	531.17000					
15	Title	0.300000	433.67000					
16	Title	0.600000	894.46000					
17	Title	0.600000	930.56000					
18	Title	0.600000	864.05000					
19	Title	1.000000	1028.22000					
20	Title	1.000000	1349.34000					
21	Title	1.000000	1304.13000					
22	Title	3.000000	2403.44000					
23	Title	3.000000	2580.16000					
24	Title	3.000000	2444.65000					

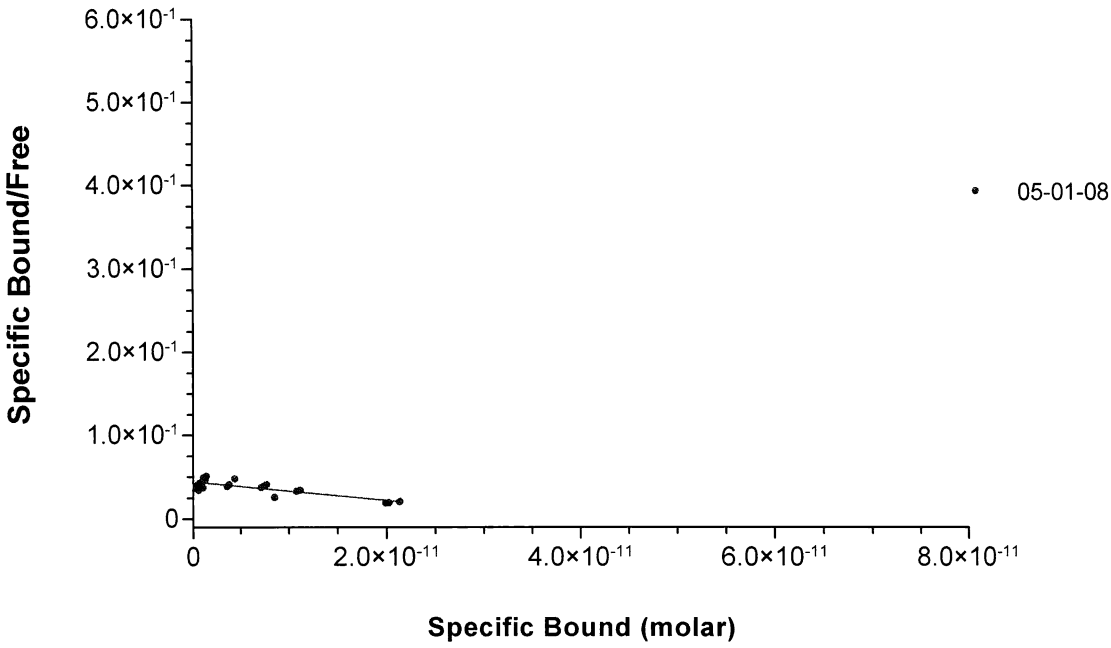
Table format: XY		X	A	B	C	D	E
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E
		X	Y	Y	Y	Y	Y
1	Title	0.030000	95.51000				
2	Title	0.030000	92.81000				
3	Title	0.030000	96.71000				
4	Title	0.060000	134.70000				
5	Title	0.060000	150.20000				
6	Title	0.060000	151.50000				
7	Title	0.080000	191.10000				
8	Title	0.080000	194.51000				
9	Title	0.080000	180.81000				
10	Title	0.100000	228.21000				
11	Title	0.100000	196.80000				
12	Title	0.100000	239.31000				
13	Title	0.300000	552.02000				
14	Title	0.300000	624.81000				
15	Title	0.300000	527.31000				
16	Title	0.600000	1025.73000				
17	Title	0.600000	1061.84000				
18	Title	0.600000	995.33000				
19	Title	1.000000	1210.34000				
20	Title	1.000000	1531.46000				
21	Title	1.000000	1486.25000				
22	Title	3.000000	2819.21000				
23	Title	3.000000	2995.92000				
24	Title	3.000000	2860.41000				

Table format: XY		X	A
		specific bound molar	05-01-08
		X	Y
1	Title	3.227080e-013	0.03940
2	Title	3.003860e-013	0.03660
3	Title	3.326280e-013	0.04060
4	Title	5.582800e-013	0.03470
5	Title	6.863790e-013	0.04300
6	Title	6.971680e-013	0.04370
7	Title	1.044060e-012	0.04820
8	Title	1.072210e-012	0.04960
9	Title	9.589880e-013	0.04410
10	Title	1.287520e-012	0.04800
11	Title	1.027850e-012	0.03790
12	Title	1.379290e-012	0.05160
13	Title	3.789450e-012	0.04170
14	Title	4.391260e-012	0.04870
15	Title	3.585210e-012	0.03940
16	Title	7.394580e-012	0.03990
17	Title	7.693070e-012	0.04150
18	Title	7.143220e-012	0.03840
19	Title	8.500440e-012	0.02640
20	Title	1.115520e-011	0.03490
21	Title	1.078140e-011	0.03370
22	Title	1.986960e-011	0.01970
23	Title	2.133050e-011	0.02120
24	Title	2.021020e-011	0.02010

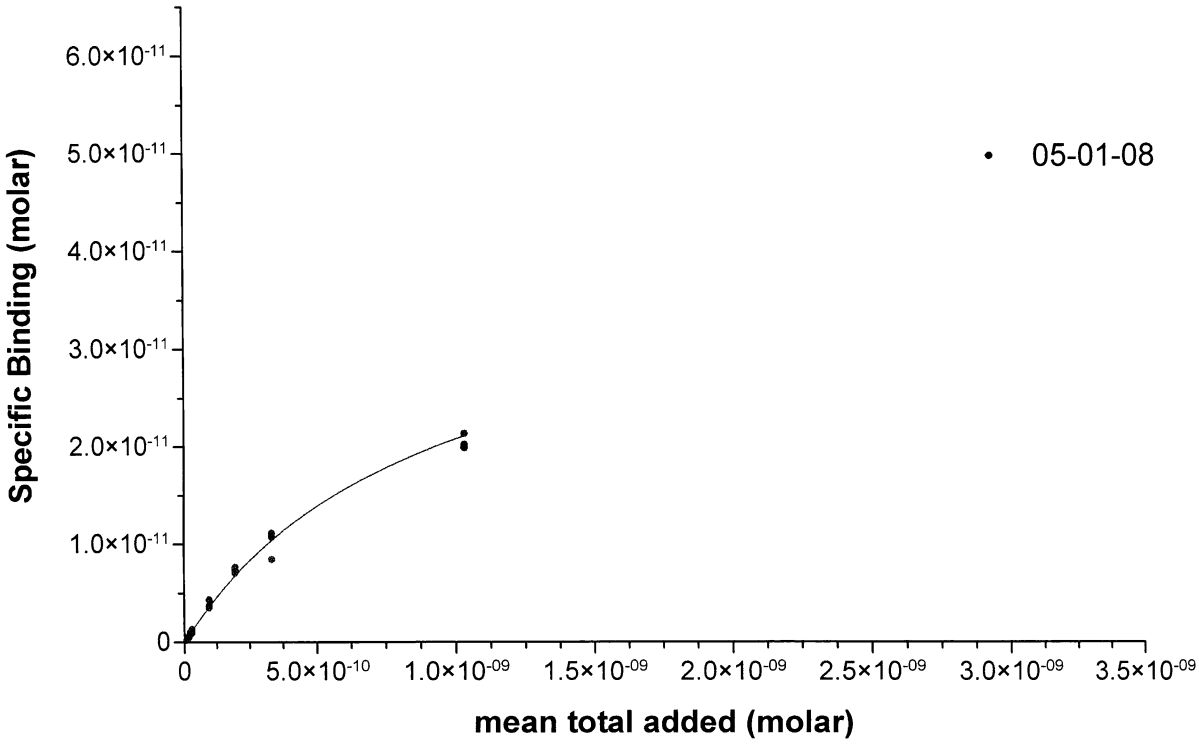
Table format: XY		X	A
		mean total added molar	05-01-08
		X	Y
1	Title	8.98000e-012	3.22710e-013
2	Title	8.98000e-012	3.00390e-013
3	Title	8.98000e-012	3.32630e-013
4	Title	1.72000e-011	5.58280e-013
5	Title	1.72000e-011	6.86380e-013
6	Title	1.72000e-011	6.97170e-013
7	Title	2.32000e-011	1.04410e-012
8	Title	2.32000e-011	1.07220e-012
9	Title	2.32000e-011	9.58990e-013
10	Title	2.87000e-011	1.28750e-012
11	Title	2.87000e-011	1.02790e-012
12	Title	2.87000e-011	1.37930e-012
13	Title	9.54000e-011	3.78940e-012
14	Title	9.54000e-011	4.39130e-012
15	Title	9.54000e-011	3.58520e-012
16	Title	1.94000e-010	7.39460e-012
17	Title	1.94000e-010	7.69310e-012
18	Title	1.94000e-010	7.14320e-012
19	Title	3.32000e-010	8.50040e-012
20	Title	3.32000e-010	1.11550e-011
21	Title	3.32000e-010	1.07810e-011
22	Title	1.03000e-009	1.98700e-011
23	Title	1.03000e-009	2.13300e-011
24	Title	1.03000e-009	2.02100e-011

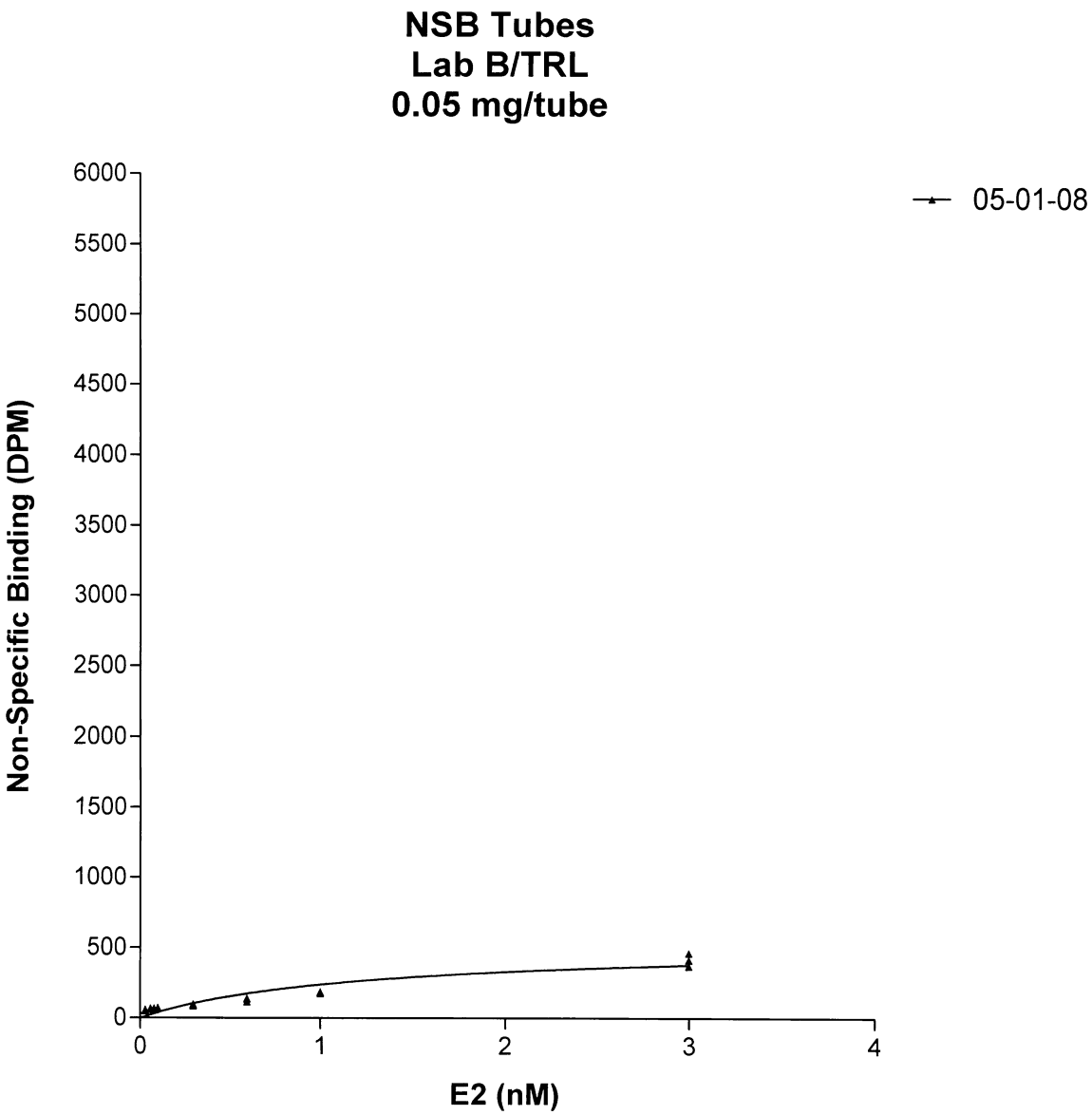

Hot Tubes
Lab B/TRL 0.05 mg/tube
05-01-08
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
1250
1500
1750
2000
E2 (fmole)

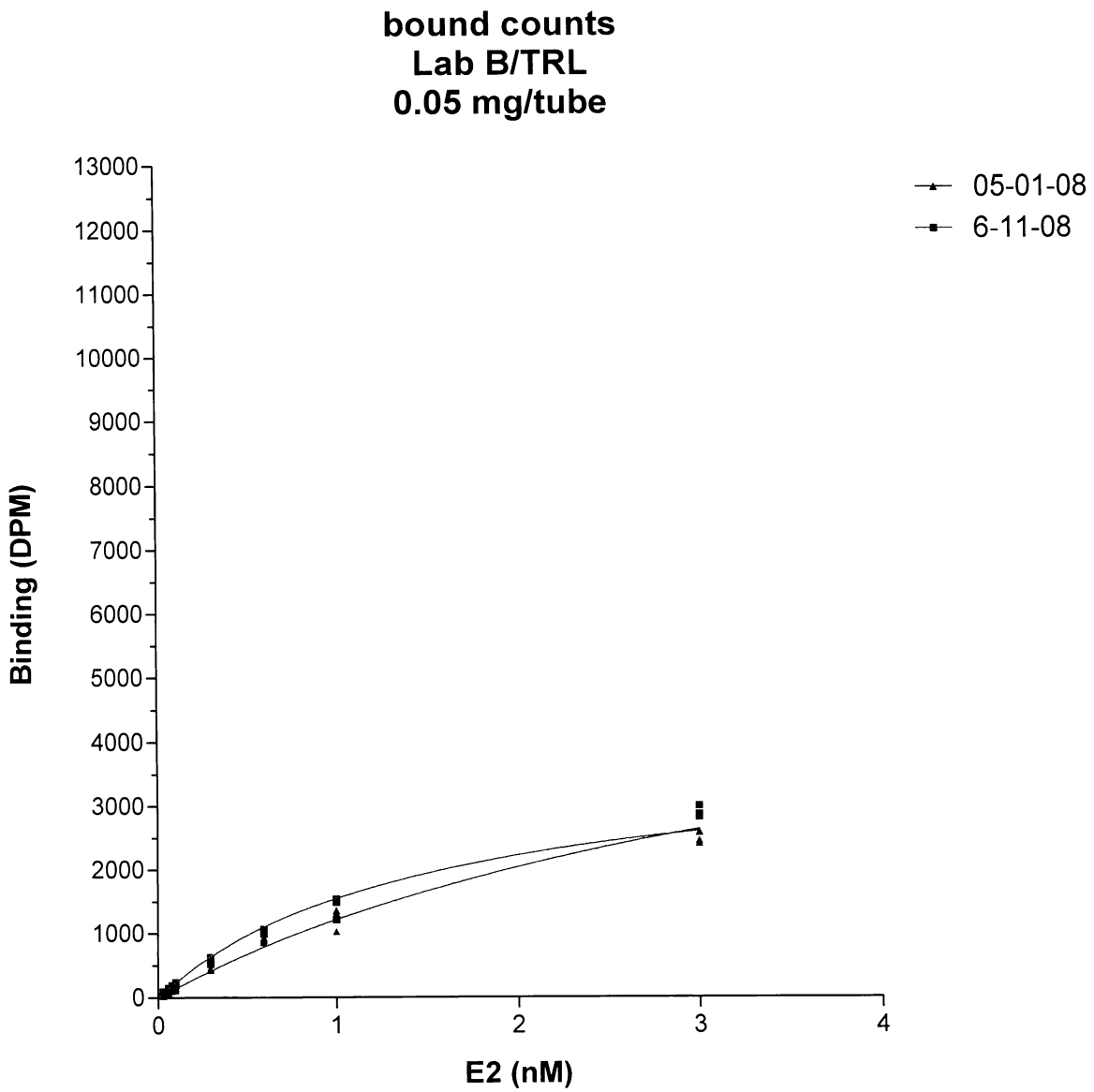
Scatchard Display
Lab B/TRL
0.04 mg/tube



Lab B/TRL
0.05 mg/tube




NSB Tubes
Lab B/TRL
0.05 mg/tube
05-01-08
Non-Specific Binding (DPM)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
E2 (nM)


bound counts
Lab B/TRL
0.05 mg/tube
05-01-08
6-11-08
Binding (DPM)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
E2 (nM)

	Nonlin fit Table of results	A 05-01-08
		Y
1	One site binding (hyperbola)	
2	Best-fit values	
3	BMAX	4.116e-011
4	KD	9.798e-010
5	Std. Error	
6	BMAX	5.397e-012
7	KD	1.463e-010
8	95% Confidence Intervals	
9	BMAX	2.996e-011 to 5.235e-011
10	KD	6.763e-010 to 1.283e-009
11	Goodness of Fit	
12	Degrees of Freedom	22
13	R^2 (unweighted)	0.9916
14	Weighted Sum of Squares ($1/Y^2$)	0.3135
15	Absolute Sum of Squares	8.644e-024
16	Sy.x	0.1194
17	Number of points	
18	Analyzed	24

APPENDIX D

Excel and Prism Files of Saturation Binding Data and Analysis for Run Identifier
TRL RUC3-03-08

Laboratory Code B

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B
Run identification:	TTN sat. binding
Assay start date:	5/1/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	108.97 Ci/mmole
Cytosol lot or vial number:	RUC-03-03-08
protein (cytosol) per tube:	50 ug
protein (cytosol) per tube:	0.05 mg
KD	9.80E-01 nM
Bmax	41.16 fmole/100 ug
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5
total volume in tubes	500 uL

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot E2 Initial Concentration (nM)	Hot E2 Volume (uL)	Hot E2 Final Concentration (nM)	Cold E2 Initial Concentration (nM)	Cold E2 Volume (uL)	Cold E2 Final Concentration (nM)	Buffer Volume (uL)	Receptor Volume (uL)	raw dpm	dpm for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Output to Curve Fitting Software: Saturation X values	Bound y values	NSB y values
1	1	H	0.3	50	0.03	—	—	—	350	100	63.67	95.505	TRUE		8.8%	0.03	95.5	56.5
2	2	H	0.3	50	0.03	—	—	—	350	100	61.87	92.805	TRUE		8.5%	0.03	92.8	56.5
3	3	H	0.3	50	0.03	—	—	—	350	100	64.47	96.705	TRUE		8.9%	0.03	96.7	56.5
4	1	H	0.6	50	0.06	—	—	—	350	100	89.8	134.7	TRUE		6.5%	0.06	134.7	67.2
5	2	H	0.6	50	0.06	—	—	—	350	100	100.13	150.195	TRUE		7.2%	0.06	150.2	67.2
6	3	H	0.6	50	0.06	—	—	—	350	100	101	151.5	TRUE		7.3%	0.06	151.5	67.2
7	1	H	0.8	50	0.08	—	—	—	350	100	127.4	191.1	TRUE		6.8%	0.08	191.1	64.8
8	2	H	0.8	50	0.08	—	—	—	350	100	129.67	194.505	TRUE		6.9%	0.08	194.5	64.8
9	3	H	0.8	50	0.08	—	—	—	350	100	120.54	180.81	TRUE		6.4%	0.08	180.8	64.8
10	1	H	1.0	50	0.10	—	—	—	350	100	152.14	228.21	TRUE		6.6%	0.1	228.2	72.5
11	2	H	1.0	50	0.10	—	—	—	350	100	131.2	196.8	TRUE		5.7%	0.1	196.8	72.5
12	3	H	1.0	50	0.10	—	—	—	350	100	159.54	239.31	TRUE		6.9%	0.1	239.3	72.5
13	1	H	3.0	50	0.30	—	—	—	350	100	368.01	552.015	TRUE		4.8%	0.3	552.0	93.6
14	2	H	3.0	50	0.30	—	—	—	350	100	416.54	624.81	TRUE		5.4%	0.3	624.8	93.6
15	3	H	3.0	50	0.30	—	—	—	350	100	351.54	527.31	TRUE		4.6%	0.3	527.3	93.6
16	1	H	6.0	50	0.60	—	—	—	350	100	683.82	1025.73	TRUE		4.4%	0.6	1025.7	131.3
17	2	H	6.0	50	0.60	—	—	—	350	100	707.89	1061.835	TRUE		4.5%	0.6	1061.8	131.3
18	3	H	6.0	50	0.60	—	—	—	350	100	663.55	995.325	TRUE		4.2%	0.6	995.3	131.3
19	1	H	10.0	50	1.00	—	—	—	350	100	806.89	1210.335	TRUE		3.0%	1	1210.3	182.1
20	2	H	10.0	50	1.00	—	—	—	350	100	1020.97	1531.455	TRUE		3.8%	1	1531.5	182.1
21	3	H	10.0	50	1.00	—	—	—	350	100	990.83	1486.245	TRUE		3.7%	1	1486.2	182.1
22	1	H	30.0	50	3.00	—	—	—	350	100	1879.47	2819.205	TRUE		2.3%	3	2819.2	415.8
23	2	H	30.0	50	3.00	—	—	—	350	100	1997.28	2995.92	TRUE		2.4%	3	2995.9	415.8
24	3	H	30.0	50	3.00	—	—	—	350	100	1906.94	2860.41	TRUE		2.3%	3	2860.4	415.8
25	1	HC	0.3	50	0.03	30	50	3	300	100	40.8	61.2	TRUE					
26	2	HC	0.3	50	0.03	30	50	3	300	100	34.2	51.3	TRUE					

27	3	HC	0.3	50	0.03	30	50	3	300	100	37.94	56.91	TRUE
28	1	HC	0.6	50	0.06	60	50	6	300	100	40.74	61.11	TRUE
29	2	HC	0.6	50	0.06	60	50	6	300	100	47.2	70.8	TRUE
30	3	HC	0.6	50	0.06	60	50	6	300	100	46.4	69.6	TRUE
31	1	HC	0.8	50	0.08	80	50	8	300	100	38.34	57.51	TRUE
32	2	HC	0.8	50	0.08	80	50	8	300	100	46.34	69.51	TRUE
33	3	HC	0.8	50	0.08	80	50	8	300	100	44.94	67.41	TRUE
34	1	HC	1.0	50	0.10	100	50	10	300	100	47.67	71.505	TRUE
35	2	HC	1.0	50	0.10	100	50	10	300	100	48.27	72.405	TRUE
36	3	HC	1.0	50	0.10	100	50	10	300	100	49	73.5	TRUE
37	1	HC	3.0	50	0.30	300	50	30	300	100	58	87	TRUE
38	2	HC	3.0	50	0.30	300	50	30	300	100	62.14	93.21	TRUE
39	3	HC	3.0	50	0.30	300	50	30	300	100	67.14	100.71	TRUE
40	1	HC	6.0	50	0.60	600	50	60	300	100	77.81	116.715	TRUE
41	2	HC	6.0	50	0.60	600	50	60	300	100	93.47	140.205	TRUE
42	3	HC	6.0	50	0.60	600	50	60	300	100	91.27	136.905	TRUE
43	1	HC	10.0	50	1.00	1000	50	100	300	100	116.61	174.915	TRUE
44	2	HC	10.0	50	1.00	1000	50	100	300	100	124.61	186.915	TRUE
45	3	HC	10.0	50	1.00	1000	50	100	300	100	123.01	184.515	TRUE
46	1	HC	30.0	50	3.00	3000	50	300	300	100	308.49	462.735	TRUE
47	2	HC	30.0	50	3.00	3000	50	300	300	100	275.75	413.625	TRUE
48	3	HC	30.0	50	3.00	3000	50	300	300	100	247.29	370.935	TRUE
49	1	Hot	0.3	50	0.03	—	—	—	—	—	726.99	1090.485	TRUE
50	2	Hot	0.3	50	0.03	—	—	—	—	—	710.53	1065.795	TRUE
51	3	Hot	0.3	50	0.03	—	—	—	—	—	736.13	1104.195	TRUE
52	1	Hot	0.6	50	0.06	—	—	—	—	—	1401.65	2102.475	TRUE
53	2	Hot	0.6	50	0.06	—	—	—	—	—	1363.65	2045.475	TRUE
54	3	Hot	0.6	50	0.06	—	—	—	—	—	1398.06	2097.09	TRUE
55	1	Hot	0.8	50	0.08	—	—	—	—	—	1933.98	2900.97	TRUE
56	2	Hot	0.8	50	0.08	—	—	—	—	—	1721.89	2582.835	TRUE
57	3	Hot	0.8	50	0.08	—	—	—	—	—	1967.92	2951.88	TRUE
58	1	Hot	1.0	50	0.10	—	—	—	—	—	2402.83	3604.245	TRUE
59	2	Hot	1.0	50	0.10	—	—	—	—	—	2072.07	3108.105	TRUE
60	3	Hot	1.0	50	0.10	—	—	—	—	—	2471.45	3707.175	TRUE
61	1	Hot	3.0	50	0.30	—	—	—	—	—	7641.11	11461.665	TRUE
62	2	Hot	3.0	50	0.30	—	—	—	—	—	7618.92	11428.38	TRUE
63	3	Hot	3.0	50	0.30	—	—	—	—	—	7821.22	11731.83	TRUE
64	1	Hot	6.0	50	0.60	—	—	—	—	—	15685.53	23528.295	TRUE
65	2	Hot	6.0	50	0.60	—	—	—	—	—	15496.81	23245.215	TRUE
66	3	Hot	6.0	50	0.60	—	—	—	—	—	15754.53	23631.795	TRUE
67	1	Hot	10.0	50	1.00	—	—	—	—	—	26133.44	39200.16	TRUE
68	2	Hot	10.0	50	1.00	—	—	—	—	—	26691.42	40037.13	TRUE
69	3	Hot	10.0	50	1.00	—	—	—	—	—	27582.83	41374.245	TRUE
70	1	Hot	30.0	50	3.00	—	—	—	—	—	83644.08	125466.12	TRUE
71	2	Hot	30.0	50	3.00	—	—	—	—	—	83912.91	125869.365	TRUE
72	3	Hot	30.0	50	3.00	—	—	—	—	—	81828.68	122743.02	TRUE

Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
specific bound/molar	bound/free %	average total added molar	specific bound/molar	total added dpm	NSB dpm	spcific bound/dpm	total bound dpm
3.22708E-13	0.0394	8.98E-12	3.2271E-13	1090.49	61.20	39.04	95.51
3.00386E-13	0.0366	8.98E-12	3.0039E-13	1065.80	51.30	36.34	92.81
3.32628E-13	0.0406	8.98E-12	3.3263E-13	1104.20	56.91	40.24	96.71
5.5828E-13	0.0347	1.72E-11	5.5828E-13	2102.48	61.11	67.53	134.70
6.86379E-13	0.0430	1.72E-11	6.8638E-13	2045.48	70.80	83.03	150.20
6.97168E-13	0.0437	1.72E-11	6.9717E-13	2097.09	69.60	84.33	151.50
1.04406E-12	0.0482	2.32E-11	1.0441E-12	2900.97	57.51	126.29	191.10
1.07221E-12	0.0496	2.32E-11	1.0722E-12	2582.84	69.51	129.70	194.51
9.58988E-13	0.0441	2.32E-11	9.5899E-13	2951.88	67.41	116.00	180.81
1.28752E-12	0.0480	2.87E-11	1.2875E-12	3604.25	71.51	155.74	228.21
1.02785E-12	0.0379	2.87E-11	1.0279E-12	3108.11	72.41	124.33	196.80
1.37929E-12	0.0516	2.87E-11	1.3793E-12	3707.18	73.50	166.84	239.31
3.78945E-12	0.0417	9.54E-11	3.7894E-12	11461.67	87.00	458.38	552.02
4.39126E-12	0.0487	9.54E-11	4.3913E-12	11428.38	93.21	531.17	624.81
3.58521E-12	0.0394	9.54E-11	3.5852E-12	11731.83	100.71	433.67	527.31
7.39458E-12	0.0399	1.94E-10	7.3946E-12	23528.30	116.72	894.46	1025.73
7.69307E-12	0.0415	1.94E-10	7.6931E-12	23245.22	140.21	930.56	1061.84
7.14322E-12	0.0384	1.94E-10	7.1432E-12	23631.80	136.91	864.05	995.33
8.50044E-12	0.0264	3.32E-10	8.5004E-12	39200.16	174.92	1028.22	1210.34
1.11552E-11	0.0349	3.32E-10	1.1155E-11	40037.13	186.92	1349.34	1531.46
1.07814E-11	0.0337	3.32E-10	1.0781E-11	41374.25	184.52	1304.13	1486.25
1.98696E-11	0.0197	1.03E-09	1.9870E-11	125466.12	462.74	2403.44	2819.21
2.13305E-11	0.0212	1.03E-09	2.1330E-11	125869.37	413.63	2580.16	2995.92
2.02102E-11	0.0201	1.03E-09	2.0210E-11	122743.02	370.94	2444.65	2860.41

	5/1/2008
One site binding (hyperbola)	
Best-fit values	
BMAX	4.12E-11
KD	9.80E-10
Std. Error	
BMAX	5.40E-12
KD	1.46E-10
95% Confidence Intervals	
BMAX	2.996e-011 to 5.235e-011
KD	6.763e-010 to 1.283e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9916
Weighted Sum of Squares ($1/Y^2$)	0.3135
Absolute Sum of Squares	8.64E-24
Sy.x	0.1194
Data	
Number of X values	24
Number of Y replicates	
Total number of values	
Number of missing values	

Bmax molar	4.12E-11	KD molar	9.80E-10
mole to molar conversion value	0.0005	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	2.42E+17	**kd nM =**	9.80E-01
Bmax molar to Bmax moles	2.058E-14		
= DPM/((DPM/mmole)*1000)	2.058E-14		
=Bmax DPM	4.98E+03		
assay date	5/1/2008		
Bmax(dpm)	4978.7485		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	108.97		
DPM/mmole	2.42E+14		
DPM/pmole	2.42E+05		
1/(DPM/mmole)	4.13E-15		
1/(DPM/pmole)	4.13E-06		
SA(dpm/pmole)	2.42E+05		
protein/tube (ug)	50		
protein./tube(mg)	0.05		
bmax pmole	0.020580		
bmax pmole/mg	0.4116		
Bmax fmole/mg	411.6		
Bmax (fmole/100 ug)	41.16		
Bmax(fmole/100 ug)/Bmax molar	1.00E+12		

Table format: XY		X	A	B	C	D	E	F	G
		X Title	05-01-08	05-01-08	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G
		X	Y	Y	Y	Y	Y	Y	Y
1	Title	0.030000	95.500	56.5000					
2	Title	0.030000	92.800	56.5000					
3	Title	0.030000	96.700	56.5000					
4	Title	0.060000	134.700	67.2000					
5	Title	0.060000	150.200	67.2000					
6	Title	0.060000	151.500	67.2000					
7	Title	0.080000	191.100	64.8000					
8	Title	0.080000	194.500	64.8000					
9	Title	0.080000	180.800	64.8000					
10	Title	0.100000	228.200	72.5000					
11	Title	0.100000	196.800	72.5000					
12	Title	0.100000	239.300	72.5000					
13	Title	0.300000	552.000	93.6000					
14	Title	0.300000	624.800	93.6000					
15	Title	0.300000	527.300	93.6000					
16	Title	0.600000	1025.700	131.3000					
17	Title	0.600000	1061.800	131.3000					
18	Title	0.600000	995.300	131.3000					
19	Title	1.000000	1210.300	182.1000					
20	Title	1.000000	1531.500	182.1000					
21	Title	1.000000	1486.200	182.1000					
22	Title	3.000000	2819.200	415.8000					
23	Title	3.000000	2995.900	415.8000					
24	Title	3.000000	2860.400	415.8000					

	H	I	J	K	L
	Data Set-H	Data Set-I	Data Set-J	Data Set-K	Data Set-L
	Y	Y	Y	Y	Y
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	15.000000	1090.490					
2	Title	15.000000	1065.800					
3	Title	15.000000	1104.200					
4	Title	30.000000	2102.480					
5	Title	30.000000	2045.480					
6	Title	30.000000	2097.090					
7	Title	40.000000	2900.970					
8	Title	40.000000	2582.840					
9	Title	40.000000	2951.880					
10	Title	50.000000	3604.250					
11	Title	50.000000	3108.110					
12	Title	50.000000	3707.180					
13	Title	150.000000	11461.670					
14	Title	150.000000	11428.380					
15	Title	150.000000	11731.830					
16	Title	300.000000	23528.300					
17	Title	300.000000	23245.220					
18	Title	300.000000	23631.800					
19	Title	500.000000	39200.160					
20	Title	500.000000	40037.130					
21	Title	500.000000	41374.250					
22	Title	1500.000000	125466.100					
23	Title	1500.000000	125869.400					
24	Title	1500.000000	122743.000					

Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	61.20					
2	Title	0.030000	51.30					
3	Title	0.030000	56.91					
4	Title	0.060000	61.11					
5	Title	0.060000	70.80					
6	Title	0.060000	69.60					
7	Title	0.080000	57.51					
8	Title	0.080000	69.51					
9	Title	0.080000	67.41					
10	Title	0.100000	71.51					
11	Title	0.100000	72.41					
12	Title	0.100000	73.50					
13	Title	0.300000	87.00					
14	Title	0.300000	93.21					
15	Title	0.300000	100.71					
16	Title	0.600000	116.72					
17	Title	0.600000	140.21					
18	Title	0.600000	136.91					
19	Title	1.000000	174.92					
20	Title	1.000000	186.92					
21	Title	1.000000	184.52					
22	Title	3.000000	462.74					
23	Title	3.000000	413.63					
24	Title	3.000000	370.94					

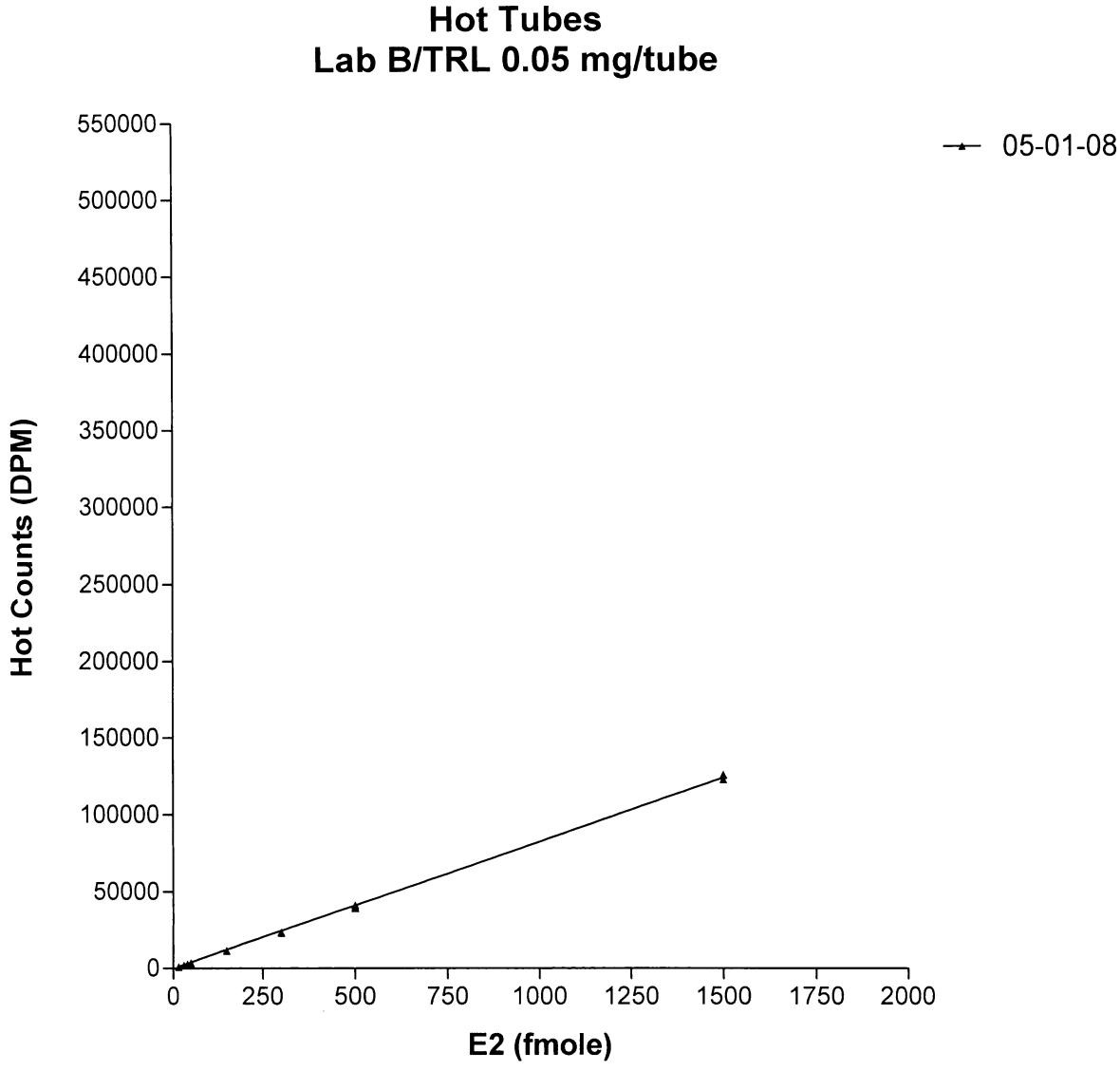
Table format: XY		X	A	B	C	D	E	F
		X Title	05-01-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	39.04000					
2	Title	0.030000	36.34000					
3	Title	0.030000	40.24000					
4	Title	0.060000	67.53000					
5	Title	0.060000	83.03000					
6	Title	0.060000	84.33000					
7	Title	0.080000	126.29000					
8	Title	0.080000	129.70000					
9	Title	0.080000	116.00000					
10	Title	0.100000	155.74000					
11	Title	0.100000	124.33000					
12	Title	0.100000	166.84000					
13	Title	0.300000	458.38000					
14	Title	0.300000	531.17000					
15	Title	0.300000	433.67000					
16	Title	0.600000	894.46000					
17	Title	0.600000	930.56000					
18	Title	0.600000	864.05000					
19	Title	1.000000	1028.22000					
20	Title	1.000000	1349.34000					
21	Title	1.000000	1304.13000					
22	Title	3.000000	2403.44000					
23	Title	3.000000	2580.16000					
24	Title	3.000000	2444.65000					

Table format: XY		X	A	B	C	D	E
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E
		X	Y	Y	Y	Y	Y
1	Title	0.030000	95.51000				
2	Title	0.030000	92.81000				
3	Title	0.030000	96.71000				
4	Title	0.060000	134.70000				
5	Title	0.060000	150.20000				
6	Title	0.060000	151.50000				
7	Title	0.080000	191.10000				
8	Title	0.080000	194.51000				
9	Title	0.080000	180.81000				
10	Title	0.100000	228.21000				
11	Title	0.100000	196.80000				
12	Title	0.100000	239.31000				
13	Title	0.300000	552.02000				
14	Title	0.300000	624.81000				
15	Title	0.300000	527.31000				
16	Title	0.600000	1025.73000				
17	Title	0.600000	1061.84000				
18	Title	0.600000	995.33000				
19	Title	1.000000	1210.34000				
20	Title	1.000000	1531.46000				
21	Title	1.000000	1486.25000				
22	Title	3.000000	2819.21000				
23	Title	3.000000	2995.92000				
24	Title	3.000000	2860.41000				

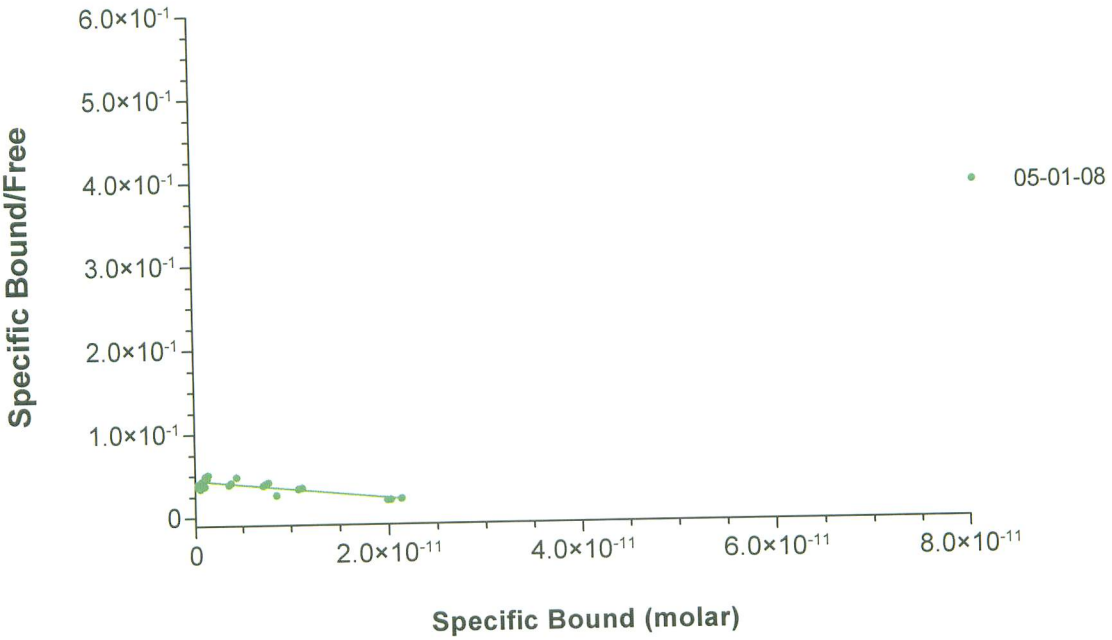
Table format: XY		X	A
		specific bound molar	05-01-08
		X	Y
1	Title	3.227080e-013	0.03940
2	Title	3.003860e-013	0.03660
3	Title	3.326280e-013	0.04060
4	Title	5.582800e-013	0.03470
5	Title	6.863790e-013	0.04300
6	Title	6.971680e-013	0.04370
7	Title	1.044060e-012	0.04820
8	Title	1.072210e-012	0.04960
9	Title	9.589880e-013	0.04410
10	Title	1.287520e-012	0.04800
11	Title	1.027850e-012	0.03790
12	Title	1.379290e-012	0.05160
13	Title	3.789450e-012	0.04170
14	Title	4.391260e-012	0.04870
15	Title	3.585210e-012	0.03940
16	Title	7.394580e-012	0.03990
17	Title	7.693070e-012	0.04150
18	Title	7.143220e-012	0.03840
19	Title	8.500440e-012	0.02640
20	Title	1.115520e-011	0.03490
21	Title	1.078140e-011	0.03370
22	Title	1.986960e-011	0.01970
23	Title	2.133050e-011	0.02120
24	Title	2.021020e-011	0.02010

Table format: XY		X	A
		mean total added molar	05-01-08
		X	Y
1	Title	8.98000e-012	3.22710e-013
2	Title	8.98000e-012	3.00390e-013
3	Title	8.98000e-012	3.32630e-013
4	Title	1.72000e-011	5.58280e-013
5	Title	1.72000e-011	6.86380e-013
6	Title	1.72000e-011	6.97170e-013
7	Title	2.32000e-011	1.04410e-012
8	Title	2.32000e-011	1.07220e-012
9	Title	2.32000e-011	9.58990e-013
10	Title	2.87000e-011	1.28750e-012
11	Title	2.87000e-011	1.02790e-012
12	Title	2.87000e-011	1.37930e-012
13	Title	9.54000e-011	3.78940e-012
14	Title	9.54000e-011	4.39130e-012
15	Title	9.54000e-011	3.58520e-012
16	Title	1.94000e-010	7.39460e-012
17	Title	1.94000e-010	7.69310e-012
18	Title	1.94000e-010	7.14320e-012
19	Title	3.32000e-010	8.50040e-012
20	Title	3.32000e-010	1.11550e-011
21	Title	3.32000e-010	1.07810e-011
22	Title	1.03000e-009	1.98700e-011
23	Title	1.03000e-009	2.13300e-011
24	Title	1.03000e-009	2.02100e-011

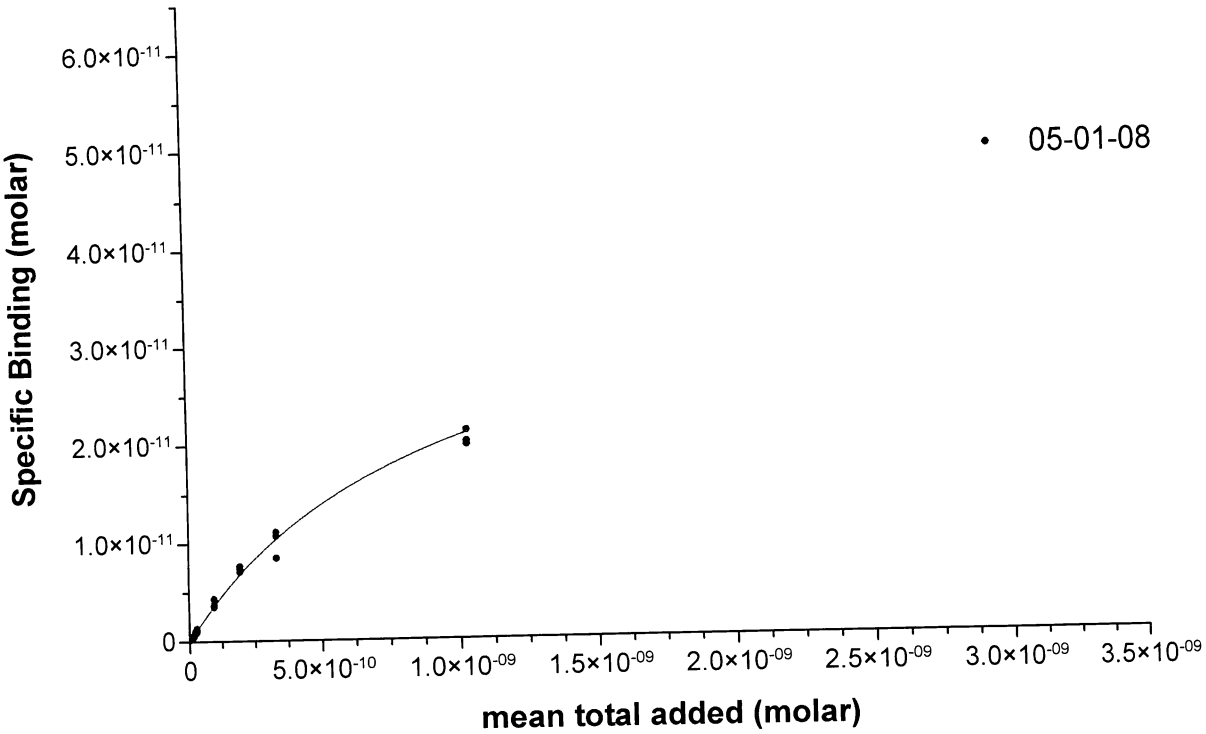
	Nonlin fit Table of results	A 05-01-08
		Y
1	One site binding (hyperbola)	
2	Best-fit values	
3	BMAX	4.116e-011
4	KD	9.798e-010
5	Std. Error	
6	BMAX	5.397e-012
7	KD	1.463e-010
8	95% Confidence Intervals	
9	BMAX	2.996e-011 to 5.235e-011
10	KD	6.763e-010 to 1.283e-009
11	Goodness of Fit	
12	Degrees of Freedom	22
13	R^2 (unweighted)	0.9916
14	Weighted Sum of Squares ($1/Y^2$)	0.3135
15	Absolute Sum of Squares	8.644e-024
16	Sy.x	0.1194
17	Number of points	
18	Analyzed	24

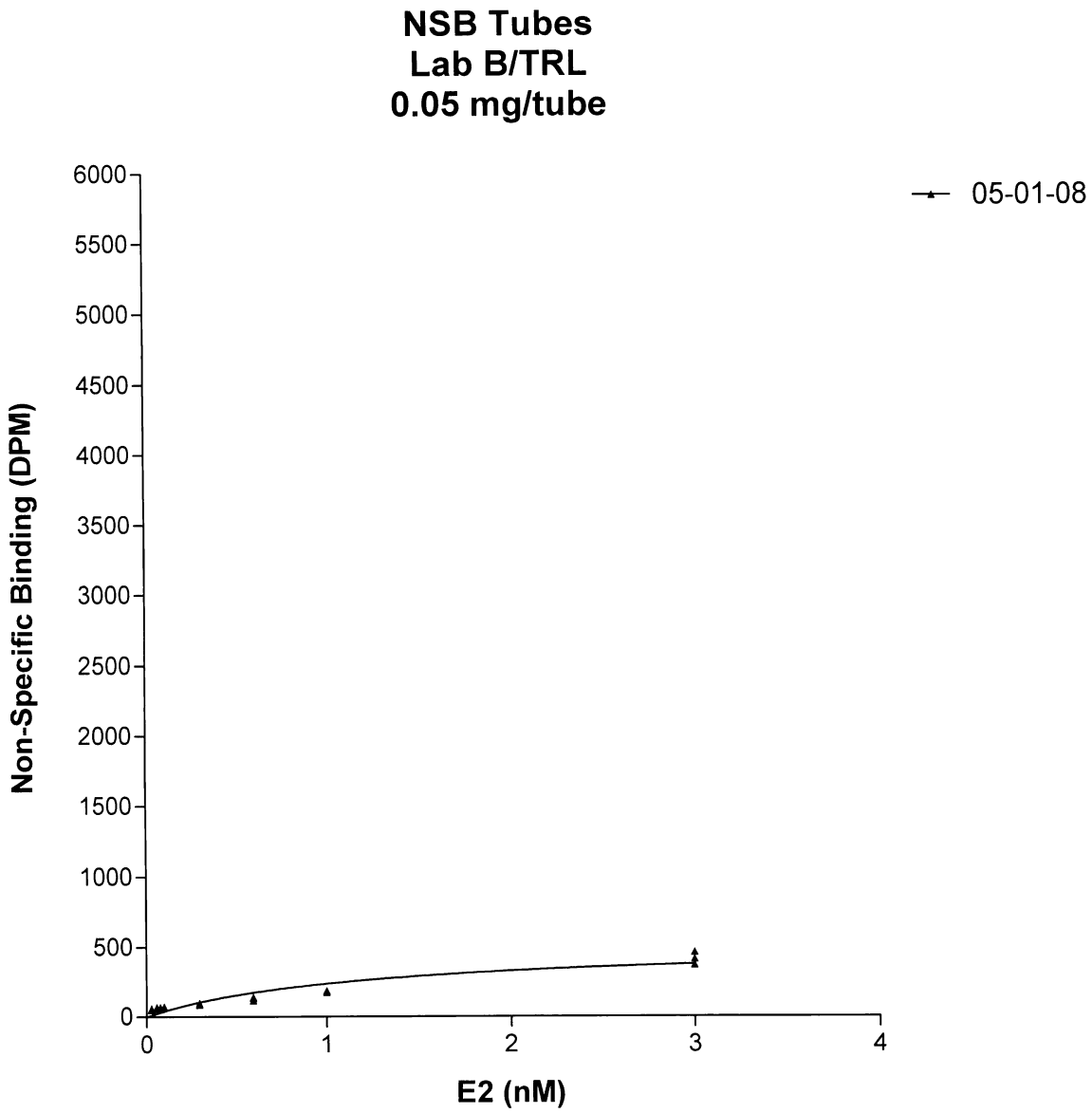

Hot Tubes
Lab B/TRL 0.05 mg/tube
05-01-08
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
1250
1500
1750
2000
E2 (fmole)

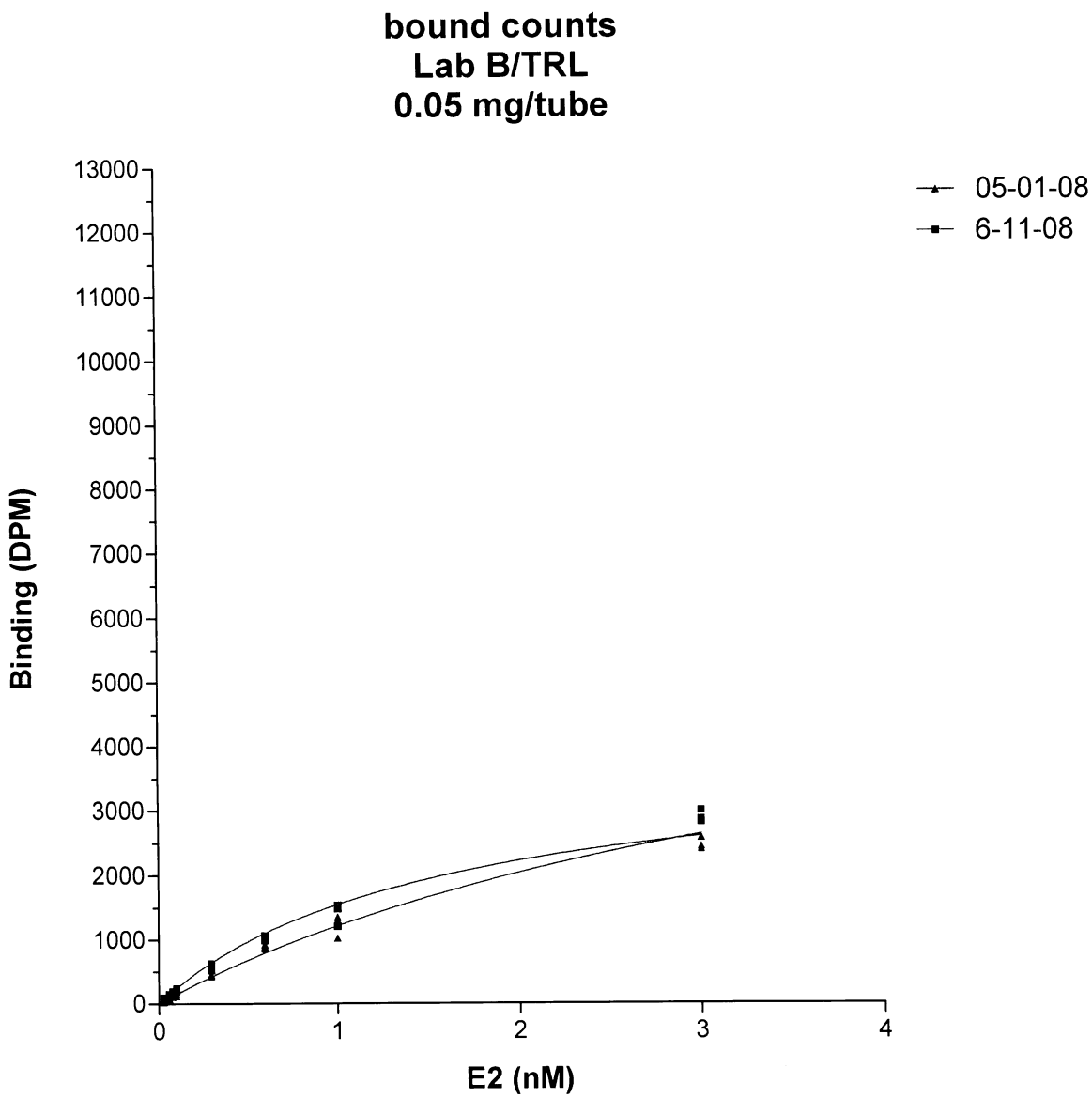
Scatchard Display
Lab B/TRL
0.04 mg/tube



Lab B/TRL
0.05 mg/tube




NSB Tubes
Lab B/TRL
0.05 mg/tube
05-01-08
Non-Specific Binding (DPM)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
E2 (nM)


bound counts
Lab B/TRL
0.05 mg/tube
05-01-08
6-11-08
Binding (DPM)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
E2 (nM)

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R;
the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	TTN022	
Assay start date:	6/19/2008	
Tracer lot number:	3589481/1	
Specific activity on day of assay:	108.16	Ci/mmole
Cytosol vial or lot identification:	RUC 06-06-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.84	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-6.06	M
RBA, norethynodrel (Weak Positive):	1.675E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-4.22	35.0
RBA, Unknown 1:	2.410E-05	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-6.63	40.0
RBA, Unknown 2:	6.252E-03	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-7.57	42.0
RBA, Unknown 3:	5.433E-02	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	0.00	43.0
RBA, Unknown 4:	1.462E-09	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 5.33%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	5	7385.4	352.63	Total Binding, solvent control, tubes
Hot	6	138662.5	4602.64	Total hot E2 added to each tube
NSB	6	1349.8	41.13	Nonspecific Binding
Specific EtOH	5	6035.7	352.63	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.05	less than 0.1?
R1881 / EtOH	#DIV/0!	close to 1.0 ?
NSB / EtOH	0.18	around 0.25 ?

at 1.0 E-6 M only

Values for analysis by nonlinear regression

- Standard Curve
- Negative Control
- Weak Positive
- Unknown

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.3
R1881	#N/A	not applicable
norethynodrel	-4.0	-1.4
Unknown 1	-4.0	38.0
Unknown 2	-4.0	-1.5
Unknown 3	-4.0	-3.3
Unknown 4	-4.0	-4.6

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

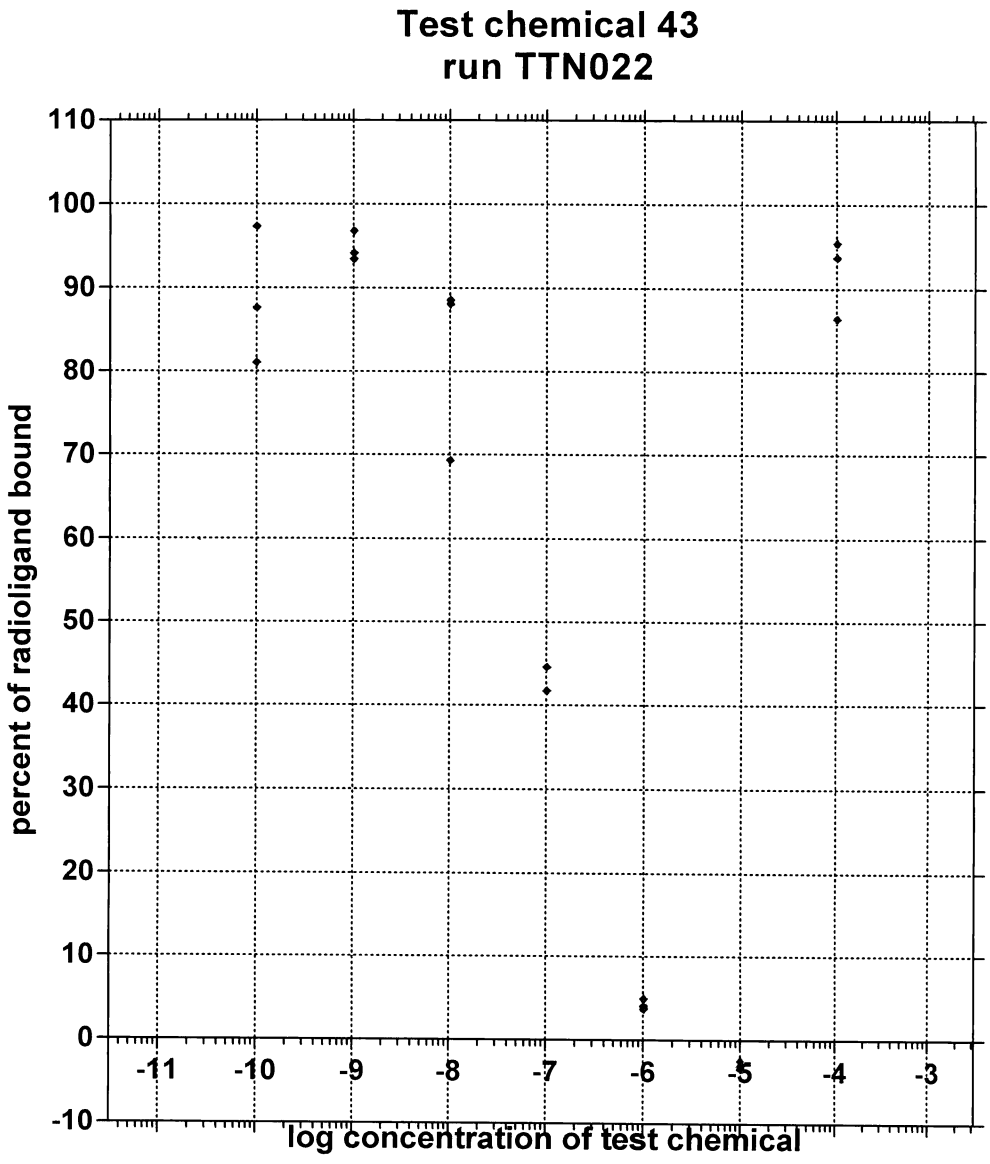
Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5091.00	7636.50	TRUE			—	104.2	7636.5	6286.8	132375.8	104.2	102.9	104.2
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5206.00	7809.00	TRUE			—	107.0	7809	6459.3	132203.3	107.0		107.0
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4822.00	7233.00	TRUE			—	97.5	7233	5883.3	132779.3	97.5		97.5
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	935.00	1402.50	TRUE			-7.0	0.9	1402.5	52.8	138609.8	0.9	0.3	0.9
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	882.00	1323.00	TRUE			-7.0	-0.4	1323	-26.8	138689.3	-0.4		-0.4
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	921.00	1381.50	TRUE			-7.0	0.5	1381.5	31.8	138630.8	0.5		0.5
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1357.00	2035.50	TRUE			-8.0	11.4	2035.5	685.8	137976.8	11.4	11.7	11.4
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1379.00	2068.50	TRUE			-8.0	11.9	2068.5	718.8	137943.8	11.9		11.9
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1373.00	2059.50	TRUE			-8.0	11.8	2059.5	709.8	137952.8	11.8		11.8
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1988.00	2982.00	TRUE			-8.5	27.0	2982	1632.3	137030.3	27.0	30.4	27.0
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2125.00	3187.50	TRUE			-8.5	30.4	3187.5	1837.8	136824.8	30.4		30.4
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2255.00	3382.50	TRUE			-8.5	33.7	3382.5	2032.8	136629.8	33.7		33.7
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3269.00	4903.50	TRUE			-9.0	58.9	4903.5	3553.8	135108.8	58.9	59.9	58.9
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3396.00	5094.00	TRUE			-9.0	62.0	5094	3744.3	134918.3	62.0		62.0
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3265.00	4897.50	TRUE			-9.0	58.8	4897.5	3547.8	135114.8	58.8		58.8
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4110.00	6165.00	TRUE			-9.5	79.8	6165	4815.3	133847.3	79.8	84.1	79.8
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4324.00	6486.00	TRUE			-9.5	85.1	6486	5136.3	133526.3	85.1		85.1
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4412.00	6618.00	TRUE			-9.5	87.3	6618	5268.3	133394.3	87.3		87.3
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4573.00	6859.50	TRUE			-10.0	91.3	6859.5	5509.8	133152.8	91.3	94.0	91.3
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4734.00	7101.00	TRUE			-10.0	95.3	7101	5751.3	132911.3	95.3		95.3
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4740.00	7110.00	TRUE			-10.0	95.4	7110	5760.3	132902.3	95.4		95.4
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4796.00	7194.00	TRUE			-11.0	96.8	7194	5844.3	132818.3	96.8	101.3	96.8
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5058.00	7587.00	TRUE			-11.0	103.3	7587	6237.3	132425.3	103.3		103.3
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5068.00	7602.00	TRUE			-11.0	103.6	7602	6252.3	132410.3	103.6		103.6
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE			-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE			-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE			-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE			-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE			-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE			-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE			-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE			-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE			-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE			-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE			-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE			-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE			-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE			-8.0							#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	5.33%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
75		42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A		
76		43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A		
77		44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
78		45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
79		46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A		
80		47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
81		48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
82		49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	780.00	1170.00	TRUE		norethynodrel	-4.0	-3.0	1170	-179.8	138842.3	-3.0	-1.4	-3.0		
83		50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	913.00	1369.50	TRUE		norethynodrel	-4.0	0.3	1369.5	19.8	138642.8	0.3		0.3		
84		51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	832.00	1248.00	TRUE		norethynodrel	-4.0	-1.7	1248	-101.8	138764.3	-1.7		-1.7		
85		52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	781.00	1171.50	TRUE		norethynodrel	-4.5	-3.0	1171.5	-178.3	138840.8	-3.0	-0.8	-3.0		
86		53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	908.00	1362.00	TRUE		norethynodrel	-4.5	0.2	1362	12.3	138650.3	0.2		0.2		
87		54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	917.00	1375.50	TRUE		norethynodrel	-4.5	0.4	1375.5	25.8	138636.8	0.4		0.4		
88		55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1794.00	2691.00	TRUE		norethynodrel	-5.5	22.2	2691	1341.3	137321.3	22.2	24.2	22.2		
89		56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1905.00	2857.50	TRUE		norethynodrel	-5.5	25.0	2857.5	1507.8	137154.8	25.0		25.0		
90		57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1916.00	2874.00	TRUE		norethynodrel	-5.5	25.3	2874	1524.3	137138.3	25.3		25.3		
91		58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2695.00	4042.50	TRUE		norethynodrel	-6.0	44.6	4042.5	2692.8	135969.8	44.6	45.9	44.6		
92		59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2772.00	4158.00	TRUE		norethynodrel	-6.0	46.5	4158	2808.3	135854.3	46.5		46.5		
93		60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2769.00	4153.50	TRUE		norethynodrel	-6.0	46.5	4153.5	2803.8	135858.8	46.5		46.5		
94		61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3688.00	5532.00	TRUE		norethynodrel	-6.5	69.3	5532	4182.3	134480.3	69.3	73.0	69.3		
95		62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3894.00	5841.00	TRUE		norethynodrel	-6.5	74.4	5841	4491.3	134171.3	74.4		74.4		
96		63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3935.00	5902.50	TRUE		norethynodrel	-6.5	75.4	5902.5	4552.8	134109.8	75.4		75.4		
97		64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3912.00	5868.00	TRUE		norethynodrel	-7.0	74.9	5868	4518.3	134144.3	74.9	83.5	74.9		
98		65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4474.00	6711.00	TRUE		norethynodrel	-7.0	88.8	6711	5361.3	133301.3	88.8		88.8		
99		66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4399.00	6598.50	TRUE		norethynodrel	-7.0	87.0	6598.5	5248.8	133413.8	87.0		87.0		
100		67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4548.00	6822.00	TRUE		norethynodrel	-7.5	90.7	6822	5472.3	133190.3	90.7	94.3	90.7		
101		68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4765.00	7147.50	TRUE		norethynodrel	-7.5	96.1	7147.5	5797.8	132864.8	96.1		96.1		
102		69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4765.00	7147.50	TRUE		norethynodrel	-7.5	96.1	7147.5	5797.8	132864.8	96.1		96.1		
103		70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4875.00	7312.50	TRUE		norethynodrel	-8.5	98.8	7312.5	5962.8	132699.8	98.8	99.2	98.8		
104		71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4956.00	7434.00	TRUE		norethynodrel	-8.5	100.8	7434	6084.3	132578.3	100.8		100.8		
105		72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4843.00	7264.50	TRUE		norethynodrel	-8.5	98.0	7264.5	5914.8	132747.8	98.0		98.0		
106		73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4835.00	7252.50	TRUE		Unknown 1	-3.0	97.8	7252.5	5902.8	132759.8	97.8	96.3			
107		74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4175.00	6262.50	TRUE		Unknown 1	-3.0	81.4	6262.5	4912.8	133749.8	81.4				
108		75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	5315.00	7972.50	TRUE		Unknown 1	-3.0	109.7	7972.5	6622.8	132039.8	109.7				
109		76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2380.00	3570.00	TRUE		Unknown 1	-4.0	36.8	3570	2220.3	136442.3	36.8	38.0	36.8		
110		77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2535.00	3802.50	TRUE		Unknown 1	-4.0	40.6	3802.5	2452.8	136209.8	40.6		40.6		
111		78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2374.00	3561.00	TRUE		Unknown 1	-4.0	36.6	3561	2211.3	136451.3	36.6		36.6		
112		79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4069.00	6103.50	TRUE		Unknown 1	-5.0	78.8	6103.5	4753.8	133908.8	78.8	81.3	78.8		
113		80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4344.00	6516.00	TRUE		Unknown 1	-5.0	85.6	6516	5166.3	133496.3	85.6		85.6		
114		81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4106.00	6159.00	TRUE		Unknown 1	-5.0	79.7	6159	4809.3	133853.3	79.7		79.7		
115		82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4539.00	6808.50	TRUE		Unknown 1	-6.0	90.4	6808.5	5458.8	133203.8	90.4	95.9	90.4		
116		83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4960.00	7440.00	TRUE		Unknown 1	-6.0	100.9	7440	6090.3	132572.3	100.9		100.9		
117		84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4774.00	7161.00	TRUE		Unknown 1	-6.0	96.3	7161	5811.3	132851.3	96.3		96.3		
118		85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4746.00	7119.00	TRUE		Unknown 1	-7.0	95.6	7119	5769.3	132893.3	95.6	100.1	95.6		
119		86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4935.00	7402.50	TRUE		Unknown 1	-7.0	100.3	7402.5	6052.8	132609.8	100.3		100.3		
120		87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5101.00	7651.50	TRUE		Unknown 1	-7.0	104.4	7651.5	6301.8	132360.8	104.4		104.4		
121		88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4653.00	6979.50	TRUE		Unknown 1	-8.0	93.3	6979.5	5629.8	133032.8	93.3	99.1	93.3		
122		89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4934.00	7401.00	TRUE		Unknown 1	-8.0	100.3	7401	6051.3	132611.3	100.3		100.3		
123		90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5071.00	7606.50	TRUE		Unknown 1	-8.0	103.7	7606.5	6256.8	132405.8	103.7		103.7		
124		91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4831.00	7246.50	TRUE		Unknown 1	-9.0	97.7	7246.5	5896.8	132765.8	97.7	100.5	97.7		
125		92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5115.00	7672.50	TRUE		Unknown 1	-9.0	104.8	7672.5	6322.8	132339.8	104.8		104.8		
126		93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4880.00	7320.00	TRUE		Unknown 1	-9.0	98.9	7320	5970.3	132692.3	98.9		98.9		
127		94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4808.00	7212.00	TRUE		Unknown 1	-10.0	97.1	7212	5862.3	132800.3	97.1	100.5	97.1		
128		95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4922.00	7383.00	TRUE		Unknown 1	-10.0	100.0	7383	6033.3	132629.3	100.0		100.0		
129		96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5105.00	7657.50	TRUE		Unknown 1	-10.0	104.5	7657.5	6307.8	132354.8	104.5		104.5		
130		97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2334.00	3501.00	TRUE		Unknown 2	-3.0	35.6	3501	2151.3	136511.3	35.6	35.1			
131		98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2380.00	3570.00	TRUE		Unknown 2	-3.0	36.8	3570	2220.3	136442.3	36.8				
132		99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2226.00	3339.00	TRUE		Unknown 2	-3.0	33.0	3339	1989.3	136673.3	33.0				
133		100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	811.00	1216.50	TRUE		Unknown 2	-4.0	-2.2	1216.5	-133.3	138795.8	-2.2	-1.5	-2.2		
134		101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	870.00	1305.00	TRUE		Unknown 2	-4.0	-0.7	1305	-44.8	138707.3	-0.7		-0.7		
135		102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	833.00	1249.50	TRUE		Unknown 2	-4.0	-1.7	1249.5	-100.3	138762.8	-1.7		-1.7		
136		103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	923.00	1384.50	TRUE		Unknown 2	-5.0	0.6	1384.5	34.8	138627.8	0.6	1.2	0.6		
137		104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	953.00	1429.50	TRUE		Unknown 2	-5.0	1.3	1429.5	79.8	138582.8	1.3		1.3		
138		105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	967.00	1450.50	TRUE		Unknown 2	-5.0	1.7	1450.5	100.8	138561.8	1.7		1.7		
139		106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1653.00	2479.50	TRUE		Unknown 2	-6.0	18.7	2479.5	1129.8	137532.8	18.7	19.2	18.7		
140		107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1657.00	2485.50	TRUE		Unknown 2	-6.0	18.8	2485.5	1135.8	137526.8	18.8		18.8		
141		108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1711.00	2566.50	TRUE		Unknown 2	-6.0	20.2	2566.5	1216.8	137445.8	20.2		20.2		
142		109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3686.00	5529.00	TRUE		Unknown 2	-7.0	69.2	5529	4179.3	134483.3	69.2	70.2	69.2		
143		110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3717.00	5575.50	TRUE		Unknown 2	-7.0	70.0	5575.5	4225.8	134436.8	70.0		70.0		
144		111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3776.00	5664.00	TRUE		Unknown 2	-7.0	71.5	5664	4314.3	134348.3	71.5		71.5		
145		112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4641.00	6961.50	TRUE		Unknown 2	-8.0	93.0	6961.5	5611.8	133050.8	93.0	83.1	93.0		
146		113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3825.00	5737.50	TRUE		Unknown 2	-8.0	72.7	5737.5	4387.8	134274.8	72.7		72.7		
147		114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4262.00	6393.00	TRUE		Unknown 2	-8.0	83.6	6393	5043.3	133619.3	83.6		83.6		
148		115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4865.00	7297.50	TRUE		Unknown 2	-9.0	98.5	7297.5	5947.8	132714.8	98.5	100.4	98.5		
149		116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4997.00	7495.50	TRUE		Unknown 2	-9.0	101.8	7495.5	6145.8	132516.8	101.8		101.8		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	5.33%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
150		117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4961.00	7441.50	TRUE		Unknown 2	-9.0	100.9	7441.5	6091.8	132570.8	100.9		100.9		
151		118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4727.00	7090.50	TRUE		Unknown 2	-10.0	95.1	7090.5	5740.8	132921.8	95.1	95.3	95.1		
152		119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4818.00	7227.00	TRUE		Unknown 2	-10.0	97.4	7227	5877.3	132785.3	97.4		97.4		
153		120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4657.00	6985.50	TRUE		Unknown 2	-10.0	93.4	6985.5	5635.8	133026.8	93.4		93.4		
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7303.00	10954.50	TRUE		Unknown 3	-3.0	159.1	10954.5	9604.8	129057.8	159.1	178.4			
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	9402.00	14103.00	TRUE		Unknown 3	-3.0	211.3	14103	12753.3	125909.3	211.3				
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7526.00	11289.00	TRUE		Unknown 3	-3.0	164.7	11289	9939.3	128723.3	164.7				
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	749.00	1123.50	TRUE		Unknown 3	-4.0	-3.7	1123.5	-226.3	138888.8	-3.7	-3.3	-3.7		
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	763.00	1144.50	TRUE		Unknown 3	-4.0	-3.4	1144.5	-205.3	138867.8	-3.4		-3.4		
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	784.00	1176.00	TRUE		Unknown 3	-4.0	-2.9	1176	-173.8	138836.3	-2.9		-2.9		
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	802.00	1203.00	TRUE		Unknown 3	-5.0	-2.4	1203	-146.8	138809.3	-2.4	-1.6	-2.4		
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	869.00	1303.50	TRUE		Unknown 3	-5.0	-0.8	1303.5	-46.3	138708.8	-0.8		-0.8		
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	840.00	1260.00	TRUE		Unknown 3	-5.0	-1.5	1260	-89.8	138752.3	-1.5		-1.5		
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1058.00	1587.00	TRUE		Unknown 3	-6.0	3.9	1587	237.3	138425.3	3.9	5.8	3.9		
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1176.00	1764.00	TRUE		Unknown 3	-6.0	6.9	1764	414.3	138248.3	6.9		6.9		
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1162.00	1743.00	TRUE		Unknown 3	-6.0	6.5	1743	393.3	138269.3	6.5		6.5		
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2711.00	4066.50	TRUE		Unknown 3	-7.0	45.0	4066.5	2716.8	135945.8	45.0	46.0	45.0		
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2781.00	4171.50	TRUE		Unknown 3	-7.0	46.8	4171.5	2821.8	135840.8	46.8		46.8		
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2756.00	4134.00	TRUE		Unknown 3	-7.0	46.1	4134	2784.3	135878.3	46.1		46.1		
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4407.00	6610.50	TRUE		Unknown 3	-8.0	87.2	6610.5	5260.8	133401.8	87.2	83.8	87.2		
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4133.00	6199.50	TRUE		Unknown 3	-8.0	80.4	6199.5	4849.8	133812.8	80.4		80.4		
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3.00	4.50	FALSE	mis	Unknown 3	-8.0									
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4453.00	6679.50	TRUE		Unknown 3	-9.0	88.3	6679.5	5329.8	133332.8	88.3	94.4	88.3		
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4798.00	7197.00	TRUE		Unknown 3	-9.0	96.9	7197	5847.3	132815.3	96.9		96.9		
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4847.00	7270.50	TRUE		Unknown 3	-9.0	98.1	7270.5	5920.8	132741.8	98.1		98.1		
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4497.00	6745.50	TRUE		Unknown 3	-10.0	89.4	6745.5	5395.8	133266.8	89.4	94.4	89.4		
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4719.00	7078.50	TRUE		Unknown 3	-10.0	94.9	7078.5	5728.8	132933.8	94.9		94.9		
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4876.00	7314.00	TRUE		Unknown 3	-10.0	98.8	7314	5964.3	132698.3	98.8		98.8		
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7512.00	11268.00	TRUE		Unknown 4	-3.0	164.3	11268	9918.3	128744.3	164.3	179.6			
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	9101.00	13651.50	TRUE		Unknown 4	-3.0	203.8	13651.5	12301.8	126360.8	203.8				
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7772.00	11658.00	TRUE		Unknown 4	-3.0	170.8	11658	10308.3	128354.3	170.8				
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	696.00	1044.00	TRUE		Unknown 4	-4.0	-5.1	1044	-305.8	138968.3	-5.1	-4.6	93.7		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	743.00	1114.50	TRUE		Unknown 4	-4.0	-3.9	1114.5	-235.3	138897.8	-3.9		95.4		
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	709.00	1063.50	TRUE		Unknown 4	-4.0	-4.7	1063.5	-286.3	138948.8	-4.7		86.4		
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	759.00	1138.50	TRUE		Unknown 4	-5.0	-3.5	1138.5	-211.3	138873.8	-3.5	-3.1	-3.5		
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	772.00	1158.00	TRUE		Unknown 4	-5.0	-3.2	1158	-191.8	138854.3	-3.2		-3.2		
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	797.00	1195.50	TRUE		Unknown 4	-5.0	-2.6	1195.5	-154.3	138816.8	-2.6		-2.6		
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1046.00	1569.00	TRUE		Unknown 4	-6.0	3.6	1569	219.3	138443.3	3.6	4.1	3.6		
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1059.00	1588.50	TRUE		Unknown 4	-6.0	4.0	1588.5	238.8	138423.8	4.0		4.0		
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1094.00	1641.00	TRUE		Unknown 4	-6.0	4.8	1641	291.3	138371.3	4.8		4.8		
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2577.00	3865.50	TRUE		Unknown 4	-7.0	41.7	3865.5	2515.8	136146.8	41.7	42.6	41.7		
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2691.00	4036.50	TRUE		Unknown 4	-7.0	44.5	4036.5	2686.8	135975.8	44.5		44.5		
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2577.00	3865.50	TRUE		Unknown 4	-7.0	41.7	3865.5	2515.8	136146.8	41.7		41.7		
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3688.00	5532.00	TRUE		Unknown 4	-8.0	69.3	5532	4182.3	134480.3	69.3	82.0	69.3		
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4463.00	6694.50	TRUE		Unknown 4	-8.0	88.6	6694.5	5344.8	133317.8	88.6		88.6		
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4444.00	6666.00	TRUE		Unknown 4	-8.0	88.1	6666	5316.3	133346.3	88.1		88.1		
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4689.00	7033.50	TRUE		Unknown 4	-9.0	94.2	7033.5	5683.8	132978.8	94.2	94.8	94.2		
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4795.00	7192.50	TRUE		Unknown 4	-9.0	96.8	7192.5	5842.8	132819.8	96.8		96.8		
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4660.00	6990.00	TRUE		Unknown 4	-9.0	93.4	6990	5640.3	133022.3	93.4		93.4		
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4426.00	6639.00	TRUE		Unknown 4	-10.0	87.6	6639	5289.3	133373.3	87.6	88.7	87.6		
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4817.00	7225.50	TRUE		Unknown 4	-10.0	97.4	7225.5	5875.8	132786.8	97.4		97.4		
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4160.00	6240.00	TRUE		Unknown 4	-10.0	81.0	6240	4890.3	133772.3	81.0		81.0		
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	916.00	1374.00	TRUE		cold E2	-7.0	0.4	1374	24.3	138638.3	0.4	-0.3	0.4		
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	872.00	1308.00	TRUE		cold E2	-7.0	-0.7	1308	-41.8	138704.3	-0.7		-0.7		
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	873.00	1309.50	TRUE		cold E2	-7.0	-0.7	1309.5	-40.3	138702.8	-0.7		-0.7		
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4604.00	6906.00	TRUE			—	92.1	6906	5556.3	133106.3	92.1	95.7	92.1		
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1290.00	1935.00	FALSE	mis		—									
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4895.00	7342.50	TRUE			—	99.3	7342.5	5992.8	132669.8	99.3		99.3		
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average			
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
211		178	1	none	Hot		—	—	—	10	—	—	—	—	94557.00	141835.50	TRUE					141835.5	140485.8						
212		179	2	none	Hot		—	—	—	10	—	—	—	—	94584.00	141876.00	TRUE					141876	140526.3						
213		180	3	none	Hot		—	—	—	10	—	—	—	—	94080.00	141120.00	TRUE					141120	139770.3						
214		181	1	none	Hot		—	—	—	10	—	—	—	—	87745.00	131617.50	TRUE					131617.5	130267.8						
215		182	2	none	Hot		—	—	—	10	—	—	—	—	89337.00	134005.50	TRUE					134005.5	132655.8						
216		183	3	none	Hot		—	—	—	10	—	—	—	—	94347.00	141520.50	TRUE					141520.5	140170.8						

Table format: XY		X	A			B		
		log concentration	Test chemical			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0	93.6729	95.3859	86.3787			
3	Title	-5.0	-3.5000	-3.1770	-2.5556			
4	Title	-6.0	3.6326	3.9557	4.8255			
5	Title	-7.0	41.6815	44.5147	41.6815			
6	Title	-8.0	69.2925	88.5530	88.0808			
7	Title	-9.0	94.1696	96.8040	93.4489			
8	Title	-10.0	87.6335	97.3507	81.0228			

Nonlin fit Table of results		A Test chemical	B Data Set-B
		Y	Y
1	logIC50	Interrupted	
2	Best-fit values		
3	BOTTOM	5.088e+009	
4	TOP	50.00	
5	LOGIC50	-5.174	
6	HILLSLOPE	-3.429	
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	21	


Test chemical 43
run TTN022
percent of radioligand bound
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

APPENDIX E

Excel and Prism Files of Saturation Binding Data and Analysis for Run Identifier
TRL RUC06-06-08

Laboratory Code B

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,
include the DPM value in column N, and
provide a reason in column Q;
the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background if the total binding exceeds 10% of the hot added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	B	
Run identification:	TTN sat. binding	
Assay start date:	6/11/2008	
Tracer lot number:	3589481/1	
Specific activity on day of assay:	108.29	Ci/mmole
Cytosol lot or vial number:	RUC-06-06-08	
protein (cytosol) per tube:	50	ug
protein (cytosol) per tube:	0.05	mg
KD	8.22E-01	nM
Bmax	113.2	fmole/100 ug
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	
total volume in tubes	500	uL

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot E2 Initial Concentration (nM)*	*Hot E2 Volume (uL)*	*Hot E2 Final Concentration (nM)*	*Cold E2 Initial Concentration (nM)*	*Cold E2 Volume (uL)*	*Cold E2 Final Concentration (nM)*	*Buffer Volume (uL)*	*Receptor Volume (uL)*	*raw dpm*	*dpm for 1.5 mL*	*Use this value?*	*Notes to explain why "Use this value" is set to "FALSE"*	*Ten Percent Rule*	Output to Curve Fitting Software: *Saturation X values*	*Bound y values*	*NSB y values*
1	1	H	0.3	50	0.03	—	—	—	350	100	246	369	TRUE		16.5%	0.03	369.0	87.5
2	2	H	0.3	50	0.03	—	—	—	350	100	259	388.5	TRUE		17.4%	0.03	388.5	87.5
3	3	H	0.3	50	0.03	—	—	—	350	100	270	405	TRUE		18.1%	0.03	405.0	87.5
4	1	H	0.6	50	0.06	—	—	—	350	100	447	670.5	TRUE		13.4%	0.06	670.5	106.0
5	2	H	0.6	50	0.06	—	—	—	350	100	285	427.5	TRUE		8.6%	0.06	427.5	106.0
6	3	H	0.6	50	0.06	—	—	—	350	100	408	612	TRUE		12.3%	0.06	612.0	106.0
7	1	H	0.8	50	0.08	—	—	—	350	100	737	1105.5	TRUE		16.2%	0.08	1105.5	123.5
8	2	H	0.8	50	0.08	—	—	—	350	100	717	1075.5	TRUE		15.8%	0.08	1075.5	123.5
9	3	H	0.8	50	0.08	—	—	—	350	100	885	1327.5	TRUE		19.5%	0.08	1327.5	123.5
10	1	H	1.0	50	0.10	—	—	—	350	100	856	1284	TRUE		14.7%	0.1	1284.0	119.0
11	2	H	1.0	50	0.10	—	—	—	350	100	531	796.5	TRUE		9.1%	0.1	796.5	119.0
12	3	H	1.0	50	0.10	—	—	—	350	100	1056	1584	TRUE		18.1%	0.1	1584.0	119.0
13	1	H	3.0	50	0.30	—	—	—	350	100	2133	3199.5	TRUE		12.3%	0.3	3199.5	186.0
14	2	H	3.0	50	0.30	—	—	—	350	100	2576	3864	TRUE		14.8%	0.3	3864.0	186.0
15	3	H	3.0	50	0.30	—	—	—	350	100	2460	3690	TRUE		14.2%	0.3	3690.0	186.0
16	1	H	6.0	50	0.60	—	—	—	350	100	4123	6184.5	TRUE		10.3%	0.6	6184.5	306.0
17	2	H	6.0	50	0.60	—	—	—	350	100	2259	3388.5	TRUE		5.7%	0.6	3388.5	306.0
18	3	H	6.0	50	0.60	—	—	—	350	100	3179	4768.5	TRUE		8.0%	0.6	4768.5	306.0
19	1	H	10.0	50	1.00	—	—	—	350	100	5487	8230.5	TRUE		8.1%	1	8230.5	465.5
20	2	H	10.0	50	1.00	—	—	—	350	100	5593	8389.5	TRUE		8.3%	1	8389.5	465.5
21	3	H	10.0	50	1.00	—	—	—	350	100	4878	7317	TRUE		7.2%	1	7317.0	465.5
22	1	H	30.0	50	3.00	—	—	—	350	100	6961	10441.5	TRUE		3.5%	3	10441.5	1296.5
23	2	H	30.0	50	3.00	—	—	—	350	100	7375	11062.5	TRUE		3.7%	3	11062.5	1296.5
24	3	H	30.0	50	3.00	—	—	—	350	100	7786	11679	TRUE		3.9%	3	11679.0	1296.5
25	1	HC	0.3	50	0.03	30	50	3	300	100	54	81	TRUE					
26	2	HC	0.3	50	0.03	30	50	3	300	100	60	90	TRUE					
27	3	HC	0.3	50	0.03	30	50	3	300	100	61	91.5	TRUE					

28	1	HC	0.6	50	0.06	60	50	6	300	100
29	2	HC	0.6	50	0.06	60	50	6	300	100
30	3	HC	0.6	50	0.06	60	50	6	300	100
31	1	HC	0.8	50	0.08	80	50	8	300	100
32	2	HC	0.8	50	0.08	80	50	8	300	100
33	3	HC	0.8	50	0.08	80	50	8	300	100
34	1	HC	1.0	50	0.10	100	50	10	300	100
35	2	HC	1.0	50	0.10	100	50	10	300	100
36	3	HC	1.0	50	0.10	100	50	10	300	100
37	1	HC	3.0	50	0.30	300	50	30	300	100
38	2	HC	3.0	50	0.30	300	50	30	300	100
39	3	HC	3.0	50	0.30	300	50	30	300	100
40	1	HC	6.0	50	0.60	600	50	60	300	100
41	2	HC	6.0	50	0.60	600	50	60	300	100
42	3	HC	6.0	50	0.60	600	50	60	300	100
43	1	HC	10.0	50	1.00	1000	50	100	300	100
44	2	HC	10.0	50	1.00	1000	50	100	300	100
45	3	HC	10.0	50	1.00	1000	50	100	300	100
46	1	HC	30.0	50	3.00	3000	50	300	300	100
47	2	HC	30.0	50	3.00	3000	50	300	300	100
48	3	HC	30.0	50	3.00	3000	50	300	300	100
49	1	Hot	0.3	50	0.03	—	—	—	—	—
50	2	Hot	0.3	50	0.03	—	—	—	—	—
51	3	Hot	0.3	50	0.03	—	—	—	—	—
52	1	Hot	0.6	50	0.06	—	—	—	—	—
53	2	Hot	0.6	50	0.06	—	—	—	—	—
54	3	Hot	0.6	50	0.06	—	—	—	—	—
55	1	Hot	0.8	50	0.08	—	—	—	—	—
56	2	Hot	0.8	50	0.08	—	—	—	—	—
57	3	Hot	0.8	50	0.08	—	—	—	—	—
58	1	Hot	1.0	50	0.10	—	—	—	—	—
59	2	Hot	1.0	50	0.10	—	—	—	—	—
60	3	Hot	1.0	50	0.10	—	—	—	—	—
61	1	Hot	3.0	50	0.30	—	—	—	—	—
62	2	Hot	3.0	50	0.30	—	—	—	—	—
63	3	Hot	3.0	50	0.30	—	—	—	—	—
64	1	Hot	6.0	50	0.60	—	—	—	—	—
65	2	Hot	6.0	50	0.60	—	—	—	—	—
66	3	Hot	6.0	50	0.60	—	—	—	—	—
67	1	Hot	10.0	50	1.00	—	—	—	—	—
68	2	Hot	10.0	50	1.00	—	—	—	—	—
69	3	Hot	10.0	50	1.00	—	—	—	—	—
70	1	Hot	30.0	50	3.00	—	—	—	—	—
71	2	Hot	30.0	50	3.00	—	—	—	—	—
72	3	Hot	30.0	50	3.00	—	—	—	—	—

71	106.5	TRUE
74	111	TRUE
67	100.5	TRUE
86	129	TRUE
83	124.5	TRUE
78	117	TRUE
77	115.5	TRUE
80	120	TRUE
81	121.5	TRUE
122	183	TRUE
130	195	TRUE
120	180	TRUE
205	307.5	TRUE
211	316.5	TRUE
196	294	TRUE
328	492	TRUE
303	454.5	TRUE
300	450	TRUE
832	1248	TRUE
918	1377	TRUE
843	1264.5	TRUE
1525	2287.5	TRUE
1475	2212.5	TRUE
1463	2194.5	TRUE
3323	4984.5	TRUE
3354	5031	TRUE
3310	4965	TRUE
4533	6799.5	TRUE
4535	6802.5	TRUE
4577	6865.5	TRUE
5910	8865	TRUE
5818	8727	TRUE
5793	8689.5	TRUE
17695	26542.5	TRUE
17219	25828.5	TRUE
17202	25803	TRUE
39974	59961	TRUE
39854	59781	TRUE
39912	59868	TRUE
67761	101641.5	TRUE
68010	102015	TRUE
66801	100201.5	TRUE
200827	301240.5	TRUE
201548	302322	TRUE
202835	304252.5	TRUE

Prism input for bound/free		Prism input for specific bound		HOT tubes	NSB Tubes	Specific bound	Total Bound
specific bound/molar	bound/free %	*average total added molar*	*specific bound/molar*	*total added dpm*	*NSB dpm*	*spcific bound/dpm*	*total bound dpm*
2.34191E-12	0.1511	1.86E-11	2.3419E-12	2287.50	81.00	281.50	369.00
2.50414E-12	0.1633	1.86E-11	2.5041E-12	2212.50	90.00	301.00	388.50
2.64141E-12	0.1738	1.86E-11	2.6414E-12	2194.50	91.50	317.50	405.00
4.69629E-12	0.1306	4.15E-11	4.6963E-12	4984.50	106.50	564.50	670.50
2.67468E-12	0.0704	4.15E-11	2.6747E-12	5031.00	111.00	321.50	427.50
4.20961E-12	0.1155	4.15E-11	4.2096E-12	4965.00	100.50	506.00	612.00
8.16964E-12	0.1718	5.68E-11	8.1696E-12	6799.50	129.00	982.00	1105.50
7.92006E-12	0.1657	5.68E-11	7.9201E-12	6802.50	124.50	952.00	1075.50
1.00165E-11	0.2191	5.68E-11	1.0017E-11	6865.50	117.00	1204.00	1327.50
9.69209E-12	0.1558	7.29E-11	9.6921E-12	8865.00	115.50	1165.00	1284.00
5.63638E-12	0.0851	7.29E-11	5.6364E-12	8727.00	120.00	677.50	796.50
1.21879E-11	0.2041	7.29E-11	1.2188E-11	8689.50	121.50	1465.00	1584.00
2.50705E-11	0.1318	2.17E-10	2.5070E-11	26542.50	183.00	3013.50	3199.50
3.05987E-11	0.1657	2.17E-10	3.0599E-11	25828.50	195.00	3678.00	3864.00
2.91511E-11	0.1567	2.17E-10	2.9151E-11	25803.00	180.00	3504.00	3690.00
4.89055E-11	0.1095	4.98E-10	4.8906E-11	59961.00	307.50	5878.50	6184.50
2.56445E-11	0.0546	4.98E-10	2.5645E-11	59781.00	316.50	3082.50	3388.50
3.71253E-11	0.0810	4.98E-10	3.7125E-11	59868.00	294.00	4462.50	4768.50
6.46E-11	0.0834	8.43E-10	6.4600E-11	101641.50	492.00	7765.00	8230.50
6.59228E-11	0.0853	8.43E-10	6.5923E-11	102015.00	454.50	7924.00	8389.50
5.70003E-11	0.0729	8.43E-10	5.7000E-11	100201.50	450.00	6851.50	7317.00
7.60808E-11	0.0313	2.52E-09	7.6081E-11	301240.50	1248.00	9145.00	10441.50
8.12471E-11	0.0335	2.52E-09	8.1247E-11	302322.00	1377.00	9766.00	11062.50
8.6376E-11	0.0357	2.52E-09	8.6376E-11	304252.50	1264.50	10382.50	11679.00

Saturation, Lab B

	6/12/2008
One site binding (hyperbola)	
Best-fit values	
BMAX	1.13E-10
KD	8.22E-10
Std. Error	
BMAX	
KD	
95% Confidence Intervals	
BMAX	
KD	
Goodness of Fit	
Degrees of Freedom	
R^2 (unweighted)	
Weighted Sum of Squares ($1/Y^2$)	
Absolute Sum of Squares	
Sy.x	
Data	
Number of X values	
Number of Y replicates	
Total number of values	
Number of missing values	

Bmax molar	1.13E-10	KD molar	8.22E-10
mole to molar conversion value	0.0005	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	2.40E+17	**kd nM =**	8.22E-01
Bmax molar to Bmax moles	5.66E-14		
= DPM/((DPM/mmole)*1000)	5.66E-14		
=Bmax DPM	1.36E+04		
assay date	6/11/2008		
Bmax(dpm)	13606.77207		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	108.29		
DPM/mmole	2.40E+14		
DPM/pmole	2.40E+05		
1/(DPM/mmole)	4.16E-15		
1/(DPM/pmole)	4.16E-06		
SA(dpm/pmole)	2.40E+05		
protein/tube (ug)	50		
protein./tube(mg)	0.05		
bmax pmole	0.056600		
bmax pmole/mg	1.132		
Bmax fmole/mg	1132		
Bmax (fmole/100 ug)	113.2		
Bmax(fmole/100 ug)/Bmax molar	1.00E+12		

Table format: XY		X	A	B	C	D	E	F	G
		X Title	6-11-08	6-11-08	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G
		X	Y	Y	Y	Y	Y	Y	Y
1	Title	0.030000	369.000	87.5000					
2	Title	0.030000	388.500	87.5000					
3	Title	0.030000	405.000	87.5000					
4	Title	0.060000	670.500	106.0000					
5	Title	0.060000	427.500	106.0000					
6	Title	0.060000	612.000	106.0000					
7	Title	0.080000	1105.500	123.5000					
8	Title	0.080000	1075.500	123.5000					
9	Title	0.080000	1327.500	123.5000					
10	Title	0.100000	1284.000	119.0000					
11	Title	0.100000	796.500	119.0000					
12	Title	0.100000	1584.000	119.0000					
13	Title	0.300000	3199.500	186.0000					
14	Title	0.300000	3864.000	186.0000					
15	Title	0.300000	3690.000	186.0000					
16	Title	0.600000	6184.500	306.0000					
17	Title	0.600000	3388.500	306.0000					
18	Title	0.600000	4768.500	306.0000					
19	Title	1.000000	8230.500	465.5000					
20	Title	1.000000	8389.500	465.5000					
21	Title	1.000000	7317.000	465.5000					
22	Title	3.000000	10441.500	1296.5000					
23	Title	3.000000	11062.500	1296.5000					
24	Title	3.000000	11679.000	1296.5000					

	H	I	J	K	L
	Data Set-H	Data Set-I	Data Set-J	Data Set-K	Data Set-L
	Y	Y	Y	Y	Y
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Table format: XY		X	A	B	C	D	E	F
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	15.000000	2287.500					
2	Title	15.000000	2212.500					
3	Title	15.000000	2194.500					
4	Title	30.000000	4984.500					
5	Title	30.000000	5031.000					
6	Title	30.000000	4965.000					
7	Title	40.000000	6799.500					
8	Title	40.000000	6802.500					
9	Title	40.000000	6865.500					
10	Title	50.000000	8865.000					
11	Title	50.000000	8727.000					
12	Title	50.000000	8689.500					
13	Title	150.000000	26542.500					
14	Title	150.000000	25828.500					
15	Title	150.000000	25803.000					
16	Title	300.000000	59961.000					
17	Title	300.000000	59781.000					
18	Title	300.000000	59868.000					
19	Title	500.000000	101641.500					
20	Title	500.000000	102015.000					
21	Title	500.000000	100201.500					
22	Title	1500.000000	301240.500					
23	Title	1500.000000	302322.000					
24	Title	1500.000000	304252.500					

Table format: XY		X	A	B	C	D	E	F
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	81.00					
2	Title	0.030000	90.00					
3	Title	0.030000	91.50					
4	Title	0.060000	106.50					
5	Title	0.060000	111.00					
6	Title	0.060000	100.50					
7	Title	0.080000	129.00					
8	Title	0.080000	124.50					
9	Title	0.080000	117.00					
10	Title	0.100000	115.50					
11	Title	0.100000	120.00					
12	Title	0.100000	121.50					
13	Title	0.300000	183.00					
14	Title	0.300000	195.00					
15	Title	0.300000	180.00					
16	Title	0.600000	307.50					
17	Title	0.600000	316.50					
18	Title	0.600000	294.00					
19	Title	1.000000	492.00					
20	Title	1.000000	454.50					
21	Title	1.000000	450.00					
22	Title	3.000000	1248.00					
23	Title	3.000000	1377.00					
24	Title	3.000000	1264.50					

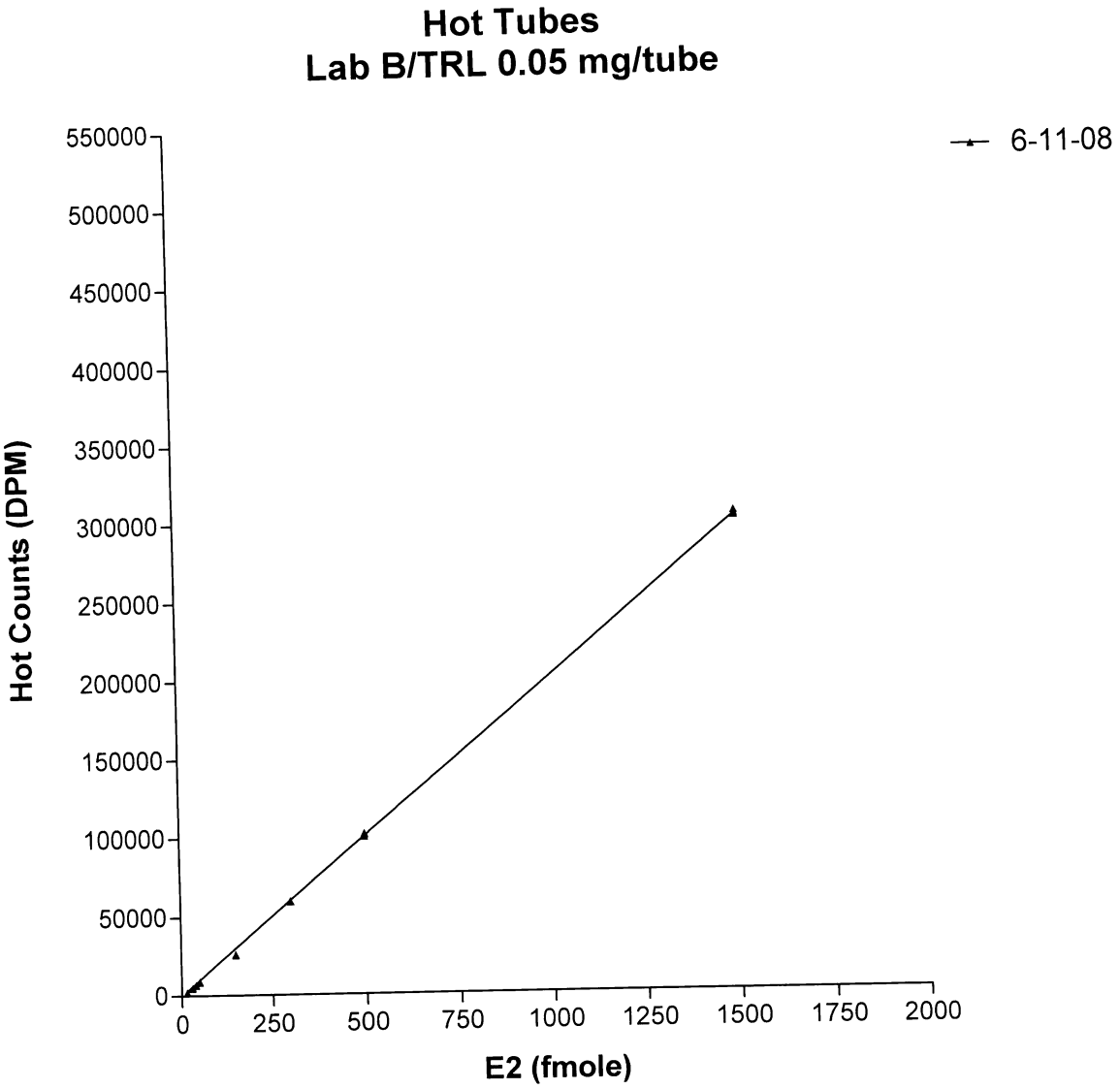
Table format: XY		X	A	B	C	D	E	F
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
		X	Y	Y	Y	Y	Y	Y
1	Title	0.030000	281.50000					
2	Title	0.030000	301.00000					
3	Title	0.030000	317.50000					
4	Title	0.060000	564.50000					
5	Title	0.060000	321.50000					
6	Title	0.060000	506.00000					
7	Title	0.080000	982.00000					
8	Title	0.080000	952.00000					
9	Title	0.080000	1204.00000					
10	Title	0.100000	1165.00000					
11	Title	0.100000	677.50000					
12	Title	0.100000	1465.00000					
13	Title	0.300000	3013.50000					
14	Title	0.300000	3678.00000					
15	Title	0.300000	3504.00000					
16	Title	0.600000	5878.50000					
17	Title	0.600000	3082.50000					
18	Title	0.600000	4462.50000					
19	Title	1.000000	7765.00000					
20	Title	1.000000	7924.00000					
21	Title	1.000000	6851.50000					
22	Title	3.000000	9145.00000					
23	Title	3.000000	9766.00000					
24	Title	3.000000	0382.50000					

Table format: XY		X	A	B	C	D	E
		X Title	6-11-08	Data Set-B	Data Set-C	Data Set-D	Data Set-E
		X	Y	Y	Y	Y	Y
1	Title	0.030000	369.00000				
2	Title	0.030000	388.50000				
3	Title	0.030000	405.00000				
4	Title	0.060000	670.50000				
5	Title	0.060000	427.50000				
6	Title	0.060000	612.00000				
7	Title	0.080000	1105.50000				
8	Title	0.080000	1075.50000				
9	Title	0.080000	1327.50000				
10	Title	0.100000	1284.00000				
11	Title	0.100000	796.50000				
12	Title	0.100000	1584.00000				
13	Title	0.300000	3199.50000				
14	Title	0.300000	3864.00000				
15	Title	0.300000	3690.00000				
16	Title	0.600000	6184.50000				
17	Title	0.600000	3388.50000				
18	Title	0.600000	4768.50000				
19	Title	1.000000	8230.50000				
20	Title	1.000000	8389.50000				
21	Title	1.000000	7317.00000				
22	Title	3.000000	0441.50000				
23	Title	3.000000	1062.50000				
24	Title	3.000000	1679.00000				

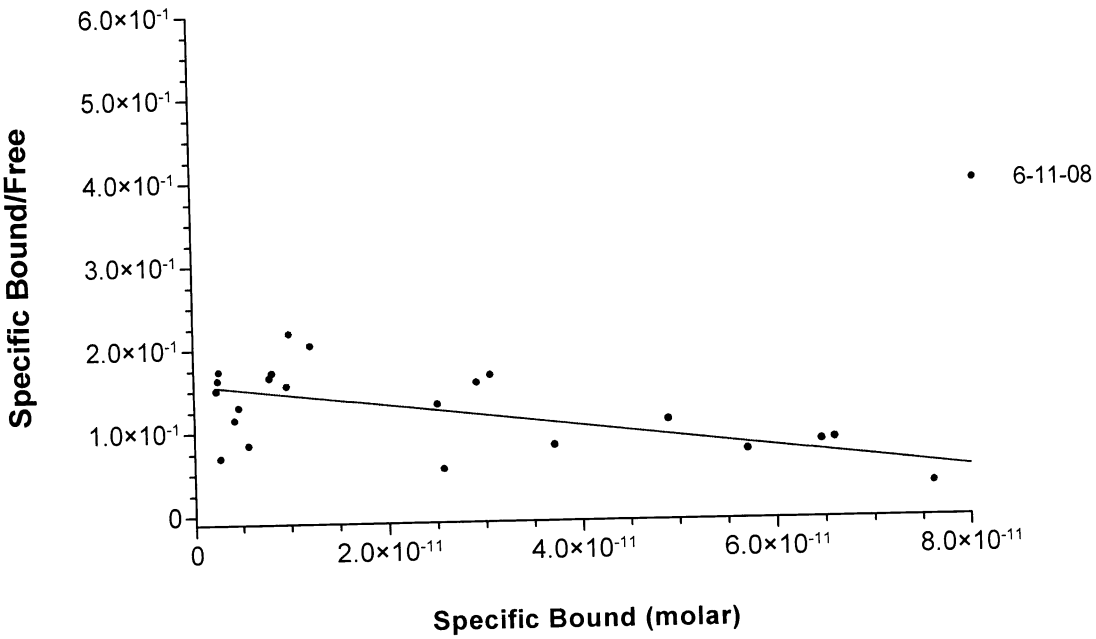
Table format: XY		X	A
		specific bound molar	6-11-08
		X	Y
1	Title	2.341910e-012	0.15110
2	Title	2.504140e-012	0.16330
3	Title	2.641410e-012	0.17380
4	Title	4.696290e-012	0.13060
5	Title	2.674680e-012	0.07040
6	Title	4.209610e-012	0.11550
7	Title	8.169640e-012	0.17180
8	Title	7.920060e-012	0.16570
9	Title	1.001650e-011	0.21910
10	Title	9.692090e-012	0.15580
11	Title	5.636380e-012	0.08510
12	Title	1.218790e-011	0.20410
13	Title	2.507050e-011	0.13180
14	Title	3.059870e-011	0.16570
15	Title	2.915110e-011	0.15670
16	Title	4.890550e-011	0.10950
17	Title	2.564450e-011	0.05460
18	Title	3.712530e-011	0.08100
19	Title	6.460000e-011	0.08340
20	Title	6.592280e-011	0.08530
21	Title	5.700030e-011	0.07290
22	Title	7.608080e-011	0.03130
23	Title	8.124710e-011	0.03350
24	Title	8.637600e-011	0.03570

Table format: XY		X	A
		mean total added molar	6-11-08
		X	Y
1	Title	1.86000e-011	2.34190e-012
2	Title	1.86000e-011	2.50410e-012
3	Title	1.86000e-011	2.64140e-012
4	Title	4.15000e-011	4.69630e-012
5	Title	4.15000e-011	2.67470e-012
6	Title	4.15000e-011	4.20960e-012
7	Title	5.68000e-011	8.16960e-012
8	Title	5.68000e-011	7.92010e-012
9	Title	5.68000e-011	1.00170e-011
10	Title	7.29000e-011	9.69210e-012
11	Title	7.29000e-011	5.63640e-012
12	Title	7.29000e-011	1.21880e-011
13	Title	2.17000e-010	2.50700e-011
14	Title	2.17000e-010	3.05990e-011
15	Title	2.17000e-010	2.91510e-011
16	Title	4.98000e-010	4.89060e-011
17	Title	4.98000e-010	2.56450e-011
18	Title	4.98000e-010	3.71250e-011
19	Title	8.43000e-010	6.46000e-011
20	Title	8.43000e-010	6.59230e-011
21	Title	8.43000e-010	5.70000e-011
22	Title	2.52000e-009	7.60810e-011
23	Title	2.52000e-009	8.12470e-011
24	Title	2.52000e-009	8.63760e-011

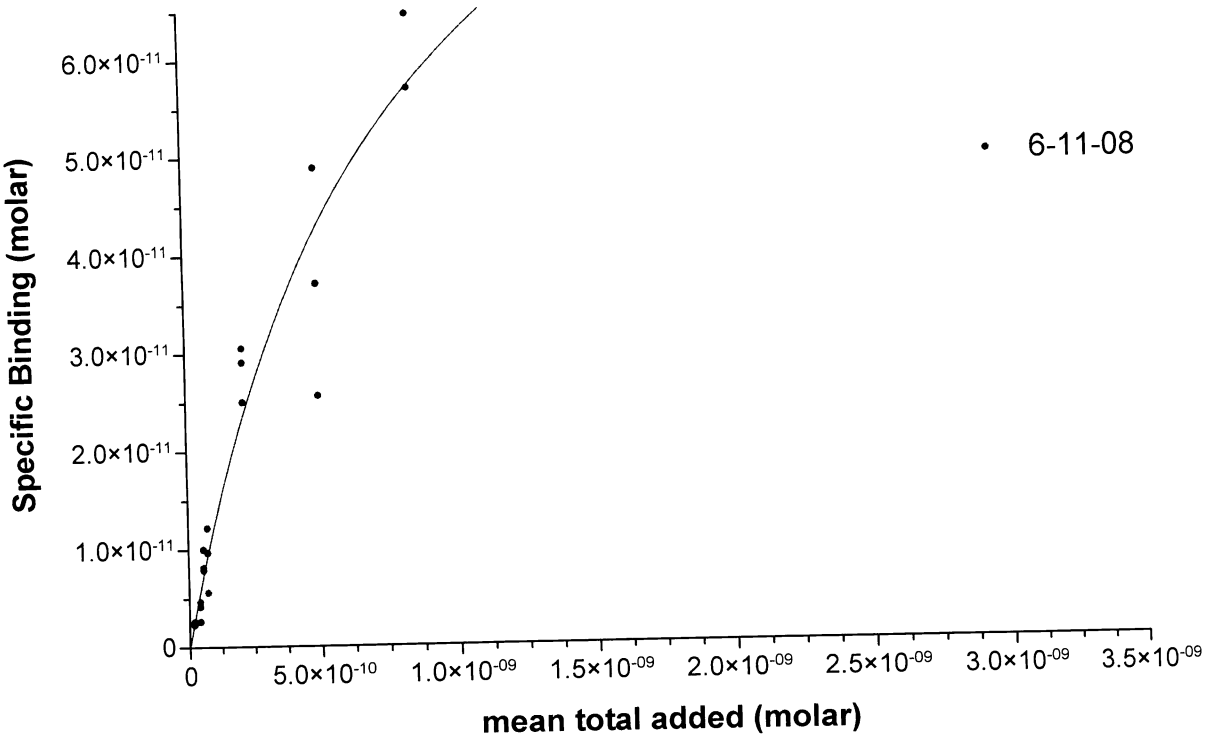
	Nonlin fit Table of results	A
		6-11-08
		Y
1	One site binding (hyperbola)	
2	Best-fit values	
3	BMAX	1.132e-010
4	KD	8.216e-010
5	Std. Error	
6	BMAX	1.661e-011
7	KD	1.541e-010
8	95% Confidence Intervals	
9	BMAX	7.870e-011 to 1.476e-010
10	KD	5.019e-010 to 1.141e-009
11	Goodness of Fit	
12	Degrees of Freedom	22
13	R^2 (unweighted)	0.9617
14	Weighted Sum of Squares ($1/Y^2$)	1.145
15	Absolute Sum of Squares	7.124e-022
16	Sy.x	0.2281
17	Number of points	
18	Analyzed	24

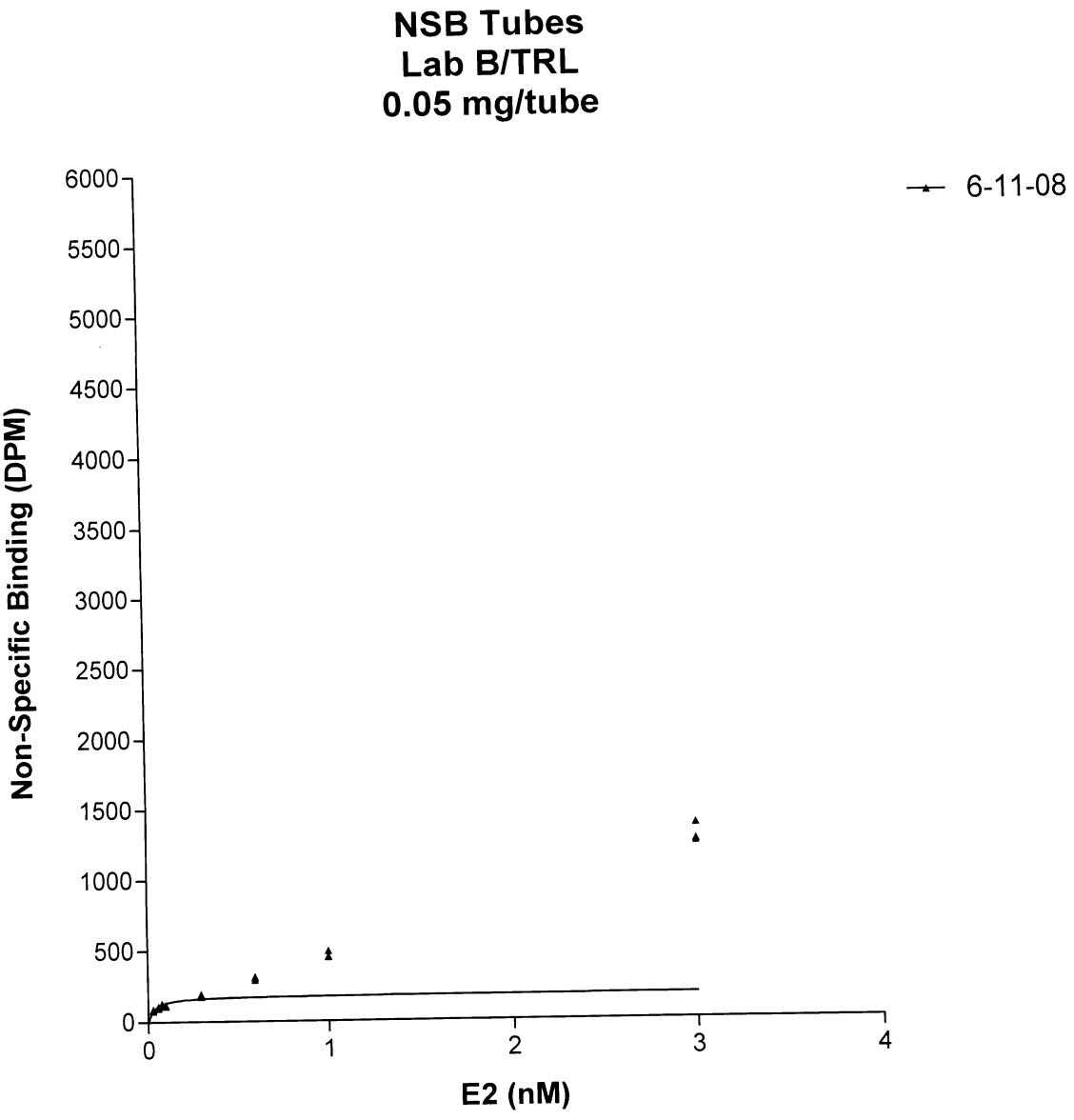

Hot Tubes
Lab B/TRL 0.05 mg/tube
6-11-08
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
1250
1500
1750
2000
E2 (fmole)

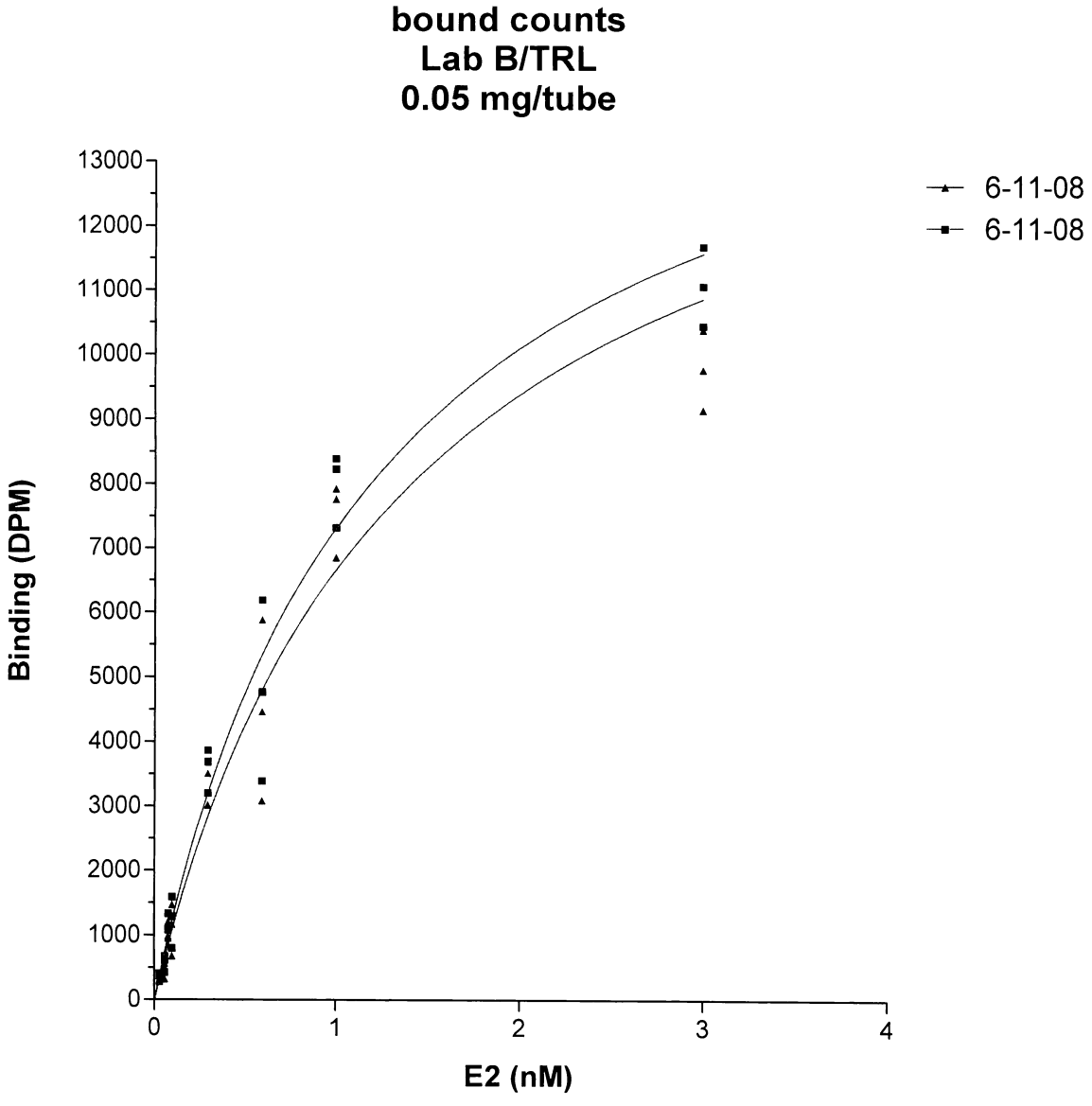
Scatchard Display
Lab B/TRL
0.04 mg/tube



Lab B/TRL
0.05 mg/tube




NSB Tubes
Lab B/TRL
0.05 mg/tube
6-11-08
Non-Specific Binding (DPM)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
E2 (nM)


bound counts
Lab B/TRL
0.05 mg/tube
Binding (DPM)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
E2 (nM)
6-11-08
6-11-08

APPENDIX F

Excel and Prism Files of the Run Identifier TTN001

ER Competitive Assay - Two Unknowns

159 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	TTN-001	
Assay start date:	3/11/2008	
Tracer lot number:	3589221/1	
Specific activity on day of assay:	105.74	Ci/mmole
Cytosol vial or lot identification:	RUC-2-18-08	
Protein (cytosol):	40	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.78	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.89	M
RBA, norethynodrel (Weak Positive):	1.271E-03	relative to E2=1.0
Max Concentration, Unknown 1:	5.00E-02	M
log(IC50), Unknown 1:	-6.97	12.0
RBA, Unknown 1:	1.552E-02	relative to E2=1.0
Max Concentration, Unknown 2:	5.00E-02	M
log(IC50), Unknown 2:	n/a	19.0
RBA, Unknown 2:	n/a	relative to E2=1.0
Max Concentration, Unknown 3:		M
log(IC50), Unknown 3:	0.00	Chem code
RBA, Unknown 3:	1.652E-09	relative to E2=1.0

Provide information in all blue cells in column O, and cells AE43 : AU43 and AE48 : AU48.

If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AV contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 21 will contain output parameters from the nonlinear regression software. and the maximum concentrations for each of the unknowns.

Summary values	n	Mean	SD	
EtOH	5	8879.4	243.02	Total Binding, solvent control, tubes
Hot	6	155912.8	5639.14	Total hot E2 added to each tube
NSB	6	497.0	44.54	Nonspecific Binding
Specific EtOH	5	8382.4	243.02	Specific Total Binding
R1881	6	7722.5	2250.12	Negative control (at 1.0 E-6 M only)

Assay Characterization Values			
EtOH / Hot	0.06	less than 0.1?	
R1881 / EtOH	0.87	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.06	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	-0.4
R1881	-4.0	31.8
norethynodrel	-4.5	6.9
Unknown 1	-4.0	-1.3
Unknown 2	-3.0	7.2
Unknown 3	#N/A	not applicable

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

volume of ethanol counted: 1 mL

multiply DPM in sample by : 1.5

Check the 10% rule: 5.70%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Three Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4482.00	6723.00	FALSE	mispipet		—							102.2	
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	6181.00	9271.50	TRUE			—	104.7	9271.5	8774.5	147138.3	104.7			104.7
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5905.00	8857.50	TRUE			—	99.7	8857.5	8360.5	147552.3	99.7			99.7
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	307.00	460.50	TRUE		cold E2	-7.0	-0.4	460.5	-36.5	155949.3	-0.4	-0.4		-0.4
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	292.00	438.00	TRUE		cold E2	-7.0	-0.7	438	-59.0	155971.8	-0.7			-0.7
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	320.00	480.00	TRUE		cold E2	-7.0	-0.2	480	-17.0	155929.8	-0.2			-0.2
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1070.00	1605.00	TRUE		cold E2	-8.0	13.2	1605	1108.0	154804.8	13.2	12.7		13.2
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1049.00	1573.50	TRUE		cold E2	-8.0	12.8	1573.5	1076.5	154836.3	12.8			12.8
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1008.00	1512.00	TRUE		cold E2	-8.0	12.1	1512	1015.0	154897.8	12.1			12.1
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2207.00	3310.50	TRUE		cold E2	-8.5	33.6	3310.5	2813.5	153099.3	33.6	32.9		33.6
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2138.00	3207.00	TRUE		cold E2	-8.5	32.3	3207	2710.0	153202.8	32.3			32.3
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2161.00	3241.50	TRUE		cold E2	-8.5	32.7	3241.5	2744.5	153168.3	32.7			32.7
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3912.00	5868.00	TRUE		cold E2	-9.0	64.1	5868	5371.0	150541.8	64.1	63.6		64.1
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3831.00	5746.50	TRUE		cold E2	-9.0	62.6	5746.5	5249.5	150663.3	62.6			62.6
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3907.00	5860.50	TRUE		cold E2	-9.0	64.0	5860.5	5363.5	150549.3	64.0			64.0
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	5276.00	7914.00	TRUE		cold E2	-9.5	88.5	7914	7417.0	148495.8	88.5	90.0		88.5
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	5452.00	8178.00	TRUE		cold E2	-9.5	91.6	8178	7681.0	148231.8	91.6			91.6
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	5350.00	8025.00	TRUE		cold E2	-9.5	89.8	8025	7528.0	148384.8	89.8			89.8
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5698.00	8547.00	TRUE		cold E2	-10.0	96.0	8547	8050.0	147862.8	96.0	100.2		96.0
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5886.00	8829.00	TRUE		cold E2	-10.0	99.4	8829	8332.0	147580.8	99.4			99.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	6213.00	9319.50	TRUE		cold E2	-10.0	105.3	9319.5	8822.5	147090.3	105.3			105.3
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	6464.00	9696.00	TRUE		cold E2	-11.0	109.7	9696	9199.0	146713.8	109.7	108.9		109.7
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	6512.00	9768.00	TRUE		cold E2	-11.0	110.6	9768	9271.0	146641.8	110.6			110.6
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	6267.00	9400.50	TRUE		cold E2	-11.0	106.2	9400.5	8903.5	147009.3	106.2			106.2
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2112.00	3168.00	TRUE		R1881	-3.0	31.9	3168	2671.0	153241.8	31.9	32.3		31.9
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2178.00	3267.00	TRUE		R1881	-3.0	33.0	3267	2770.0	153142.8	33.0			33.0
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2126.00	3189.00	TRUE		R1881	-3.0	32.1	3189	2692.0	153220.8	32.1			32.1
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2119.00	3178.50	TRUE		R1881	-4.0	32.0	3178.5	2681.5	153231.3	32.0	31.8		32.0
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2217.00	3325.50	TRUE		R1881	-4.0	33.7	3325.5	2828.5	153084.3	33.7			33.7
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1990.00	2985.00	TRUE		R1881	-4.0	29.7	2985	2488.0	153424.8	29.7			29.7
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4277.00	6415.50	TRUE		R1881	-5.0	70.6	6415.5	5918.5	149994.3	70.6	67.4		70.6
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4564.00	6846.00	TRUE		R1881	-5.0	75.7	6846	6349.0	149563.8	75.7			75.7
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3457.00	5185.50	TRUE		R1881	-5.0	55.9	5185.5	4688.5	151224.3	55.9			55.9
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5616.00	8424.00	TRUE		R1881	-6.0	94.6	8424	7927.0	147985.8	94.6	86.2		94.6
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	6542.00	9813.00	TRUE		R1881	-6.0	111.1	9813	9316.0	146596.8	111.1			111.1
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3287.00	4930.50	TRUE		R1881	-6.0	52.9	4930.5	4433.5	151479.3	52.9			52.9
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5999.00	8998.50	TRUE		R1881	-7.0	101.4	8998.5	8501.5	147411.3	101.4	104.8		101.4
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6311.00	9466.50	TRUE		R1881	-7.0	107.0	9466.5	8969.5	146943.3	107.0			107.0

Com... Assay Tube Layout - Three Test Chemicals

Check the 10% rule: 5.70%

If the ratio of EtOH ... 10% then there are problems v... assay

% binding >105 or < 0.95 highlighted in red font; points in the ... a U-shaped curve (binding > 10% higher than at avg minimum) are flagged ... w cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6249.00	9373.50	TRUE		R1881	-7.0	105.9	9373.5	8876.5	147036.3	105.9		105.9
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5907.00	8860.50	TRUE		R1881	-8.0	99.8	8860.5	8363.5	147549.3	99.8	98.2	99.8
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5768.00	8652.00	TRUE		R1881	-8.0	97.3	8652	8155.0	147757.8	97.3		97.3
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5786.00	8679.00	TRUE		R1881	-8.0	97.6	8679	8182.0	147730.8	97.6		97.6
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6593.00	9889.50	TRUE		R1881	-9.0	112.1	9889.5	9392.5	146520.3	112.1	111.9	112.1
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6441.00	9661.50	TRUE		R1881	-9.0	109.3	9661.5	9164.5	146748.3	109.3		109.3
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6728.00	10092.00	TRUE		R1881	-9.0	114.5	10092	9595.0	146317.8	114.5		114.5
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6403.00	9604.50	TRUE		R1881	-10.0	108.7	9604.5	9107.5	146805.3	108.7	95.6	108.7
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6380.00	9570.00	TRUE		R1881	-10.0	108.2	9570	9073.0	146839.8	108.2		108.2
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4242.00	6363.00	TRUE		R1881	-10.0	70.0	6363	5866.0	150046.8	70.0		70.0
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2715.00	4072.50	TRUE		norethynodrel	-4.0	42.7	4072.5	3575.5	152337.3	42.7	48.3	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	3125.00	4687.50	TRUE		norethynodrel	-4.0	50.0	4687.5	4190.5	151722.3	50.0		
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	3256.00	4884.00	TRUE		norethynodrel	-4.0	52.3	4884	4387.0	151525.8	52.3		
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	657.00	985.50	TRUE		norethynodrel	-4.5	5.8	985.5	488.5	155424.3	5.8	6.9	5.8
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	760.00	1140.00	TRUE		norethynodrel	-4.5	7.7	1140	643.0	155269.8	7.7		7.7
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	741.00	1111.50	TRUE		norethynodrel	-4.5	7.3	1111.5	614.5	155298.3	7.3		7.3
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1951.00	2926.50	TRUE		norethynodrel	-5.5	29.0	2926.5	2429.5	153483.3	29.0	28.7	29.0
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1972.00	2958.00	TRUE		norethynodrel	-5.5	29.4	2958	2461.0	153451.8	29.4		29.4
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1882.00	2823.00	TRUE		norethynodrel	-5.5	27.7	2823	2326.0	153586.8	27.7		27.7
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3477.00	5215.50	TRUE		norethynodrel	-6.0	56.3	5215.5	4718.5	151194.3	56.3	55.8	56.3
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3416.00	5124.00	TRUE		norethynodrel	-6.0	55.2	5124	4627.0	151285.8	55.2		55.2
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3453.00	5179.50	TRUE		norethynodrel	-6.0	55.9	5179.5	4682.5	151230.3	55.9		55.9
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	5654.00	8481.00	TRUE		norethynodrel	-6.5	95.2	8481	7984.0	147928.8	95.2	93.2	95.2
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	5591.00	8386.50	TRUE		norethynodrel	-6.5	94.1	8386.5	7889.5	148023.3	94.1		94.1
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	5382.00	8073.00	TRUE		norethynodrel	-6.5	90.4	8073	7576.0	148336.8	90.4		90.4
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	6276.00	9414.00	TRUE		norethynodrel	-7.0	106.4	9414	8917.0	146995.8	106.4	103.5	106.4
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	6151.00	9226.50	TRUE		norethynodrel	-7.0	104.1	9226.5	8729.5	147183.3	104.1		104.1
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5920.00	8880.00	TRUE		norethynodrel	-7.0	100.0	8880	8383.0	147529.8	100.0		100.0
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	6703.00	10054.50	TRUE		norethynodrel	-7.5	114.0	10054.5	9557.5	146355.3	114.0	114.2	114.0
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	6849.00	10273.50	TRUE		norethynodrel	-7.5	116.6	10273.5	9776.5	146136.3	116.6		116.6
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	6593.00	9889.50	TRUE		norethynodrel	-7.5	112.1	9889.5	9392.5	146520.3	112.1		112.1
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	6836.00	10254.00	TRUE		norethynodrel	-8.5	116.4	10254	9757.0	146155.8	116.4	113.5	116.4
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	6627.00	9940.50	TRUE		norethynodrel	-8.5	112.7	9940.5	9443.5	146469.3	112.7		112.7
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	6562.00	9843.00	TRUE		norethynodrel	-8.5	111.5	9843	9346.0	146566.8	111.5		111.5
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3139.00	4708.50	TRUE		Unknown 1	-3.0	50.2	4708.5	4211.5	151701.3	50.2	56.7	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3842.00	5763.00	TRUE		Unknown 1	-3.0	62.8	5763	5266.0	150646.8	62.8		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3521.00	5281.50	TRUE		Unknown 1	-3.0	57.1	5281.5	4784.5	151128.3	57.1		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	247.00	370.50	TRUE		Unknown 1	-4.0	-1.5	370.5	-126.5	156039.3	-1.5	-1.3	-1.5
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	278.00	417.00	TRUE		Unknown 1	-4.0	-1.0	417	-80.0	155992.8	-1.0		-1.0
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	255.00	382.50	TRUE		Unknown 1	-4.0	-1.4	382.5	-114.5	156027.3	-1.4		-1.4
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	266.00	399.00	TRUE		Unknown 1	-5.0	-1.2	399	-98.0	156010.8	-1.2	0.7	-1.2
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	439.00	658.50	TRUE		Unknown 1	-5.0	1.9	658.5	161.5	155751.3	1.9		1.9
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	409.00	613.50	TRUE		Unknown 1	-5.0	1.4	613.5	116.5	155796.3	1.4		1.4
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	779.00	1168.50	TRUE		Unknown 1	-6.0	8.0	1168.5	671.5	155241.3	8.0	7.5	8.0
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	748.00	1122.00	TRUE		Unknown 1	-6.0	7.5	1122	625.0	155287.8	7.5		7.5
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	717.00	1075.50	TRUE		Unknown 1	-6.0	6.9	1075.5	578.5	155334.3	6.9		6.9
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3368.00	5052.00	TRUE		Unknown 1	-7.0	54.3	5052	4555.0	151357.8	54.3	51.7	54.3
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3295.00	4942.50	TRUE		Unknown 1	-7.0	53.0	4942.5	4445.5	151467.3	53.0		53.0
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3004.00	4506.00	TRUE		Unknown 1	-7.0	47.8	4506	4009.0	151903.8	47.8		47.8
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	6146.00	9219.00	TRUE		Unknown 1	-8.0	104.1	9219	8722.0	147190.8	104.1	101.6	104.1
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	6043.00	9064.50	TRUE		Unknown 1	-8.0	102.2	9064.5	8567.5	147345.3	102.2		102.2
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5846.00	8769.00	TRUE		Unknown 1	-8.0	98.7	8769	8272.0	147640.8	98.7		98.7
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6379.00	9568.50	TRUE		Unknown 1	-9.0	108.2	9568.5	9071.5	146841.3	108.2	110.4	108.2
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6541.00	9811.50	TRUE		Unknown 1	-9.0	111.1	9811.5	9314.5	146598.3	111.1		111.1
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6574.00	9861.00	TRUE		Unknown 1	-9.0	111.7	9861	9364.0	146548.8	111.7		111.7
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6717.00	10075.50	TRUE		Unknown 1	-10.0	114.3	10075.5	9578.5	146334.3	114.3	112.2	114.3
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6693.00	10039.50	TRUE		Unknown 1	-10.0	113.8	10039.5	9542.5	146370.3	113.8		113.8
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6392.00	9588.00	TRUE		Unknown 1	-10.0	108.5	9588	9091.0	146821.8	108.5		108.5
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	748.00	1122.00	TRUE		Unknown 2	-3.0	7.5	1122	625.0	155287.8	7.5	7.2	7.5
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	910.00	1365.00	TRUE		Unknown 2	-3.0	10.4	1365	868.0	155044.8	10.4		10.4
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	538.00	807.00	TRUE		Unknown 2	-3.0	3.7	807	310.0	155602.8	3.7		3.7
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	5172.00	7758.00	TRUE		Unknown 2	-4.0	86.6	7758	7261.0	148651.8	86.6	113.5	86.6
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4905.00	7357.50	TRUE		Unknown 2	-4.0	81.8	7357.5	6860.5	149052.3	81.8		81.8
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	9943.00	14914.50	TRUE		Unknown 2	-4.0	172.0	14914.5	14417.5	141495.3	172.0		172.0
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	6366.00	9549.00	TRUE		Unknown 2	-5.0	108.0	9549	9052.0	146860.8	108.0	98.0	108.0
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4530.00	6795.00	TRUE		Unknown 2	-5.0	75.1	6795	6298.0	149614.8	75.1		75.1
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	6524.00	9786.00	TRUE		Unknown 2	-5.0	110.8	9786	9289.0	146623.8	110.8		110.8
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	6409.00	9613.50	TRUE		Unknown 2	-6.0	108.8	9613.5	9116.5	146796.3	108.8	109.9	108.8
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	6570.00	9855.00	TRUE		Unknown 2	-6.0	111.6	9855	9358.0	146554.8	111.6		111.6
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	6446.00	9669.00	TRUE		Unknown 2	-6.0	109.4	9669	9172.0	146740.8	109.4		109.4
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6035.00	9052.50	TRUE		Unknown 2	-7.0	102.1	9052.5	8555.5	147357.3	102.1	103.5	102.1
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6154.00	9231.00	TRUE		Unknown 2	-7.0	104.2	9231	8734.0	147178.8	104.2		104.2
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6153.00	9229.50	TRUE		Unknown 2	-7.0	104.2	9229.5	8732.5	147180.3	104.2		104.2
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	6587.00	9880.50	TRUE		Unknown 2	-8.0	111.9	9880.5	9383.5	146529.3	111.9	112.0	111.9
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	6672.00	10008.00	TRUE		Unknown 2	-8.0	113.5	10008	9511.0	146401.8	113.5		113.4
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	6506.00	9759.00	TRUE		Unknown 2	-8.0	110.5	9759	9262.0	146650.8	110.5		110.5
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6618.00	9927.00	TRUE		Unknown 2	-9.0	112.5	9927	9430.0	146482.8	112.5	108.7	112.5
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6486.00	9729.00	TRUE		Unknown 2	-9.0	110.1	9729	9232.0	146680.8	110.1		110.1
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6112.00	9168.00	TRUE		Unknown 2	-9.0	103.4	9168	8671.0	147241.8	103.4		103.4
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6453.00	9679.50	TRUE		Unknown 2	-10.0	109.5	9679.5	9182.5	146730.3	109.5	109.8	109.5
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6686.00	10029.00	TRUE		Unknown 2	-10.0	113.7	10029	9532.0	146380.8	113.7		113.7
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	6268.00	9402.00	TRUE		Unknown 2	-10.0	106.2	9402	8905.0	147007.8	106.2		106.2

Competitive Assay Tube Layout - Three Test Chemicals

Check the 10% rule: 5.70% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
121	1	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
122	2	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
123	3	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
124	1	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
125	2	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
126	3	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
127	1	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
128	2	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
129	3	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
130	1	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
131	2	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
132	3	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
133	1	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
134	2	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
135	3	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
136	1	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
137	2	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
138	3	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
139	1	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
140	2	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
141	3	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
142	1	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
143	2	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
144	3	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
145	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	343.00	514.50	TRUE		cold E2	-7.0	0.2	514.5	17.5	155895.3	0.2	0.4	0.2
146	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	363.00	544.50	TRUE		cold E2	-7.0	0.6	544.5	47.5	155865.3	0.6		0.6
147	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	363.00	544.50	TRUE		cold E2	-7.0	0.6	544.5	47.5	155865.3	0.6		0.6
148	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5940.00	8910.00	TRUE			—	100.4	8910	8413.0	147499.8	100.4	98.5	100.4
149	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5799.00	8698.50	TRUE			—	97.8	8698.5	8201.5	147711.3	97.8		97.8
150	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5773.00	8659.50	TRUE			—	97.4	8659.5	8162.5	147750.3	97.4		97.4
151	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	5616.00	8424.00	TRUE			-6.0	94.6	8424	7927.0	147985.8	94.6	86.2	94.6
152	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	6542.00	9813.00	TRUE			-6.0	111.1	9813	9316.0	146596.8	111.1		111.1
153	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	3287.00	4930.50	TRUE			-6.0	52.9	4930.5	4433.5	151479.3	52.9		52.9
154	1	none	Hot		—	—	—	10	—	—	—	—	105355.00	158032.50	TRUE					158032.5	157535.5				
155	2	none	Hot		—	—	—	10	—	—	—	—	103192.00	154788.00	TRUE					154788	154291.0				
156	3	none	Hot		—	—	—	10	—	—	—	—	99338.00	149007.00	TRUE					149007	148510.0				
157	1	none	Hot		—	—	—	10	—	—	—	—	101060.00	151590.00	TRUE					151590	151093.0				
158	2	none	Hot		—	—	—	10	—	—	—	—	104584.00	156876.00	TRUE					156876	156379.0				
159	3	none	Hot		—	—	—	10	—	—	—	—	110122.00	165183.00	TRUE					165183	164686.0				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				12.0				19.0				Chem code				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	-0.4354	-0.7039	-0.2028	-4.0				-3.0				-3.0	7.4561	10.3550	3.6982	#NUM!	#NUM!	#NUM!	#NUM!	-3.0	31.8644	33.0454	32.1149
2	2	-7.0	0.2088	0.5667	0.5667	-4.5	5.8277	7.6708	7.3308	-4.0	-1.5091	-0.9544	-1.3660	-4.0	86.6220	81.8441	171.9973	#NUM!	#NUM!	#NUM!	#NUM!	-4.0	31.9896	33.7433	29.6812
3	3	-8.0	13.2182	12.8424	12.1087	-5.5	28.9833	29.3591	27.7486	-5.0	-1.1691	1.9267	1.3898	-5.0	107.9882	75.1336	110.8155	#NUM!	#NUM!	#NUM!	#NUM!	-5.0	70.6063	75.7420	55.9327
4	1	-8.5	33.5644	32.3296	32.7412	-6.0	56.2906	55.1990	55.8611	-6.0	8.0108	7.4561	6.9014	-6.0	108.7576	111.6387	109.4197	#NUM!	#NUM!	#NUM!	#NUM!	-6.0	94.5672	111.1376	52.8906
5	2	-9.0	64.0747	62.6253	63.9853	-6.5	95.2472	94.1198	90.3798	-7.0	54.3400	53.0337	47.8264	-7.0	102.0650	104.1945	104.1766	#NUM!	#NUM!	#NUM!	#NUM!	-7.0	101.4208	107.0040	105.8945
6	3	-9.5	88.4830	91.6325	89.8072	-7.0	106.3776	104.1408	100.0072	-8.0	104.0513	102.2082	98.6830	-8.0	111.9429	113.4639	110.4934	#NUM!	#NUM!	#NUM!	#NUM!	-8.0	99.7745	97.2872	97.6093
7	1	-10.0	96.0345	99.3987	105.2503	-7.5	114.0187	116.6313	112.0502	-9.0	108.2208	111.1197	111.7103	-9.0	112.4976	110.1355	103.4429	#NUM!	#NUM!	#NUM!	#NUM!	-9.0	112.0502	109.3303	114.4660
8	2	-11.0	109.7418	110.6008	106.2166	-8.5	116.3986	112.6587	111.4955	-10.0	114.2692	113.8397	108.4534	-10.0	109.5450	113.7144	106.2345	#NUM!	#NUM!	#NUM!	#NUM!	-10.0	108.6503	108.2387	69.9800
9	3																								
10	1	Standard Curve		Prism run TTN 001 03/12/08		Positive Control		Prism run TTN 001 03/12/08		12.0		Prism run TTN 001 03/12/08		19.0		Prism run TTN 001 03/12/08		Chem code		Prism run ID & date		Negative Control		Prism run TTN 001 03/12/08	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values			
13	1	BOTTOM		-1.579		BOTTOM		5.59		BOTTOM		-0.7165		BOTTOM		~ -239.6		BOTTOM				BOTTOM		28.86	
14	2	TOP		108.9		TOP		114.3		TOP		111.9		TOP		107.9		TOP				TOP		107.1	
15	3	LOGIC50		-8.782		LOGIC50		-5.886		LOGIC50		-6.973		LOGIC50				LOGIC50				LOGIC50		-4.515	
16	1	HILLSLOPE		-0.995		HILLSLOPE		-1.153		HILLSLOPE		-1.048		HILLSLOPE				HILLSLOPE				HILLSLOPE		-0.7982	
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		0.9284		BOTTOM		2.05		BOTTOM		0.875		BOTTOM				BOTTOM				BOTTOM		10.13	
19	1	TOP		1.072		TOP		1.336		TOP		0.9175		TOP				TOP				TOP		4.598	
20	2	LOGIC50		0.01243		LOGIC50		0.01974		LOGIC50		0.01718		LOGIC50				LOGIC50				LOGIC50		0.2768	
21	3	HILLSLOPE		0.03787		HILLSLOPE		0.07419		HILLSLOPE		0.05608		HILLSLOPE				HILLSLOPE				HILLSLOPE		0.3733	
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																								

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.2

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Estradiol
Run ID:	TTN 001
Assay start date:	3/11/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-7.0	-0.5
1	1	2	-7.0	-0.7
1	1	3	-7.0	-0.2
1	2	1	-8.0	13.8
1	2	2	-8.0	13.4
1	2	3	-8.0	12.7
1	3	1	-8.5	35.1
1	3	2	-8.5	33.8
1	3	3	-8.5	34.2
1	4	1	-9.0	66.9
1	4	2	-9.0	65.4
1	4	3	-9.0	66.9
1	5	1	-9.5	92.4
1	5	2	-9.5	95.7
1	5	3	-9.5	93.8
1	6	1	-10.0	100.3
1	6	2	-10.0	103.9
1	6	3	-10.0	110.0
1	7	1	-11.0	114.7
1	7	2	-11.0	115.6
1	7	3	-11.0	111.0
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.8

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Norethynodrel
Run ID:	TTN 001
Assay start date:	3/11/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-4.0	44.6
1	1	2	-4.0	52.2
1	1	3	-4.0	54.7
1	2	1	-4.5	6.1
1	2	2	-4.5	8.0
1	2	3	-4.5	7.7
1	3	1	-5.5	30.3
1	3	2	-5.5	30.7
1	3	3	-5.5	29.0
1	4	1	-6.0	58.8
1	4	2	-6.0	57.7
1	4	3	-6.0	58.4
1	5	1	-6.5	99.5
1	5	2	-6.5	98.3
1	5	3	-6.5	94.4
1	6	1	-7.0	111.1
1	6	2	-7.0	108.8
1	6	3	-7.0	104.5
1	7	1	-7.5	119.1
1	7	2	-7.5	121.9
1	7	3	-7.5	117.1
1	8	1	-8.5	121.6
1	8	2	-8.5	117.7
1	8	3	-8.5	116.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 14.40773

Provide information in all blue cells.

		run	xgroup	rep	x	y
Laboratory Code:	B					
Compound ID:	R1881	1	1	1	-3	33.29179
Run ID:	TTN 001	1	1	2	-3	34.52574
Assay start date:	3/12/08	1	1	3	-3	33.55353
Technician ID:	ttn	1	2	1	-4	33.42266
		1	2	2	-4	35.25489
		1	2	3	-4	31.01084
		1	3	1	-5	73.76916
		1	3	2	-5	79.13499
		1	3	3	-5	58.43824
		1	4	1	-6	98.80344
		1	4	2	-6	116.1162
		1	4	3	-6	55.25988
		1	5	1	-7	105.9641
		1	5	2	-7	111.7973
		1	5	3	-7	110.6382
		1	6	1	-8	104.244
		1	6	2	-8	101.6453
		1	6	3	-8	101.9818
		1	7	1	-9	117.0697
		1	7	2	-9	114.2278
		1	7	3	-9	119.5937
		1	8	1	-10	113.5174
		1	8	2	-10	113.0874
		1	8	3	-10	73.11479
		1	9	1		
		1	9	2		
		1	9	3		
		1	10	1		
		1	10	2		
		1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.2

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 12
Run ID: TTN 001
Assay start date: 3/11/08
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	52.5
1	1	2	-3.0	65.6
1	1	3	-3.0	59.6
1	2	1	-4.0	-1.6
1	2	2	-4.0	-1.0
1	2	3	-4.0	-1.4
1	3	1	-5.0	-1.2
1	3	2	-5.0	2.0
1	3	3	-5.0	1.5
1	4	1	-6.0	8.4
1	4	2	-6.0	7.8
1	4	3	-6.0	7.2
1	5	1	-7.0	56.8
1	5	2	-7.0	55.4
1	5	3	-7.0	50.0
1	6	1	-8.0	108.7
1	6	2	-8.0	106.8
1	6	3	-8.0	103.1
1	7	1	-9.0	113.1
1	7	2	-9.0	116.1
1	7	3	-9.0	116.7
1	8	1	-10.0	119.4
1	8	2	-10.0	118.9
1	8	3	-10.0	113.3
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 20.3

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 19
Run ID: TTN 001
Assay start date: 3/11/08
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	7.8
1	1	2	-3.0	10.8
1	1	3	-3.0	3.9
1	2	1	-4.0	90.5
1	2	2	-4.0	85.5
1	2	3	-4.0	179.7
1	3	1	-5.0	112.8
1	3	2	-5.0	78.5
1	3	3	-5.0	115.8
1	4	1	-6.0	113.6
1	4	2	-6.0	116.6
1	4	3	-6.0	114.3
1	5	1	-7.0	106.6
1	5	2	-7.0	108.9
1	5	3	-7.0	108.8
1	6	1	-8.0	117.0
1	6	2	-8.0	118.5
1	6	3	-8.0	115.4
1	7	1	-9.0	117.5
1	7	2	-9.0	115.1
1	7	3	-9.0	108.1
1	8	1	-10.0	114.5
1	8	2	-10.0	118.8
1	8	3	-10.0	111.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	Estradiol standard TTN 001		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-0.4354	-0.7039	-0.2028
2	Title	-7.0	0.2088	0.5667	0.5667
3	Title	-8.0	13.2182	12.8424	12.1087
4	Title	-8.5	33.5644	32.3296	32.7412
5	Title	-9.0	64.0747	62.6253	63.9853
6	Title	-9.5	88.4830	91.6325	89.8072
7	Title	-10.0	96.0345	99.3987	105.2503
8	Title	-11.0	109.7418	110.6008	106.2166

	Nonlin fit Table of results	A Estradiol standard TTN 001
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.579
4	TOP	108.9
5	LOGIC50	-8.782
6	HILLSLOPE	-0.9950
7	Std. Error	
8	BOTTOM	0.9284
9	TOP	1.072
10	LOGIC50	0.01243
11	HILLSLOPE	0.03787
12	95% Confidence Intervals	
13	BOTTOM	-3.516 to 0.3572
14	TOP	106.7 to 111.2
15	LOGIC50	-8.808 to -8.756
16	HILLSLOPE	-1.074 to -0.9160
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9983
20	Absolute Sum of Squares	74.85
21	Sy.x	1.935
22	Number of points	
23	Analyzed	24

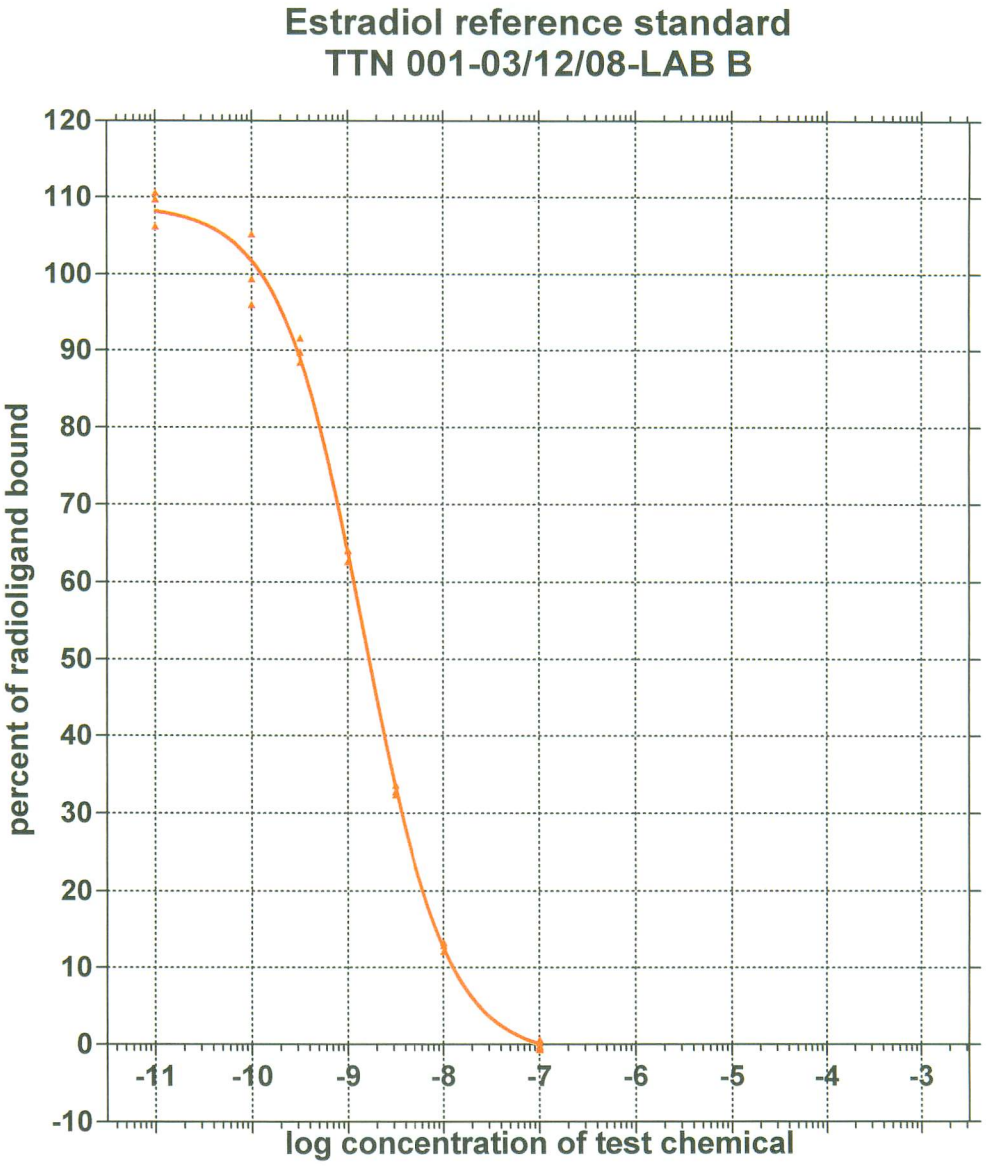

Estradiol reference standard
TTN 001-03/12/08-LAB B
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Norethynodre TTN 001		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	5.8277	7.6708	7.3308
3	Title	-5.5	28.9833	29.3591	27.7486
4	Title	-6.0	56.2906	55.1990	55.8611
5	Title	-6.5	95.2472	94.1198	90.3798
6	Title	-7.0	106.3776	104.1408	100.0072
7	Title	-7.5	114.0187	116.6313	112.0502
8	Title	-8.5	116.3986	112.6587	111.4955

	Nonlin fit Table of results	A Norethynodre TTN 001
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	5.590
4	TOP	114.3
5	LOGIC50	-5.886
6	HILLSLOPE	-1.153
7	Std. Error	
8	BOTTOM	2.050
9	TOP	1.336
10	LOGIC50	0.01974
11	HILLSLOPE	0.07419
12	95% Confidence Intervals	
13	BOTTOM	1.264 to 9.917
14	TOP	111.4 to 117.1
15	LOGIC50	-5.928 to -5.844
16	HILLSLOPE	-1.310 to -0.9970
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9954
20	Absolute Sum of Squares	156.3
21	Sy.x	3.032
22	Number of points	
23	Analyzed	21

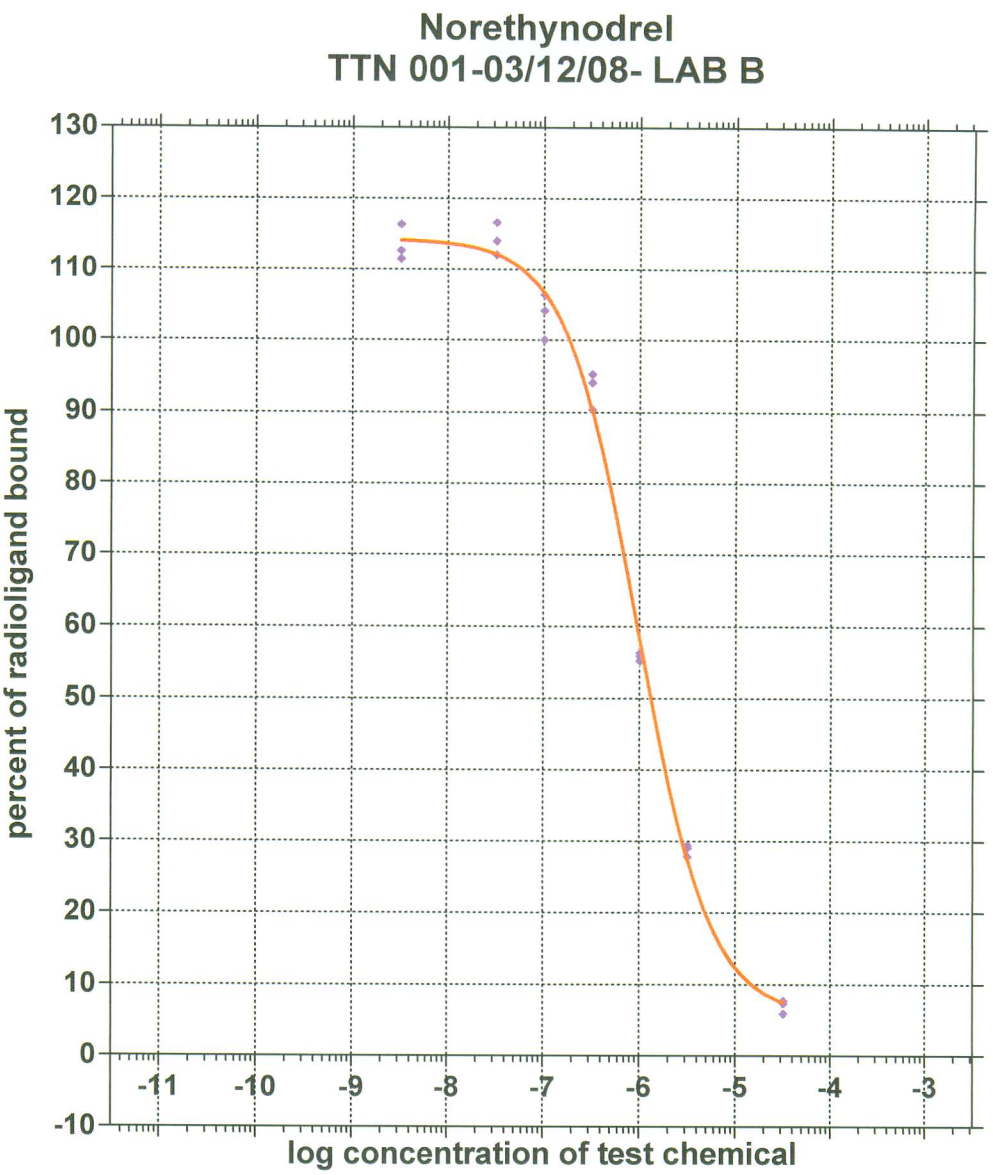

Norethynodrel
TTN 001-03/12/08- LAB B
percent of radioligand bound
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	R1881 TTN 001		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	33.2918	34.5257	33.5535
2	Title	-4.0	33.4227	35.2549	31.0108
3	Title	-5.0	73.7692	79.1350	58.4382
4	Title	-6.0	98.8034	116.1162	55.2599
5	Title	-7.0	105.9641	111.7973	110.6382
6	Title	-8.0	104.2440	101.6453	101.9818
7	Title	-9.0	117.0697	114.2278	119.5937
8	Title	-10.0	113.5174	113.0874	73.1148

	Nonlin fit Table of results	A R1881 TTN 001
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	28.86
4	TOP	107.1
5	LOGIC50	-4.515
6	HILLSLOPE	-0.7982
7	Std. Error	
8	BOTTOM	10.13
9	TOP	4.598
10	LOGIC50	0.2768
11	HILLSLOPE	0.3733
12	95% Confidence Intervals	
13	BOTTOM	7.730 to 49.98
14	TOP	97.50 to 116.7
15	LOGIC50	-5.092 to -3.937
16	HILLSLOPE	-1.577 to -0.01946
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.8417
20	Absolute Sum of Squares	4148
21	Sy.x	14.40
22	Number of points	
23	Analyzed	24

R1881
TTN 001-03/12/08-LAB B

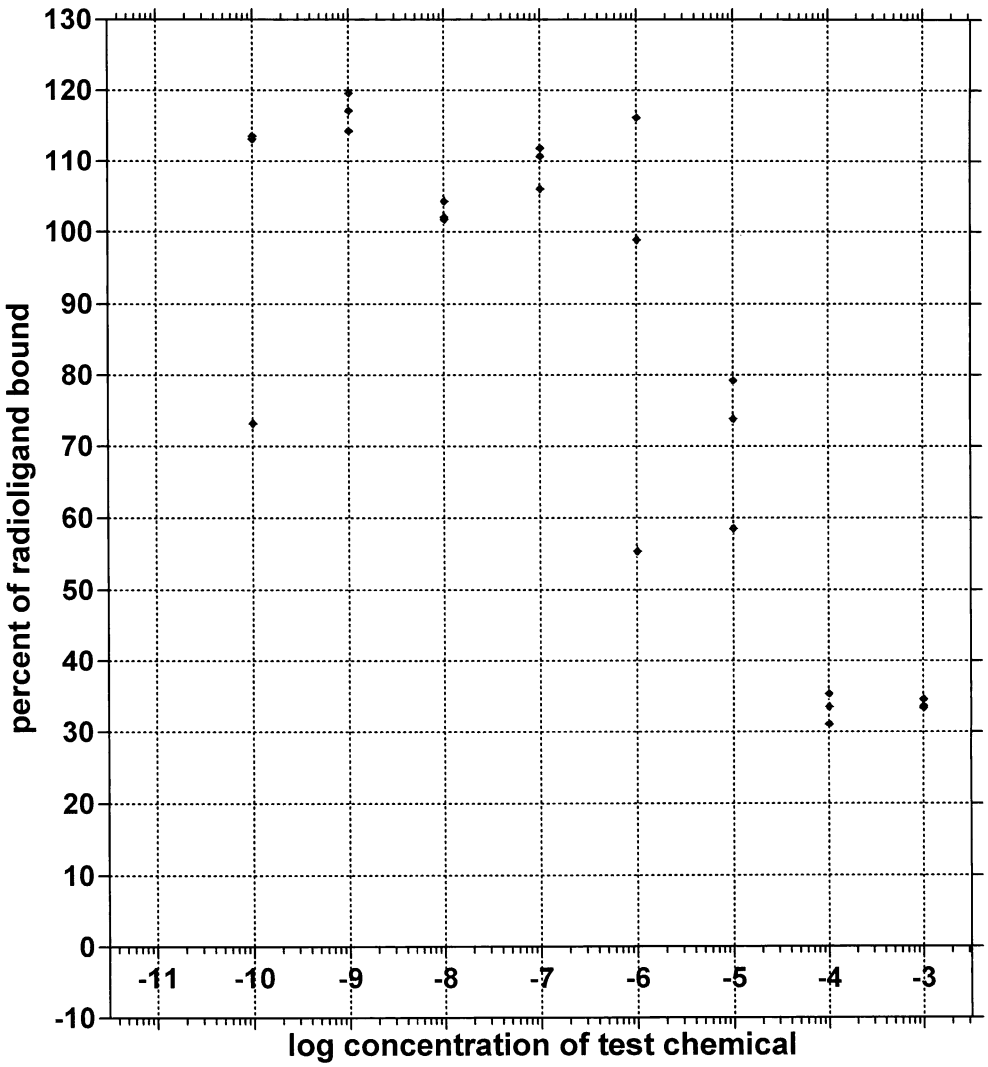


Table format: XY		X	A		
		log concentration	Test chemical CODE 12 TTN 001		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-1.5091	-0.9544	-1.3660
3	Title	-5.0	-1.1691	1.9267	1.3898
4	Title	-6.0	8.0108	7.4561	6.9014
5	Title	-7.0	54.3400	53.0337	47.8264
6	Title	-8.0	104.0513	102.2082	98.6830
7	Title	-9.0	108.2208	111.1197	111.7103
8	Title	-10.0	114.2692	113.8397	108.4534

	Nonlin fit Table of results	A Test chemical CODE 12 TTN 001
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.7165
4	TOP	111.9
5	LOGIC50	-6.973
6	HILLSLOPE	-1.048
7	Std. Error	
8	BOTTOM	0.8750
9	TOP	0.9175
10	LOGIC50	0.01718
11	HILLSLOPE	0.05608
12	95% Confidence Intervals	
13	BOTTOM	-2.563 to 1.130
14	TOP	110.0 to 113.8
15	LOGIC50	-7.010 to -6.937
16	HILLSLOPE	-1.167 to -0.9300
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9985
20	Absolute Sum of Squares	76.74
21	Sy.x	2.125
22	Number of points	
23	Analyzed	21

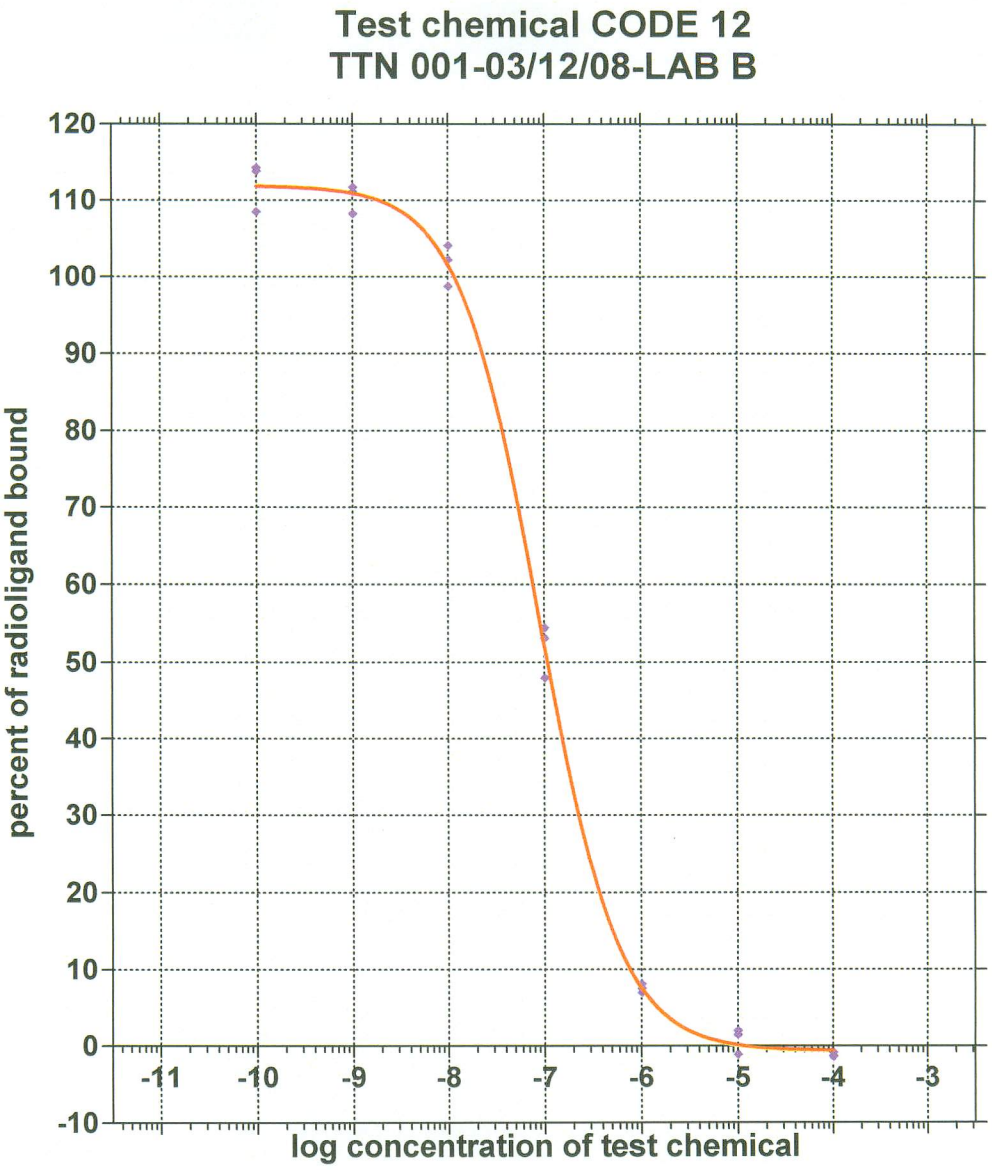
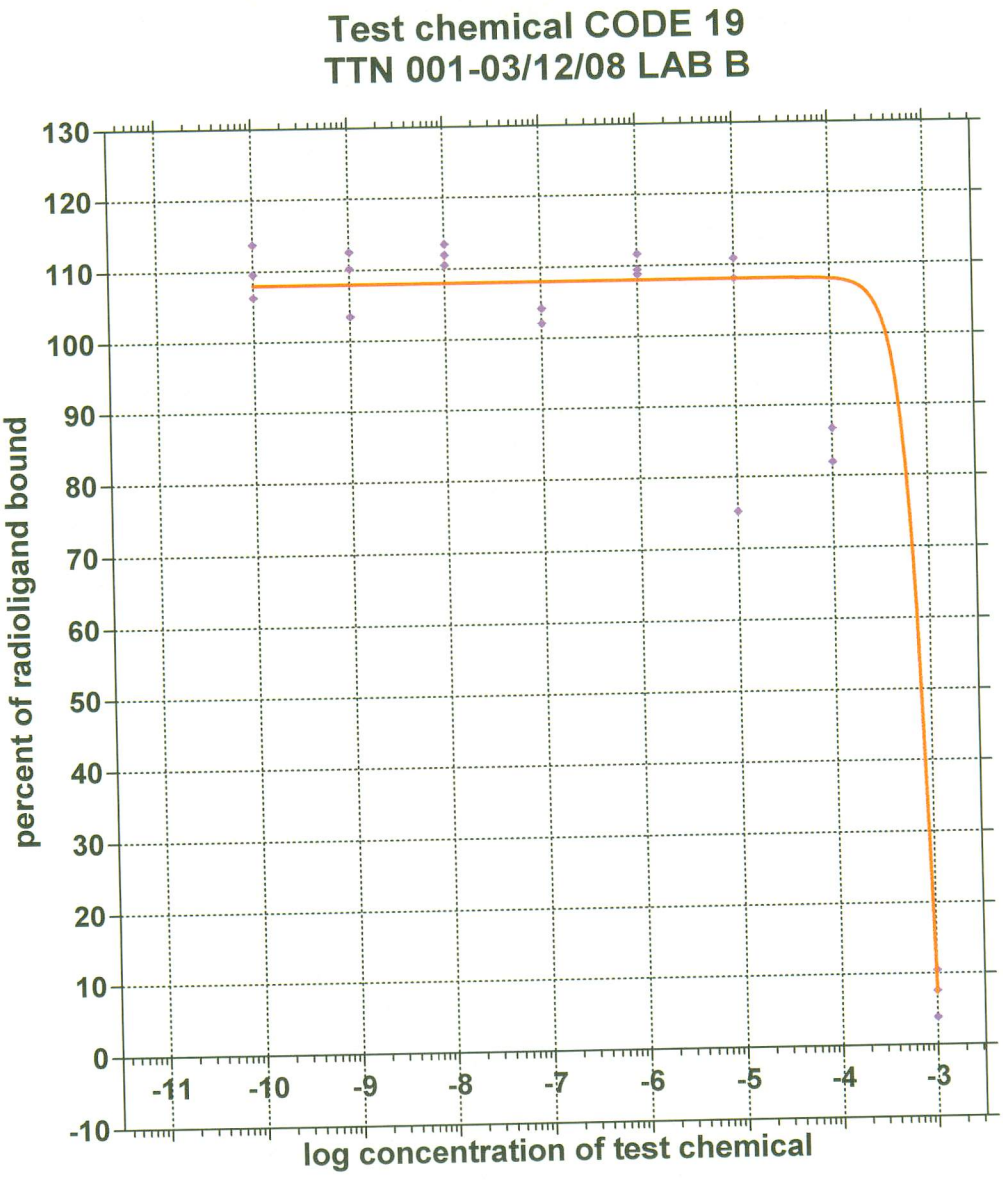

Test chemical CODE 12
TTN 001-03/12/08-LAB B
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical CODE 19 TTN 001		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	7.4561	10.3550	3.6982
2	Title	-4.0	86.6220	81.8441	171.9973
3	Title	-5.0	107.9882	75.1336	110.8155
4	Title	-6.0	108.7576	111.6387	109.4197
5	Title	-7.0	102.0650	104.1945	104.1766
6	Title	-8.0	111.9429	113.4639	110.4934
7	Title	-9.0	112.4976	110.1355	103.4429
8	Title	-10.0	109.5450	113.7144	106.2345

	Nonlin fit Table of results	A Test chemical CODE 19 TTN 001
		Y
1	logIC50	Ambiguous
2	Best-fit values	
3	BOTTOM	~ -239.6
4	TOP	107.9
5	LOGIC50	~ -3.104
6	HILLSLOPE	~ -2.970
7	Std. Error	
8	BOTTOM	~ 6.009e+007
9	TOP	4.681
10	LOGIC50	~ 4191
11	HILLSLOPE	~ 30638
12	95% Confidence Intervals	
13	BOTTOM	(Very wide)
14	TOP	98.16 to 117.7
15	LOGIC50	(Very wide)
16	HILLSLOPE	(Very wide)
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.8022
20	Absolute Sum of Squares	6568
21	Sy.x	18.12
22	Number of points	
23	Analyzed	24


Test chemical CODE 19
TTN 001-03/12/08 LAB B
percent of radioligand bound
130
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

APPENDIX G

Excel and Prism Files of the Run Identifier TTN002

ER Competitive Assay - Two Unknowns

159 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AU43 and AE48 : AU48.

If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AV contain values to be analyzed by nonlinear regression software

Column O, Rows 10 through 21 will contain output parameters from the nonlinear regression software. and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	TTN-002	
Assay start date:	3/17/2008	
Tracer lot number:	3589221/1	
Specific activity on day of assay:	105.64	Ci/mmole
Cytosol vial or lot identification:	RUC-2-18-08	
Protein (cytosol):	40	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-9.09	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-6.07	M
RBA, norethynodrel (Weak Positive):	9.594E-04	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-5.57	11.0
RBA, Unknown 1:	3.006E-04	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-5.43	15.0
RBA, Unknown 2:	2.163E-04	relative to E2=1.0
Max Concentration, Unknown 3:		M
log(IC50), Unknown 3:	0.00	Chem code
RBA, Unknown 3:	8.091E-10	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Summary values

	n	Mean	SD	
EtOH	5	8322.0	550.54	Total Binding, solvent control, tubes
Hot	6	184073.3	3917.31	Total hot E2 added to each tube
NSB	6	442.8	19.84	Nonspecific Binding
Specific EtOH	5	7879.3	550.54	Specific Total Binding
R1881	6	4969.0	193.95	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.05	less than 0.1?
R1881 / EtOH	0.60	close to 1.0 ?
NSB / EtOH	0.05	around 0.25 ?

at 1.0 E-6 M only

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.0
R1881	-3.0	27.3
norethynodrel	-4.5	3.5
Unknown 1	-4.0	3.2
Unknown 2	-3.0	-2.2
Unknown 3	#N/A	not applicable

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

Check the 10% rule: 4.52% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Three Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5465.00	8197.50	TRUE			—	98.4	8197.5	7754.8	176318.5	98.4	96.1	98.4
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4158.00	6237.00	FALSE	mispipet		—							
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5218.00	7827.00	TRUE			—	93.7	7827	7384.3	176689.0	93.7		93.7
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	293.00	439.50	TRUE		cold E2	-7.0	0.0	439.5	-3.3	184076.5	0.0	0.0	0.0
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	282.00	423.00	TRUE		cold E2	-7.0	-0.3	423	-19.8	184093.0	-0.3		-0.3
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	309.00	463.50	TRUE		cold E2	-7.0	0.3	463.5	20.8	184052.5	0.3		0.3
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	772.00	1158.00	TRUE		cold E2	-8.0	9.1	1158	715.3	183358.0	9.1	9.4	9.1
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	816.00	1224.00	TRUE		cold E2	-8.0	9.9	1224	781.3	183292.0	9.9		9.9
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	780.00	1170.00	TRUE		cold E2	-8.0	9.2	1170	727.3	183346.0	9.2		9.2
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1741.00	2611.50	TRUE		cold E2	-8.5	27.5	2611.5	2168.8	181904.5	27.5	26.1	27.5
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1747.00	2620.50	TRUE		cold E2	-8.5	27.6	2620.5	2177.8	181895.5	27.6		27.6
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1516.00	2274.00	TRUE		cold E2	-8.5	23.2	2274	1831.3	182242.0	23.2		23.2
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2074.00	3111.00	TRUE		cold E2	-9.0	33.9	3111	2668.3	181405.0	33.9	34.1	33.9
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1974.00	2961.00	TRUE		cold E2	-9.0	32.0	2961	2518.3	181555.0	32.0		32.0
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2208.00	3312.00	TRUE		cold E2	-9.0	36.4	3312	2869.3	181204.0	36.4		36.4
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4597.00	6895.50	TRUE		cold E2	-9.5	81.9	6895.5	6452.8	177620.5	81.9	81.2	81.9
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4549.00	6823.50	TRUE		cold E2	-9.5	81.0	6823.5	6380.8	177692.5	81.0		81.0
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4543.00	6814.50	TRUE		cold E2	-9.5	80.9	6814.5	6371.8	177701.5	80.9		80.9
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5005.00	7507.50	TRUE		cold E2	-10.0	89.7	7507.5	7064.8	177008.5	89.7	90.7	89.7
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5095.00	7642.50	TRUE		cold E2	-10.0	91.4	7642.5	7199.8	176873.5	91.4		91.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5077.00	7615.50	TRUE		cold E2	-10.0	91.0	7615.5	7172.8	176900.5	91.0		91.0
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5580.00	8370.00	TRUE		cold E2	-11.0	100.6	8370	7927.3	176146.0	100.6	98.1	100.6
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5516.00	8274.00	TRUE		cold E2	-11.0	99.4	8274	7831.3	176242.0	99.4		99.4
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5250.00	7875.00	TRUE		cold E2	-11.0	94.3	7875	7432.3	176641.0	94.3		94.3
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1564.00	2346.00	TRUE		R1881	-3.0	24.2	2346	1903.3	182170.0	24.2	27.3	24.2
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2018.00	3027.00	TRUE		R1881	-3.0	32.8	3027	2584.3	181489.0	32.8		32.8
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1600.00	2400.00	TRUE		R1881	-3.0	24.8	2400	1957.3	182116.0	24.8		24.8
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1979.00	2968.50	TRUE		R1881	-4.0	32.1	2968.5	2525.8	181547.5	32.1	33.8	32.1
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2143.00	3214.50	TRUE		R1881	-4.0	35.2	3214.5	2771.8	181301.5	35.2		35.2
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2083.00	3124.50	TRUE		R1881	-4.0	34.0	3124.5	2681.8	181391.5	34.0		34.0
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1918.00	2877.00	TRUE		R1881	-5.0	30.9	2877	2434.3	181639.0	30.9	37.2	30.9
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2301.00	3451.50	TRUE		R1881	-5.0	38.2	3451.5	3008.8	181064.5	38.2		38.2
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2529.00	3793.50	TRUE		R1881	-5.0	42.5	3793.5	3350.8	180722.5	42.5		42.5
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3458.00	5187.00	TRUE		R1881	-6.0	60.2	5187	4744.3	179329.0	60.2	56.8	60.2
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3134.00	4701.00	TRUE		R1881	-6.0	54.0	4701	4258.3	179815.0	54.0		54.0
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3237.00	4855.50	TRUE		R1881	-6.0	56.0	4855.5	4412.8	179660.5	56.0		56.0
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5147.00	7720.50	TRUE		R1881	-7.0	92.4	7720.5	7277.8	176795.5	92.4	95.6	92.4
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5429.00	8143.50	TRUE		R1881	-7.0	97.7	8143.5	7700.8	176372.5	97.7		97.7
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5374.00	8061.00	TRUE		R1881	-7.0	96.7	8061	7618.3	176455.0	96.7		96.7
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5393.00	8089.50	TRUE		R1881	-8.0	97.0	8089.5	7646.8	176426.5	97.0	100.5	97.0
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5809.00	8713.50	TRUE		R1881	-8.0	105.0	8713.5	8270.8	175802.5	105.0		105.0
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5525.00	8287.50	TRUE		R1881	-8.0	99.6	8287.5	7844.8	176228.5	99.6		99.6

Competi... y Tube Layout - Three Test Chemicals

Check the 10% rule: 4.52%

If the ratio of EtOH / Hot is > 10 there are problems with the ass...

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-sh... (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5714.00	8571.00	TRUE		R1881	-9.0	103.2	8571	8128.3	175945.0	103.2	103.3	103.2
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5747.00	8620.50	TRUE		R1881	-9.0	103.8	8620.5	8177.8	175895.5	103.8		103.8
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5705.00	8557.50	TRUE		R1881	-9.0	103.0	8557.5	8114.8	175958.5	103.0		103.0
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5494.00	8241.00	TRUE		R1881	-10.0	99.0	8241	7798.3	176275.0	99.0	101.4	99.0
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5684.00	8526.00	TRUE		R1881	-10.0	102.6	8526	8083.3	175990.0	102.6		102.6
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5682.00	8523.00	TRUE		R1881	-10.0	102.6	8523	8080.3	175993.0	102.6		102.6
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2436.00	3654.00	TRUE		norethynodrel	-4.0	40.8	3654	3211.3	180862.0	40.8	41.4	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2415.00	3622.50	TRUE		norethynodrel	-4.0	40.4	3622.5	3179.8	180893.5	40.4		
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2566.00	3849.00	TRUE		norethynodrel	-4.0	43.2	3849	3406.3	180667.0	43.2		
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	485.00	727.50	TRUE		norethynodrel	-4.5	3.6	727.5	284.8	183788.5	3.6	3.5	3.6
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	500.00	750.00	TRUE		norethynodrel	-4.5	3.9	750	307.3	183766.0	3.9		3.9
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	456.00	684.00	TRUE		norethynodrel	-4.5	3.1	684	241.3	183832.0	3.1		3.1
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1420.00	2130.00	TRUE		norethynodrel	-5.5	21.4	2130	1687.3	182386.0	21.4	25.4	21.4
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1821.00	2731.50	TRUE		norethynodrel	-5.5	29.0	2731.5	2288.8	181784.5	29.0		29.0
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1647.00	2470.50	TRUE		norethynodrel	-5.5	25.7	2470.5	2027.8	182045.5	25.7		25.7
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2182.00	3273.00	TRUE		norethynodrel	-6.0	35.9	3273	2830.3	181243.0	35.9	38.3	35.9
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2026.00	3039.00	TRUE		norethynodrel	-6.0	33.0	3039	2596.3	181477.0	33.0		33.0
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2706.00	4059.00	TRUE		norethynodrel	-6.0	45.9	4059	3616.3	180457.0	45.9		45.9
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4637.00	6955.50	TRUE		norethynodrel	-6.5	82.7	6955.5	6512.8	177560.5	82.7	83.5	82.7
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4718.00	7077.00	TRUE		norethynodrel	-6.5	84.2	7077	6634.3	177439.0	84.2		84.2
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4693.00	7039.50	TRUE		norethynodrel	-6.5	83.7	7039.5	6596.8	177476.5	83.7		83.7
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5178.00	7767.00	TRUE		norethynodrel	-7.0	93.0	7767	7324.3	176749.0	93.0	93.3	93.0
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5222.00	7833.00	TRUE		norethynodrel	-7.0	93.8	7833	7390.3	176683.0	93.8		93.8
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5196.00	7794.00	TRUE		norethynodrel	-7.0	93.3	7794	7351.3	176722.0	93.3		93.3
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5723.00	8584.50	TRUE		norethynodrel	-7.5	103.3	8584.5	8141.8	175931.5	103.3	103.8	103.3
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5818.00	8727.00	TRUE		norethynodrel	-7.5	105.1	8727	8284.3	175789.0	105.1		105.1
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5707.00	8560.50	TRUE		norethynodrel	-7.5	103.0	8560.5	8117.8	175955.5	103.0		103.0
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5831.00	8746.50	TRUE		norethynodrel	-8.5	105.4	8746.5	8303.8	175769.5	105.4	101.7	105.4
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5756.00	8634.00	TRUE		norethynodrel	-8.5	104.0	8634	8191.3	175882.0	104.0		104.0
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5325.00	7987.50	TRUE		norethynodrel	-8.5	95.8	7987.5	7544.8	176528.5	95.8		95.8
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2201.00	3301.50	TRUE		Unknown 1	-3.0	36.3	3301.5	2858.8	181214.5	36.3	39.7	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2837.00	4255.50	TRUE		Unknown 1	-3.0	48.4	4255.5	3812.8	180260.5	48.4		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2110.00	3165.00	TRUE		Unknown 1	-3.0	34.5	3165	2722.3	181351.0	34.5		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	450.00	675.00	TRUE		Unknown 1	-4.0	2.9	675	232.3	183841.0	2.9	3.2	2.9
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	479.00	718.50	TRUE		Unknown 1	-4.0	3.5	718.5	275.8	183797.5	3.5		3.5
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	454.00	681.00	TRUE		Unknown 1	-4.0	3.0	681	238.3	183835.0	3.0		3.0
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1479.00	2218.50	TRUE		Unknown 1	-5.0	22.5	2218.5	1775.8	182297.5	22.5	23.8	22.5
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1590.00	2385.00	TRUE		Unknown 1	-5.0	24.7	2385	1942.3	182131.0	24.7		24.7
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1567.00	2350.50	TRUE		Unknown 1	-5.0	24.2	2350.5	1907.8	182165.5	24.2		24.2
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4051.00	6076.50	TRUE		Unknown 1	-6.0	71.5	6076.5	5633.8	178439.5	71.5	70.8	71.5
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4096.00	6144.00	TRUE		Unknown 1	-6.0	72.4	6144	5701.3	178372.0	72.4		72.4
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3895.00	5842.50	TRUE		Unknown 1	-6.0	68.5	5842.5	5399.8	178673.5	68.5		68.5
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5242.00	7863.00	TRUE		Unknown 1	-7.0	94.2	7863	7420.3	176653.0	94.2	96.0	94.2
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5476.00	8214.00	TRUE		Unknown 1	-7.0	98.6	8214	7771.3	176302.0	98.6		98.6
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5288.00	7932.00	TRUE		Unknown 1	-7.0	95.1	7932	7489.3	176584.0	95.1		95.1
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5459.00	8188.50	TRUE		Unknown 1	-8.0	98.3	8188.5	7745.8	176327.5	98.3	100.0	98.3
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5505.00	8257.50	TRUE		Unknown 1	-8.0	99.2	8257.5	7814.8	176258.5	99.2		99.2
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5675.00	8512.50	TRUE		Unknown 1	-8.0	102.4	8512.5	8069.8	176003.5	102.4		102.4
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5686.00	8529.00	TRUE		Unknown 1	-9.0	102.6	8529	8086.3	175987.0	102.6	104.1	102.6
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5791.00	8686.50	TRUE		Unknown 1	-9.0	104.6	8686.5	8243.8	175829.5	104.6		104.6
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5809.00	8713.50	TRUE		Unknown 1	-9.0	105.0	8713.5	8270.8	175802.5	105.0		105.0
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5377.00	8065.50	TRUE		Unknown 1	-10.0	96.7	8065.5	7622.8	176450.5	96.7	101.9	96.7
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5956.00	8934.00	TRUE		Unknown 1	-10.0	107.8	8934	8491.3	175582.0	107.8		107.8
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5613.00	8419.50	TRUE		Unknown 1	-10.0	101.2	8419.5	7976.8	176096.5	101.2		101.2
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	153.00	229.50	TRUE		Unknown 2	-3.0	-2.7	229.5	-213.3	184286.5	-2.7	-2.2	-2.7
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	226.00	339.00	TRUE		Unknown 2	-3.0	-1.3	339	-103.8	184177.0	-1.3		-1.3
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	163.00	244.50	TRUE		Unknown 2	-3.0	-2.5	244.5	-198.3	184271.5	-2.5		-2.5
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	492.00	738.00	TRUE		Unknown 2	-4.0	3.7	738	295.3	183778.0	3.7	3.2	3.7
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	456.00	684.00	TRUE		Unknown 2	-4.0	3.1	684	241.3	183832.0	3.1		3.1
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	435.00	652.50	TRUE		Unknown 2	-4.0	2.7	652.5	209.8	183863.5	2.7		2.7
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1769.00	2653.50	TRUE		Unknown 2	-5.0	28.1	2653.5	2210.8	181862.5	28.1	27.8	28.1
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1755.00	2632.50	TRUE		Unknown 2	-5.0	27.8	2632.5	2189.8	181883.5	27.8		27.8
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1746.00	2619.00	TRUE		Unknown 2	-5.0	27.6	2619	2176.3	181897.0	27.6		27.6
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4224.00	6336.00	TRUE		Unknown 2	-6.0	74.8	6336	5893.3	178180.0	74.8	78.1	74.8
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4434.00	6651.00	TRUE		Unknown 2	-6.0	78.8	6651	6208.3	177865.0	78.8		78.8
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4537.00	6805.50	TRUE		Unknown 2	-6.0	80.8	6805.5	6362.8	177710.5	80.8		80.8
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5546.00	8319.00	TRUE		Unknown 2	-7.0	100.0	8319	7876.3	176197.0	100.0	103.7	100.0
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5941.00	8911.50	TRUE		Unknown 2	-7.0	107.5	8911.5	8468.8	175604.5	107.5		107.5
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5734.00	8601.00	TRUE		Unknown 2	-7.0	103.5	8601	8158.3	175915.0	103.5		103.5
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5540.00	8310.00	TRUE		Unknown 2	-8.0	99.8	8310	7867.3	176206.0	99.8	98.5	99.8
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5753.00	8629.50	TRUE		Unknown 2	-8.0	103.9	8629.5	8186.8	175886.5	103.9		103.9
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5110.00	7665.00	TRUE		Unknown 2	-8.0	91.7	7665	7222.3	176851.0	91.7		91.7
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5800.00	8700.00	TRUE		Unknown 2	-9.0	104.8	8700	8257.3	175816.0	104.8	108.9	104.8
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	6289.00	9433.50	TRUE		Unknown 2	-9.0	114.1	9433.5	8990.8	175082.5	114.1		114.1
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5963.00	8944.50	TRUE		Unknown 2	-9.0	107.9	8944.5	8501.8	175571.5	107.9		107.9
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5370.00	8055.00	TRUE		Unknown 2	-10.0	96.6	8055	7612.3	176461.0	96.6	100.4	96.6
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5570.00	8355.00	TRUE		Unknown 2	-10.0	100.4	8355	7912.3	176161.0	100.4		100.4
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5771.00	8656.50	TRUE		Unknown 2	-10.0	104.2	8656.5	8213.8	175859.5	104.2		104.2
121	1	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
122	2	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
123	3	Unknown 3	U3	1	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
124	1	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
125	2	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
126	3	Unknown 3	U3	2	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
127	1	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
128	2	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!

Competitive Assay Tube Layout - Three Test Chemicals

Check the 10% rule: 4.52% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
129	3	Unknown 3	U3	3	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
130	1	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
131	2	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
132	3	Unknown 3	U3	4	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
133	1	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
134	2	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
135	3	Unknown 3	U3	5	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
136	1	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
137	2	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
138	3	Unknown 3	U3	6	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
139	1	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
140	2	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
141	3	Unknown 3	U3	7	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
142	1	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!						no average	#NUM!
143	2	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
144	3	Unknown 3	U3	8	0.00E+00	100	380	10	10	250	500	0.0E+00		0.00	TRUE		Unknown 3	#NUM!							#NUM!
145	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	286.00	429.00	TRUE		cold E2	-7.0	-0.2	429	-13.8	184087.0	-0.2	0.0	-0.2
146	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	314.00	471.00	TRUE		cold E2	-7.0	0.4	471	28.3	184045.0	0.4		0.4
147	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	287.00	430.50	TRUE		cold E2	-7.0	-0.2	430.5	-12.3	184085.5	-0.2		-0.2
148	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5743.00	8614.50	TRUE			—	103.7	8614.5	8171.8	175901.5	103.7	102.6	103.7
149	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5232.00	7848.00	TRUE			—	94.0	7848	7405.3	176668.0	94.0		94.0
150	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	6082.00	9123.00	TRUE			—	110.2	9123	8680.3	175393.0	110.2		110.2
151	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	3373.00	5059.50	TRUE			-6.0	58.6	5059.5	4616.8	179456.5	58.6	58.1	58.6
152	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	3237.00	4855.50	TRUE			-6.0	56.0	4855.5	4412.8	179660.5	56.0		56.0
153	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06	3437.00	5155.50	TRUE			-6.0	59.8	5155.5	4712.8	179360.5	59.8		59.8
154	1	none	Hot		—	—	—	10	—	—	—	—	120813.00	181219.50	TRUE					181219.5	180776.8				
155	2	none	Hot		—	—	—	10	—	—	—	—	123359.00	185038.50	TRUE					185038.5	184595.8				
156	3	none	Hot		—	—	—	10	—	—	—	—	127190.00	190785.00	TRUE					190785	190342.3				
157	1	none	Hot		—	—	—	10	—	—	—	—	120988.00	181482.00	TRUE					181482	181039.3				
158	2	none	Hot		—	—	—	10	—	—	—	—	120246.00	180369.00	TRUE					180369	179926.3				
159	3	none	Hot		—	—	—	10	—	—	—	—	123697.00	185545.50	TRUE					185545.5	185102.8				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				11.0				15.0				Chem code				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	-0.0412	-0.2507	0.2633	-4.0				-3.0				-3.0	-2.7065	-1.3167	-2.5161	#NUM!	#NUM!	#NUM!	#NUM!	-3.0	24.1552	32.7982	24.8406
2	2	-7.0	-0.1745	0.3585	-0.1555	-4.5	3.6139	3.8995	3.0618	-4.0	2.9476	3.4997	3.0238	-4.0	3.7472	3.0618	2.6621	#NUM!	#NUM!	#NUM!	#NUM!	-4.0	32.0557	35.1778	34.0356
3	3	-8.0	9.0776	9.9153	9.2299	-5.5	21.4138	29.0478	25.7353	-5.0	22.5370	24.6502	24.2123	-5.0	28.0579	27.7914	27.6200	#NUM!	#NUM!	#NUM!	#NUM!	-5.0	30.8944	38.1857	42.5263
4	1	-8.5	27.5248	27.6391	23.2414	-6.0	35.9203	32.9505	45.8959	-6.0	71.5011	72.3578	68.5313	-6.0	74.7946	78.7924	80.7532	#NUM!	#NUM!	#NUM!	#NUM!	-6.0	60.2119	54.0438	56.0047
5	2	-9.0	33.8643	31.9605	36.4153	-6.5	82.6570	84.1990	83.7231	-7.0	94.1746	98.6293	95.0503	-7.0	99.9619	107.4817	103.5409	#NUM!	#NUM!	#NUM!	#NUM!	-7.0	92.3660	97.7346	96.6875
6	3	-9.5	81.8955	80.9817	80.8675	-7.0	92.9562	93.7938	93.2989	-8.0	98.3057	99.1814	102.4177	-8.0	99.8477	103.9027	91.6616	#NUM!	#NUM!	#NUM!	#NUM!	-8.0	97.0492	104.9687	99.5621
7	1	-10.0	89.6627	91.3761	91.0334	-7.5	103.3315	105.1401	103.0269	-9.0	102.6272	104.6261	104.9687	-9.0	104.7974	114.1067	107.9005	#NUM!	#NUM!	#NUM!	#NUM!	-9.0	103.1602	103.7884	102.9889
8	2	-11.0	100.6092	99.3908	94.3269	-8.5	105.3876	103.9598	95.7547	-10.0	96.7446	107.7672	101.2374	-10.0	96.6114	100.4188	104.2453	#NUM!	#NUM!	#NUM!	#NUM!	-10.0	98.9720	102.5891	102.5510
9	3																								
10	1	Standard Curve		Prism run TTN 002 03/18/08		Positive Control		Prism run TTN 002 03/18/08		11.0		Prism run TTN 002 03/18/08		15.0		Prism run TTN 002 03/18/08		Chem code		Prism run ID & date		Negative Control		Prism run TTN 002 03/18/08	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values			
13	1	BOTTOM		1.807		BOTTOM		5.809		BOTTOM		-1.626		BOTTOM		-2.127		BOTTOM				BOTTOM		32.85	
14	2	TOP		100.4		TOP		103.4		TOP		102.3		TOP		103.7		TOP				TOP		107	
15	3	LOGIC50		-9.092		LOGIC50		-6.074		LOGIC50		-5.57		LOGIC50		-5.427		LOGIC50				LOGIC50		-5.734	
16	1	HILLSLOPE		-1.124		HILLSLOPE		-1.28		HILLSLOPE		-0.8412		HILLSLOPE		-0.9262		HILLSLOPE				HILLSLOPE		-1.113	
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		2.42		BOTTOM		3.571		BOTTOM		2.385		BOTTOM		2.377		BOTTOM				BOTTOM			
19	1	TOP		3.476		TOP		2.566		TOP		0.8733		TOP		1.476		TOP				TOP			
20	2	LOGIC50		0.04017		LOGIC50		0.03798		LOGIC50		0.02803		LOGIC50		0.04489		LOGIC50				LOGIC50		0.06781	
21	3	HILLSLOPE		0.1446		HILLSLOPE		0.1729		HILLSLOPE		0.0559		HILLSLOPE		0.08826		HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.9

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Estradiol
Run ID:	TTN 002
Assay start date:	3/17/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-7.0	0.0
1	1	2	-7.0	-0.3
1	1	3	-7.0	0.3
1	2	1	-8.0	9.5
1	2	2	-8.0	10.4
1	2	3	-8.0	9.7
1	3	1	-8.5	28.8
1	3	2	-8.5	28.9
1	3	3	-8.5	24.3
1	4	1	-9.0	35.4
1	4	2	-9.0	33.4
1	4	3	-9.0	38.1
1	5	1	-9.5	85.7
1	5	2	-9.5	84.7
1	5	3	-9.5	84.6
1	6	1	-10.0	93.8
1	6	2	-10.0	95.6
1	6	3	-10.0	95.2
1	7	1	-11.0	105.3
1	7	2	-11.0	104.0
1	7	3	-11.0	98.7
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
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   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.6

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Norethynodrel
Run ID:	TTN 002
Assay start date:	3/18/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-4.0	42.6
1	1	2	-4.0	42.2
1	1	3	-4.0	45.2
1	2	1	-4.5	3.8
1	2	2	-4.5	4.1
1	2	3	-4.5	3.2
1	3	1	-5.5	22.4
1	3	2	-5.5	30.4
1	3	3	-5.5	26.9
1	4	1	-6.0	37.6
1	4	2	-6.0	34.5
1	4	3	-6.0	48.0
1	5	1	-6.5	86.5
1	5	2	-6.5	88.1
1	5	3	-6.5	87.6
1	6	1	-7.0	97.2
1	6	2	-7.0	98.1
1	6	3	-7.0	97.6
1	7	1	-7.5	108.1
1	7	2	-7.5	110.0
1	7	3	-7.5	107.8
1	8	1	-8.5	110.2
1	8	2	-8.5	108.8
1	8	3	-8.5	100.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
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   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.7

Provide information in all blue cells.

Laboratory Code:	B
Compound ID:	R1881
Run ID:	TTN 002
Assay start date:	3/18/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-3	25.3
1	1	2	-3	34.3
1	1	3	-3	26.0
1	2	1	-4	33.5
1	2	2	-4	36.8
1	2	3	-4	35.6
1	3	1	-5	32.3
1	3	2	-5	39.9
1	3	3	-5	44.5
1	4	1	-6	63.0
1	4	2	-6	56.5
1	4	3	-6	58.6
1	5	1	-7	96.6
1	5	2	-7	102.2
1	5	3	-7	101.1
1	6	1	-8	101.5
1	6	2	-8	109.8
1	6	3	-8	104.2
1	7	1	-9	107.9
1	7	2	-9	108.6
1	7	3	-9	107.7
1	8	1	-10	103.5
1	8	2	-10	107.3
1	8	3	-10	107.3
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
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1. SDwithin-run is estimated separately for each run.
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   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.8

Provide information in all blue cells.

Laboratory Code:	B
Compound ID:	Chem Code 11
Run ID:	TTN 002
Assay start date:	3/17/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-3.0	38.0
1	1	2	-3.0	50.6
1	1	3	-3.0	36.1
1	2	1	-4.0	3.1
1	2	2	-4.0	3.7
1	2	3	-4.0	3.2
1	3	1	-5.0	23.6
1	3	2	-5.0	25.8
1	3	3	-5.0	25.3
1	4	1	-6.0	74.8
1	4	2	-6.0	75.7
1	4	3	-6.0	71.7
1	5	1	-7.0	98.5
1	5	2	-7.0	103.2
1	5	3	-7.0	99.4
1	6	1	-8.0	102.8
1	6	2	-8.0	103.8
1	6	3	-8.0	107.1
1	7	1	-9.0	107.4
1	7	2	-9.0	109.5
1	7	3	-9.0	109.8
1	8	1	-10.0	101.2
1	8	2	-10.0	112.7
1	8	3	-10.0	105.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
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How to use this spreadsheet:
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2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.7

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Chem Code 15
Run ID:	TTN 002
Assay start date:	3/17/08
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-3.0	-2.8
1	1	2	-3.0	-1.4
1	1	3	-3.0	-2.6
1	2	1	-4.0	3.9
1	2	2	-4.0	3.2
1	2	3	-4.0	2.8
1	3	1	-5.0	29.4
1	3	2	-5.0	29.1
1	3	3	-5.0	28.9
1	4	1	-6.0	78.2
1	4	2	-6.0	82.4
1	4	3	-6.0	84.5
1	5	1	-7.0	104.6
1	5	2	-7.0	112.4
1	5	3	-7.0	108.3
1	6	1	-8.0	104.5
1	6	2	-8.0	108.7
1	6	3	-8.0	95.9
1	7	1	-9.0	109.6
1	7	2	-9.0	119.4
1	7	3	-9.0	112.9
1	8	1	-10.0	101.1
1	8	2	-10.0	105.1
1	8	3	-10.0	109.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	Estradiol standard TTN 002		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-0.0412	-0.2507	0.2633
2	Title	-7.0	-0.1745	0.3585	-0.1555
3	Title	-8.0	9.0776	9.9153	9.2299
4	Title	-8.5	27.5248	27.6391	23.2414
5	Title	-9.0	33.8643	31.9605	36.4153
6	Title	-9.5	81.8955	80.9817	80.8675
7	Title	-10.0	89.6627	91.3761	91.0334
8	Title	-11.0	100.6092	99.3908	94.3269

	Nonlin fit Table of results	A Estradiol standard TTN 002
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.807
4	TOP	100.4
5	LOGIC50	-9.092
6	HILLSLOPE	-1.124
7	Std. Error	
8	BOTTOM	2.420
9	TOP	3.476
10	LOGIC50	0.04017
11	HILLSLOPE	0.1446
12	95% Confidence Intervals	
13	BOTTOM	-3.242 to 6.855
14	TOP	93.16 to 107.7
15	LOGIC50	-9.176 to -9.008
16	HILLSLOPE	-1.426 to -0.8229
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9797
20	Absolute Sum of Squares	728.0
21	Sy.x	6.033
22	Number of points	
23	Analyzed	24

Estradiol reference standard
TTN 002-03/18/08-LAB B

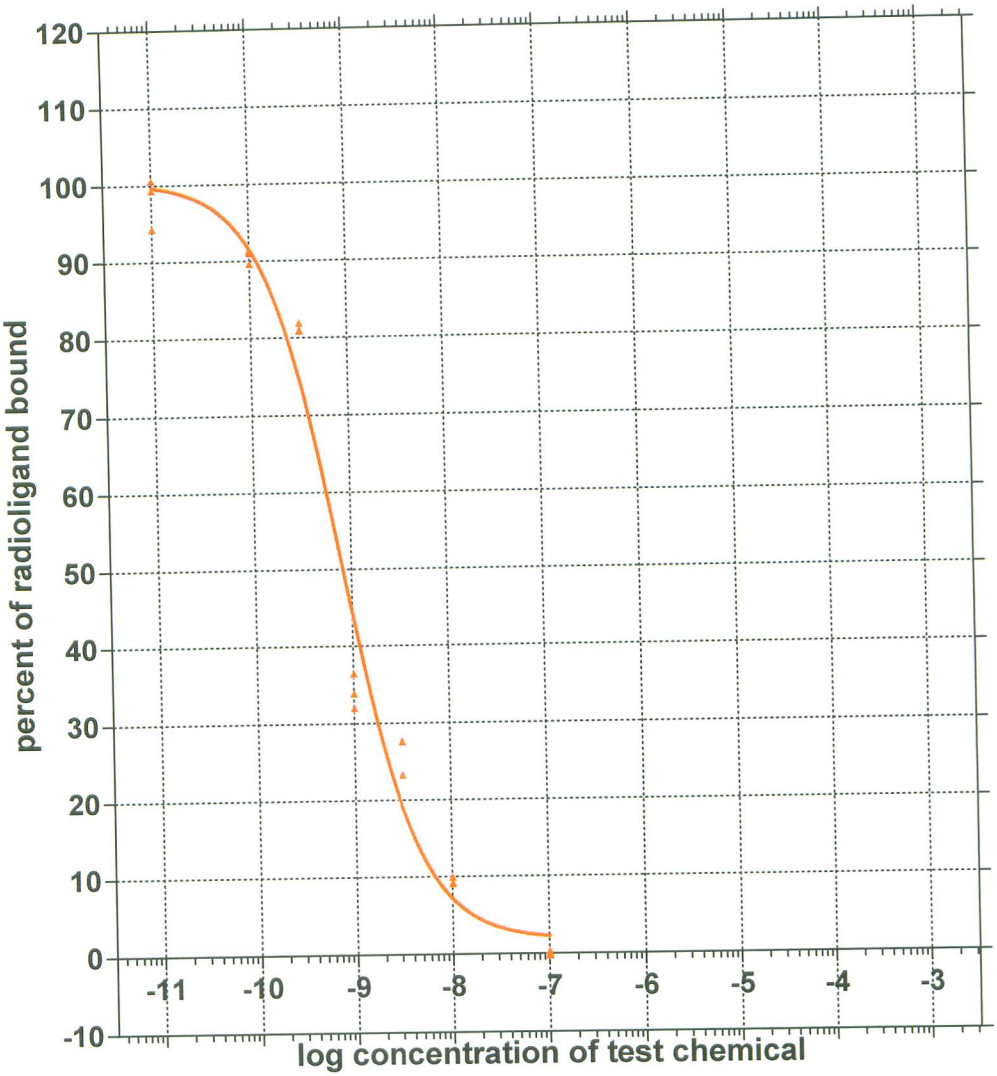


Table format: XY		X	A		
		log concentration	Norethynodre TTN 002		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	3.6139	3.8995	3.0618
3	Title	-5.5	21.4138	29.0478	25.7353
4	Title	-6.0	35.9203	32.9505	45.8959
5	Title	-6.5	82.6570	84.1990	83.7231
6	Title	-7.0	92.9562	93.7938	93.2989
7	Title	-7.5	103.3315	105.1401	103.0269
8	Title	-8.5	105.3876	103.9598	95.7547

	Nonlin fit Table of results	A Norethynodre TTN 002
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	5.809
4	TOP	103.4
5	LOGIC50	-6.074
6	HILLSLOPE	-1.280
7	Std. Error	
8	BOTTOM	3.571
9	TOP	2.566
10	LOGIC50	0.03798
11	HILLSLOPE	0.1729
12	95% Confidence Intervals	
13	BOTTOM	-1.725 to 13.34
14	TOP	97.95 to 108.8
15	LOGIC50	-6.154 to -5.994
16	HILLSLOPE	-1.645 to -0.9156
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9804
20	Absolute Sum of Squares	596.4
21	Sy.x	5.923
22	Number of points	
23	Analyzed	21

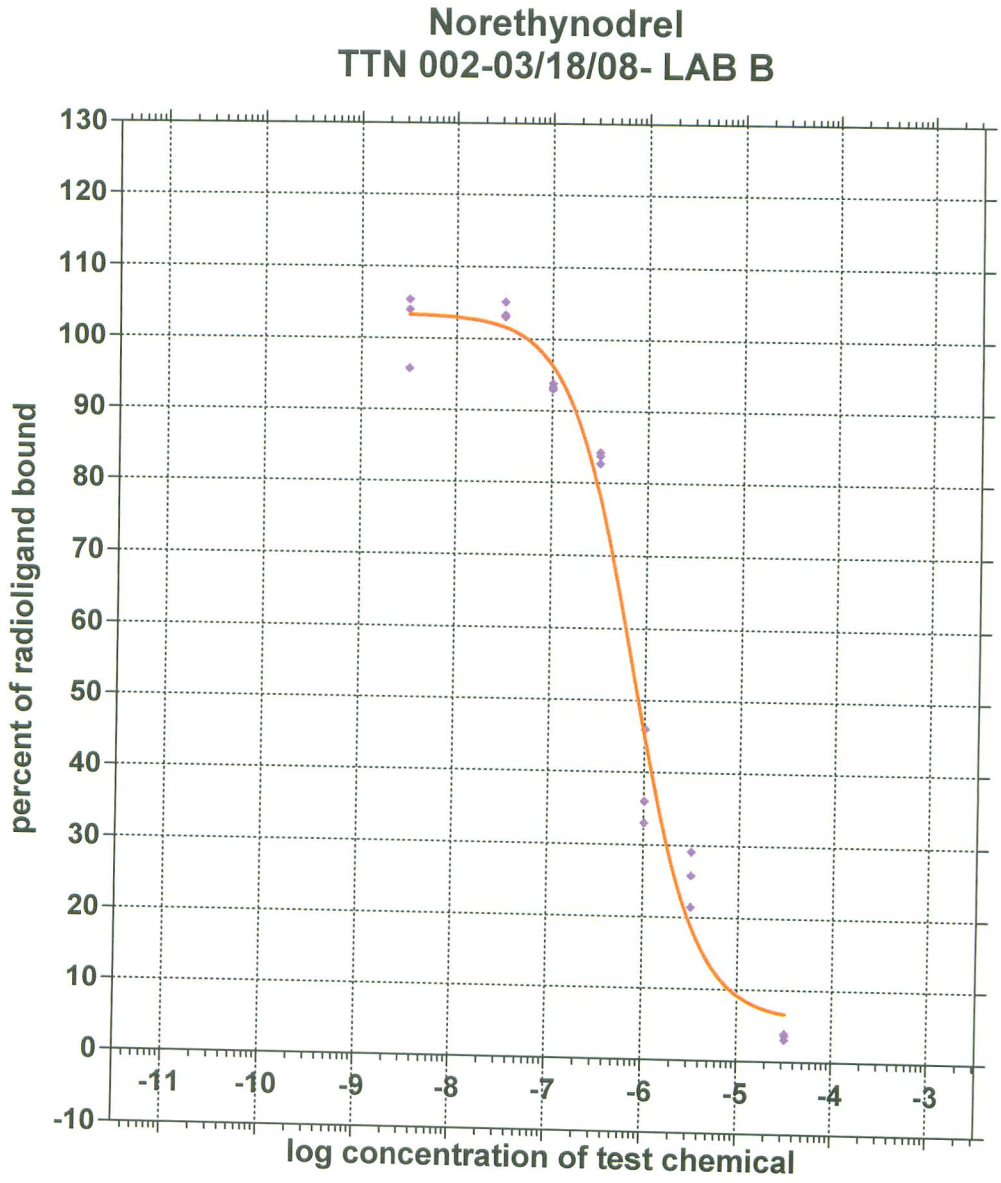

Norethynodrel
TTN 002-03/18/08- LAB B
percent of radioligand bound
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	R1881 TTN 002		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	25.2697	34.3114	25.9867
2	Title	-4.0	33.5347	36.8009	35.6059
3	Title	-5.0	32.3198	39.9476	44.4883
4	Title	-6.0	62.9900	56.5373	58.5886
5	Title	-7.0	96.6276	102.2438	101.1485
6	Title	-8.0	101.5269	109.8118	104.1557
7	Title	-9.0	107.9198	108.5770	107.7406
8	Title	-10.0	103.5384	107.3223	107.2825

	Nonlin fit Table of results	A R1881 TTN 002
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	32.85
4	TOP	107.0
5	LOGIC50	-5.734
6	HILLSLOPE	-1.113
7	Std. Error	
8	BOTTOM	1.543
9	TOP	1.435
10	LOGIC50	0.06781
11	HILLSLOPE	0.1545
12	95% Confidence Intervals	
13	BOTTOM	29.63 to 36.07
14	TOP	104.0 to 110.0
15	LOGIC50	-5.876 to -5.593
16	HILLSLOPE	-1.436 to -0.7912
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9875
20	Absolute Sum of Squares	332.8
21	Sy.x	4.079
22	Number of points	
23	Analyzed	24

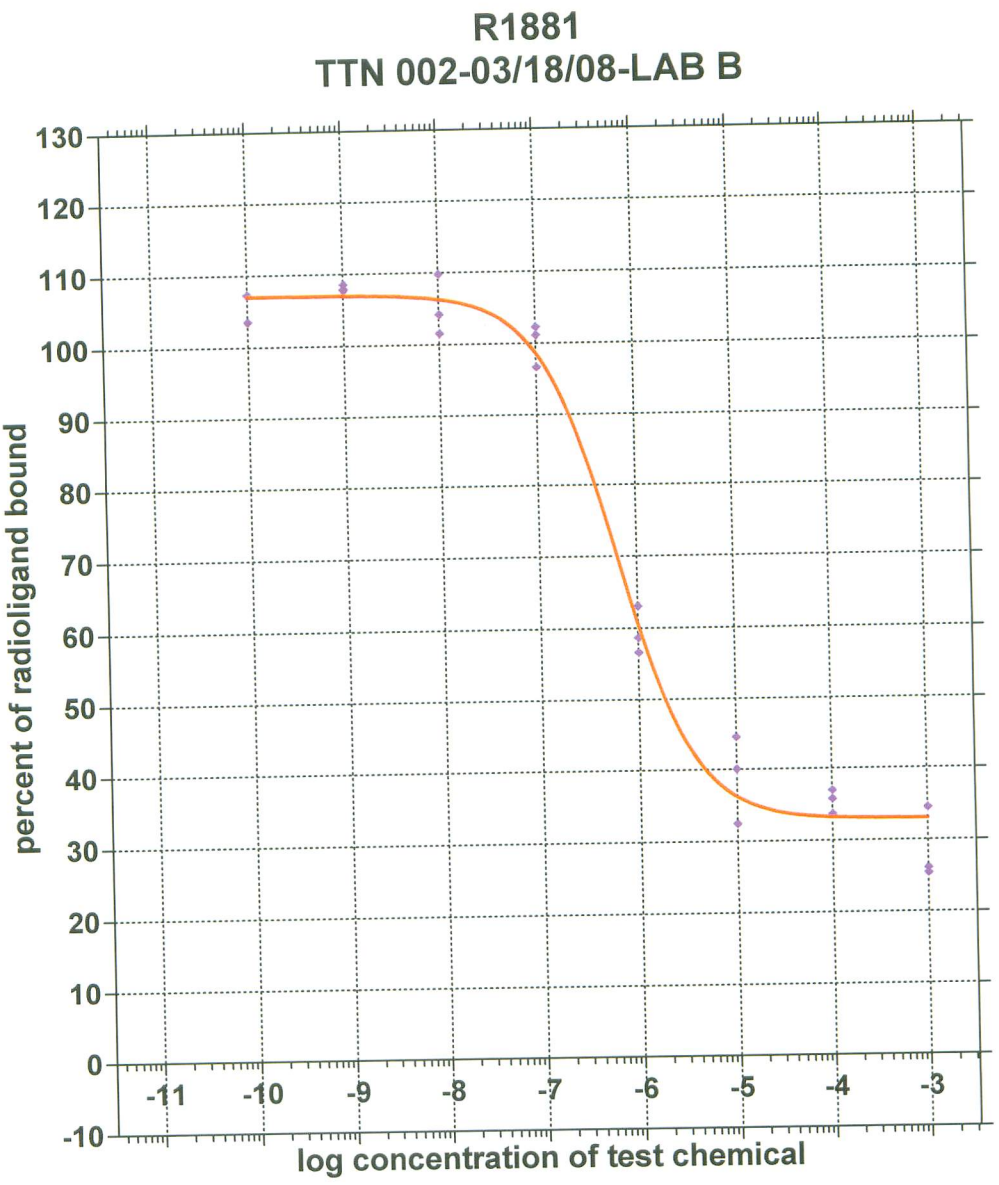

R1881
TTN 002-03/18/08-LAB B
percent of radioligand bound
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical CODE 11 TTN 002		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	2.9476	3.4997	3.0238
3	Title	-5.0	22.5370	24.6502	24.2123
4	Title	-6.0	71.5011	72.3578	68.5313
5	Title	-7.0	94.1746	98.6293	95.0503
6	Title	-8.0	98.3057	99.1814	102.4177
7	Title	-9.0	102.6272	104.6261	104.9687
8	Title	-10.0	96.7446	107.7672	101.2374

	Nonlin fit Table of results	A Test chemical CODE 11 TTN 002
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.626
4	TOP	102.3
5	LOGIC50	-5.570
6	HILLSLOPE	-0.8412
7	Std. Error	
8	BOTTOM	2.385
9	TOP	0.8733
10	LOGIC50	0.02803
11	HILLSLOPE	0.05590
12	95% Confidence Intervals	
13	BOTTOM	-6.659 to 3.408
14	TOP	100.5 to 104.2
15	LOGIC50	-5.629 to -5.511
16	HILLSLOPE	-0.9591 to -0.7232
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9964
20	Absolute Sum of Squares	113.5
21	Sy.x	2.583
22	Number of points	
23	Analyzed	21

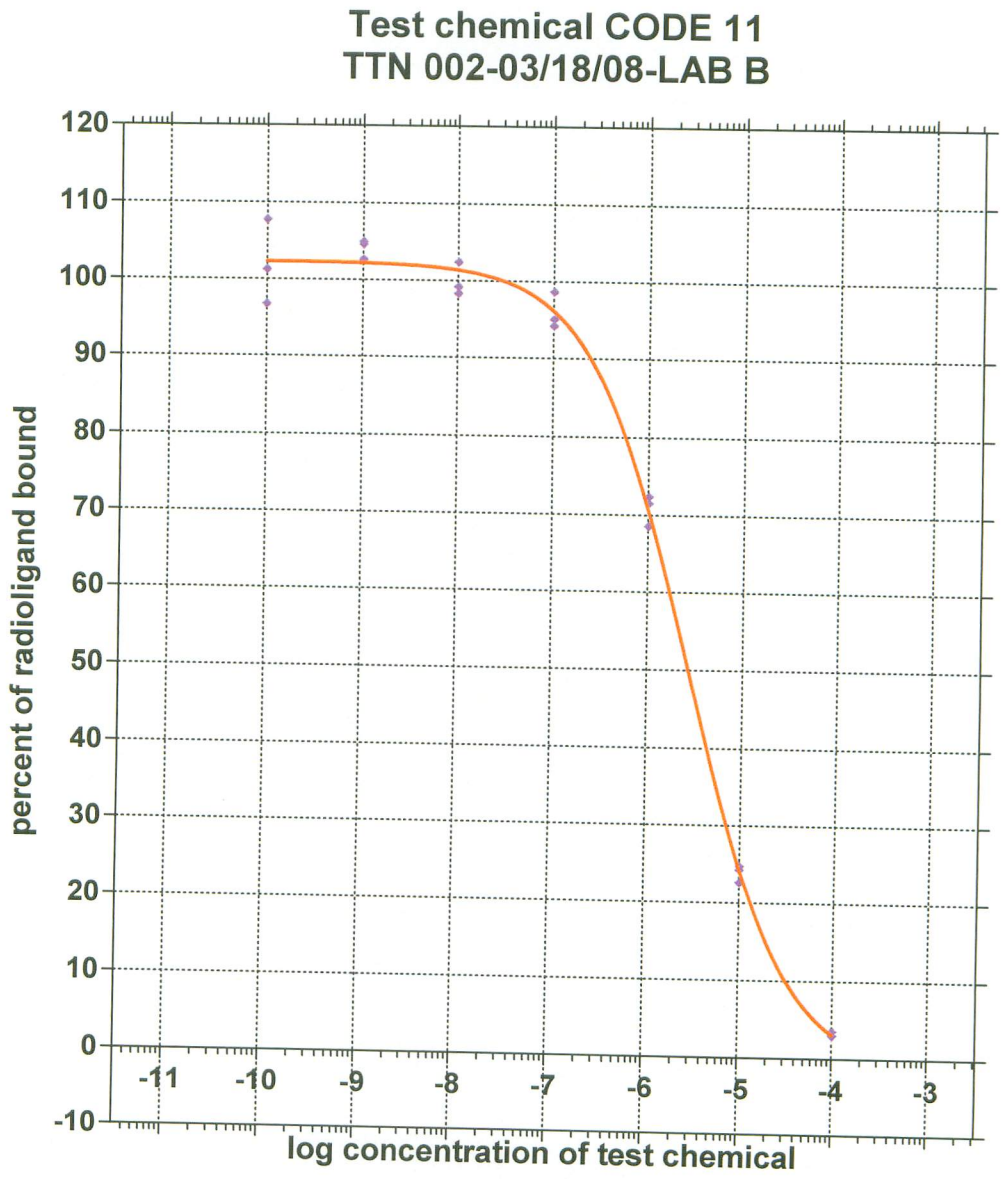
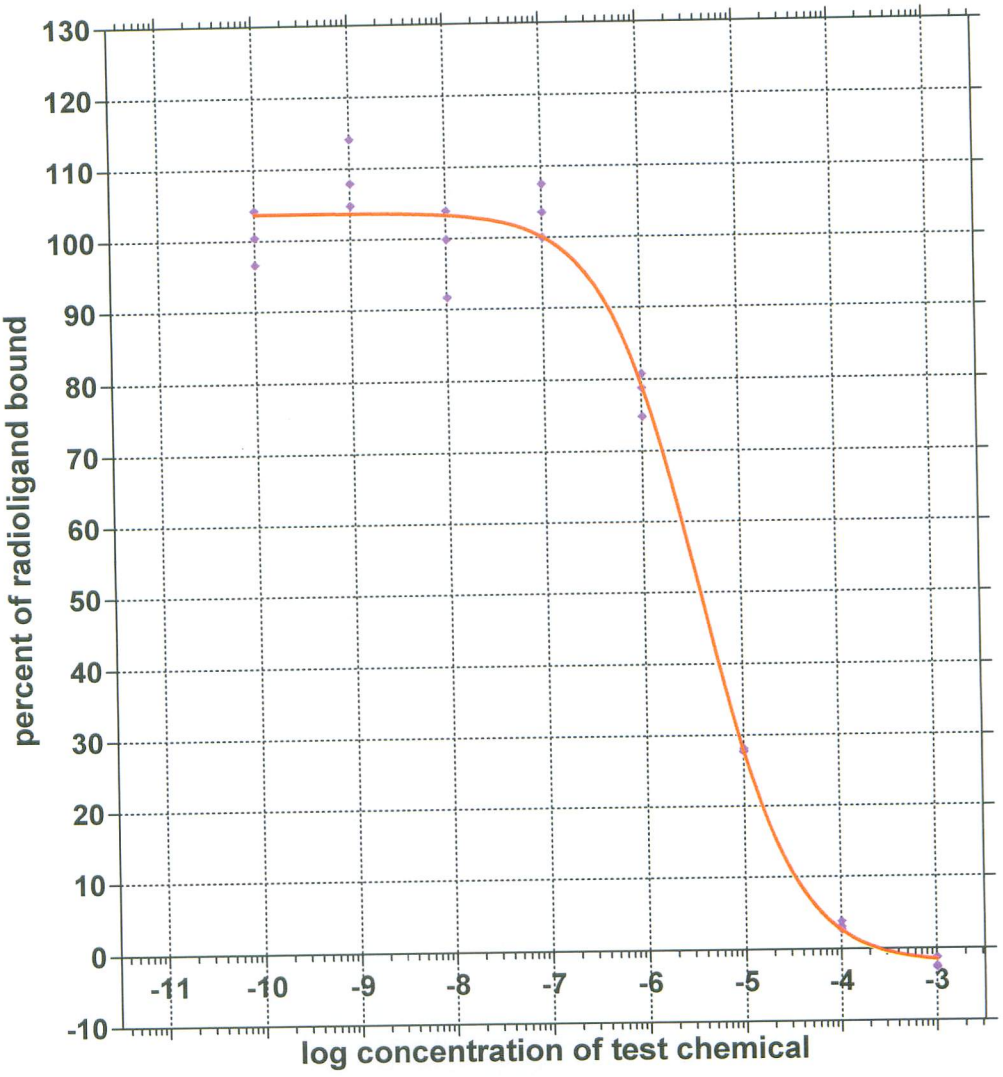

Test chemical CODE 11
TTN 002-03/18/08-LAB B
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical CODE 15 TTN 002		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	-2.7065	-1.3167	-2.5161
2	Title	-4.0	3.7472	3.0618	2.6621
3	Title	-5.0	28.0579	27.7914	27.6200
4	Title	-6.0	74.7946	78.7924	80.7532
5	Title	-7.0	99.9619	107.4817	103.5409
6	Title	-8.0	99.8477	103.9027	91.6616
7	Title	-9.0	104.7974	114.1067	107.9005
8	Title	-10.0	96.6114	100.4188	104.2453

	Nonlin fit Table of results	A Test chemical CODE 15 TTN 002
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.127
4	TOP	103.7
5	LOGIC50	-5.427
6	HILLSLOPE	-0.9262
7	Std. Error	
8	BOTTOM	2.377
9	TOP	1.476
10	LOGIC50	0.04489
11	HILLSLOPE	0.08826
12	95% Confidence Intervals	
13	BOTTOM	-7.086 to 2.832
14	TOP	100.7 to 106.8
15	LOGIC50	-5.521 to -5.333
16	HILLSLOPE	-1.110 to -0.7421
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9909
20	Absolute Sum of Squares	429.7
21	Sy.x	4.635
22	Number of points	
23	Analyzed	24

Test chemical CODE 15
TTN 002-03/18/08 LAB B



APPENDIX H

Excel and Prism Files of the Run Identifier TTN006

ER Competitive Assay - Four Unknowns

159 assay tubes No R1881

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	TTN006	
Assay start date:	4/16/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	109.23	Ci/mmole
Cytosol vial or lot identification:	RUC02-22-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.96	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-6.08	M
RBA, norethynodrel (Weak Positive):	1.315E-03	relative to E2=1.0
Max Concentration, Unknown 1:	5.00E-02	M
log(IC50), Unknown 1:	-5.66	11.0
RBA, Unknown 1:	5.047E-04	relative to E2=1.0
Max Concentration, Unknown 2:	5.00E-02	M
log(IC50), Unknown 2:	-6.81	12.0
RBA, Unknown 2:	7.145E-03	relative to E2=1.0
Max Concentration, Unknown 3:	5.00E-02	M
log(IC50), Unknown 3:	-5.14	15.0
RBA, Unknown 3:	1.517E-04	relative to E2=1.0
Max Concentration, Unknown 4:	5.00E-02	M
log(IC50), Unknown 4:	0.00	21.0
RBA, Unknown 4:	N/A	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 6.23%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	7045.3	196.38	Total Binding, solvent control, tubes
Hot	6	113143.0	5448.23	Total hot E2 added to each tube
NSB	6	431.8	123.18	Nonspecific Binding
Specific EtOH	6	6613.5	196.38	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.06	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.06	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	-1.4
R1881	#N/A	not applicable
norethynodrel	-4.5	-0.7
Unknown 1	-4.0	1.0
Unknown 2	-4.0	-2.9
Unknown 3	-3.0	-0.5
Unknown 4	-7.0	97.4

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4759.00	7138.50	TRUE			—	101.4	7138.5	6706.8	106436.3	101.4	101.1	101.4
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4806.00	7209.00	TRUE			—	102.5	7209	6777.3	106365.8	102.5		102.5
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4675.00	7012.50	TRUE			—	99.5	7012.5	6580.8	106562.3	99.5		99.5
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	226.00	339.00	TRUE		cold E2	-7.0	-1.4	339	-92.8	113235.8	-1.4	-1.4	-1.4
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	228.00	342.00	TRUE		cold E2	-7.0	-1.4	342	-89.8	113232.8	-1.4		-1.4
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	219.00	328.50	TRUE		cold E2	-7.0	-1.6	328.5	-103.3	113246.3	-1.6		-1.6
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	656.00	984.00	TRUE		cold E2	-8.0	8.4	984	552.3	112590.8	8.4	8.3	8.4
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	665.00	997.50	TRUE		cold E2	-8.0	8.6	997.5	565.8	112577.3	8.6		8.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	636.00	954.00	TRUE		cold E2	-8.0	7.9	954	522.3	112620.8	7.9		7.9
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1378.00	2067.00	TRUE		cold E2	-8.5	24.7	2067	1635.3	111507.8	24.7	27.8	24.7
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1534.00	2301.00	TRUE		cold E2	-8.5	28.3	2301	1869.3	111273.8	28.3		28.3
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1632.00	2448.00	TRUE		cold E2	-8.5	30.5	2448	2016.3	111126.8	30.5		30.5
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2465.00	3697.50	TRUE		cold E2	-9.0	49.4	3697.5	3265.8	109877.3	49.4	51.4	49.4
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2651.00	3976.50	TRUE		cold E2	-9.0	53.6	3976.5	3544.8	109598.3	53.6		53.6
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2544.00	3816.00	TRUE		cold E2	-9.0	51.2	3816	3384.3	109758.8	51.2		51.2
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3764.00	5646.00	TRUE		cold E2	-9.5	78.8	5646	5214.3	107928.8	78.8	77.6	78.8
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3699.00	5548.50	TRUE		cold E2	-9.5	77.4	5548.5	5116.8	108026.3	77.4		77.4
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3670.00	5505.00	TRUE		cold E2	-9.5	76.7	5505	5073.3	108069.8	76.7		76.7
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4346.00	6519.00	TRUE		cold E2	-10.0	92.0	6519	6087.3	107055.8	92.0	91.9	92.0
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4273.00	6409.50	TRUE		cold E2	-10.0	90.4	6409.5	5977.8	107165.3	90.4		90.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4406.00	6609.00	TRUE		cold E2	-10.0	93.4	6609	6177.3	106965.8	93.4		93.4
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4915.00	7372.50	TRUE		cold E2	-11.0	104.9	7372.5	6940.8	106202.3	104.9	100.4	104.9
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4364.00	6546.00	TRUE		cold E2	-11.0	92.5	6546	6114.3	107028.8	92.5		92.5
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4870.00	7305.00	TRUE		cold E2	-11.0	103.9	7305	6873.3	106269.8	103.9		103.9
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 6.23%

If the ratio of EtOH / Hot is > 10% th there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	1544.00	2316.00	TRUE		norethynodrel	-4.0	28.5	2316	1884.3	111258.8	28.5	32.2	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	425.00	637.50	FALSE	pipet error	norethynodrel	-4.0							
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	1871.00	2806.50	TRUE		norethynodrel	-4.0	35.9	2806.5	2374.8	110768.3	35.9		
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	253.00	379.50	TRUE		norethynodrel	-4.5	-0.8	379.5	-52.3	113195.3	-0.8	-0.7	-0.8
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	254.00	381.00	TRUE		norethynodrel	-4.5	-0.8	381	-50.8	113193.8	-0.8		-0.8
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	264.00	396.00	TRUE		norethynodrel	-4.5	-0.5	396	-35.8	113178.8	-0.5		-0.5
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1107.00	1660.50	TRUE		norethynodrel	-5.5	18.6	1660.5	1228.8	111914.3	18.6	19.5	18.6
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1117.00	1675.50	TRUE		norethynodrel	-5.5	18.8	1675.5	1243.8	111899.3	18.8		18.8
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1213.00	1819.50	TRUE		norethynodrel	-5.5	21.0	1819.5	1387.8	111755.3	21.0		21.0
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2414.00	3621.00	TRUE		norethynodrel	-6.0	48.2	3621	3189.3	109953.8	48.2	48.6	48.2
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2426.00	3639.00	TRUE		norethynodrel	-6.0	48.5	3639	3207.3	109935.8	48.5		48.5
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2454.00	3681.00	TRUE		norethynodrel	-6.0	49.1	3681	3249.3	109893.8	49.1		49.1
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3395.00	5092.50	TRUE		norethynodrel	-6.5	70.5	5092.5	4660.8	108482.3	70.5	69.0	70.5
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3302.00	4953.00	TRUE		norethynodrel	-6.5	68.4	4953	4521.3	108621.8	68.4		68.4
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3293.00	4939.50	TRUE		norethynodrel	-6.5	68.2	4939.5	4507.8	108635.3	68.2		68.2
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4252.00	6378.00	TRUE		norethynodrel	-7.0	89.9	6378	5946.3	107196.8	89.9	91.0	89.9
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4245.00	6367.50	TRUE		norethynodrel	-7.0	89.8	6367.5	5935.8	107207.3	89.8		89.8
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4409.00	6613.50	TRUE		norethynodrel	-7.0	93.5	6613.5	6181.8	106961.3	93.5		93.5
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4509.00	6763.50	TRUE		norethynodrel	-7.5	95.7	6763.5	6331.8	106811.3	95.7	96.5	95.7
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4517.00	6775.50	TRUE		norethynodrel	-7.5	95.9	6775.5	6343.8	106799.3	95.9		95.9
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4604.00	6906.00	TRUE		norethynodrel	-7.5	97.9	6906	6474.3	106668.8	97.9		97.9
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4683.00	7024.50	TRUE		norethynodrel	-8.0	99.7	7024.5	6592.8	106550.3	99.7	103.4	99.7
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4929.00	7393.50	TRUE		norethynodrel	-8.0	105.3	7393.5	6961.8	106181.3	105.3		105.3
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4932.00	7398.00	TRUE		norethynodrel	-8.0	105.3	7398	6966.3	106176.8	105.3		105.3
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4208.00	6312.00	TRUE		Unknown 1	-3.0	88.9	6312	5880.3	107262.8	88.9	97.6	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	5106.00	7659.00	TRUE		Unknown 1	-3.0	109.3	7659	7227.3	105915.8	109.3		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4459.00	6688.50	TRUE		Unknown 1	-3.0	94.6	6688.5	6256.8	106886.3	94.6		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	315.00	472.50	TRUE		Unknown 1	-4.0	0.6	472.5	40.8	113102.3	0.6	1.0	0.6
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	362.00	543.00	TRUE		Unknown 1	-4.0	1.7	543	111.3	113031.8	1.7		1.7
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	324.00	486.00	TRUE		Unknown 1	-4.0	0.8	486	54.3	113088.8	0.8		0.8
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	855.00	1282.50	TRUE		Unknown 1	-5.0	12.9	1282.5	850.8	112292.3	12.9	19.0	12.9
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1275.00	1912.50	TRUE		Unknown 1	-5.0	22.4	1912.5	1480.8	111662.3	22.4		22.4
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1247.00	1870.50	TRUE		Unknown 1	-5.0	21.8	1870.5	1438.8	111704.3	21.8		21.8
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3258.00	4887.00	TRUE		Unknown 1	-6.0	67.4	4887	4455.3	108687.8	67.4	66.7	67.4
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3287.00	4930.50	TRUE		Unknown 1	-6.0	68.0	4930.5	4498.8	108644.3	68.0		68.0
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3135.00	4702.50	TRUE		Unknown 1	-6.0	64.6	4702.5	4270.8	108872.3	64.6		64.6
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4388.00	6582.00	TRUE		Unknown 1	-7.0	93.0	6582	6150.3	106992.8	93.0	97.1	93.0
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4635.00	6952.50	TRUE		Unknown 1	-7.0	98.6	6952.5	6520.8	106622.3	98.6		98.6
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4683.00	7024.50	TRUE		Unknown 1	-7.0	99.7	7024.5	6592.8	106550.3	99.7		99.7
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4424.00	6636.00	TRUE		Unknown 1	-8.0	93.8	6636	6204.3	106938.8	93.8	98.5	93.8
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4702.00	7053.00	TRUE		Unknown 1	-8.0	100.1	7053	6621.3	106521.8	100.1		100.1
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4772.00	7158.00	TRUE		Unknown 1	-8.0	101.7	7158	6726.3	106416.8	101.7		101.7
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3460.00	5190.00	TRUE		Unknown 1	-9.0	71.9	5190	4758.3	108384.8	71.9	92.0	71.9
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4792.00	7188.00	TRUE		Unknown 1	-9.0	102.2	7188	6756.3	106386.8	102.2		102.2
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4781.00	7171.50	TRUE		Unknown 1	-9.0	101.9	7171.5	6739.8	106403.3	101.9		101.9
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4490.00	6735.00	TRUE		Unknown 1	-10.0	95.3	6735	6303.3	106839.8	95.3	99.3	95.3
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4714.00	7071.00	TRUE		Unknown 1	-10.0	100.4	7071	6639.3	106503.8	100.4		100.4
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4789.00	7183.50	TRUE		Unknown 1	-10.0	102.1	7183.5	6751.8	106391.3	102.1		102.1
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3566.00	5349.00	TRUE		Unknown 2	-3.0	74.4	5349	4917.3	108225.8	74.4	67.7	
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3190.00	4785.00	TRUE		Unknown 2	-3.0	65.8	4785	4353.3	108789.8	65.8		
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3065.00	4597.50	TRUE		Unknown 2	-3.0	63.0	4597.5	4165.8	108977.3	63.0		
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	197.00	295.50	TRUE		Unknown 2	-4.0	-2.1	295.5	-136.3	113279.3	-2.1	-2.9	-2.1
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	154.00	231.00	TRUE		Unknown 2	-4.0	-3.0	231	-200.8	113343.8	-3.0		-3.0
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	126.00	189.00	TRUE		Unknown 2	-4.0	-3.7	189	-242.8	113385.8	-3.7		-3.7
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	497.00	745.50	TRUE		Unknown 2	-5.0	4.7	745.5	313.8	112829.3	4.7	1.2	4.7
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	288.00	432.00	TRUE		Unknown 2	-5.0	0.0	432	0.3	113142.8	0.0		0.0
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	239.00	358.50	TRUE		Unknown 2	-5.0	-1.1	358.5	-73.3	113216.3	-1.1		-1.1
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	869.00	1303.50	TRUE		Unknown 2	-6.0	13.2	1303.5	871.8	112271.3	13.2	14.3	13.2
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	888.00	1332.00	TRUE		Unknown 2	-6.0	13.6	1332	900.3	112242.8	13.6		13.6
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	992.00	1488.00	TRUE		Unknown 2	-6.0	16.0	1488	1056.3	112086.8	16.0		16.0
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2857.00	4285.50	TRUE		Unknown 2	-7.0	58.3	4285.5	3853.8	109289.3	58.3	59.7	58.3
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2941.00	4411.50	TRUE		Unknown 2	-7.0	60.2	4411.5	3979.8	109163.3	60.2		60.2
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2966.00	4449.00	TRUE		Unknown 2	-7.0	60.7	4449	4017.3	109125.8	60.7		60.7
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4625.00	6937.50	TRUE		Unknown 2	-8.0	98.4	6937.5	6505.8	106637.3	98.4	96.1	98.4
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4512.00	6768.00	TRUE		Unknown 2	-8.0	95.8	6768	6336.3	106806.8	95.8		95.8
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4440.00	6660.00	TRUE		Unknown 2	-8.0	94.2	6660	6228.3	106914.8	94.2		94.2
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4684.00	7026.00	TRUE		Unknown 2	-9.0	99.7	7026	6594.3	106548.8	99.7	103.5	99.7
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4927.00	7390.50	TRUE		Unknown 2	-9.0	105.2	7390.5	6958.8	106184.3	105.2		105.2
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4949.00	7423.50	TRUE		Unknown 2	-9.0	105.7	7423.5	6991.8	106151.3	105.7		105.7
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4741.00	7111.50	TRUE		Unknown 2	-10.0	101.0	7111.5	6679.8	106463.3	101.0	102.2	101.0
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4858.00	7287.00	TRUE		Unknown 2	-10.0	103.7	7287	6855.3	106287.8	103.7		103.7
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4776.00	7164.00	TRUE		Unknown 2	-10.0	101.8	7164	6732.3	106410.8	101.8		101.8
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	353.00	529.50	TRUE		Unknown 3	-3.0	1.5	529.5	97.8	113045.3	1.5	-0.5	1.5
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	207.00	310.50	TRUE		Unknown 3	-3.0	-1.8	310.5	-121.3	113264.3	-1.8		-1.8
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	231.00	346.50	TRUE		Unknown 3	-3.0	-1.3	346.5	-85.3	113228.3	-1.3		-1.3
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	725.00	1087.50	TRUE		Unknown 3	-4.0	9.9	1087.5	655.8	112487.3	9.9	11.1	9.9
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	805.00	1207.50	TRUE		Unknown 3	-4.0	11.7	1207.5	775.8	112367.3	11.7		11.7
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	802.00	1203.00	TRUE		Unknown 3	-4.0	11.7	1203	771.3	112371.8	11.7		11.7
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2586.00	3879.00	TRUE		Unknown 3	-5.0	52.1	3879	3447.3	109695.8	52.1	40.2	52.1

Competitive Assay Tube Layout - Four Test Chemicals														Check the 10% rule:	6.23%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown							% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.	
Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1759.00	2638.50	TRUE		Unknown 3	-5.0	33.4	2638.5	2206.8	110936.3	33.4		33.4	
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1838.00	2757.00	TRUE		Unknown 3	-5.0	35.2	2757	2325.3	110817.8	35.2		35.2	
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4110.00	6165.00	TRUE		Unknown 3	-6.0	86.7	6165	5733.3	107409.8	86.7	91.3	86.7	
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4501.00	6751.50	TRUE		Unknown 3	-6.0	95.6	6751.5	6319.8	106823.3	95.6		95.6	
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4324.00	6486.00	TRUE		Unknown 3	-6.0	91.5	6486	6054.3	107088.8	91.5		91.5	
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4472.00	6708.00	TRUE		Unknown 3	-7.0	94.9	6708	6276.3	106866.8	94.9	97.1	94.9	
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4612.00	6918.00	TRUE		Unknown 3	-7.0	98.1	6918	6486.3	106656.8	98.1		98.1	
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4617.00	6925.50	TRUE		Unknown 3	-7.0	98.2	6925.5	6493.8	106649.3	98.2		98.2	
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4556.00	6834.00	TRUE		Unknown 3	-8.0	96.8	6834	6402.3	106740.8	96.8	100.1	96.8	
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4715.00	7072.50	TRUE		Unknown 3	-8.0	100.4	7072.5	6640.8	106502.3	100.4		100.4	
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4832.00	7248.00	TRUE		Unknown 3	-8.0	103.1	7248	6816.3	106326.8	103.1		103.1	
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4534.00	6801.00	TRUE		Unknown 3	-9.0	96.3	6801	6369.3	106773.8	96.3	99.7	96.3	
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4852.00	7278.00	TRUE		Unknown 3	-9.0	103.5	7278	6846.3	106296.8	103.5		103.5	
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4669.00	7003.50	TRUE		Unknown 3	-9.0	99.4	7003.5	6571.8	106571.3	99.4		99.4	
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4690.00	7035.00	TRUE		Unknown 3	-10.0	99.8	7035	6603.3	106539.8	99.8	102.2	99.8	
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4851.00	7276.50	TRUE		Unknown 3	-10.0	103.5	7276.5	6844.8	106298.3	103.5		103.5	
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4845.00	7267.50	TRUE		Unknown 3	-10.0	103.4	7267.5	6835.8	106307.3	103.4		103.4	
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4649.00	6973.50	TRUE		Unknown 4	-3.0	98.9	6973.5	6541.8	106601.3	98.9	102.9	98.9	
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4832.00	7248.00	TRUE		Unknown 4	-3.0	103.1	7248	6816.3	106326.8	103.1		103.1	
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4999.00	7498.50	TRUE		Unknown 4	-3.0	106.9	7498.5	7066.8	106076.3	106.9		106.9	
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4589.00	6883.50	TRUE		Unknown 4	-4.0	97.6	6883.5	6451.8	106691.3	97.6	102.2	97.6	
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4889.00	7333.50	TRUE		Unknown 4	-4.0	104.4	7333.5	6901.8	106241.3	104.4		104.4	
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4899.00	7348.50	TRUE		Unknown 4	-4.0	104.6	7348.5	6916.8	106226.3	104.6		104.6	
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4492.00	6738.00	TRUE		Unknown 4	-5.0	95.4	6738	6306.3	106836.8	95.4	98.8	95.4	
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4759.00	7138.50	TRUE		Unknown 4	-5.0	101.4	7138.5	6706.8	106436.3	101.4		101.4	
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4676.00	7014.00	TRUE		Unknown 4	-5.0	99.5	7014	6582.3	106560.8	99.5		99.5	
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4551.00	6826.50	TRUE		Unknown 4	-6.0	96.7	6826.5	6394.8	106748.3	96.7	101.6	96.7	
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4928.00	7392.00	TRUE		Unknown 4	-6.0	105.2	7392	6960.3	106182.8	105.2		105.2	
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4819.00	7228.50	TRUE		Unknown 4	-6.0	102.8	7228.5	6796.8	106346.3	102.8		102.8	
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4308.00	6462.00	TRUE		Unknown 4	-7.0	91.2	6462	6030.3	107112.8	91.2	97.4	91.2	
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4887.00	7330.50	TRUE		Unknown 4	-7.0	104.3	7330.5	6898.8	106244.3	104.3		104.3	
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4557.00	6835.50	TRUE		Unknown 4	-7.0	96.8	6835.5	6403.8	106739.3	96.8		96.8	
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4367.00	6550.50	TRUE		Unknown 4	-8.0	92.5	6550.5	6118.8	107024.3	92.5	98.0	92.5	
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4737.00	7105.50	TRUE		Unknown 4	-8.0	100.9	7105.5	6673.8	106469.3	100.9		100.9	
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4718.00	7077.00	TRUE		Unknown 4	-8.0	100.5	7077	6645.3	106497.8	100.5		100.5	
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4354.00	6531.00	TRUE		Unknown 4	-9.0	92.2	6531	6099.3	107043.8	92.2	99.4	92.2	
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4827.00	7240.50	TRUE		Unknown 4	-9.0	103.0	7240.5	6808.8	106334.3	103.0		103.0	
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4831.00	7246.50	TRUE		Unknown 4	-9.0	103.0	7246.5	6814.8	106328.3	103.0		103.0	
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4484.00	6726.00	TRUE		Unknown 4	-10.0	95.2	6726	6294.3	106848.8	95.2	98.9	95.2	
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4678.00	7017.00	TRUE		Unknown 4	-10.0	99.6	7017	6585.3	106557.8	99.6		99.6	
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4778.00	7167.00	TRUE		Unknown 4	-10.0	101.8	7167	6735.3	106407.8	101.8		101.8	
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	276.00	414.00	TRUE		cold E2	-7.0	-0.3	414	-17.8	113160.8	-0.3	1.4	-0.3	
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	411.00	616.50	TRUE		cold E2	-7.0	2.8	616.5	184.8	112958.3	2.8		2.8	
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	367.00	550.50	TRUE		cold E2	-7.0	1.8	550.5	118.8	113024.3	1.8		1.8	
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4536.00	6804.00	TRUE			—	96.4	6804	6372.3	106770.8	96.4	98.9	96.4	
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4850.00	7275.00	TRUE			—	103.5	7275	6843.3	106299.8	103.5		103.5	
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4555.00	6832.50	TRUE			—	96.8	6832.5	6400.8	106742.3	96.8		96.8	
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average		
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
178	1	none	Hot		—	—	—	10	—	—	—	—	74587.00	111880.50	TRUE					111880.5	111448.8					
179	2	none	Hot		—	—	—	10	—	—	—	—	77002.00	115503.00	TRUE					115503	115071.3					
180	3	none	Hot		—	—	—	10	—	—	—	—	77449.00	116173.50	TRUE					116173.5	115741.8					
181	1	none	Hot		—	—	—	10	—	—	—	—	68655.00	102982.50	TRUE					102982.5	102550.8					
182	2	none	Hot		—	—	—	10	—	—	—	—	78977.00	118465.50	TRUE					118465.5	118033.8					
183	3	none	Hot		—	—	—	10	—	—	—	—	75902.00	113853.00	TRUE					113853	113421.3					

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				11.0				12.0				15.0				21.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	-1.4024	-1.3571	-1.5612	-4.0				-3.0				-3.0				-3.0	1.4780	-1.8334	-1.2890	-3.0	98.9151	103.0657	106.8534	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.2684	2.7935	1.7956	-4.5	-0.7901	-0.7674	-0.5406	-4.0	0.6162	1.6822	0.8203	-4.0	-2.0602	-3.0355	-3.6705	-4.0	9.9153	11.7298	11.6618	-4.0	97.5542	104.3585	104.5853	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	8.3503	8.5545	7.8967	-5.5	18.5794	18.8062	20.9836	-5.0	12.8638	22.3898	21.7547	-5.0	4.7441	0.0038	-1.1076	-5.0	52.1244	33.3674	35.1591	-5.0	95.3542	101.4100	99.5275	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	24.7259	28.2642	30.4869	-6.0	48.2233	48.4955	49.1306	-6.0	67.3660	68.0237	64.5762	-6.0	13.1814	13.6123	15.9711	-6.0	86.6901	95.5583	91.5438	-6.0	96.6924	105.2431	102.7708	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	49.3801	53.5987	51.1718	-6.5	70.4733	68.3640	68.1598	-7.0	92.9954	98.5976	99.6862	-7.0	58.2710	60.1762	60.7432	-7.0	94.9006	98.0759	98.1893	-7.0	91.1809	104.3131	96.8285	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	78.8425	77.3683	76.7105	-7.0	89.9108	89.7520	93.4717	-8.0	93.8119	100.1172	101.7048	-8.0	98.3708	95.8078	94.1748	-8.0	96.8058	100.4120	103.0657	-8.0	92.5191	100.9110	100.4801	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	92.0428	90.3871	93.4036	-7.5	95.7398	95.9212	97.8945	-9.0	71.9475	102.1585	101.9090	-9.0	99.7089	105.2204	105.7194	-9.0	96.3068	103.5193	99.3687	-9.0	92.2242	102.9523	103.0430	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	104.9482	92.4510	103.9276	-8.5	99.6862	105.2657	105.3338	-10.0	95.3088	100.3894	102.0904	-10.0	101.0017	103.6554	101.7956	-10.0	99.8450	103.4966	103.3606	-10.0	95.1728	99.5728	101.8409	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		Prism ttn006-04-17-08		Positive Control		Prism ttn006-04-17-08		11.0		Prism ttn006-04-17-08		12.0		Prism ttn006-04-17-08		15.0		Prism ttn006-04-17-08		21.0		Prism ttn006-04-17-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values		"bad initial values"		Best-fit values			
13	1	BOTTOM		-1.711		BOTTOM		-5.913		BOTTOM		-0.5095		BOTTOM		-2.095		BOTTOM		1.713		BOTTOM		97.44		BOTTOM			
14	2	TOP		101.7		TOP		103.7		TOP		97.61		TOP		103.7		TOP		100.5		TOP		102.9		TOP			
15	3	LOGIC50		-8.958		LOGIC50		-6.077		LOGIC50		-5.661		LOGIC50		-6.812		LOGIC50		-5.139		LOGIC50				LOGIC50			
16	1	HILLSLOPE		-0.9434		HILLSLOPE		-0.864		HILLSLOPE		-1.003		HILLSLOPE		-0.905		HILLSLOPE		-1.05		HILLSLOPE				HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.367		BOTTOM		2.217		BOTTOM		5.549		BOTTOM		1.047		BOTTOM		2.47		BOTTOM				BOTTOM			
19	1	TOP		1.747		TOP		1.436		TOP		2.335		TOP		0.9251		TOP		1.416		TOP				TOP			
20	2	LOGIC50		0.02018		LOGIC50		0.01845		LOGIC50		0.07621		LOGIC50		0.0211		LOGIC50		0.04336		LOGIC50				LOGIC50			
21	3	HILLSLOPE		0.05742		HILLSLOPE		0.05234		HILLSLOPE		0.1922		HILLSLOPE		0.04688		HILLSLOPE		0.1299		HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.04

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 006
Assay start date: 4/17/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	-1.4
1	1	2	-7.0	-1.4
1	1	3	-7.0	-1.6
1	2	1	-8.0	8.4
1	2	2	-8.0	8.6
1	2	3	-8.0	7.9
1	3	1	-8.5	24.7
1	3	2	-8.5	28.3
1	3	3	-8.5	30.5
1	4	1	-9.0	49.4
1	4	2	-9.0	53.6
1	4	3	-9.0	51.2
1	5	1	-9.5	78.8
1	5	2	-9.5	77.4
1	5	3	-9.5	76.7
1	6	1	-10.0	92.0
1	6	2	-10.0	90.4
1	6	3	-10.0	93.4
1	7	1	-11.0	104.9
1	7	2	-11.0	92.5
1	7	3	-11.0	103.9
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.1

Provide information in all blue cells.

		run	xgroup	rep	x	y
Laboratory Code:	B					
Compound ID:	Norethynodrel	1	1	1	-4.0	28.5
Run ID:	TTN 006	1	1	2		
Assay start date:	4/17/08	1	1	3	-4.0	35.9
Technician ID:	ttn	1	2	1	-4.5	-0.8
		1	2	2	-4.5	-0.8
		1	2	3	-4.5	-0.5
		1	3	1	-5.5	18.6
		1	3	2	-5.5	18.8
		1	3	3	-5.5	21.0
		1	4	1	-6.0	48.2
		1	4	2	-6.0	48.5
		1	4	3	-6.0	49.1
		1	5	1	-6.5	70.5
		1	5	2	-6.5	68.4
		1	5	3	-6.5	68.2
		1	6	1	-7.0	89.9
		1	6	2	-7.0	89.8
		1	6	3	-7.0	93.5
		1	7	1	-7.5	95.7
		1	7	2	-7.5	95.9
		1	7	3	-7.5	97.9
		1	8	1	-8.5	99.7
		1	8	2	-8.5	105.3
		1	8	3	-8.5	105.3
		1	9	1		
		1	9	2		
		1	9	3		
		1	10	1		
		1	10	2		
		1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.80

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 11
Run ID: TTN 006
Assay start date: 4/17/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	88.9
1	1	2	-3.0	109.3
1	1	3	-3.0	94.6
1	2	1	-4.0	0.6
1	2	2	-4.0	1.7
1	2	3	-4.0	0.8
1	3	1	-5.0	12.9
1	3	2	-5.0	22.4
1	3	3	-5.0	21.8
1	4	1	-6.0	67.4
1	4	2	-6.0	68.0
1	4	3	-6.0	64.6
1	5	1	-7.0	93.0
1	5	2	-7.0	98.6
1	5	3	-7.0	99.7
1	6	1	-8.0	93.8
1	6	2	-8.0	100.1
1	6	3	-8.0	101.7
1	7	1	-9.0	71.9
1	7	2	-9.0	102.2
1	7	3	-9.0	101.9
1	8	1	-10.0	95.3
1	8	2	-10.0	100.4
1	8	3	-10.0	102.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.89

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 12
Run ID: TTN 006
Assay start date: 4/17/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	74.4
1	1	2	-3.0	65.8
1	1	3	-3.0	63.0
1	2	1	-4.0	-2.1
1	2	2	-4.0	-3.0
1	2	3	-4.0	-3.7
1	3	1	-5.0	4.7
1	3	2	-5.0	0.0
1	3	3	-5.0	-1.1
1	4	1	-6.0	13.2
1	4	2	-6.0	13.6
1	4	3	-6.0	16.0
1	5	1	-7.0	58.3
1	5	2	-7.0	60.2
1	5	3	-7.0	60.7
1	6	1	-8.0	98.4
1	6	2	-8.0	95.8
1	6	3	-8.0	94.2
1	7	1	-9.0	99.7
1	7	2	-9.0	105.2
1	7	3	-9.0	105.7
1	8	1	-10.0	101.0
1	8	2	-10.0	103.7
1	8	3	-10.0	101.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.50

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 15
Run ID: TTN 006
Assay start date: 4/17/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	1.5
1	1	2	-3.0	-1.8
1	1	3	-3.0	-1.3
1	2	1	-4.0	9.9
1	2	2	-4.0	11.7
1	2	3	-4.0	11.7
1	3	1	-5.0	52.1
1	3	2	-5.0	33.4
1	3	3	-5.0	35.2
1	4	1	-6.0	86.7
1	4	2	-6.0	95.6
1	4	3	-6.0	91.5
1	5	1	-7.0	94.9
1	5	2	-7.0	98.1
1	5	3	-7.0	98.2
1	6	1	-8.0	96.8
1	6	2	-8.0	100.4
1	6	3	-8.0	103.1
1	7	1	-9.0	96.3
1	7	2	-9.0	103.5
1	7	3	-9.0	99.4
1	8	1	-10.0	99.8
1	8	2	-10.0	103.5
1	8	3	-10.0	103.4
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.70

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 21
Run ID: TTN 006
Assay start date: 4/17/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	98.9
1	1	2	-3.0	103.1
1	1	3	-3.0	106.9
1	2	1	-4.0	97.6
1	2	2	-4.0	104.4
1	2	3	-4.0	104.6
1	3	1	-5.0	95.4
1	3	2	-5.0	101.4
1	3	3	-5.0	99.5
1	4	1	-6.0	96.7
1	4	2	-6.0	105.2
1	4	3	-6.0	102.8
1	5	1	-7.0	91.2
1	5	2	-7.0	104.3
1	5	3	-7.0	96.8
1	6	1	-8.0	92.5
1	6	2	-8.0	100.9
1	6	3	-8.0	100.5
1	7	1	-9.0	92.2
1	7	2	-9.0	103.0
1	7	3	-9.0	103.0
1	8	1	-10.0	95.2
1	8	2	-10.0	99.6
1	8	3	-10.0	101.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

Table format: XY		X	A		
		log concentration	Estradiol standard TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-1.4024	-1.3571	-1.5612
2	Title	-7.0	-0.2684	2.7935	1.7956
3	Title	-8.0	8.3503	8.5545	7.8967
4	Title	-8.5	24.7259	28.2642	30.4869
5	Title	-9.0	49.3801	53.5987	51.1718
6	Title	-9.5	78.8425	77.3683	76.7105
7	Title	-10.0	92.0428	90.3871	93.4036
8	Title	-11.0	104.9482	92.4510	103.9276

	Nonlin fit Table of results	A Estradiol standard TTN 006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.711
4	TOP	101.7
5	LOGIC50	-8.958
6	HILLSLOPE	-0.9434
7	Std. Error	
8	BOTTOM	1.367
9	TOP	1.747
10	LOGIC50	0.02018
11	HILLSLOPE	0.05742
12	95% Confidence Intervals	
13	BOTTOM	-4.562 to 1.140
14	TOP	98.04 to 105.3
15	LOGIC50	-9.000 to -8.916
16	HILLSLOPE	-1.063 to -0.8236
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9955
20	Absolute Sum of Squares	164.8
21	Sy.x	2.870
22	Number of points	
23	Analyzed	24

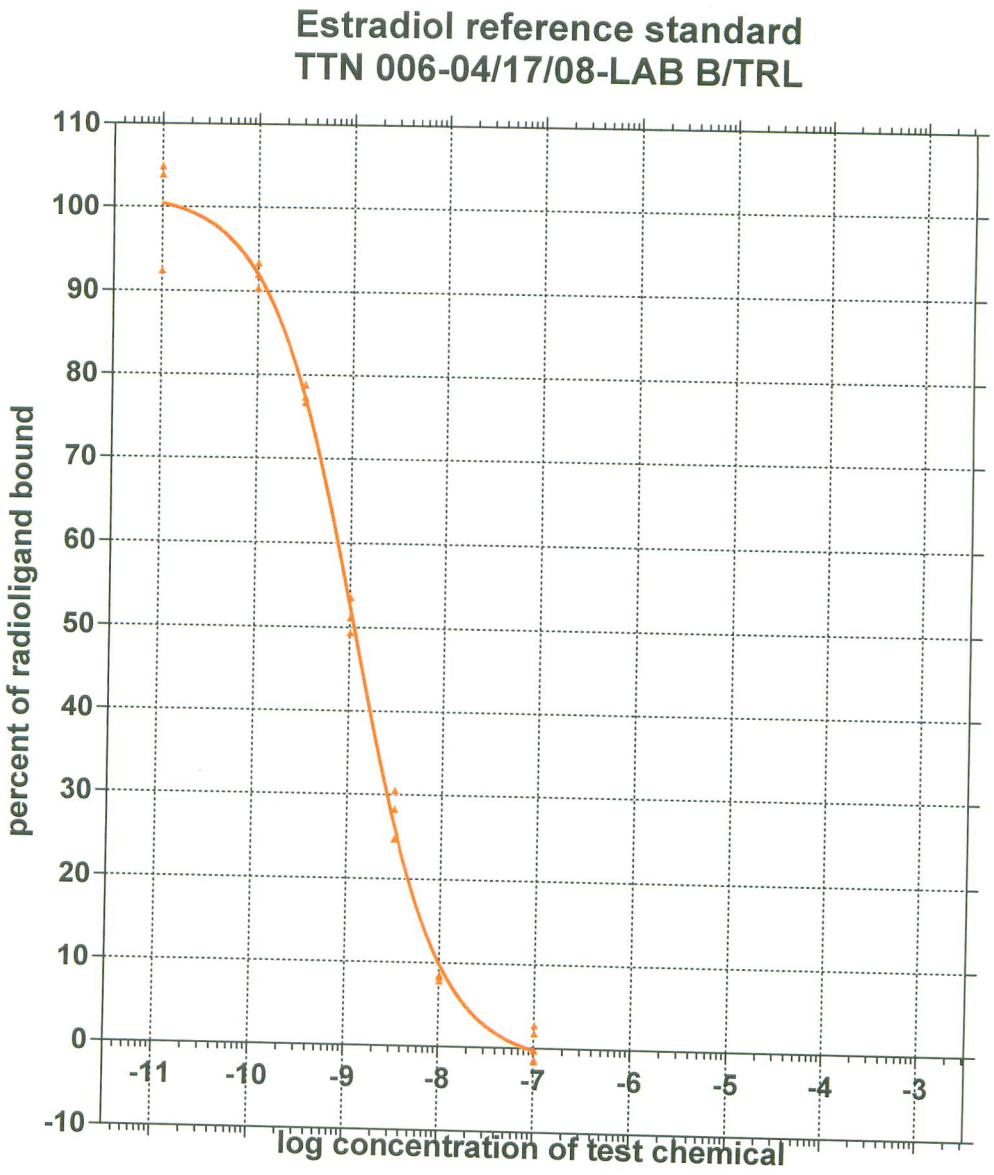

Estradiol reference standard
TTN 006-04/17/08-LAB B/TRL
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Norethynodre TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	-0.7901	-0.7674	-0.5406
3	Title	-5.5	18.5794	18.8062	20.9836
4	Title	-6.0	48.2233	48.4955	49.1306
5	Title	-6.5	70.4733	68.3640	68.1598
6	Title	-7.0	89.9108	89.7520	93.4717
7	Title	-7.5	95.7398	95.9212	97.8945
8	Title	-8.5	99.6862	105.2657	105.3338

	Nonlin fit Table of results	A Norethynodre TTN 006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-5.913
4	TOP	103.7
5	LOGIC50	-6.077
6	HILLSLOPE	-0.8640
7	Std. Error	
8	BOTTOM	2.217
9	TOP	1.436
10	LOGIC50	0.01845
11	HILLSLOPE	0.05234
12	95% Confidence Intervals	
13	BOTTOM	-10.59 to -1.236
14	TOP	100.7 to 106.8
15	LOGIC50	-6.116 to -6.038
16	HILLSLOPE	-0.9745 to -0.7536
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9962
20	Absolute Sum of Squares	112.0
21	Sy.x	2.567
22	Number of points	
23	Analyzed	21

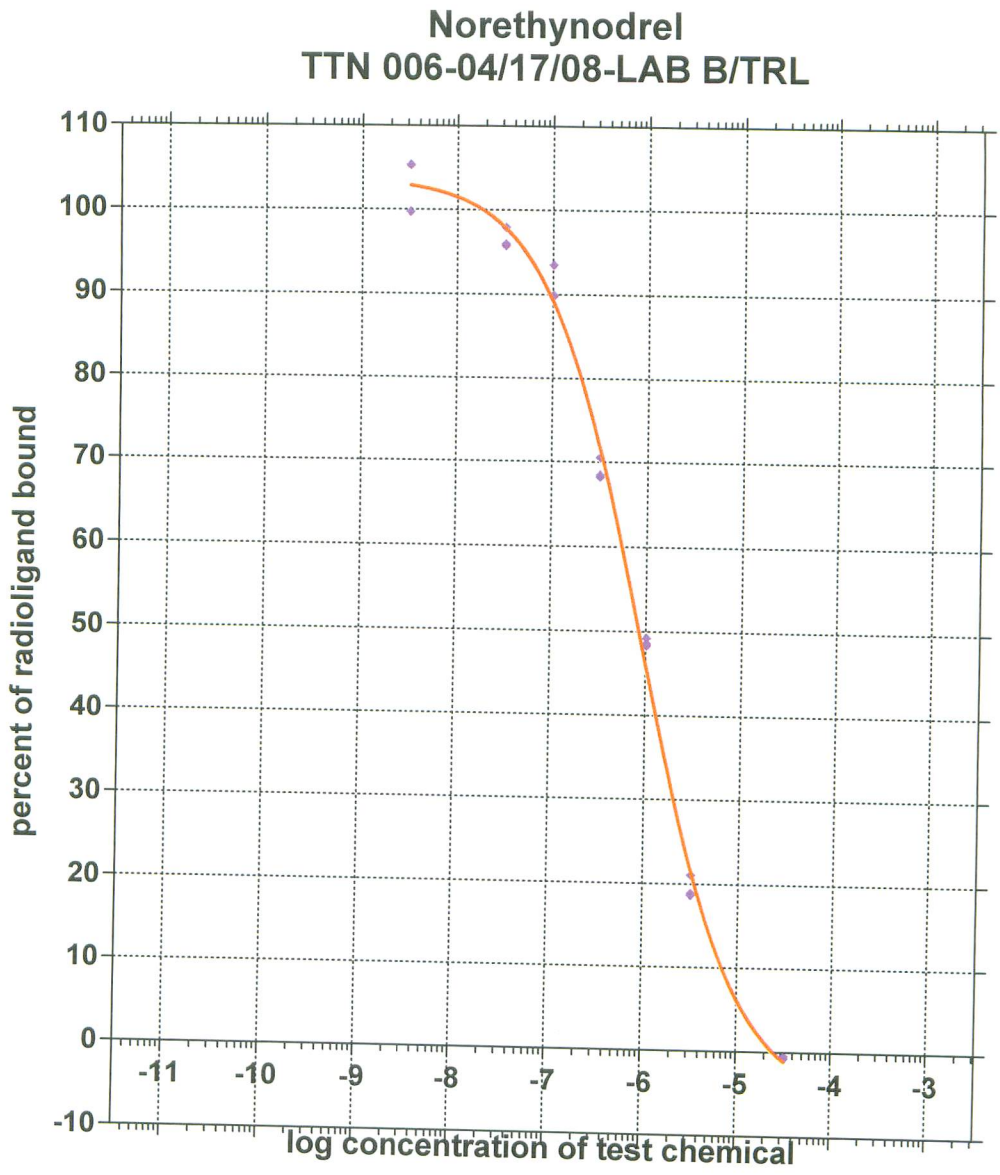

Norethynodrel
TTN 006-04/17/08-LAB B/TRL
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical CODE 11 TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	0.6162	1.6822	0.8203
3	Title	-5.0	12.8638	22.3898	21.7547
4	Title	-6.0	67.3660	68.0237	64.5762
5	Title	-7.0	92.9954	98.5976	99.6862
6	Title	-8.0	93.8119	100.1172	101.7048
7	Title	-9.0	71.9475	102.1585	101.9090
8	Title	-10.0	95.3088	100.3894	102.0904

	Nonlin fit Table of results	A Test chemical CODE 11 TTN 006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.5095
4	TOP	97.61
5	LOGIC50	-5.661
6	HILLSLOPE	-1.003
7	Std. Error	
8	BOTTOM	5.549
9	TOP	2.335
10	LOGIC50	0.07621
11	HILLSLOPE	0.1922
12	95% Confidence Intervals	
13	BOTTOM	-12.22 to 11.20
14	TOP	92.69 to 102.5
15	LOGIC50	-5.822 to -5.500
16	HILLSLOPE	-1.408 to -0.5974
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9711
20	Absolute Sum of Squares	907.7
21	Sy.x	7.307
22	Number of points	
23	Analyzed	21

Test chemical CODE 11
TTN 006-04/17/08-LAB B/TRL

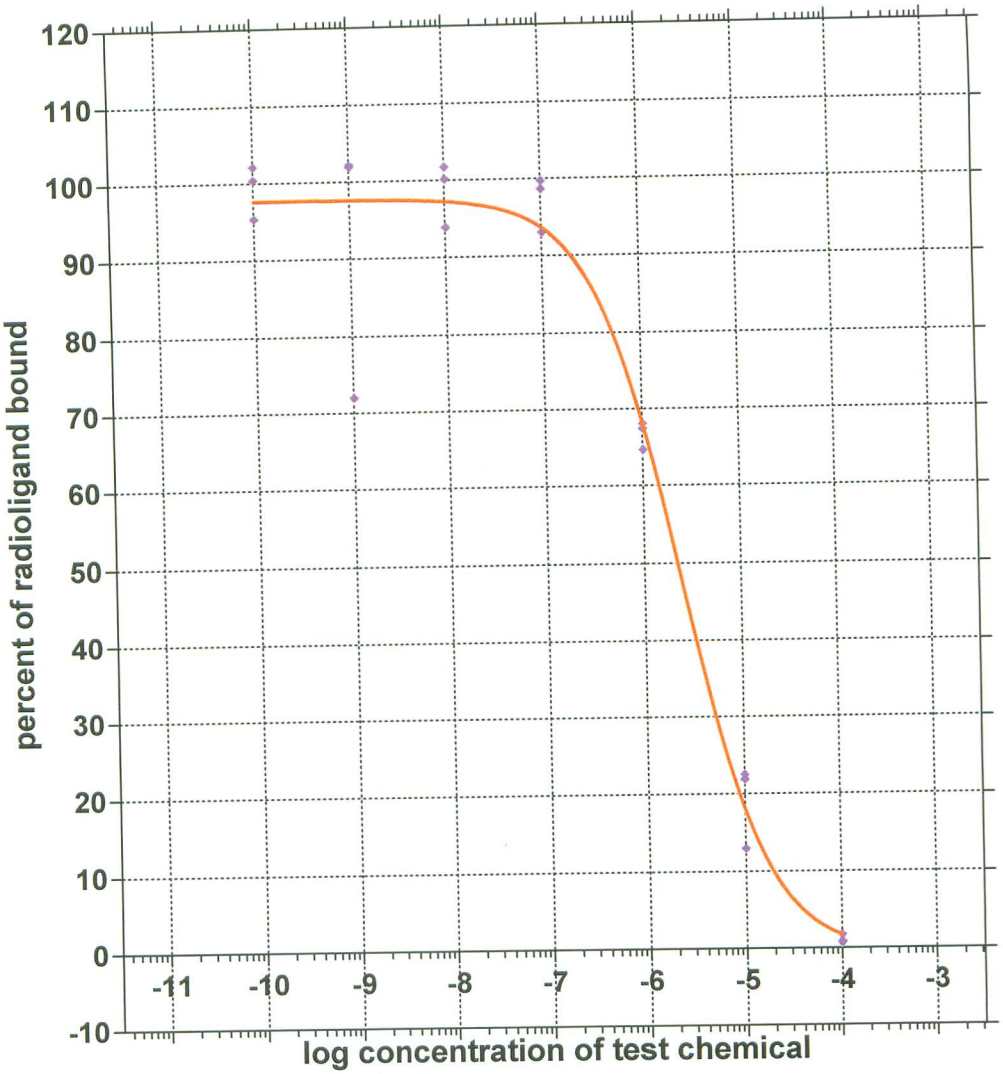


Table format: XY		X	A		
		log concentration	Test chemical CODE 12 TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-2.0602	-3.0355	-3.6705
3	Title	-5.0	4.7441	0.0038	-1.1076
4	Title	-6.0	13.1814	13.6123	15.9711
5	Title	-7.0	58.2710	60.1762	60.7432
6	Title	-8.0	98.3708	95.8078	94.1748
7	Title	-9.0	99.7089	105.2204	105.7194
8	Title	-10.0	101.0017	103.6554	101.7956

	Nonlin fit Table of results	A Test chemical CODE 12 TTN 006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.095
4	TOP	103.7
5	LOGIC50	-6.812
6	HILLSLOPE	-0.9050
7	Std. Error	
8	BOTTOM	1.047
9	TOP	0.9251
10	LOGIC50	0.02110
11	HILLSLOPE	0.04688
12	95% Confidence Intervals	
13	BOTTOM	-4.305 to 0.1140
14	TOP	101.8 to 105.7
15	LOGIC50	-6.857 to -6.768
16	HILLSLOPE	-1.004 to -0.8061
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9981
20	Absolute Sum of Squares	82.36
21	Sy.x	2.201
22	Number of points	
23	Analyzed	21

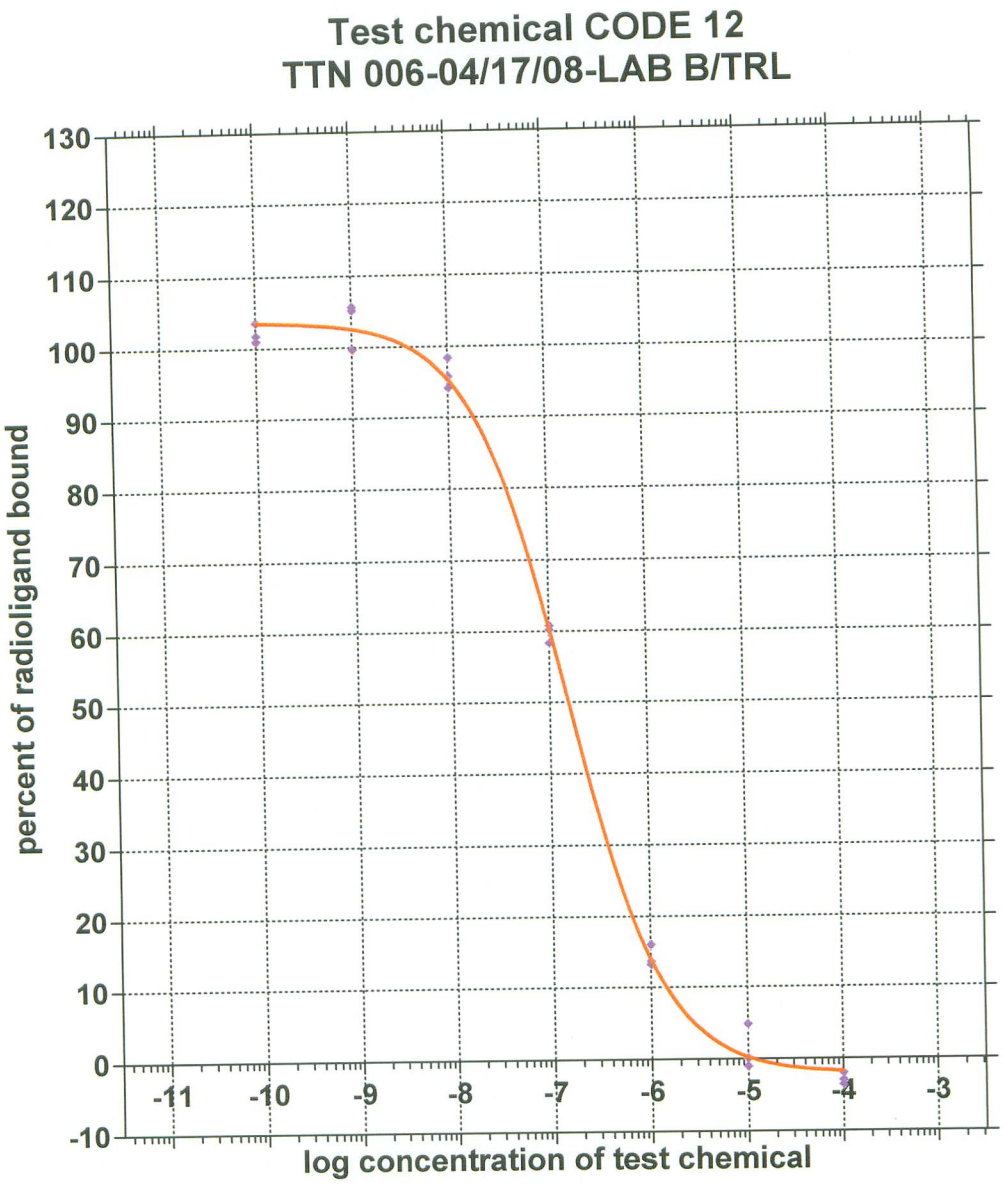

Test chemical CODE 12
TTN 006-04/17/08-LAB B/TRL
percent of radioligand bound
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical CODE 15 TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	1.4780	-1.8334	-1.2890
2	Title	-4.0	9.9153	11.7298	11.6618
3	Title	-5.0	52.1244	33.3674	35.1591
4	Title	-6.0	86.6901	95.5583	91.5438
5	Title	-7.0	94.9006	98.0759	98.1893
6	Title	-8.0	96.8058	100.4120	103.0657
7	Title	-9.0	96.3068	103.5193	99.3687
8	Title	-10.0	99.8450	103.4966	103.3606

	Nonlin fit Table of results	A Test chemical CODE 15 TTN 006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.713
4	TOP	100.5
5	LOGIC50	-5.139
6	HILLSLOPE	-1.050
7	Std. Error	
8	BOTTOM	2.470
9	TOP	1.416
10	LOGIC50	0.04336
11	HILLSLOPE	0.1299
12	95% Confidence Intervals	
13	BOTTOM	-3.439 to 6.866
14	TOP	97.52 to 103.4
15	LOGIC50	-5.230 to -5.049
16	HILLSLOPE	-1.321 to -0.7786
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9891
20	Absolute Sum of Squares	437.2
21	Sy.x	4.676
22	Number of points	
23	Analyzed	24

Test chemical CODE 15
TTN 006-04/17/08-LAB B/TRL

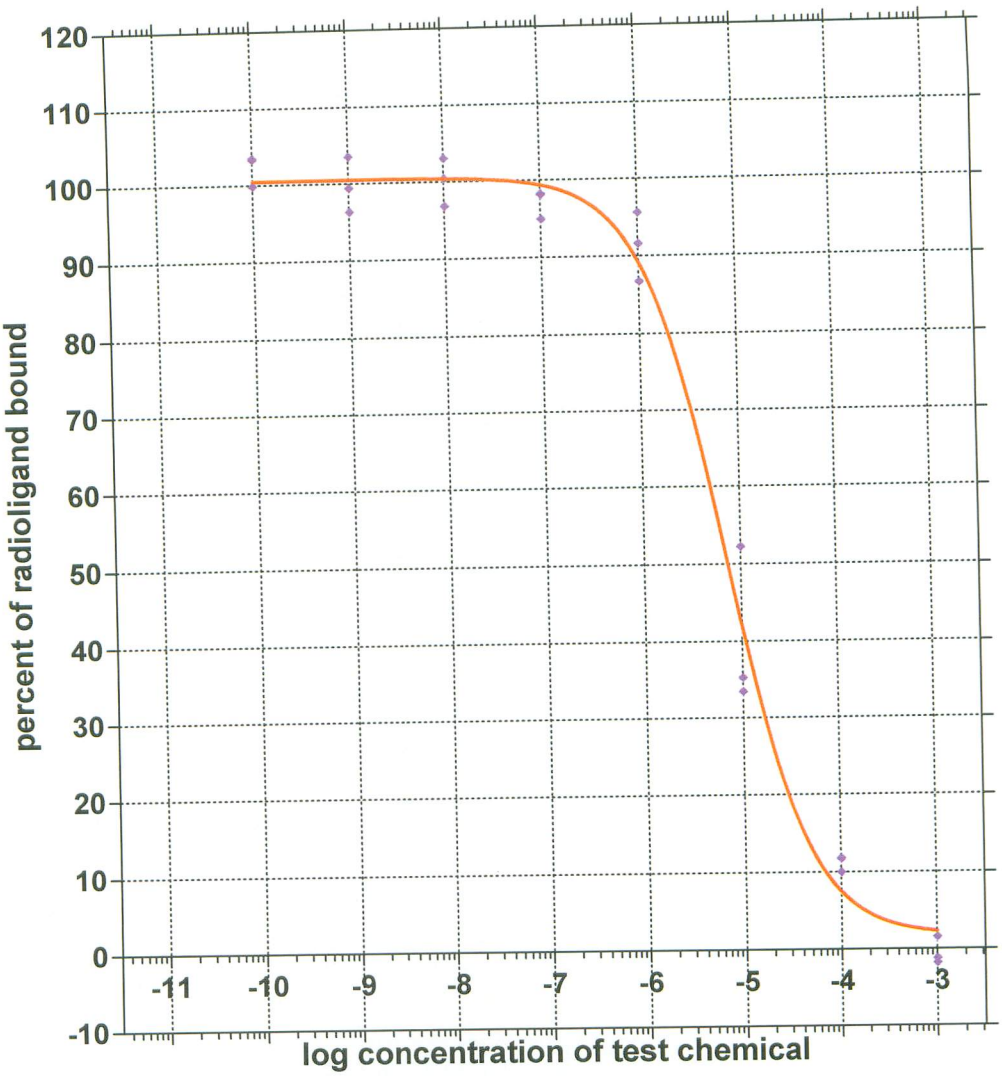
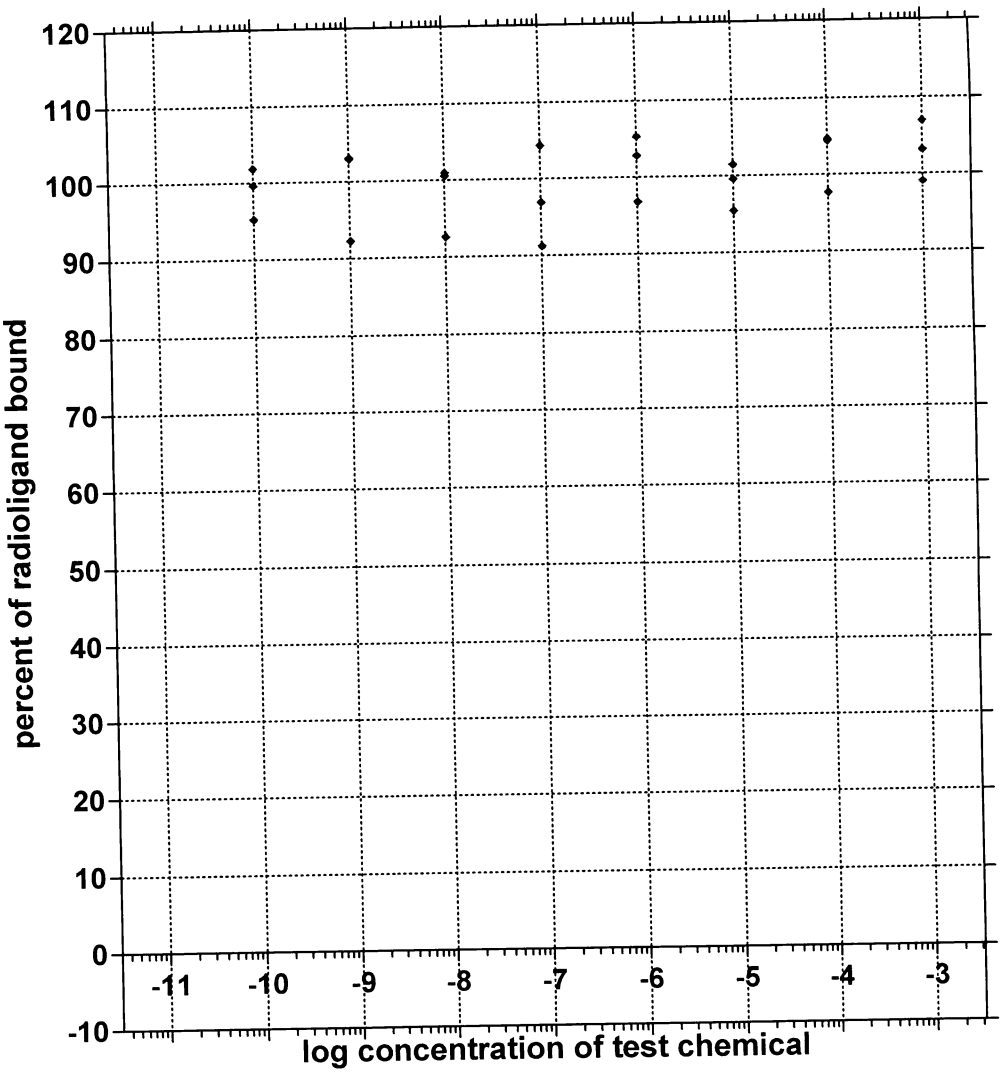


Table format: XY		X	A		
		log concentration	Test chemical CODE 21 TTN 006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	98.9151	103.0657	106.8534
2	Title	-4.0	97.5542	104.3585	104.5853
3	Title	-5.0	95.3542	101.4100	99.5275
4	Title	-6.0	96.6924	105.2431	102.7708
5	Title	-7.0	91.1809	104.3131	96.8285
6	Title	-8.0	92.5191	100.9110	100.4801
7	Title	-9.0	92.2242	102.9523	103.0430
8	Title	-10.0	95.1728	99.5728	101.8409

	Nonlin fit Table of results	A Test chemical CODE 21 TTN 006
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	97.44
4	TOP	102.9
5	LOGIC50	-7.909
6	HILLSLOPE	1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	24

Test chemical CODE 21
TTN 006-04/17/08-LAB B/TRL



APPENDIX I

Excel and Prism Files of the Run Identifier TTN007

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	TTN007	
Assay start date:	4/21/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	109.14	Ci/mmole
Cytosol vial or lot identification:	RUC2-22-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.88	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.95	M
RBA, norethynodrel (Weak Positive):	1.183E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-6.45	5.0
RBA, Unknown 1:	3.707E-03	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-5.04	16.0
RBA, Unknown 2:	1.459E-04	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-3.55	19.0
RBA, Unknown 3:	4.853E-06	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	n/a	22.0
RBA, Unknown 4:	n/a	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Summary values

	n	Mean	SD	
EtOH	6	5800.5	1107.90	Total Binding, solvent control, tubes
Hot	6	82962.2	1263.62	Total hot E2 added to each tube
NSB	6	371.5	23.56	Nonspecific Binding
Specific EtOH	6	5429.0	1107.90	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.07	less than 0.1?
R1881 / EtOH	#DIV/0!	close to 1.0 ? at 1.0 E-6 M only
NSB / EtOH	0.06	around 0.25 ?

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.1
R1881	#N/A	not applicable
norethynodrel	-4.0	1.9
Unknown 1	-4.0	1.6
Unknown 2	-4.0	-2.5
Unknown 3	-3.0	12.2
Unknown 4	-5.0	72.5

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

Check the 10% rule: 6.99%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3477.00	5215.50	TRUE			—	89.2	5215.5	4844.0	78118.2	89.2	96.7	89.2
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3529.00	5293.50	TRUE			—	90.7	5293.5	4922.0	78040.2	90.7		90.7
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4232.00	6348.00	TRUE			—	110.1	6348	5976.5	76985.7	110.1		110.1
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	265.00	397.50	TRUE		cold E2	-7.0	0.5	397.5	26.0	82936.2	0.5	0.1	0.5
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	229.00	343.50	TRUE		cold E2	-7.0	-0.5	343.5	-28.0	82990.2	-0.5		-0.5
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	264.00	396.00	TRUE		cold E2	-7.0	0.5	396	24.5	82937.7	0.5		0.5
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	723.00	1084.50	TRUE		cold E2	-8.0	13.1	1084.5	713.0	82249.2	13.1	12.7	13.1
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	689.00	1033.50	TRUE		cold E2	-8.0	12.2	1033.5	662.0	82300.2	12.2		12.2
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	715.00	1072.50	TRUE		cold E2	-8.0	12.9	1072.5	701.0	82261.2	12.9		12.9
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1370.00	2055.00	TRUE		cold E2	-8.5	31.0	2055	1683.5	81278.7	31.0	32.2	31.0
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1495.00	2242.50	TRUE		cold E2	-8.5	34.5	2242.5	1871.0	81091.2	34.5		34.5
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1378.00	2067.00	TRUE		cold E2	-8.5	31.2	2067	1695.5	81266.7	31.2		31.2
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2022.00	3033.00	TRUE		cold E2	-9.0	49.0	3033	2661.5	80300.7	49.0	55.4	49.0
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2381.00	3571.50	TRUE		cold E2	-9.0	58.9	3571.5	3200.0	79762.2	58.9		58.9
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2356.00	3534.00	TRUE		cold E2	-9.0	58.3	3534	3162.5	79799.7	58.3		58.3
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3104.00	4656.00	TRUE		cold E2	-9.5	78.9	4656	4284.5	78677.7	78.9	77.7	78.9
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2993.00	4489.50	TRUE		cold E2	-9.5	75.9	4489.5	4118.0	78844.2	75.9		75.9
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3084.00	4626.00	TRUE		cold E2	-9.5	78.4	4626	4254.5	78707.7	78.4		78.4
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3345.00	5017.50	TRUE		cold E2	-10.0	85.6	5017.5	4646.0	78316.2	85.6	88.8	85.6
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3510.00	5265.00	TRUE		cold E2	-10.0	90.1	5265	4893.5	78068.7	90.1		90.1
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3529.00	5293.50	TRUE		cold E2	-10.0	90.7	5293.5	4922.0	78040.2	90.7		90.7
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3665.00	5497.50	TRUE		cold E2	-11.0	94.4	5497.5	5126.0	77836.2	94.4	96.2	94.4
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3590.00	5385.00	TRUE		cold E2	-11.0	92.3	5385	5013.5	77948.7	92.3		92.3
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3934.00	5901.00	TRUE		cold E2	-11.0	101.9	5901	5529.5	77432.7	101.9		101.9
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 6.99%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Unknown	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	282.00	423.00	TRUE		norethynodrel	-4.0	0.9	423	51.5	82910.7	0.9	1.9	0.9
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	292.00	438.00	TRUE		norethynodrel	-4.0	1.2	438	66.5	82895.7	1.2		1.2
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	371.00	556.50	TRUE		norethynodrel	-4.0	3.4	556.5	185.0	82777.2	3.4		3.4
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	322.00	483.00	TRUE		norethynodrel	-4.5	2.1	483	111.5	82850.7	2.1	2.0	2.1
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	313.00	469.50	TRUE		norethynodrel	-4.5	1.8	469.5	98.0	82864.2	1.8		1.8
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	327.00	490.50	TRUE		norethynodrel	-4.5	2.2	490.5	119.0	82843.2	2.2		2.2
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1271.00	1906.50	TRUE		norethynodrel	-5.5	28.3	1906.5	1535.0	81427.2	28.3	28.3	28.3
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1285.00	1927.50	TRUE		norethynodrel	-5.5	28.7	1927.5	1556.0	81406.2	28.7		28.7
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1265.00	1897.50	TRUE		norethynodrel	-5.5	28.1	1897.5	1526.0	81436.2	28.1		28.1
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2216.00	3324.00	TRUE		norethynodrel	-6.0	54.4	3324	2952.5	80009.7	54.4	52.0	54.4
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1959.00	2938.50	TRUE		norethynodrel	-6.0	47.3	2938.5	2567.0	80395.2	47.3		47.3
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2209.00	3313.50	TRUE		norethynodrel	-6.0	54.2	3313.5	2942.0	80020.2	54.2		54.2
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2992.00	4488.00	TRUE		norethynodrel	-6.5	75.8	4488	4116.5	78845.7	75.8	73.9	75.8
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2882.00	4323.00	TRUE		norethynodrel	-6.5	72.8	4323	3951.5	79010.7	72.8		72.8
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2896.00	4344.00	TRUE		norethynodrel	-6.5	73.2	4344	3972.5	78989.7	73.2		73.2
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3444.00	5166.00	TRUE		norethynodrel	-7.0	88.3	5166	4794.5	78167.7	88.3	89.5	88.3
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3493.00	5239.50	TRUE		norethynodrel	-7.0	89.7	5239.5	4868.0	78094.2	89.7		89.7
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3529.00	5293.50	TRUE		norethynodrel	-7.0	90.7	5293.5	4922.0	78040.2	90.7		90.7
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3380.00	5070.00	TRUE		norethynodrel	-7.5	86.5	5070	4698.5	78263.7	86.5	90.9	86.5
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3661.00	5491.50	TRUE		norethynodrel	-7.5	94.3	5491.5	5120.0	77842.2	94.3		94.3
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3574.00	5361.00	TRUE		norethynodrel	-7.5	91.9	5361	4989.5	77972.7	91.9		91.9
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3693.00	5539.50	TRUE		norethynodrel	-8.5	95.2	5539.5	5168.0	77794.2	95.2	93.0	95.2
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3697.00	5545.50	TRUE		norethynodrel	-8.5	95.3	5545.5	5174.0	77788.2	95.3		95.3
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3449.00	5173.50	TRUE		norethynodrel	-8.5	88.5	5173.5	4802.0	78160.2	88.5		88.5
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2534.00	3801.00	TRUE		Unknown 1	-3.0	63.2	3801	3429.5	79532.7	63.2	60.9	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2416.00	3624.00	TRUE		Unknown 1	-3.0	59.9	3624	3252.5	79709.7	59.9		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2406.00	3609.00	TRUE		Unknown 1	-3.0	59.6	3609	3237.5	79724.7	59.6		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	322.00	483.00	TRUE		Unknown 1	-4.0	2.1	483	111.5	82850.7	2.1	1.6	2.1
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	306.00	459.00	TRUE		Unknown 1	-4.0	1.6	459	87.5	82874.7	1.6		1.6
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	291.00	436.50	TRUE		Unknown 1	-4.0	1.2	436.5	65.0	82897.2	1.2		1.2
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	461.00	691.50	TRUE		Unknown 1	-5.0	5.9	691.5	320.0	82642.2	5.9	7.0	5.9
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	517.00	775.50	TRUE		Unknown 1	-5.0	7.4	775.5	404.0	82558.2	7.4		7.4
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	523.00	784.50	TRUE		Unknown 1	-5.0	7.6	784.5	413.0	82549.2	7.6		7.6
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1306.00	1959.00	TRUE		Unknown 1	-6.0	29.2	1959	1587.5	81374.7	29.2	28.9	29.2
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1275.00	1912.50	TRUE		Unknown 1	-6.0	28.4	1912.5	1541.0	81421.2	28.4		28.4
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1300.00	1950.00	TRUE		Unknown 1	-6.0	29.1	1950	1578.5	81383.7	29.1		29.1
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2619.00	3928.50	TRUE		Unknown 1	-7.0	65.5	3928.5	3557.0	79405.2	65.5	73.5	65.5
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2963.00	4444.50	TRUE		Unknown 1	-7.0	75.0	4444.5	4073.0	78889.2	75.0		75.0
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3143.00	4714.50	TRUE		Unknown 1	-7.0	80.0	4714.5	4343.0	78619.2	80.0		80.0
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3119.00	4678.50	TRUE		Unknown 1	-8.0	79.3	4678.5	4307.0	78655.2	79.3	83.2	79.3
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3272.00	4908.00	TRUE		Unknown 1	-8.0	83.6	4908	4536.5	78425.7	83.6		83.6
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3388.00	5082.00	TRUE		Unknown 1	-8.0	86.8	5082	4710.5	78251.7	86.8		86.8
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3639.00	5458.50	TRUE		Unknown 1	-9.0	93.7	5458.5	5087.0	77875.2	93.7	94.3	93.7
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3698.00	5547.00	TRUE		Unknown 1	-9.0	95.3	5547	5175.5	77786.7	95.3		95.3
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3643.00	5464.50	TRUE		Unknown 1	-9.0	93.8	5464.5	5093.0	77869.2	93.8		93.8
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3556.00	5334.00	TRUE		Unknown 1	-10.0	91.4	5334	4962.5	77999.7	91.4	88.0	91.4
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3552.00	5328.00	TRUE		Unknown 1	-10.0	91.3	5328	4956.5	78005.7	91.3		91.3
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3191.00	4786.50	TRUE		Unknown 1	-10.0	81.3	4786.5	4415.0	78547.2	81.3		81.3
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	338.00	507.00	TRUE		Unknown 2	-3.0	2.5	507	135.5	82826.7	2.5	2.1	2.5
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	300.00	450.00	TRUE		Unknown 2	-3.0	1.4	450	78.5	82883.7	1.4		1.4
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	337.00	505.50	TRUE		Unknown 2	-3.0	2.5	505.5	134.0	82828.2	2.5		2.5
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	149.00	223.50	TRUE		Unknown 2	-4.0	-2.7	223.5	-148.0	83110.2	-2.7	-2.5	-2.7
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	187.00	280.50	TRUE		Unknown 2	-4.0	-1.7	280.5	-91.0	83053.2	-1.7		-1.7
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	131.00	196.50	TRUE		Unknown 2	-4.0	-3.2	196.5	-175.0	83137.2	-3.2		-3.2
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1585.00	2377.50	TRUE		Unknown 2	-5.0	36.9	2377.5	2006.0	80956.2	36.9	37.6	36.9
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1636.00	2454.00	TRUE		Unknown 2	-5.0	38.4	2454	2082.5	80879.7	38.4		38.4
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1602.00	2403.00	TRUE		Unknown 2	-5.0	37.4	2403	2031.5	80930.7	37.4		37.4
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3575.00	5362.50	TRUE		Unknown 2	-6.0	91.9	5362.5	4991.0	77971.2	91.9	95.4	91.9
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3808.00	5712.00	TRUE		Unknown 2	-6.0	98.4	5712	5340.5	77621.7	98.4		98.4
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3714.00	5571.00	TRUE		Unknown 2	-6.0	95.8	5571	5199.5	77762.7	95.8		95.8
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3601.00	5401.50	TRUE		Unknown 2	-7.0	92.7	5401.5	5030.0	77932.2	92.7	94.6	92.7
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3893.00	5839.50	TRUE		Unknown 2	-7.0	100.7	5839.5	5468.0	77494.2	100.7		100.7
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3525.00	5287.50	TRUE		Unknown 2	-7.0	90.6	5287.5	4916.0	78046.2	90.6		90.6
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3706.00	5559.00	TRUE		Unknown 2	-8.0	95.6	5559	5187.5	77774.7	95.6	96.0	95.6
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3674.00	5511.00	TRUE		Unknown 2	-8.0	94.7	5511	5139.5	77822.7	94.7		94.7
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3785.00	5677.50	TRUE		Unknown 2	-8.0	97.7	5677.5	5306.0	77656.2	97.7		97.7
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3865.00	5797.50	TRUE		Unknown 2	-9.0	99.9	5797.5	5426.0	77536.2	99.9	91.6	99.9
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3835.00	5752.50	TRUE		Unknown 2	-9.0	99.1	5752.5	5381.0	77581.2	99.1		99.1
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2994.00	4491.00	TRUE		Unknown 2	-9.0	75.9	4491	4119.5	78842.7	75.9		75.9
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3740.00	5610.00	TRUE		Unknown 2	-10.0	96.5	5610	5238.5	77723.7	96.5	96.2	96.5
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3575.00	5362.50	TRUE		Unknown 2	-10.0	91.9	5362.5	4991.0	77971.2	91.9		91.9

Competitive Assay Tube Layout - Four Test Chemicals															Check the 10% rule:	6.99%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown		% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.					
Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3872.00	5808.00	TRUE		Unknown 2	-10.0	100.1	5808	5436.5	77525.7	100.1		100.1
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	821.00	1231.50	TRUE		Unknown 3	-3.0	15.8	1231.5	860.0	82102.2	15.8	12.2	15.8
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	599.00	898.50	TRUE		Unknown 3	-3.0	9.7	898.5	527.0	82435.2	9.7		9.7
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	651.00	976.50	TRUE		Unknown 3	-3.0	11.1	976.5	605.0	82357.2	11.1		11.1
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3346.00	5019.00	TRUE		Unknown 3	-4.0	85.6	5019	4647.5	78314.7	85.6	86.7	85.6
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3453.00	5179.50	TRUE		Unknown 3	-4.0	88.6	5179.5	4808.0	78154.2	88.6		88.6
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3358.00	5037.00	TRUE		Unknown 3	-4.0	85.9	5037	4665.5	78296.7	85.9		85.9
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3672.00	5508.00	TRUE		Unknown 3	-5.0	94.6	5508	5136.5	77825.7	94.6	95.0	94.6
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3681.00	5521.50	TRUE		Unknown 3	-5.0	94.9	5521.5	5150.0	77812.2	94.9		94.9
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3708.00	5562.00	TRUE		Unknown 3	-5.0	95.6	5562	5190.5	77771.7	95.6		95.6
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3713.00	5569.50	TRUE		Unknown 3	-6.0	95.7	5569.5	5198.0	77764.2	95.7	97.3	95.7
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3818.00	5727.00	TRUE		Unknown 3	-6.0	98.6	5727	5355.5	77606.7	98.6		98.6
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3780.00	5670.00	TRUE		Unknown 3	-6.0	97.6	5670	5298.5	77663.7	97.6		97.6
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3555.00	5332.50	TRUE		Unknown 3	-7.0	91.4	5332.5	4961.0	78001.2	91.4	93.6	91.4
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3732.00	5598.00	TRUE		Unknown 3	-7.0	96.3	5598	5226.5	77735.7	96.3		96.3
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3621.00	5431.50	TRUE		Unknown 3	-7.0	93.2	5431.5	5060.0	77902.2	93.2		93.2
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3778.00	5667.00	TRUE		Unknown 3	-8.0	97.5	5667	5295.5	77666.7	97.5	93.1	97.5
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3435.00	5152.50	TRUE		Unknown 3	-8.0	88.1	5152.5	4781.0	78181.2	88.1		88.1
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3637.00	5455.50	TRUE		Unknown 3	-8.0	93.6	5455.5	5084.0	77878.2	93.6		93.6
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3703.00	5554.50	TRUE		Unknown 3	-9.0	95.5	5554.5	5183.0	77779.2	95.5	95.9	95.5
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3695.00	5542.50	TRUE		Unknown 3	-9.0	95.2	5542.5	5171.0	77791.2	95.2		95.2
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3762.00	5643.00	TRUE		Unknown 3	-9.0	97.1	5643	5271.5	77690.7	97.1		97.1
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3633.00	5449.50	TRUE		Unknown 3	-10.0	93.5	5449.5	5078.0	77884.2	93.5	94.9	93.5
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3758.00	5637.00	TRUE		Unknown 3	-10.0	97.0	5637	5265.5	77696.7	97.0		97.0
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3655.00	5482.50	TRUE		Unknown 3	-10.0	94.1	5482.5	5111.0	77851.2	94.1		94.1
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3207.00	4810.50	TRUE		Unknown 4	-3.0	81.8	4810.5	4439.0	78523.2	81.8	79.3	81.8
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3200.00	4800.00	TRUE		Unknown 4	-3.0	81.6	4800	4428.5	78533.7	81.6		81.6
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2949.00	4423.50	TRUE		Unknown 4	-3.0	74.6	4423.5	4052.0	78910.2	74.6		74.6
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3638.00	5457.00	TRUE		Unknown 4	-4.0	93.7	5457	5085.5	77876.7	93.7	91.8	93.7
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3700.00	5550.00	TRUE		Unknown 4	-4.0	95.4	5550	5178.5	77783.7	95.4		95.4
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3374.00	5061.00	TRUE		Unknown 4	-4.0	86.4	5061	4689.5	78272.7	86.4		86.4
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2937.00	4405.50	TRUE		Unknown 4	-5.0	74.3	4405.5	4034.0	78928.2	74.3	72.5	74.3
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3194.00	4791.00	TRUE		Unknown 4	-5.0	81.4	4791	4419.5	78542.7	81.4		81.4
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2488.00	3732.00	TRUE		Unknown 4	-5.0	61.9	3732	3360.5	79601.7	61.9		61.9
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3553.00	5329.50	TRUE		Unknown 4	-6.0	91.3	5329.5	4958.0	78004.2	91.3	96.5	91.3
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3822.00	5733.00	TRUE		Unknown 4	-6.0	98.8	5733	5361.5	77600.7	98.8		98.8
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3844.00	5766.00	TRUE		Unknown 4	-6.0	99.4	5766	5394.5	77567.7	99.4		99.4
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3648.00	5472.00	TRUE		Unknown 4	-7.0	93.9	5472	5100.5	77861.7	93.9	93.7	93.9
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3587.00	5380.50	TRUE		Unknown 4	-7.0	92.3	5380.5	5009.0	77953.2	92.3		92.3
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3683.00	5524.50	TRUE		Unknown 4	-7.0	94.9	5524.5	5153.0	77809.2	94.9		94.9
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3634.00	5451.00	TRUE		Unknown 4	-8.0	93.6	5451	5079.5	77882.7	93.6	96.2	93.6
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3685.00	5527.50	TRUE		Unknown 4	-8.0	95.0	5527.5	5156.0	77806.2	95.0		95.0
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3872.00	5808.00	TRUE		Unknown 4	-8.0	100.1	5808	5436.5	77525.7	100.1		100.1
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3591.00	5386.50	TRUE		Unknown 4	-9.0	92.4	5386.5	5015.0	77947.2	92.4	94.0	92.4
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3701.00	5551.50	TRUE		Unknown 4	-9.0	95.4	5551.5	5180.0	77782.2	95.4		95.4
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3656.00	5484.00	TRUE		Unknown 4	-9.0	94.2	5484	5112.5	77849.7	94.2		94.2
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3574.00	5361.00	TRUE		Unknown 4	-10.0	91.9	5361	4989.5	77972.7	91.9	95.0	91.9
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3743.00	5614.50	TRUE		Unknown 4	-10.0	96.6	5614.5	5243.0	77719.2	96.6		96.6
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3736.00	5604.00	TRUE		Unknown 4	-10.0	96.4	5604	5232.5	77729.7	96.4		96.4
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	230.00	345.00	TRUE		cold E2	-7.0	-0.5	345	-26.5	82988.7	-0.5	-0.1	-0.5
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	250.00	375.00	TRUE		cold E2	-7.0	0.1	375	3.5	82958.7	0.1		0.1
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	248.00	372.00	TRUE		cold E2	-7.0	0.0	372	0.5	82961.7	0.0		0.0
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3311.00	4966.50	TRUE			—	84.6	4966.5	4595.0	78367.2	84.6	103.3	84.6
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5220.00	7830.00	TRUE			—	137.4	7830	7458.5	75503.7	137.4		137.4
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3433.00	5149.50	TRUE			—	88.0	5149.5	4778.0	78184.2	88.0		88.0
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	80698.00	80698.00	TRUE					80698	80326.5				
179	2	none	Hot		—	—	—	10	—	—	—	—	84088.00	84088.00	TRUE					84088	83716.5				
180	3	none	Hot		—	—	—	10	—	—	—	—	84191.00	84191.00	TRUE					84191	83819.5				
181	1	none	Hot		—	—	—	10	—	—	—	—	83133.00	83133.00	TRUE					83133	82761.5				
182	2	none	Hot		—	—	—	10	—	—	—	—	82741.00	82741.00	TRUE					82741	82369.5				
183	3	none	Hot		—	—	—	10	—	—	—	—	82922.00	82922.00	TRUE					82922	82550.5				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				5.0				16.0				19.0				22.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	0.4789	-0.5157	0.4513	-4.0	0.9486	1.2249	3.4076	-3.0				-3.0	2.4959	1.4459	2.4682	-3.0	15.8409	9.7071	11.1439	-3.0	81.7646	81.5712	74.6362	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.4881	0.0645	0.0092	-4.5	2.0538	1.8051	2.1919	-4.0	2.0538	1.6117	1.1973	-4.0	-2.7261	-1.6762	-3.2234	-4.0	85.6051	88.5614	85.9366	-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	13.1332	12.1938	12.9121	-5.5	28.2741	28.6609	28.1083	-5.0	5.8943	7.4415	7.6073	-5.0	36.9497	38.3588	37.4194	-5.0	94.6123	94.8609	95.6069	-5.0	74.3047	81.4054	61.8991	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	31.0094	34.4631	31.2304	-6.0	54.3839	47.2831	54.1905	-6.0	29.2411	28.3846	29.0753	-6.0	91.9322	98.3699	95.7727	-6.0	95.7451	98.6462	97.5962	-6.0	91.3244	98.7567	99.3645	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	49.0238	58.9427	58.2520	-6.5	75.8243	72.7850	73.1719	-7.0	65.5185	75.0230	79.9963	-7.0	92.6506	100.7184	90.5507	-7.0	91.3796	96.2700	93.2032	-7.0	93.9492	92.2638	94.9162	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	78.9188	75.8519	78.3662	-7.0	88.3128	89.6666	90.6613	-8.0	79.3332	83.5605	86.7655	-8.0	95.5517	94.6675	97.7344	-8.0	97.5410	88.0641	93.6452	-8.0	93.5624	94.9714	100.1381	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	85.5775	90.1363	90.6613	-7.5	86.5445	94.3083	91.9046	-9.0	93.7005	95.3306	93.8110	-9.0	99.9447	99.1159	75.8795	-9.0	95.4688	95.2477	97.0989	-9.0	92.3743	95.4135	94.1702	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	94.4189	92.3467	101.8512	-8.5	95.1925	95.3030	88.4509	-10.0	91.4073	91.2967	81.3225	-10.0	96.4911	91.9322	100.1381	-10.0	93.5347	96.9884	94.1426	-10.0	91.9046	96.5740	96.3805	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		TTN007 4-21-08		Positive Control		TTN007 4-21-08		5.0		TTN007 4-21-08		16.0		TTN007 4-21-08		19.0		TTN007 4-21-08		22.0		TTN007 4-21-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values	"bad initial values"			Best-fit values			
13	1	BOTTOM		-2.422		BOTTOM		-0.3814		BOTTOM		1.576		BOTTOM		-0.2026		BOTTOM		8.426		BOTTOM		72.54		BOTTOM			
14	2	TOP		97.39		TOP		93.98		TOP		89.77		TOP		94.76		TOP		94.99		TOP		96.48		TOP			
15	3	LOGIC50		-8.877		LOGIC50		-5.95		LOGIC50		-6.446		LOGIC50		-5.041		LOGIC50		-3.563		LOGIC50	n/a			LOGIC50			
16	1	HILLSLOPE		-0.8839		HILLSLOPE		-0.9852		HILLSLOPE		-0.9349		HILLSLOPE		-5.665		HILLSLOPE		-2.308		HILLSLOPE	n/a			HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.457		BOTTOM		1.471		BOTTOM		2.365		BOTTOM		2.287		BOTTOM		146.7		BOTTOM				BOTTOM			
19	1	TOP		1.644		TOP		1.265		TOP		1.695		TOP		1.521		TOP		0.6668		TOP				TOP			
20	2	LOGIC50		0.02061		LOGIC50		0.02086		LOGIC50		0.053		LOGIC50		~ 32.52		LOGIC50		2.677		LOGIC50				LOGIC50			
21	3	HILLSLOPE		0.055		HILLSLOPE		0.06667		HILLSLOPE		0.1097		HILLSLOPE		~ 4547		HILLSLOPE		15.8		HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.2

Provide information in all blue cells.

Laboratory Code:	TRL B
Compound ID:	Estradiol
Run ID:	TTN007
Assay start date:	4/21/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-7.0	0.5
1	1	2	-7.0	-0.5
1	1	3	-7.0	0.5
1	2	1	-8.0	13.1
1	2	2	-8.0	12.2
1	2	3	-8.0	12.9
1	3	1	-8.5	31.0
1	3	2	-8.5	34.5
1	3	3	-8.5	31.2
1	4	1	-9.0	49.0
1	4	2	-9.0	58.9
1	4	3	-9.0	58.3
1	5	1	-9.5	78.9
1	5	2	-9.5	75.9
1	5	3	-9.5	78.4
1	6	1	-10.0	85.6
1	6	2	-10.0	90.1
1	6	3	-10.0	90.7
1	7	1	-11.0	94.4
1	7	2	-11.0	92.3
1	7	3	-11.0	101.9
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.6

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Norethynodrel
Run ID: TTN007
Assay start date: 4/21/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-4.0	0.9
1	1	2	-4.0	1.2
1	1	3	-4.0	3.4
1	2	1	-4.5	2.1
1	2	2	-4.5	1.8
1	2	3	-4.5	2.2
1	3	1	-5.5	28.3
1	3	2	-5.5	28.7
1	3	3	-5.5	28.1
1	4	1	-6.0	54.4
1	4	2	-6.0	47.3
1	4	3	-6.0	54.2
1	5	1	-6.5	75.8
1	5	2	-6.5	72.8
1	5	3	-6.5	73.2
1	6	1	-7.0	88.3
1	6	2	-7.0	89.7
1	6	3	-7.0	90.7
1	7	1	-7.5	86.5
1	7	2	-7.5	94.3
1	7	3	-7.5	91.9
1	8	1	-8.5	95.2
1	8	2	-8.5	95.3
1	8	3	-8.5	88.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.67

Provide information in all blue cells.

Laboratory Code: TRL B

Compound ID: Chem Code 5
Run ID: TTN007
Assay start date: 4/21/2008
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	63.17001289
1	1	2	-3.0	59.90974397
1	1	3	-3.0	59.63344999
1	2	1	-4.0	2.053785227
1	2	2	-4.0	1.611714865
1	2	3	-4.0	1.197273899
1	3	1	-5.0	5.894271505
1	3	2	-5.0	7.441517775
1	3	3	-5.0	7.607294161
1	4	1	-6.0	29.24111254
1	4	2	-6.0	28.38460122
1	4	3	-6.0	29.07533616
1	5	1	-7.0	65.5185117
1	5	2	-7.0	75.0230245
1	5	3	-7.0	79.99631608
1	6	1	-8.0	79.33321054
1	6	2	-8.0	83.56050838
1	6	3	-8.0	86.76551851
1	7	1	-9.0	93.70
1	7	2	-9.0	95.33
1	7	3	-9.0	93.81
1	8	1	-10.0	91.41
1	8	2	-10.0	91.30
1	8	3	-10.0	81.32
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.6

Provide information in all blue cells.
Laboratory Code: TRL B
Compound ID: Chem Code 16
Run ID: TTN007
Assay start date: 4/21/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	2.5
1	1	2	-3.0	1.4
1	1	3	-3.0	2.5
1	2	1	-4.0	-2.7
1	2	2	-4.0	-1.7
1	2	3	-4.0	-3.2
1	3	1	-5.0	36.9
1	3	2	-5.0	38.4
1	3	3	-5.0	37.4
1	4	1	-6.0	91.9
1	4	2	-6.0	98.4
1	4	3	-6.0	95.8
1	5	1	-7.0	92.7
1	5	2	-7.0	100.7
1	5	3	-7.0	90.6
1	6	1	-8.0	95.6
1	6	2	-8.0	94.7
1	6	3	-8.0	97.7
1	7	1	-9.0	99.9
1	7	2	-9.0	99.1
1	7	3	-9.0	75.9
1	8	1	-10.0	96.5
1	8	2	-10.0	91.9
1	8	3	-10.0	100.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.5

Provide information in all blue cells.
Laboratory Code: TRL B
Compound ID: Chem Code 19
Run ID: TTN007
Assay start date: 4/21/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	15.8
1	1	2	-3.0	9.7
1	1	3	-3.0	11.1
1	2	1	-4.0	85.6
1	2	2	-4.0	88.6
1	2	3	-4.0	85.9
1	3	1	-5.0	94.6
1	3	2	-5.0	94.9
1	3	3	-5.0	95.6
1	4	1	-6.0	95.7
1	4	2	-6.0	98.6
1	4	3	-6.0	97.6
1	5	1	-7.0	91.4
1	5	2	-7.0	96.3
1	5	3	-7.0	93.2
1	6	1	-8.0	97.5
1	6	2	-8.0	88.1
1	6	3	-8.0	93.6
1	7	1	-9.0	95.5
1	7	2	-9.0	95.2
1	7	3	-9.0	97.1
1	8	1	-10.0	93.5
1	8	2	-10.0	97.0
1	8	3	-10.0	94.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.7

Provide information in all blue cells.

Laboratory Code:	TRL B
Compound ID:	Chem Code 22
Run ID:	TTN007
Assay start date:	4/21/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-3.0	81.8
1	1	2	-3.0	81.6
1	1	3	-3.0	74.6
1	2	1	-4.0	93.7
1	2	2	-4.0	95.4
1	2	3	-4.0	86.4
1	3	1	-5.0	74.3
1	3	2	-5.0	81.4
1	3	3	-5.0	61.9
1	4	1	-6.0	91.3
1	4	2	-6.0	98.8
1	4	3	-6.0	99.4
1	5	1	-7.0	93.9
1	5	2	-7.0	92.3
1	5	3	-7.0	94.9
1	6	1	-8.0	93.6
1	6	2	-8.0	95.0
1	6	3	-8.0	100.1
1	7	1	-9.0	92.4
1	7	2	-9.0	95.4
1	7	3	-9.0	94.2
1	8	1	-10.0	91.9
1	8	2	-10.0	96.6
1	8	3	-10.0	96.4
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run TTN007 4-21-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.4789	-0.5157	0.4513
2	Title	-7.0	-0.4881	0.0645	0.0092
3	Title	-8.0	13.1332	12.1938	12.9121
4	Title	-8.5	31.0094	34.4631	31.2304
5	Title	-9.0	49.0238	58.9427	58.2520
6	Title	-9.5	78.9188	75.8519	78.3662
7	Title	-10.0	85.5775	90.1363	90.6613
8	Title	-11.0	94.4189	92.3467	101.8512

	Nonlin fit Table of results	A run TTN007 4-21-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.422
4	TOP	97.39
5	LOGIC50	-8.877
6	HILLSLOPE	-0.8839
7	Std. Error	
8	BOTTOM	1.457
9	TOP	1.644
10	LOGIC50	0.02061
11	HILLSLOPE	0.05500
12	95% Confidence Intervals	
13	BOTTOM	-5.462 to 0.6178
14	TOP	93.96 to 100.8
15	LOGIC50	-8.920 to -8.834
16	HILLSLOPE	-0.9986 to -0.7692
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9956
20	Absolute Sum of Squares	146.5
21	Sy.x	2.706
22	Number of points	
23	Analyzed	24

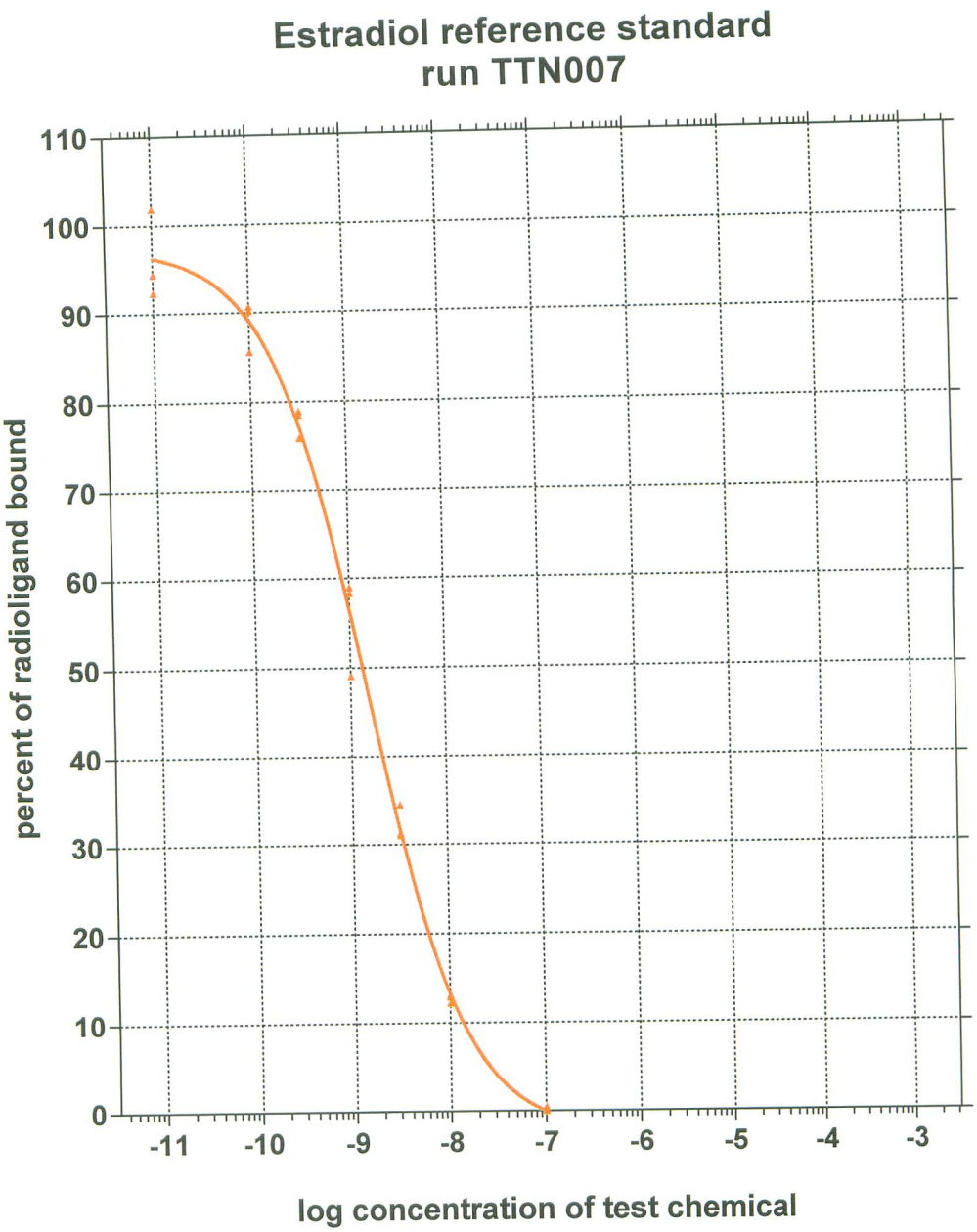

Estradiol reference standard
run TTN007
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run TTN007 4-21-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0	0.9486	1.2249	3.4076
2	Title	-4.5	2.0538	1.8051	2.1919
3	Title	-5.5	28.2741	28.6609	28.1083
4	Title	-6.0	54.3839	47.2831	54.1905
5	Title	-6.5	75.8243	72.7850	73.1719
6	Title	-7.0	88.3128	89.6666	90.6613
7	Title	-7.5	86.5445	94.3083	91.9046
8	Title	-8.5	95.1925	95.3030	88.4509

	Nonlin fit Table of results	A run TTN007 4-21-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.3814
4	TOP	93.98
5	LOGIC50	-5.950
6	HILLSLOPE	-0.9852
7	Std. Error	
8	BOTTOM	1.471
9	TOP	1.265
10	LOGIC50	0.02086
11	HILLSLOPE	0.06667
12	95% Confidence Intervals	
13	BOTTOM	-3.450 to 2.687
14	TOP	91.34 to 96.62
15	LOGIC50	-5.993 to -5.906
16	HILLSLOPE	-1.124 to -0.8461
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9953
20	Absolute Sum of Squares	149.3
21	Sy.x	2.732
22	Number of points	
23	Analyzed	24

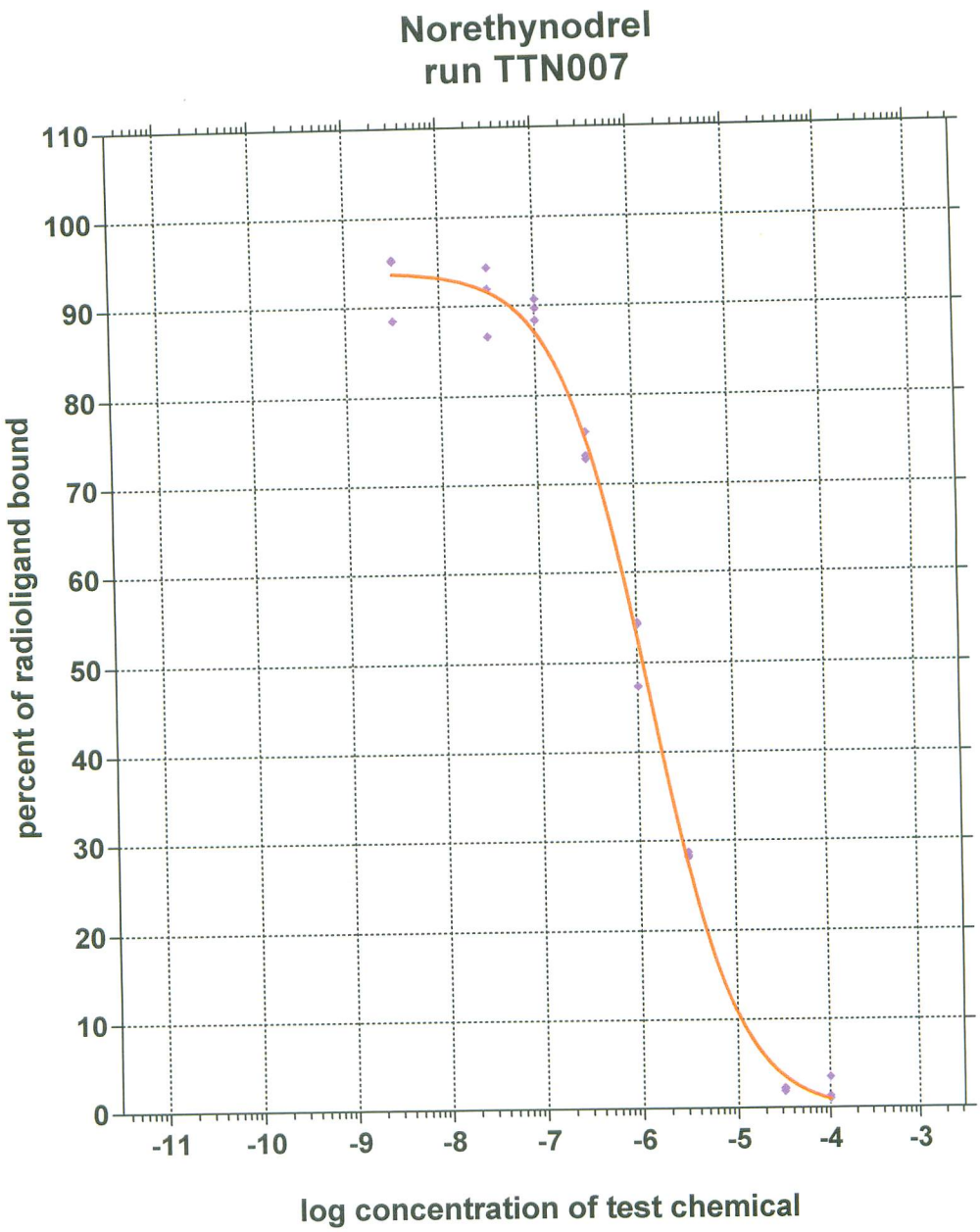

Norethynodrel
run TTN007
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 5 run TTN007		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	2.0538	1.6117	1.1973
3	Title	-5.0	5.8943	7.4415	7.6073
4	Title	-6.0	29.2411	28.3846	29.0753
5	Title	-7.0	65.5185	75.0230	79.9963
6	Title	-8.0	79.3332	83.5605	86.7655
7	Title	-9.0	93.7005	95.3306	93.8110
8	Title	-10.0	91.4073	91.2967	81.3225

Nonlin fit Table of results		A Test chemical 5 run TTN007
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.576
4	TOP	89.77
5	LOGIC50	-6.446
6	HILLSLOPE	-0.9349
7	Std. Error	
8	BOTTOM	2.365
9	TOP	1.695
10	LOGIC50	0.05300
11	HILLSLOPE	0.1097
12	95% Confidence Intervals	
13	BOTTOM	-3.416 to 6.567
14	TOP	86.19 to 93.34
15	LOGIC50	-6.558 to -6.334
16	HILLSLOPE	-1.166 to -0.7035
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9881
20	Absolute Sum of Squares	345.2
21	Sy.x	4.506
22	Number of points	
23	Analyzed	21

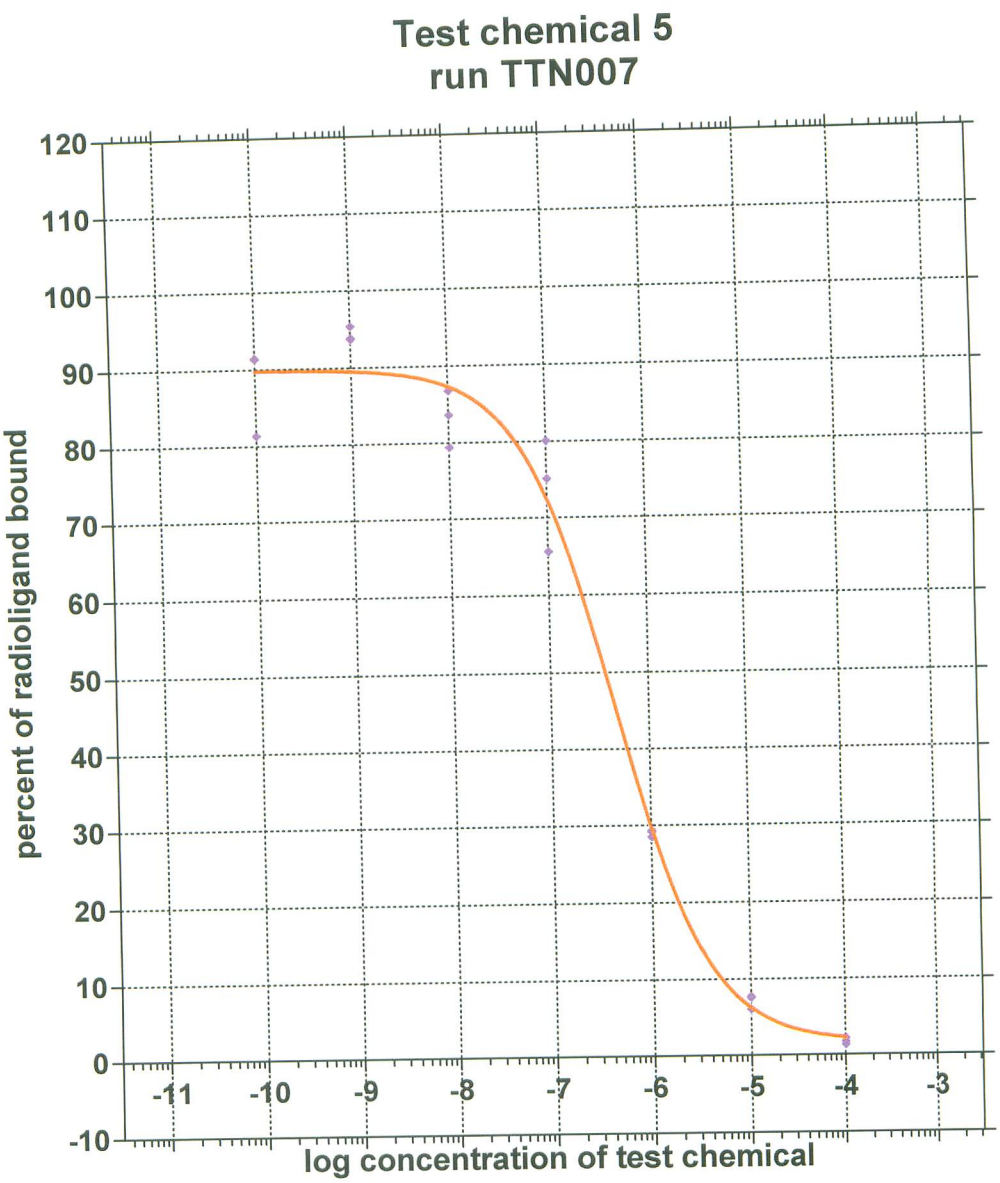

Test chemical 5
run TTN007
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 16 run TTN007		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	2.4959	1.4459	2.4682
2	Title	-4.0	-2.7261	-1.6762	-3.2234
3	Title	-5.0	36.9497	38.3588	37.4194
4	Title	-6.0	91.9322	98.3699	95.7727
5	Title	-7.0	92.6506	100.7184	90.5507
6	Title	-8.0	95.5517	94.6675	97.7344
7	Title	-9.0	99.9447	99.1159	75.8795
8	Title	-10.0	96.4911	91.9322	100.1381

	Nonlin fit Table of results	A Test chemical 16 run TTN007
		Y
1	logIC50	Ambiguous
2	Best-fit values	
3	BOTTOM	-0.2026
4	TOP	94.76
5	LOGIC50	~ -5.041
6	HILLSLOPE	~ -5.665
7	Std. Error	
8	BOTTOM	2.287
9	TOP	1.521
10	LOGIC50	~ 32.52
11	HILLSLOPE	~ 4547
12	95% Confidence Intervals	
13	BOTTOM	-4.974 to 4.569
14	TOP	91.59 to 97.94
15	LOGIC50	(Very wide)
16	HILLSLOPE	(Very wide)
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9864
20	Absolute Sum of Squares	567.3
21	Sy.x	5.326
22	Number of points	
23	Analyzed	24

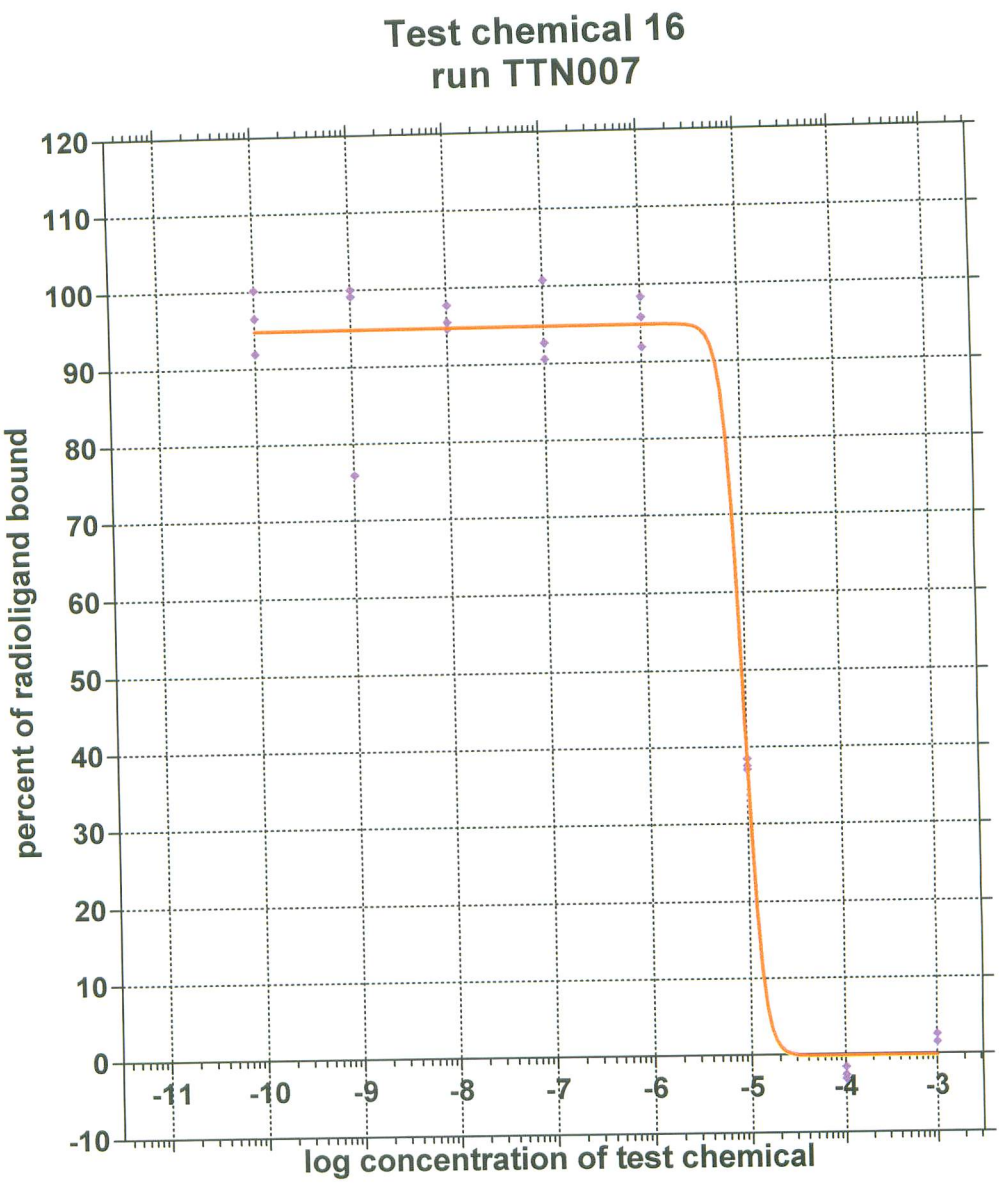

Test chemical 16
run TTN007
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 19 run TTN007		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	15.8409	9.7071	11.1439
2	Title	-4.0	85.6051	88.5614	85.9366
3	Title	-5.0	94.6123	94.8609	95.6069
4	Title	-6.0	95.7451	98.6462	97.5962
5	Title	-7.0	91.3796	96.2700	93.2032
6	Title	-8.0	97.5410	88.0641	93.6452
7	Title	-9.0	95.4688	95.2477	97.0989
8	Title	-10.0	93.5347	96.9884	94.1426

	Nonlin fit Table of results	A Test chemical 19 run TTN007
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	8.426
4	TOP	94.99
5	LOGIC50	-3.563
6	HILLSLOPE	-2.308
7	Std. Error	
8	BOTTOM	146.7
9	TOP	0.6668
10	LOGIC50	2.677
11	HILLSLOPE	15.80
12	95% Confidence Intervals	
13	BOTTOM	-297.6 to 314.5
14	TOP	93.60 to 96.38
15	LOGIC50	-9.146 to 2.020
16	HILLSLOPE	-35.27 to 30.66
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9925
20	Absolute Sum of Squares	132.8
21	Sy.x	2.577
22	Number of points	
23	Analyzed	24

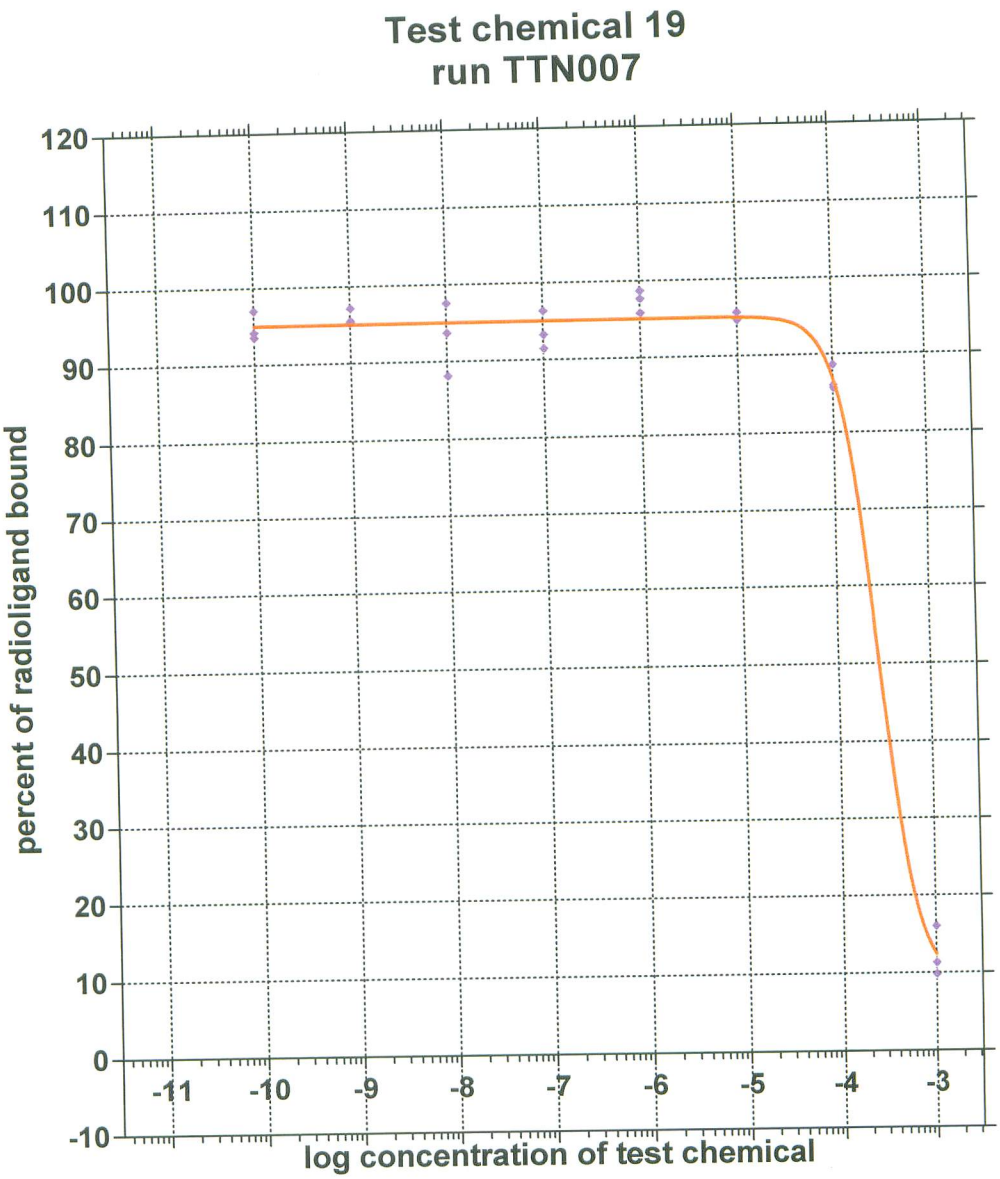
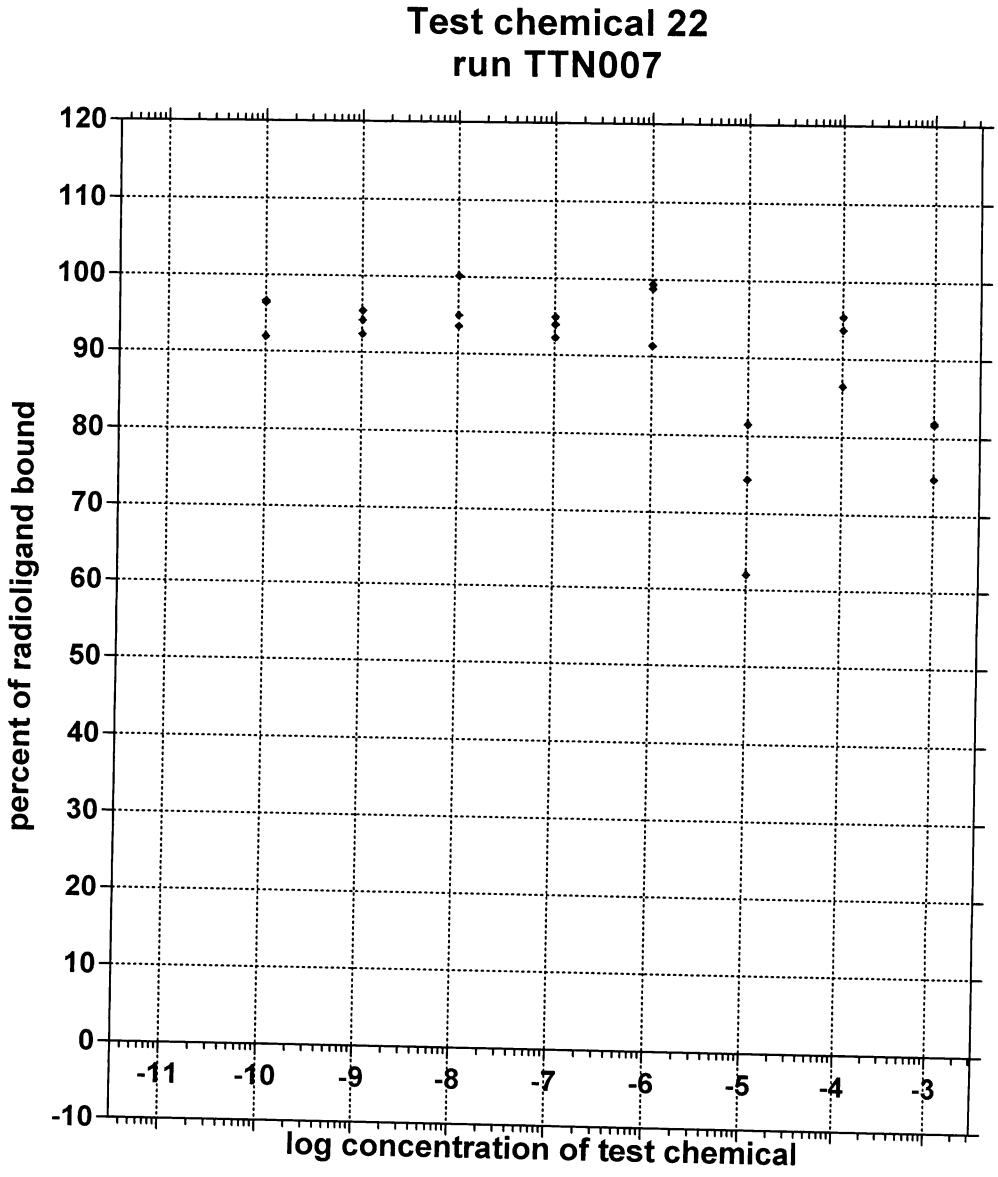

Test chemical 19
run TTN007
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 22 run TTN007		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	81.7646	81.5712	74.6362
2	Title	-4.0	93.6729	95.3859	86.3787
3	Title	-5.0	74.3047	81.4054	61.8991
4	Title	-6.0	91.3244	98.7567	99.3645
5	Title	-7.0	93.9492	92.2638	94.9162
6	Title	-8.0	93.5624	94.9714	100.1381
7	Title	-9.0	92.3743	95.4135	94.1702
8	Title	-10.0	91.9046	96.5740	96.3805

	Nonlin fit Table of results	A Test chemical 22 run TTN007
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	72.54
4	TOP	96.48
5	LOGIC50	-3.415
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	24


Test chemical 22
run TTN007
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

APPENDIX J

Excel and Prism Files of the Run Identifier TTN008

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B
Run identification:	TTN008
Assay start date:	4/22/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	109.12 Ci/mmole
Cytosol vial or lot identification:	
Protein (cytosol):	50 microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.86 M
Weak Positive, Max Concentration:	5.00E-03 M
log(IC50), norethynodrel (Weak Positive):	-5.91 M
RBA, norethynodrel (Weak Positive):	1.130E-03 relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03 M
log(IC50), Unknown 1:	-6.51 5.0
RBA, Unknown 1:	4.436E-03 relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03 M
log(IC50), Unknown 2:	-5.11 16.0
RBA, Unknown 2:	1.778E-04 relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03 M
log(IC50), Unknown 3:	-5.38 17.0
RBA, Unknown 3:	3.311E-04 relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03 M
log(IC50), Unknown 4:	-3.27 22.0
RBA, Unknown 4:	2.547E-06 relative to E2=1.0
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Summary values

	n	Mean	SD	
EtOH	6	8043.1	939.90	Total Binding, solvent control, tubes
Hot	6	84956.9	5257.52	Total hot E2 added to each tube
NSB	6	481.9	21.49	Nonspecific Binding
Specific EtOH	6	7561.3	939.90	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.09	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.06	around 0.25 ?	

log conc'n at min hot binding		min hot (%)
cold E2	-7.0	0.0
R1881	#N/A	not applicable
norethynodrel	-4.5	0.9
Unknown 1	-4.0	0.7
Unknown 2	-4.0	-2.1
Unknown 3	-4.0	-0.7
Unknown 4	-3.0	41.4

Values for analysis by nonlinear regression

- Standard Curve
- Negative Control
- Weak Positive
- Unknown

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 9.47% — If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5564.70	8347.05	TRUE			—	104.0	8347.05	7865.2	77091.7	104.0	110.4	104.0
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5981.19	8971.79	TRUE			—	112.3	8971.785	8489.9	76467.0	112.3		112.3
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	6111.56	9167.34	TRUE			—	114.9	9167.34	8685.5	76271.4	114.9		114.9
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	333.63	500.45	TRUE		cold E2	-7.0	0.2	500.445	18.6	84938.3	0.2	0.0	0.2
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	332.08	498.12	TRUE		cold E2	-7.0	0.2	498.12	16.2	84940.7	0.2		0.2
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	304.17	456.26	TRUE		cold E2	-7.0	-0.3	456.255	-25.6	84982.5	-0.3		-0.3
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	839.02	1258.53	TRUE		cold E2	-8.0	10.3	1258.53	776.6	84180.2	10.3	11.2	10.3
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	949.33	1424.00	TRUE		cold E2	-8.0	12.5	1423.995	942.1	84014.8	12.5		12.5
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	864.56	1296.84	TRUE		cold E2	-8.0	10.8	1296.84	815.0	84141.9	10.8		10.8
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1806.04	2709.06	TRUE		cold E2	-8.5	29.5	2709.06	2227.2	82729.7	29.5	28.7	29.5
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1766.87	2650.31	TRUE		cold E2	-8.5	28.7	2650.305	2168.4	82788.5	28.7		28.7
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1725.87	2588.81	TRUE		cold E2	-8.5	27.9	2588.805	2106.9	82850.0	27.9		27.9
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3222.50	4833.75	TRUE		cold E2	-9.0	57.6	4833.75	4351.9	80605.0	57.6	57.4	57.6
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3155.04	4732.56	TRUE		cold E2	-9.0	56.2	4732.56	4250.7	80706.2	56.2		56.2
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3271.80	4907.70	TRUE		cold E2	-9.0	58.5	4907.7	4425.8	80531.1	58.5		58.5
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4616.51	6924.77	TRUE		cold E2	-9.5	85.2	6924.765	6442.9	78514.0	85.2	85.7	85.2
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4612.95	6919.43	TRUE		cold E2	-9.5	85.1	6919.425	6437.5	78519.4	85.1		85.1
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4688.32	7032.48	TRUE		cold E2	-9.5	86.6	7032.48	6550.6	78406.3	86.6		86.6
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5028.92	7543.38	TRUE		cold E2	-10.0	93.4	7543.38	7061.5	77895.4	93.4	99.1	93.4
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5484.42	8226.63	TRUE		cold E2	-10.0	102.4	8226.63	7744.7	77212.1	102.4		102.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5429.70	8144.55	TRUE		cold E2	-10.0	101.3	8144.55	7662.7	77294.2	101.3		101.3
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5128.29	7692.44	TRUE		cold E2	-11.0	95.4	7692.435	7210.5	77746.3	95.4	101.2	95.4
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5627.51	8441.27	TRUE		cold E2	-11.0	105.3	8441.265	7959.4	76997.5	105.3		105.9
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5511.42	8267.13	TRUE		cold E2	-11.0	103.0	8267.13	7785.2	77171.6	103.0		103.0
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

Competitive Ass... Layout - Four Test Chemicals

Check the 10% rule: 9.47%

If the ratio of EtOH / Hot is > 10% there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2342.31	3513.47	TRUE		norethynodrel	-4.0	40.1	3513.465	3031.6	81925.3	40.1	41.4	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2498.88	3748.32	TRUE		norethynodrel	-4.0	43.2	3748.32	3266.4	81690.5	43.2		
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2381.78	3572.67	TRUE		norethynodrel	-4.0	40.9	3572.67	3090.8	81866.1	40.9		
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	363.00	544.50	TRUE		norethynodrel	-4.5	0.8	544.5	62.6	84894.3	0.8	0.9	0.8
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	352.83	529.25	TRUE		norethynodrel	-4.5	0.6	529.245	47.4	84909.5	0.6		0.6
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	379.53	569.30	TRUE		norethynodrel	-4.5	1.2	569.295	87.4	84869.5	1.2		1.2
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	2311.55	3467.33	TRUE		norethynodrel	-5.5	39.5	3467.325	2985.4	81971.5	39.5	30.2	39.5
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1629.77	2444.66	TRUE		norethynodrel	-5.5	26.0	2444.655	1962.8	82994.1	26.0		26.0
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1583.56	2375.37	TRUE		norethynodrel	-5.5	25.0	2375.37	1893.5	83063.4	25.0		25.0
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2887.33	4331.00	TRUE		norethynodrel	-6.0	50.9	4330.995	3849.1	81107.8	50.9	52.6	50.9
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2966.17	4449.26	TRUE		norethynodrel	-6.0	52.5	4449.255	3967.4	80989.5	52.5		52.5
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3064.49	4596.74	TRUE		norethynodrel	-6.0	54.4	4596.735	4114.8	80842.0	54.4		54.4
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4192.79	6289.19	TRUE		norethynodrel	-6.5	76.8	6289.185	5807.3	79149.6	76.8	80.5	76.8
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4471.40	6707.10	TRUE		norethynodrel	-6.5	82.3	6707.1	6225.2	78731.7	82.3		82.3
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4479.86	6719.79	TRUE		norethynodrel	-6.5	82.6	6719.79	6237.9	78719.0	82.5		82.5
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5085.34	7628.01	TRUE		norethynodrel	-7.0	94.5	7628.01	7146.1	77810.8	94.5	97.6	94.5
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5318.12	7977.18	TRUE		norethynodrel	-7.0	99.1	7977.18	7495.3	77461.6	99.1		99.1
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5319.21	7978.82	TRUE		norethynodrel	-7.0	99.1	7978.815	7496.9	77460.0	99.1		99.1
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5436.00	8154.00	TRUE		norethynodrel	-7.5	101.5	8154	7672.1	77284.8	101.5	104.1	101.5
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5558.89	8338.34	TRUE		norethynodrel	-7.5	103.9	8338.335	7856.4	77100.4	103.9		103.9
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5714.21	8571.32	TRUE		norethynodrel	-7.5	107.0	8571.315	8089.4	76867.5	107.0		107.0
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5604.06	8406.09	TRUE		norethynodrel	-8.5	104.8	8406.09	7924.2	77032.7	104.8	106.7	104.8
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5805.62	8708.43	TRUE		norethynodrel	-8.5	108.8	8708.43	8226.5	76730.3	108.8		108.8
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5687.30	8530.95	TRUE		norethynodrel	-8.5	106.5	8530.95	8049.1	76907.8	106.5		106.5
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2613.93	3920.90	TRUE		Unknown 1	-3.0	45.5	3920.895	3439.0	81517.9	45.5	80.7	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	5752.11	8628.17	TRUE		Unknown 1	-3.0	107.7	8628.165	8146.3	76810.6	107.7		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4806.25	7209.38	TRUE		Unknown 1	-3.0	89.0	7209.375	6727.5	78229.4	89.0		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	367.92	551.88	TRUE		Unknown 1	-4.0	0.9	551.88	70.0	84886.9	0.9	0.7	0.9
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	338.13	507.20	TRUE		Unknown 1	-4.0	0.3	507.195	25.3	84931.6	0.3		0.3
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	358.78	538.17	TRUE		Unknown 1	-4.0	0.7	538.17	56.3	84900.6	0.7		0.7
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	640.83	961.25	TRUE		Unknown 1	-5.0	6.3	961.245	479.4	84477.5	6.3	6.6	6.3
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	675.10	1012.65	TRUE		Unknown 1	-5.0	7.0	1012.65	530.8	84426.1	7.0		7.0
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	639.90	959.85	TRUE		Unknown 1	-5.0	6.3	959.85	478.0	84478.9	6.3		6.3
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1449.78	2174.67	TRUE		Unknown 1	-6.0	22.4	2174.67	1692.8	83264.1	22.4	23.3	22.4
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1493.52	2240.28	TRUE		Unknown 1	-6.0	23.3	2240.28	1758.4	83198.5	23.3		23.3
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1536.85	2305.28	TRUE		Unknown 1	-6.0	24.1	2305.275	1823.4	83133.5	24.1		24.1
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3843.28	5764.92	TRUE		Unknown 1	-7.0	69.9	5764.92	5283.0	79673.9	69.9	77.2	69.9
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4306.77	6460.16	TRUE		Unknown 1	-7.0	79.1	6460.155	5978.3	78978.6	79.1		79.1
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4484.53	6726.80	TRUE		Unknown 1	-7.0	82.6	6726.795	6244.9	78712.0	82.6		82.6
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5324.04	7986.06	TRUE		Unknown 1	-8.0	99.2	7986.06	7504.2	77452.7	99.2	101.3	99.2
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5414.47	8121.71	TRUE		Unknown 1	-8.0	101.0	8121.705	7639.8	77317.1	101.0		101.0
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5537.13	8305.70	TRUE		Unknown 1	-8.0	103.5	8305.695	7823.8	77133.1	103.5		103.5
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5381.69	8072.54	TRUE		Unknown 1	-9.0	100.4	8072.535	7590.6	77366.2	100.4	104.0	100.4
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5756.95	8635.43	TRUE		Unknown 1	-9.0	107.8	8635.425	8153.5	76803.4	107.8		107.8
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5550.00	8325.00	TRUE		Unknown 1	-9.0	103.7	8325	7843.1	77113.8	103.7		103.7
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5404.83	8107.25	TRUE		Unknown 1	-10.0	100.8	8107.245	7625.4	77331.5	100.8	101.8	100.8
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5410.52	8115.78	TRUE		Unknown 1	-10.0	101.0	8115.78	7633.9	77323.0	101.0		101.0
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5547.58	8321.37	TRUE		Unknown 1	-10.0	103.7	8321.37	7839.5	77117.4	103.7		103.7
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	328.46	492.69	TRUE		Unknown 2	-3.0	0.1	492.69	10.8	84946.1	0.1	0.4	0.1
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	328.07	492.11	TRUE		Unknown 2	-3.0	0.1	492.105	10.2	84946.7	0.1		0.1
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	370.74	556.11	TRUE		Unknown 2	-3.0	1.0	556.11	74.2	84882.7	1.0		1.0
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	217.51	326.27	TRUE		Unknown 2	-4.0	-2.1	326.265	-155.6	85112.5	-2.1	-2.1	-2.1
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	234.26	351.39	TRUE		Unknown 2	-4.0	-1.7	351.39	-130.5	85087.4	-1.7		-1.7
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	199.90	299.85	TRUE		Unknown 2	-4.0	-2.4	299.85	-182.0	85138.9	-2.4		-2.4
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2283.15	3424.73	TRUE		Unknown 2	-5.0	38.9	3424.725	2942.8	82014.1	38.9	39.1	38.9
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2345.90	3518.85	TRUE		Unknown 2	-5.0	40.2	3518.85	3037.0	81919.9	40.2		40.2
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2251.26	3376.89	TRUE		Unknown 2	-5.0	38.3	3376.89	2895.0	82061.9	38.3		38.3
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5175.87	7763.81	TRUE		Unknown 2	-6.0	96.3	7763.805	7281.9	77675.0	96.3	102.6	96.3
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5633.76	8450.64	TRUE		Unknown 2	-6.0	105.4	8450.64	7968.8	76988.1	105.4		105.4
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5671.79	8507.69	TRUE		Unknown 2	-6.0	106.1	8507.685	8025.8	76931.1	106.1		106.1
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5546.39	8319.59	TRUE		Unknown 2	-7.0	103.7	8319.585	7837.7	77119.2	103.7	106.8	103.7
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5892.62	8838.93	TRUE		Unknown 2	-7.0	110.5	8838.93	8357.0	76599.8	110.5		110.5
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5671.66	8507.49	TRUE		Unknown 2	-7.0	106.1	8507.49	8025.6	76931.3	106.1		106.1
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5910.54	8865.81	TRUE		Unknown 2	-8.0	110.9	8865.81	8383.9	76573.0	110.9	108.7	110.9
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5834.43	8751.65	TRUE		Unknown 2	-8.0	109.4	8751.645	8269.8	76687.1	109.4		109.4
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5654.46	8481.69	TRUE		Unknown 2	-8.0	105.8	8481.69	7999.8	76957.1	105.8		105.8
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5604.37	8406.56	TRUE		Unknown 2	-9.0	104.8	8406.555	7924.7	77032.2	104.8	106.9	104.8
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5674.23	8511.35	TRUE		Unknown 2	-9.0	106.2	8511.345	8029.5	76927.4	106.2		106.2
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5847.84	8771.76	TRUE		Unknown 2	-9.0	109.6	8771.76	8289.9	76667.0	109.6		109.6
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5603.43	8405.15	TRUE		Unknown 2	-10.0	104.8	8405.145	7923.3	77033.6	104.8	107.3	104.8
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5760.14	8640.21	TRUE		Unknown 2	-10.0	107.9	8640.21	8158.3	76798.6	107.9		107.9

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 9.47%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5827.18	8740.77	TRUE		Unknown 2	-10.0	109.2	8740.77	8258.9	76698.0	109.2		109.2
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1971.23	2956.85	TRUE		Unknown 3	-3.0	32.7	2956.845	2475.0	82481.9	32.7	34.5	
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2005.02	3007.53	TRUE		Unknown 3	-3.0	33.4	3007.53	2525.6	82431.2	33.4		
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2211.03	3316.55	TRUE		Unknown 3	-3.0	37.5	3316.545	2834.7	82122.2	37.5		
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	286.13	429.20	TRUE		Unknown 3	-4.0	-0.7	429.195	-52.7	85009.6	-0.7	-0.7	-0.7
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	276.86	415.29	TRUE		Unknown 3	-4.0	-0.9	415.29	-66.6	85023.5	-0.9		-0.9
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	295.92	443.88	TRUE		Unknown 3	-4.0	-0.5	443.88	-38.0	84994.9	-0.5		-0.5
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1625.74	2438.61	TRUE		Unknown 3	-5.0	25.9	2438.61	1956.7	83000.2	25.9	26.0	25.9
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1684.35	2526.53	TRUE		Unknown 3	-5.0	27.0	2526.525	2044.6	82912.3	27.0		27.0
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1581.24	2371.86	TRUE		Unknown 3	-5.0	25.0	2371.86	1890.0	83066.9	25.0		25.0
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4595.18	6892.77	TRUE		Unknown 3	-6.0	84.8	6892.77	6410.9	78546.0	84.8	86.4	84.8
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4739.29	7108.94	TRUE		Unknown 3	-6.0	87.6	7108.935	6627.0	78329.8	87.6		87.6
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4695.52	7043.28	TRUE		Unknown 3	-6.0	86.8	7043.28	6561.4	78395.5	86.8		86.8
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5767.90	8651.85	TRUE		Unknown 3	-7.0	108.1	8651.85	8170.0	76786.9	108.1	107.6	108.1
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5678.15	8517.23	TRUE		Unknown 3	-7.0	106.3	8517.225	8035.3	76921.6	106.3		106.3
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5796.17	8694.26	TRUE		Unknown 3	-7.0	108.6	8694.255	8212.4	76744.5	108.6		108.6
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5488.88	8233.32	TRUE		Unknown 3	-8.0	102.5	8233.32	7751.4	77205.5	102.5	107.9	102.5
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5879.72	8819.58	TRUE		Unknown 3	-8.0	110.3	8819.58	8337.7	76619.2	110.3		110.3
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5909.00	8863.50	TRUE		Unknown 3	-8.0	110.8	8863.5	8381.6	76575.3	110.8		110.8
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5397.74	8096.61	TRUE		Unknown 3	-9.0	100.7	8096.61	7614.7	77342.2	100.7	107.3	100.7
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5997.12	8995.68	TRUE		Unknown 3	-9.0	112.6	8995.68	8513.8	76443.1	112.6		112.6
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5795.55	8693.33	TRUE		Unknown 3	-9.0	108.6	8693.325	8211.4	76745.5	108.6		108.6
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5433.83	8150.75	TRUE		Unknown 3	-10.0	101.4	8150.745	7668.9	77288.0	101.4	106.5	101.4
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5751.11	8626.67	TRUE		Unknown 3	-10.0	107.7	8626.665	8144.8	76812.1	107.7		107.7
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5890.50	8835.75	TRUE		Unknown 3	-10.0	110.5	8835.75	8353.9	76603.0	110.5		110.5
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2425.95	3638.93	TRUE		Unknown 4	-3.0	41.8	3638.925	3157.0	81799.9	41.8	41.4	41.8
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2431.28	3646.92	TRUE		Unknown 4	-3.0	41.9	3646.92	3165.0	81791.9	41.9		41.9
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2368.06	3552.09	TRUE		Unknown 4	-3.0	40.6	3552.09	3070.2	81886.7	40.6		40.6
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	5091.35	7637.03	TRUE		Unknown 4	-4.0	94.6	7637.025	7155.1	77801.8	94.6	86.3	93.7
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3805.71	5708.57	TRUE		Unknown 4	-4.0	69.1	5708.565	5226.7	79730.2	69.1		95.4
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	5121.28	7681.92	TRUE		Unknown 4	-4.0	95.2	7681.92	7200.0	77756.9	95.2		86.4
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	5409.51	8114.27	TRUE		Unknown 4	-5.0	100.9	8114.265	7632.4	77324.5	100.9	105.1	100.9
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	5811.96	8717.94	TRUE		Unknown 4	-5.0	108.9	8717.94	8236.1	76720.8	108.9		108.9
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	5629.00	8443.50	TRUE		Unknown 4	-5.0	105.3	8443.5	7961.6	76995.3	105.3		105.3
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5469.26	8203.89	TRUE		Unknown 4	-6.0	102.1	8203.89	7722.0	77234.9	102.1	105.0	102.1
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5640.12	8460.18	TRUE		Unknown 4	-6.0	105.5	8460.18	7978.3	76978.6	105.5		105.5
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	5732.10	8598.15	TRUE		Unknown 4	-6.0	107.3	8598.15	8116.3	76840.6	107.3		107.3
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	6059.85	9089.78	TRUE		Unknown 4	-7.0	113.8	9089.775	8607.9	76349.0	113.8	107.6	113.8
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5441.37	8162.06	TRUE		Unknown 4	-7.0	101.6	8162.055	7680.2	77276.7	101.6		101.6
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5728.60	8592.90	TRUE		Unknown 4	-7.0	107.3	8592.9	8111.0	76845.9	107.3		107.3
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5043.29	7564.94	TRUE		Unknown 4	-8.0	93.7	7564.935	7083.0	77873.8	93.7	103.2	93.7
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5671.08	8506.62	TRUE		Unknown 4	-8.0	106.1	8506.62	8024.7	76932.2	106.1		106.1
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5855.70	8783.55	TRUE		Unknown 4	-8.0	109.8	8783.55	8301.7	76655.2	109.8		109.8
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5437.45	8156.18	TRUE		Unknown 4	-9.0	101.5	8156.175	7674.3	77282.6	101.5	105.9	101.5
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5925.57	8888.36	TRUE		Unknown 4	-9.0	111.2	8888.355	8406.5	76550.4	111.2		111.2
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5612.80	8419.20	TRUE		Unknown 4	-9.0	105.0	8419.2	7937.3	77019.6	105.0		105.0
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5398.75	8098.13	TRUE		Unknown 4	-10.0	100.7	8098.125	7616.2	77340.7	100.7	104.4	100.7
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5543.93	8315.90	TRUE		Unknown 4	-10.0	103.6	8315.895	7834.0	77122.9	103.6		103.6
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5813.76	8720.64	TRUE		Unknown 4	-10.0	109.0	8720.64	8238.8	76718.1	109.0		109.0
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	302.02	453.03	TRUE		cold E2	-7.0	-0.4	453.03	-28.9	84985.7	-0.4	0.0	-0.4
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	329.85	494.78	TRUE		cold E2	-7.0	0.2	494.775	12.9	84944.0	0.2		0.2
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	325.80	488.70	TRUE		cold E2	-7.0	0.1	488.7	6.8	84950.1	0.1		0.1
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4537.63	6806.45	TRUE			—	83.6	6806.445	6324.6	78632.3	83.6	89.6	83.6
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4892.30	7338.45	TRUE			—	90.7	7338.45	6856.6	78100.3	90.7		90.7
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5085.18	7627.77	TRUE			—	94.5	7627.77	7145.9	77811.0	94.5		94.5
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	91122.98	91122.98	TRUE					91122.98	90641.1				
179	2	none	Hot		—	—	—	10	—	—	—	—	91464.15	91464.15	TRUE					91464.15	90982.3				
180	3	none	Hot		—	—	—	10	—	—	—	—	78527.71	78527.71	TRUE					78527.71	78045.8				
181	1	none	Hot		—	—	—	10	—	—	—	—	82389.59	82389.59	TRUE					82389.59	81907.7				
182	2	none	Hot		—	—	—	10	—	—	—	—	81861.97	81861.97	TRUE					81861.97	81380.1				
183	3	none	Hot		—	—	—	10	—	—	—	—	84374.94	84374.94	TRUE					84374.94	83893.1				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				5.0				16.0				17.0				22.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	0.2454	0.2147	-0.3390	-4.0				-3.0				-3.0	0.1429	0.1351	0.9816	-3.0				-3.0	41.7528	41.8586	40.6044	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.3816	0.1704	0.0901	-4.5	0.8281	0.6263	1.1560	-4.0	0.9257	0.3347	0.7444	-4.0	-2.0582	-1.7259	-2.4075	-4.0	-0.6969	-0.8808	-0.5027	-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	10.2713	12.4597	10.7780	-5.5	39.4834	25.9582	25.0419	-5.0	6.3397	7.0195	6.3212	-5.0	38.9200	40.1648	38.2873	-5.0	25.8783	27.0410	24.9955	-5.0	100.9407	108.9244	105.2949	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	29.4551	28.6780	27.8647	-6.0	50.9057	52.4697	54.4202	-6.0	22.3876	23.2553	24.1149	-6.0	96.3057	105.3893	106.1438	-6.0	84.7860	87.6448	86.7765	-6.0	102.1260	105.5155	107.3402	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	57.5548	56.2165	58.5328	-6.5	76.8034	82.3304	82.4983	-7.0	69.8698	79.0645	82.5909	-7.0	103.6561	110.5246	106.1412	-7.0	108.0504	106.2699	108.6112	-7.0	113.8421	101.5727	107.2708	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	85.2091	85.1385	86.6337	-7.0	94.5098	99.1277	99.1493	-8.0	99.2451	101.0390	103.4724	-8.0	110.8801	109.3702	105.8000	-8.0	102.5152	110.2687	110.8495	-8.0	93.6756	106.1297	109.7922	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	93.3905	102.4267	101.3412	-7.5	101.4662	103.9040	106.9853	-9.0	100.3888	107.8332	103.7277	-9.0	104.8063	106.1922	109.6362	-9.0	100.7072	112.5976	108.5989	-9.0	101.4949	111.1782	104.9735	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	95.3618	105.2653	102.9623	-8.5	104.8001	108.7987	106.4514	-10.0	100.8478	100.9607	103.6797	-10.0	104.7876	107.8964	109.2264	-10.0	101.4231	107.7173	110.4825	-10.0	100.7272	103.6073	108.9602	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		TTN008 4-22-08		Positive Control		TTN008 4-22-08		5.0		TTN008 4-22-08		16.0		TTN008 4-22-08		17.0		TTN008 4-22-08		22.0		TTN008 4-22-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values			
13	1	BOTTOM		-0.4359		BOTTOM		-4.385		BOTTOM		2.121		BOTTOM		-1.483		BOTTOM		-3.839		BOTTOM		35.05		BOTTOM			
14	2	TOP		103.3		TOP		108.5		TOP		103.5		TOP		107.3		TOP		107.8		TOP		105.3		TOP			
15	3	LOGIC50		-8.861		LOGIC50		-5.914		LOGIC50		-6.508		LOGIC50		-5.111		LOGIC50		-5.381		LOGIC50		-3.267		LOGIC50			
16	1	HILLSLOPE		-1.083		HILLSLOPE		-0.8705		HILLSLOPE		-1.03		HILLSLOPE		-1.649		HILLSLOPE		-1.08		HILLSLOPE		-1.627		HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.186		BOTTOM		3.443		BOTTOM		1.467		BOTTOM		1.222		BOTTOM		2.553		BOTTOM		55.42		BOTTOM			
19	1	TOP		1.42		TOP		1.986		TOP		1.152		TOP		0.8055		TOP		1.02		TOP		1.204		TOP			
20	2	LOGIC50		0.01726		LOGIC50		0.02687		LOGIC50		0.03059		LOGIC50		0.01958		LOGIC50		0.02957		LOGIC50		0.7845		LOGIC50			
21	3	HILLSLOPE		0.05911		HILLSLOPE		0.07685		HILLSLOPE		0.06876		HILLSLOPE		0.1973		HILLSLOPE		0.07607		HILLSLOPE		3.36		HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.8

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Estradiol
Run ID: TTN008
Assay start date: 4/22/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-7.0	0.2
1	1	2	-7.0	0.2
1	1	3	-7.0	-0.3
1	2	1	-8.0	10.3
1	2	2	-8.0	12.5
1	2	3	-8.0	10.8
1	3	1	-8.5	29.5
1	3	2	-8.5	28.7
1	3	3	-8.5	27.9
1	4	1	-9.0	57.6
1	4	2	-9.0	56.2
1	4	3	-9.0	58.5
1	5	1	-9.5	85.2
1	5	2	-9.5	85.1
1	5	3	-9.5	86.6
1	6	1	-10.0	93.4
1	6	2	-10.0	102.4
1	6	3	-10.0	101.3
1	7	1	-11.0	95.4
1	7	2	-11.0	105.3
1	7	3	-11.0	103.0
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.5

Provide information in all blue cells.
Laboratory Code: TRL B
Compound ID: Norethynodrel
Run ID: TTN008
Assay start date: 4/22/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-4.0	40.1
1	1	2	-4.0	43.2
1	1	3	-4.0	40.9
1	2	1	-4.5	0.8
1	2	2	-4.5	0.6
1	2	3	-4.5	1.2
1	3	1	-5.5	39.5
1	3	2	-5.5	26.0
1	3	3	-5.5	25.0
1	4	1	-6.0	50.9
1	4	2	-6.0	52.5
1	4	3	-6.0	54.4
1	5	1	-6.5	76.8
1	5	2	-6.5	82.3
1	5	3	-6.5	82.5
1	6	1	-7.0	94.5
1	6	2	-7.0	99.1
1	6	3	-7.0	99.1
1	7	1	-7.5	101.5
1	7	2	-7.5	103.9
1	7	3	-7.5	107.0
1	8	1	-8.5	104.8
1	8	2	-8.5	108.8
1	8	3	-8.5	106.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 11.6

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID:	Chem Code 5
Run ID:	TTN008
Assay start date:	4/22/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-3.0	45.5
1	1	2	-3.0	107.7
1	1	3	-3.0	89.0
1	2	1	-4.0	0.9
1	2	2	-4.0	0.3
1	2	3	-4.0	0.7
1	3	1	-5.0	6.3
1	3	2	-5.0	7.0
1	3	3	-5.0	6.3
1	4	1	-6.0	22.4
1	4	2	-6.0	23.3
1	4	3	-6.0	24.1
1	5	1	-7.0	69.9
1	5	2	-7.0	79.1
1	5	3	-7.0	82.6
1	6	1	-8.0	99.2
1	6	2	-8.0	101.0
1	6	3	-8.0	103.5
1	7	1	-9.0	100.4
1	7	2	-9.0	107.8
1	7	3	-9.0	103.7
1	8	1	-10.0	100.8
1	8	2	-10.0	101.0
1	8	3	-10.0	103.7
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.8

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID:	Chem Code 16
Run ID:	TTN008
Assay start date:	4/22/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-3.0	0.1
1	1	2	-3.0	0.1
1	1	3	-3.0	1.0
1	2	1	-4.0	-2.1
1	2	2	-4.0	-1.7
1	2	3	-4.0	-2.4
1	3	1	-5.0	38.9
1	3	2	-5.0	40.2
1	3	3	-5.0	38.3
1	4	1	-6.0	96.3
1	4	2	-6.0	105.4
1	4	3	-6.0	106.1
1	5	1	-7.0	103.7
1	5	2	-7.0	110.5
1	5	3	-7.0	106.1
1	6	1	-8.0	110.9
1	6	2	-8.0	109.4
1	6	3	-8.0	105.8
1	7	1	-9.0	104.8
1	7	2	-9.0	106.2
1	7	3	-9.0	109.6
1	8	1	-10.0	104.8
1	8	2	-10.0	107.9
1	8	3	-10.0	109.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
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Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
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   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
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1. SDwithin-run is estimated separately for each run.
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   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.4

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Chem Code 17
Run ID: TTN008
Assay start date: 4/22/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	32.7
1	1	2	-3.0	33.4
1	1	3	-3.0	37.5
1	2	1	-4.0	-0.7
1	2	2	-4.0	-0.9
1	2	3	-4.0	-0.5
1	3	1	-5.0	25.9
1	3	2	-5.0	27.0
1	3	3	-5.0	25.0
1	4	1	-6.0	84.8
1	4	2	-6.0	87.6
1	4	3	-6.0	86.8
1	5	1	-7.0	108.1
1	5	2	-7.0	106.3
1	5	3	-7.0	108.6
1	6	1	-8.0	102.5
1	6	2	-8.0	110.3
1	6	3	-8.0	110.8
1	7	1	-9.0	100.7
1	7	2	-9.0	112.6
1	7	3	-9.0	108.6
1	8	1	-10.0	101.4
1	8	2	-10.0	107.7
1	8	3	-10.0	110.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

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   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.0

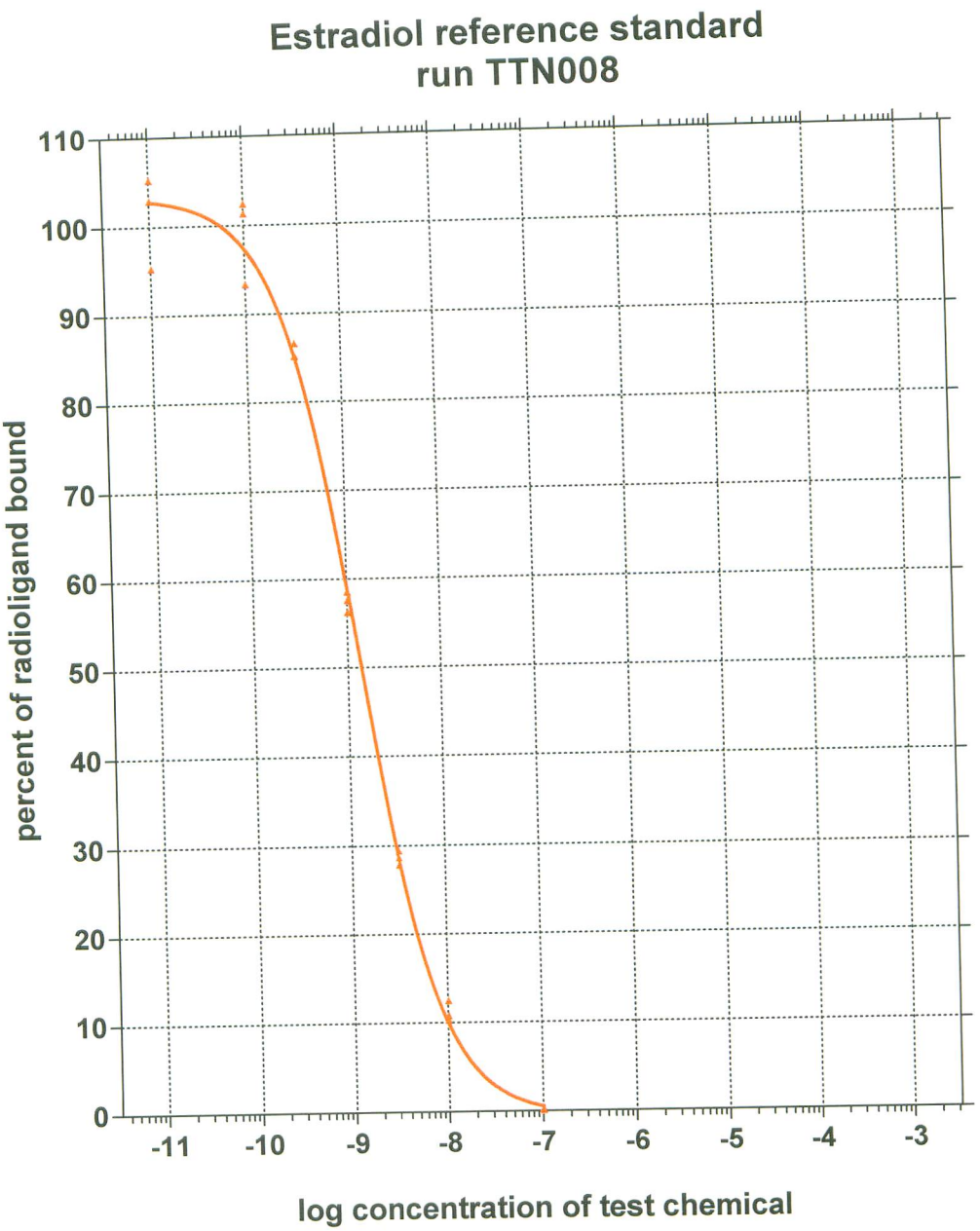
Provide information in all blue cells.

Laboratory Code:	TRL B
Compound ID:	Chem Code 22
Run ID:	TTN008
Assay start date:	4/22/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-3.0	41.8
1	1	2	-3.0	41.9
1	1	3	-3.0	40.6
1	2	1	-4.0	94.6
1	2	2	-4.0	69.1
1	2	3	-4.0	95.2
1	3	1	-5.0	100.9
1	3	2	-5.0	108.9
1	3	3	-5.0	105.3
1	4	1	-6.0	102.1
1	4	2	-6.0	105.5
1	4	3	-6.0	107.3
1	5	1	-7.0	113.8
1	5	2	-7.0	101.6
1	5	3	-7.0	107.3
1	6	1	-8.0	93.7
1	6	2	-8.0	106.1
1	6	3	-8.0	109.8
1	7	1	-9.0	101.5
1	7	2	-9.0	111.2
1	7	3	-9.0	105.0
1	8	1	-10.0	100.7
1	8	2	-10.0	103.6
1	8	3	-10.0	109.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

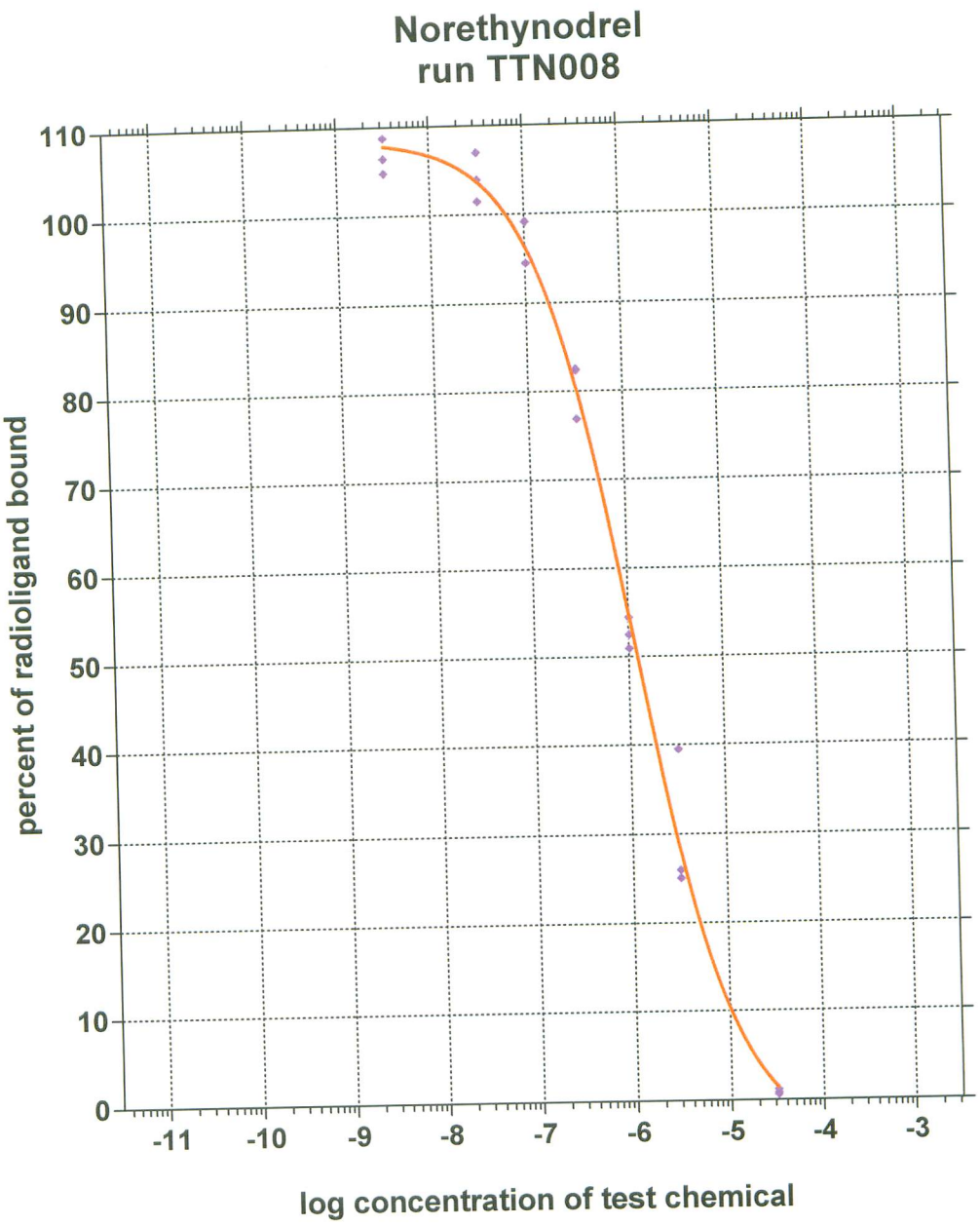
ıble format: XY		X	A		
		log concentration	run TTN008 4-22-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.2454	0.2147	-0.3390
2	Title	-7.0	-0.3816	0.1704	0.0901
3	Title	-8.0	10.2713	12.4597	10.7780
4	Title	-8.5	29.4551	28.6780	27.8647
5	Title	-9.0	57.5548	56.2165	58.5328
6	Title	-9.5	85.2091	85.1385	86.6337
7	Title	-10.0	93.3905	102.4267	101.3412
8	Title	-11.0	95.3618	105.2653	102.9623

	Nonlin fit Table of results	A run TTN008 4-22-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.4359
4	TOP	103.3
5	LOGIC50	-8.861
6	HILLSLOPE	-1.083
7	Std. Error	
8	BOTTOM	1.186
9	TOP	1.420
10	LOGIC50	0.01726
11	HILLSLOPE	0.05911
12	95% Confidence Intervals	
13	BOTTOM	-2.910 to 2.038
14	TOP	100.4 to 106.3
15	LOGIC50	-8.897 to -8.825
16	HILLSLOPE	-1.207 to -0.9602
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9964
20	Absolute Sum of Squares	142.7
21	Sy.x	2.671
22	Number of points	
23	Analyzed	24


Estradiol reference standard
run TTN008
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

ble format: XY		X	A		
		log concentration	run TTN008 4-22-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	0.8281	0.6263	1.1560
3	Title	-5.5	39.4834	25.9582	25.0419
4	Title	-6.0	50.9057	52.4697	54.4202
5	Title	-6.5	76.8034	82.3304	82.4983
6	Title	-7.0	94.5098	99.1277	99.1493
7	Title	-7.5	101.4662	103.9040	106.9853
8	Title	-8.5	104.8001	108.7987	106.4514

	Nonlin fit Table of results	A run TTN008 4-22-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-4.385
4	TOP	108.5
5	LOGIC50	-5.914
6	HILLSLOPE	-0.8705
7	Std. Error	
8	BOTTOM	3.443
9	TOP	1.986
10	LOGIC50	0.02687
11	HILLSLOPE	0.07685
12	95% Confidence Intervals	
13	BOTTOM	-11.65 to 2.880
14	TOP	104.3 to 112.7
15	LOGIC50	-5.971 to -5.858
16	HILLSLOPE	-1.033 to -0.7083
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9922
20	Absolute Sum of Squares	234.2
21	Sy.x	3.712
22	Number of points	
23	Analyzed	21


Norethynodrel
run TTN008
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

ıble format: XY		X	A		
		log concentration	Test chemical 5 run TTN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	0.9257	0.3347	0.7444
3	Title	-5.0	6.3397	7.0195	6.3212
4	Title	-6.0	22.3876	23.2553	24.1149
5	Title	-7.0	69.8698	79.0645	82.5909
6	Title	-8.0	99.2451	101.0390	103.4724
7	Title	-9.0	100.3888	107.8332	103.7277
8	Title	-10.0	100.8478	100.9607	103.6797

	Nonlin fit Table of results	A Test chemical 5 run TTN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	2.121
4	TOP	103.5
5	LOGIC50	-6.508
6	HILLSLOPE	-1.030
7	Std. Error	
8	BOTTOM	1.467
9	TOP	1.152
10	LOGIC50	0.03059
11	HILLSLOPE	0.06876
12	95% Confidence Intervals	
13	BOTTOM	-0.9739 to 5.215
14	TOP	101.0 to 105.9
15	LOGIC50	-6.573 to -6.444
16	HILLSLOPE	-1.175 to -0.8850
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9960
20	Absolute Sum of Squares	163.3
21	Sy.x	3.099
22	Number of points	
23	Analyzed	21

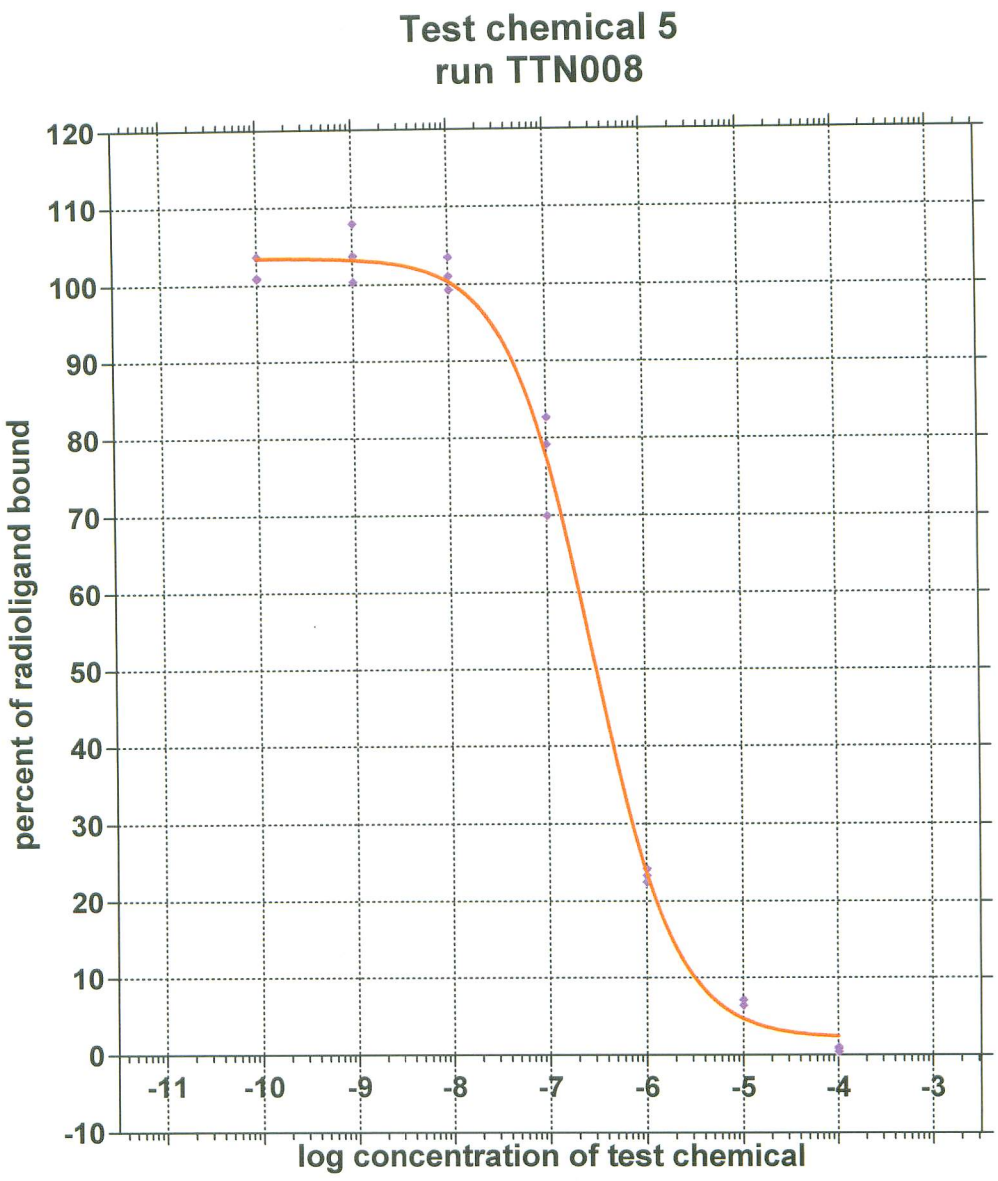
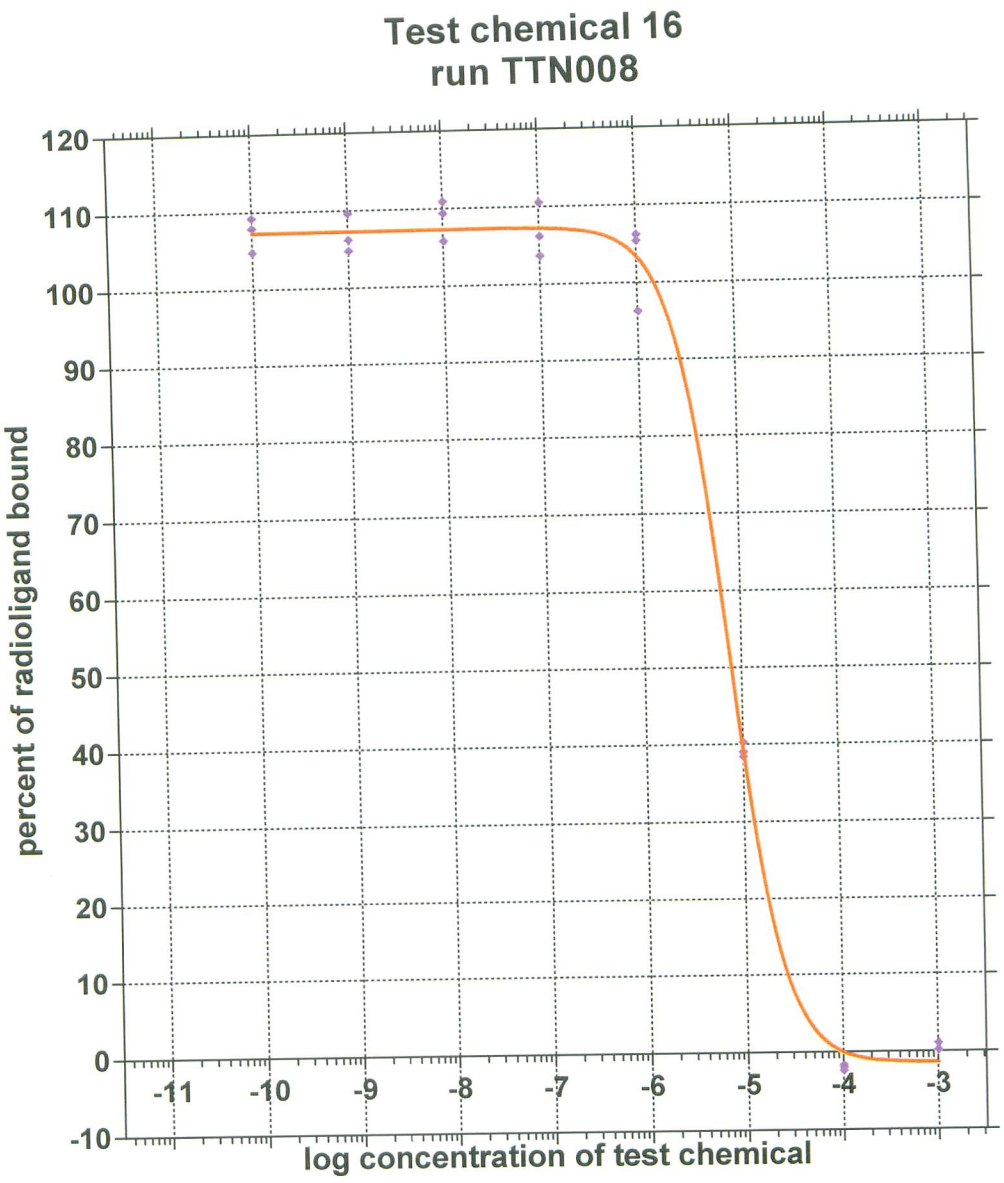

Test chemical 5
run TTN008
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

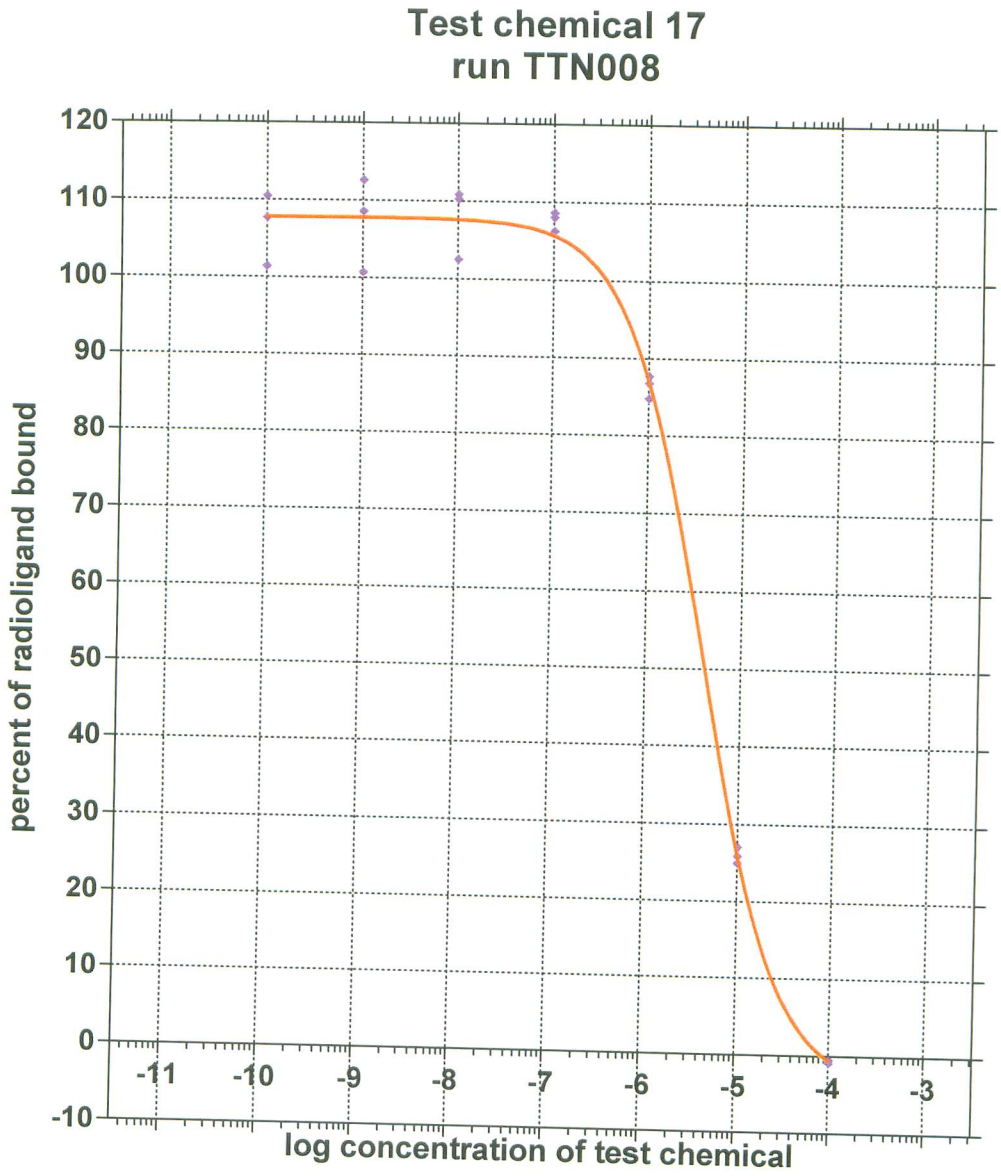
Table format: XY		X	A		
		log concentration	Test chemical 16 run TTN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	0.1429	0.1351	0.9816
2	Title	-4.0	-2.0582	-1.7259	-2.4075
3	Title	-5.0	38.9200	40.1648	38.2873
4	Title	-6.0	96.3057	105.3893	106.1438
5	Title	-7.0	103.6561	110.5246	106.1412
6	Title	-8.0	110.8801	109.3702	105.8000
7	Title	-9.0	104.8063	106.1922	109.6362
8	Title	-10.0	104.7876	107.8964	109.2264

	Nonlin fit Table of results	A Test chemical 16 run TTN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.483
4	TOP	107.3
5	LOGIC50	-5.111
6	HILLSLOPE	-1.649
7	Std. Error	
8	BOTTOM	1.222
9	TOP	0.8055
10	LOGIC50	0.01958
11	HILLSLOPE	0.1973
12	95% Confidence Intervals	
13	BOTTOM	-4.032 to 1.066
14	TOP	105.6 to 109.0
15	LOGIC50	-5.151 to -5.070
16	HILLSLOPE	-2.060 to -1.237
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9971
20	Absolute Sum of Squares	154.1
21	Sy.x	2.775
22	Number of points	
23	Analyzed	24


Test chemical 16
run TTN008
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

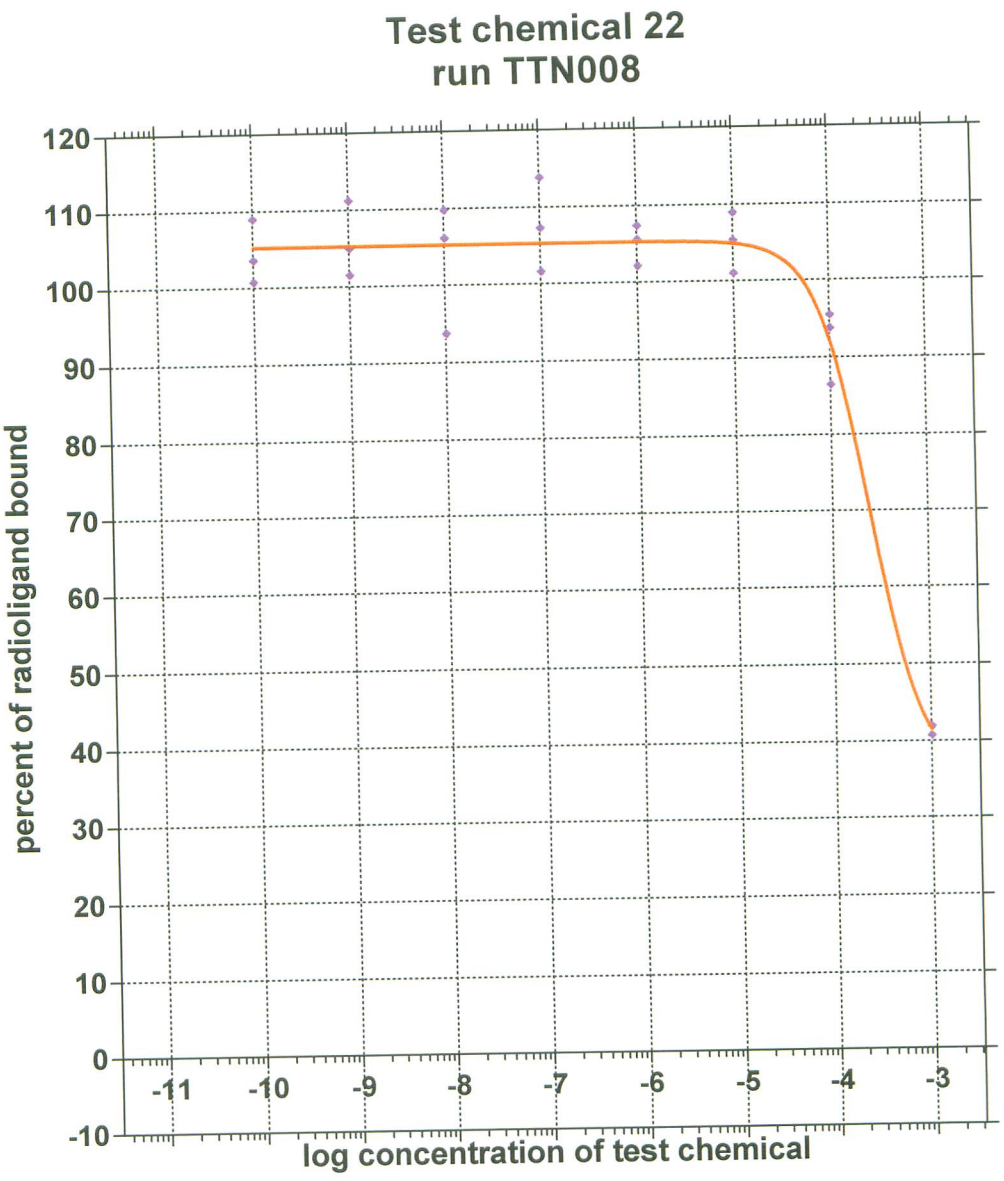
ble format: XY		X	A		
		log concentration	Test chemical 17 run TTN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-0.6969	-0.8808	-0.5027
3	Title	-5.0	25.8783	27.0410	24.9955
4	Title	-6.0	84.7860	87.6448	86.7765
5	Title	-7.0	108.0504	106.2699	108.6112
6	Title	-8.0	102.5152	110.2687	110.8495
7	Title	-9.0	100.7072	112.5976	108.5989
8	Title	-10.0	101.4231	107.7173	110.4825

	Nonlin fit Table of results	A Test chemical 17 run TTN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-3.839
4	TOP	107.8
5	LOGIC50	-5.381
6	HILLSLOPE	-1.080
7	Std. Error	
8	BOTTOM	2.553
9	TOP	1.020
10	LOGIC50	0.02957
11	HILLSLOPE	0.07607
12	95% Confidence Intervals	
13	BOTTOM	-9.225 to 1.548
14	TOP	105.7 to 110.0
15	LOGIC50	-5.444 to -5.319
16	HILLSLOPE	-1.241 to -0.9195
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9950
20	Absolute Sum of Squares	186.9
21	Sy.x	3.316
22	Number of points	
23	Analyzed	21


Test chemical 17
run TTN008
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

ole format: XY		X	A		
		log concentration	Test chemical 22 run TTN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	41.7528	41.8586	40.6044
2	Title	-4.0	93.6729	95.3859	86.3787
3	Title	-5.0	100.9407	108.9244	105.2949
4	Title	-6.0	102.1260	105.5155	107.3402
5	Title	-7.0	113.8421	101.5727	107.2708
6	Title	-8.0	93.6756	106.1297	109.7922
7	Title	-9.0	101.4949	111.1782	104.9735
8	Title	-10.0	100.7272	103.6073	108.9602

	Nonlin fit Table of results	A Test chemical 22 run TTN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	35.05
4	TOP	105.3
5	LOGIC50	-3.267
6	HILLSLOPE	-1.627
7	Std. Error	
8	BOTTOM	55.42
9	TOP	1.204
10	LOGIC50	0.7845
11	HILLSLOPE	3.360
12	95% Confidence Intervals	
13	BOTTOM	-80.56 to 150.7
14	TOP	102.7 to 107.8
15	LOGIC50	-4.903 to -1.630
16	HILLSLOPE	-8.637 to 5.383
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9610
20	Absolute Sum of Squares	426.0
21	Sy.x	4.615
22	Number of points	
23	Analyzed	24


Test chemical 22
run TTN008
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

APPENDIX K

Excel and Prism Files of the Run Identifier TTN009

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	TTN009	
Assay start date:	4/23/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	109.11	Ci/mmole
Cytosol vial or lot identification:		
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.81	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-6.33	M
RBA, norethynodrel (Weak Positive):	3.311E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-6.40	5.0
RBA, Unknown 1:	3.908E-03	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-5.12	16.0
RBA, Unknown 2:	2.056E-04	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-5.38	17.0
RBA, Unknown 3:	3.715E-04	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	n/a	22.0
RBA, Unknown 4:	n/a	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 7.69%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	6831.0	596.15	Total Binding, solvent control, tubes
Hot	6	88857.3	3987.41	Total hot E2 added to each tube
NSB	6	509.8	182.58	Nonspecific Binding
Specific EtOH	6	6321.3	596.15	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.08	less than 0.1?
R1881 / EtOH	#DIV/0!	close to 1.0 ?
NSB / EtOH	0.07	around 0.25 ?

at 1.0 E-6 M only

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	1.6
R1881	#N/A	not applicable
norethynodrel	-4.5	-0.7
Unknown 1	-4.0	-0.5
Unknown 2	-4.0	-4.7
Unknown 3	-4.0	13.7
Unknown 4	-3.0	83.1

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4751.00	7126.50	TRUE			—	104.7	7126.5	6616.8	82240.6	104.7	106.2	104.7
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4550.00	6825.00	TRUE			—	99.9	6825	6315.3	82542.1	99.9		99.9
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5150.00	7725.00	TRUE			—	114.1	7725	7215.3	81642.1	114.1		114.1
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	276.00	414.00	TRUE		cold E2	-7.0	-1.5	414	-95.8	88953.1	-1.5	1.6	-1.5
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	369.00	553.50	TRUE		cold E2	-7.0	0.7	553.5	43.8	88813.6	0.7		0.7
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	571.00	856.50	TRUE		cold E2	-7.0	5.5	856.5	346.8	88510.6	5.5		5.5
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	741.00	1111.50	TRUE		cold E2	-8.0	9.5	1111.5	601.8	88255.6	9.5	11.1	9.5
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	770.00	1155.00	TRUE		cold E2	-8.0	10.2	1155	645.3	88212.1	10.2		10.2
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	914.00	1371.00	TRUE		cold E2	-8.0	13.6	1371	861.3	87996.1	13.6		13.6
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1528.00	2292.00	TRUE		cold E2	-8.5	28.2	2292	1782.3	87075.1	28.2	29.7	28.2
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1660.00	2490.00	TRUE		cold E2	-8.5	31.3	2490	1980.3	86877.1	31.3		31.3
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1587.00	2380.50	TRUE		cold E2	-8.5	29.6	2380.5	1870.8	86986.6	29.6		29.6
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3087.00	4630.50	TRUE		cold E2	-9.0	65.2	4630.5	4120.8	84736.6	65.2	64.4	65.2
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2958.00	4437.00	TRUE		cold E2	-9.0	62.1	4437	3927.3	84930.1	62.1		62.1
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3118.00	4677.00	TRUE		cold E2	-9.0	65.9	4677	4167.3	84690.1	65.9		65.9
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4004.00	6006.00	TRUE		cold E2	-9.5	86.9	6006	5496.3	83361.1	86.9	86.3	86.9
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3862.00	5793.00	TRUE		cold E2	-9.5	83.6	5793	5283.3	83574.1	83.6		83.6
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4067.00	6100.50	TRUE		cold E2	-9.5	88.4	6100.5	5590.8	83266.6	88.4		88.4
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4767.00	7150.50	TRUE		cold E2	-10.0	105.1	7150.5	6640.8	82216.6	105.1	103.3	105.1
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4661.00	6991.50	TRUE		cold E2	-10.0	102.5	6991.5	6481.8	82375.6	102.5		102.5
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4653.00	6979.50	TRUE		cold E2	-10.0	102.3	6979.5	6469.8	82387.6	102.3		102.3
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4818.00	7227.00	TRUE		cold E2	-11.0	106.3	7227	6717.3	82140.1	106.3	105.4	106.3
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4737.00	7105.50	TRUE		cold E2	-11.0	104.3	7105.5	6595.8	82261.6	104.3		104.3
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4785.00	7177.50	TRUE		cold E2	-11.0	105.5	7177.5	6667.8	82189.6	105.5		105.5
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

Competitive Ass... Layout - Four Test Chemicals

Check the 10% rule: 7.69%

If the ratio of EtOH / Hot is > 10% there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Unknown	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	1984.00	2976.00	TRUE		norethynodrel	-4.0	39.0	2976	2466.3	86391.1	39.0	44.6	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2414.00	3621.00	TRUE		norethynodrel	-4.0	49.2	3621	3111.3	85746.1	49.2		
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2263.00	3394.50	TRUE		norethynodrel	-4.0	45.6	3394.5	2884.8	85972.6	45.6		
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	316.00	474.00	TRUE		norethynodrel	-4.5	-0.6	474	-35.8	88893.1	-0.6	-0.7	-0.6
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	291.00	436.50	TRUE		norethynodrel	-4.5	-1.2	436.5	-73.3	88930.6	-1.2		-1.2
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	326.00	489.00	TRUE		norethynodrel	-4.5	-0.3	489	-20.8	88878.1	-0.3		-0.3
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	714.00	1071.00	TRUE		norethynodrel	-5.5	8.9	1071	561.3	88296.1	8.9	7.6	8.9
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	620.00	930.00	TRUE		norethynodrel	-5.5	6.6	930	420.3	88437.1	6.6		6.6
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	644.00	966.00	TRUE		norethynodrel	-5.5	7.2	966	456.3	88401.1	7.2		7.2
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1390.00	2085.00	TRUE		norethynodrel	-6.0	24.9	2085	1575.3	87282.1	24.9	27.3	24.9
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1574.00	2361.00	TRUE		norethynodrel	-6.0	29.3	2361	1851.3	87006.1	29.3		29.3
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1503.00	2254.50	TRUE		norethynodrel	-6.0	27.6	2254.5	1744.8	87112.6	27.6		27.6
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2954.00	4431.00	TRUE		norethynodrel	-6.5	62.0	4431	3921.3	84936.1	62.0	60.6	62.0
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2802.00	4203.00	TRUE		norethynodrel	-6.5	58.4	4203	3693.3	85164.1	58.4		58.4
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2923.00	4384.50	TRUE		norethynodrel	-6.5	61.3	4384.5	3874.8	84982.6	61.3		61.3
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4752.00	7128.00	TRUE		norethynodrel	-7.0	104.7	7128	6618.3	82239.1	104.7	105.7	104.7
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4913.00	7369.50	TRUE		norethynodrel	-7.0	108.5	7369.5	6859.8	81997.6	108.5		108.5
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4723.00	7084.50	TRUE		norethynodrel	-7.0	104.0	7084.5	6574.8	82282.6	104.0		104.0
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4783.00	7174.50	TRUE		norethynodrel	-7.5	105.4	7174.5	6664.8	82192.6	105.4	105.7	105.4
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4746.00	7119.00	TRUE		norethynodrel	-7.5	104.6	7119	6609.3	82248.1	104.6		104.6
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4848.00	7272.00	TRUE		norethynodrel	-7.5	107.0	7272	6762.3	82095.1	107.0		107.0
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5076.00	7614.00	TRUE		norethynodrel	-8.5	112.4	7614	7104.3	81753.1	112.4	106.9	112.4
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5006.00	7509.00	TRUE		norethynodrel	-8.5	110.7	7509	6999.3	81858.1	110.7		110.7
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4454.00	6681.00	TRUE		norethynodrel	-8.5	97.6	6681	6171.3	82686.1	97.6		97.6
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1656.00	2484.00	TRUE		Unknown 1	-3.0	31.2	2484	1974.3	86883.1	31.2	37.5	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2045.00	3067.50	TRUE		Unknown 1	-3.0	40.5	3067.5	2557.8	86299.6	40.5		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2062.00	3093.00	TRUE		Unknown 1	-3.0	40.9	3093	2583.3	86274.1	40.9		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	287.00	430.50	TRUE		Unknown 1	-4.0	-1.3	430.5	-79.3	88936.6	-1.3	-0.5	-1.3
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	297.00	445.50	TRUE		Unknown 1	-4.0	-1.0	445.5	-64.3	88921.6	-1.0		-1.0
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	377.00	565.50	TRUE		Unknown 1	-4.0	0.9	565.5	55.8	88801.6	0.9		0.9
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	490.00	735.00	TRUE		Unknown 1	-5.0	3.6	735	225.3	88632.1	3.6	4.2	3.6
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	513.00	769.50	TRUE		Unknown 1	-5.0	4.1	769.5	259.8	88597.6	4.1		4.1
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	553.00	829.50	TRUE		Unknown 1	-5.0	5.1	829.5	319.8	88537.6	5.1		5.1
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1460.00	2190.00	TRUE		Unknown 1	-6.0	26.6	2190	1680.3	87177.1	26.6	28.3	26.6
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1551.00	2326.50	TRUE		Unknown 1	-6.0	28.7	2326.5	1816.8	87040.6	28.7		28.7
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1592.00	2388.00	TRUE		Unknown 1	-6.0	29.7	2388	1878.3	86979.1	29.7		29.7
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3753.00	5629.50	TRUE		Unknown 1	-7.0	81.0	5629.5	5119.8	83737.6	81.0	82.0	81.0
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3735.00	5602.50	TRUE		Unknown 1	-7.0	80.6	5602.5	5092.8	83764.6	80.6		80.6
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3894.00	5841.00	TRUE		Unknown 1	-7.0	84.3	5841	5331.3	83526.1	84.3		84.3
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4697.00	7045.50	TRUE		Unknown 1	-8.0	103.4	7045.5	6535.8	82321.6	103.4	107.1	103.4
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4948.00	7422.00	TRUE		Unknown 1	-8.0	109.3	7422	6912.3	81945.1	109.3		109.3
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4915.00	7372.50	TRUE		Unknown 1	-8.0	108.6	7372.5	6862.8	81994.6	108.6		108.6
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4754.00	7131.00	TRUE		Unknown 1	-9.0	104.7	7131	6621.3	82236.1	104.7	106.4	104.7
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4764.00	7146.00	TRUE		Unknown 1	-9.0	105.0	7146	6636.3	82221.1	105.0		105.0
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4950.00	7425.00	TRUE		Unknown 1	-9.0	109.4	7425	6915.3	81942.1	109.4		109.4
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4775.00	7162.50	TRUE		Unknown 1	-10.0	105.2	7162.5	6652.8	82204.6	105.2	106.4	105.2
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4713.00	7069.50	TRUE		Unknown 1	-10.0	103.8	7069.5	6559.8	82297.6	103.8		103.8
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4982.00	7473.00	TRUE		Unknown 1	-10.0	110.2	7473	6963.3	81894.1	110.2		110.2
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	295.00	442.50	TRUE		Unknown 2	-3.0	-1.1	442.5	-67.3	88924.6	-1.1	-0.2	-1.1
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	419.00	628.50	TRUE		Unknown 2	-3.0	1.9	628.5	118.8	88738.6	1.9		1.9
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	274.00	411.00	TRUE		Unknown 2	-3.0	-1.6	411	-98.8	88956.1	-1.6		-1.6
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	117.00	175.50	TRUE		Unknown 2	-4.0	-5.3	175.5	-334.3	89191.6	-5.3	-4.7	-5.3
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	158.00	237.00	TRUE		Unknown 2	-4.0	-4.3	237	-272.8	89130.1	-4.3		-4.3
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	155.00	232.50	TRUE		Unknown 2	-4.0	-4.4	232.5	-277.3	89134.6	-4.4		-4.4
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1642.00	2463.00	TRUE		Unknown 2	-5.0	30.9	2463	1953.3	86904.1	30.9	32.9	30.9
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1636.00	2454.00	TRUE		Unknown 2	-5.0	30.8	2454	1944.3	86913.1	30.8		30.8
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1897.00	2845.50	TRUE		Unknown 2	-5.0	37.0	2845.5	2335.8	86521.6	37.0		37.0
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4581.00	6871.50	TRUE		Unknown 2	-6.0	100.6	6871.5	6361.8	82495.6	100.6	106.2	100.6
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4982.00	7473.00	TRUE		Unknown 2	-6.0	110.2	7473	6963.3	81894.1	110.2		110.2
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4887.00	7330.50	TRUE		Unknown 2	-6.0	107.9	7330.5	6820.8	82036.6	107.9		107.9
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4755.00	7132.50	TRUE		Unknown 2	-7.0	104.8	7132.5	6622.8	82234.6	104.8	107.3	104.8
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4800.00	7200.00	TRUE		Unknown 2	-7.0	105.8	7200	6690.3	82167.1	105.8		105.8
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5028.00	7542.00	TRUE		Unknown 2	-7.0	111.2	7542	7032.3	81825.1	111.2		111.2
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4866.00	7299.00	TRUE		Unknown 2	-8.0	107.4	7299	6789.3	82068.1	107.4	109.2	107.4
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4921.00	7381.50	TRUE		Unknown 2	-8.0	108.7	7381.5	6871.8	81985.6	108.7		108.7
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	5034.00	7551.00	TRUE		Unknown 2	-8.0	111.4	7551	7041.3	81816.1	111.4		111.4
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4736.00	7104.00	TRUE		Unknown 2	-9.0	104.3	7104	6594.3	82263.1	104.3	105.4	104.3
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4934.00	7401.00	TRUE		Unknown 2	-9.0	109.0	7401	6891.3	81966.1	109.0		109.0
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4674.00	7011.00	TRUE		Unknown 2	-9.0	102.8	7011	6501.3	82356.1	102.8		102.8
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4774.00	7161.00	TRUE		Unknown 2	-10.0	105.2	7161	6651.3	82206.1	105.2	109.8	105.2
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5128.00	7692.00	TRUE		Unknown 2	-10.0	113.6	7692	7182.3	81675.1	113.6		113.6

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 7.69%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5002.00	7503.00	TRUE		Unknown 2	-10.0	110.6	7503	6993.3	81864.1	110.6		110.6
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7416.00	11124.00	TRUE		Unknown 3	-3.0	167.9	11124	10614.3	78243.1	167.9	176.1	
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	8104.00	12156.00	TRUE		Unknown 3	-3.0	184.2	12156	11646.3	77211.1	184.2		
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4443.00	6664.50	FALSE	mispipet	Unknown 3	-3.0							
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	848.00	1272.00	TRUE		Unknown 3	-4.0	12.1	1272	762.3	88095.1	12.1	13.7	12.1
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	944.00	1416.00	TRUE		Unknown 3	-4.0	14.3	1416	906.3	87951.1	14.3		14.3
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	960.00	1440.00	TRUE		Unknown 3	-4.0	14.7	1440	930.3	87927.1	14.7		14.7
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1069.00	1603.50	TRUE		Unknown 3	-5.0	17.3	1603.5	1093.8	87763.6	17.3	22.9	17.3
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1390.00	2085.00	TRUE		Unknown 3	-5.0	24.9	2085	1575.3	87282.1	24.9		24.9
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1459.00	2188.50	TRUE		Unknown 3	-5.0	26.6	2188.5	1678.8	87178.6	26.6		26.6
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4282.00	6423.00	TRUE		Unknown 3	-6.0	93.5	6423	5913.3	82944.1	93.5	99.6	93.5
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4511.00	6766.50	TRUE		Unknown 3	-6.0	99.0	6766.5	6256.8	82600.6	99.0		99.0
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4822.00	7233.00	TRUE		Unknown 3	-6.0	106.4	7233	6723.3	82134.1	106.4		106.4
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4614.00	6921.00	TRUE		Unknown 3	-7.0	101.4	6921	6411.3	82446.1	101.4	102.3	101.4
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4425.00	6637.50	TRUE		Unknown 3	-7.0	96.9	6637.5	6127.8	82729.6	96.9		96.9
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4910.00	7365.00	TRUE		Unknown 3	-7.0	108.4	7365	6855.3	82002.1	108.4		108.4
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4877.00	7315.50	TRUE		Unknown 3	-8.0	107.7	7315.5	6805.8	82051.6	107.7	108.3	107.7
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4922.00	7383.00	TRUE		Unknown 3	-8.0	108.7	7383	6873.3	81984.1	108.7		108.7
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4917.00	7375.50	TRUE		Unknown 3	-8.0	108.6	7375.5	6865.8	81991.6	108.6		108.6
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4782.00	7173.00	TRUE		Unknown 3	-9.0	105.4	7173	6663.3	82194.1	105.4	108.8	105.4
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5057.00	7585.50	TRUE		Unknown 3	-9.0	111.9	7585.5	7075.8	81781.6	111.9		111.9
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4931.00	7396.50	TRUE		Unknown 3	-9.0	108.9	7396.5	6886.8	81970.6	108.9		108.9
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4787.00	7180.50	TRUE		Unknown 3	-10.0	105.5	7180.5	6670.8	82186.6	105.5	109.2	105.5
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4940.00	7410.00	TRUE		Unknown 3	-10.0	109.2	7410	6900.3	81957.1	109.2		109.2
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	5092.00	7638.00	TRUE		Unknown 3	-10.0	112.8	7638	7128.3	81729.1	112.8		112.8
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4084.00	6126.00	TRUE		Unknown 4	-3.0	88.8	6126	5616.3	83241.1	88.8	83.1	88.8
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4142.00	6213.00	TRUE		Unknown 4	-3.0	90.2	6213	5703.3	83154.1	90.2		90.2
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3296.00	4944.00	TRUE		Unknown 4	-3.0	70.1	4944	4434.3	84423.1	70.1		70.1
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4634.00	6951.00	TRUE		Unknown 4	-4.0	101.9	6951	6441.3	82416.1	101.9	89.7	93.7
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4752.00	7128.00	TRUE		Unknown 4	-4.0	104.7	7128	6618.3	82239.1	104.7		95.4
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2978.00	4467.00	TRUE		Unknown 4	-4.0	62.6	4467	3957.3	84900.1	62.6		86.4
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4841.00	7261.50	TRUE		Unknown 4	-5.0	106.8	7261.5	6751.8	82105.6	106.8	109.4	106.8
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	5028.00	7542.00	TRUE		Unknown 4	-5.0	111.2	7542	7032.3	81825.1	111.2		111.2
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4984.00	7476.00	TRUE		Unknown 4	-5.0	110.2	7476	6966.3	81891.1	110.2		110.2
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4762.00	7143.00	TRUE		Unknown 4	-6.0	104.9	7143	6633.3	82224.1	104.9	102.7	104.9
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4437.00	6655.50	TRUE		Unknown 4	-6.0	97.2	6655.5	6145.8	82711.6	97.2		97.2
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4807.00	7210.50	TRUE		Unknown 4	-6.0	106.0	7210.5	6700.8	82156.6	106.0		106.0
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4509.00	6763.50	TRUE		Unknown 4	-7.0	98.9	6763.5	6253.8	82603.6	98.9	104.8	98.9
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4751.00	7126.50	TRUE		Unknown 4	-7.0	104.7	7126.5	6616.8	82240.6	104.7		104.7
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	5008.00	7512.00	TRUE		Unknown 4	-7.0	110.8	7512	7002.3	81855.1	110.8		110.8
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4867.00	7300.50	TRUE		Unknown 4	-8.0	107.4	7300.5	6790.8	82066.6	107.4	106.3	107.4
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4947.00	7420.50	TRUE		Unknown 4	-8.0	109.3	7420.5	6910.8	81946.6	109.3		109.3
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4641.00	6961.50	TRUE		Unknown 4	-8.0	102.1	6961.5	6451.8	82405.6	102.1		102.1
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4685.00	7027.50	TRUE		Unknown 4	-9.0	103.1	7027.5	6517.8	82339.6	103.1	108.2	103.1
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4951.00	7426.50	TRUE		Unknown 4	-9.0	109.4	7426.5	6916.8	81940.6	109.4		109.4
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	5057.00	7585.50	TRUE		Unknown 4	-9.0	111.9	7585.5	7075.8	81781.6	111.9		111.9
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4332.00	6498.00	TRUE		Unknown 4	-10.0	94.7	6498	5988.3	82869.1	94.7	101.0	94.7
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4821.00	7231.50	TRUE		Unknown 4	-10.0	106.3	7231.5	6721.8	82135.6	106.3		106.3
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4638.00	6957.00	TRUE		Unknown 4	-10.0	102.0	6957	6447.3	82410.1	102.0		102.0
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	231.00	346.50	TRUE		cold E2	-7.0	-2.6	346.5	-163.3	89020.6	-2.6	-1.6	-2.6
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	292.00	438.00	TRUE		cold E2	-7.0	-1.1	438	-71.8	88929.1	-1.1		-1.1
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	300.00	450.00	TRUE		cold E2	-7.0	-0.9	450	-59.8	88917.1	-0.9		-0.9
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4457.00	6685.50	TRUE			—	97.7	6685.5	6175.8	82681.6	97.7	93.8	97.7
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4479.00	6718.50	TRUE			—	98.2	6718.5	6208.8	82648.6	98.2		98.2
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3937.00	5905.50	TRUE			—	85.4	5905.5	5395.8	83461.6	85.4		85.4
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	94224.00	94224.00	TRUE					94224	93714.3				
179	2	none	Hot		—	—	—	10	—	—	—	—	90247.00	90247.00	TRUE					90247	89737.3				
180	3	none	Hot		—	—	—	10	—	—	—	—	90180.00	90180.00	TRUE					90180	89670.3				
181	1	none	Hot		—	—	—	10	—	—	—	—	86388.00	86388.00	TRUE					86388	85878.3				
182	2	none	Hot		—	—	—	10	—	—	—	—	89601.00	89601.00	TRUE					89601	89091.3				
183	3	none	Hot		—	—	—	10	—	—	—	—	82504.00	82504.00	TRUE					82504	81994.3				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				5.0				16.0				17.0				22.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	-1.5147	0.6921	5.4855	-4.0				-3.0				-3.0	-1.0639	1.8786	-1.5622	-3.0				-3.0	88.8471	90.2235	70.1483	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-2.5826	-1.1351	-0.9452	-4.5	-0.5656	-1.1588	-0.3283	-4.0	-1.2537	-1.0164	0.8819	-4.0	-5.2877	-4.3148	-4.3860	-4.0	12.0585	14.3366	14.7162	-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	9.5195	10.2076	13.6247	-5.5	8.8788	6.6482	7.2177	-5.0	3.5634	4.1092	5.0583	-5.0	30.8997	30.7574	36.9508	-5.0	17.3027	24.9199	26.5572	-5.0	106.8104	111.2478	110.2037	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	28.1946	31.3269	29.5946	-6.0	24.9199	29.2861	27.6013	-6.0	26.5810	28.7404	29.7133	-6.0	100.6407	110.1562	107.9019	-6.0	93.5456	98.9796	106.3595	-6.0	104.9357	97.2237	106.0036	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	65.1888	62.1277	65.9245	-6.5	62.0328	58.4259	61.2972	-7.0	80.9927	80.5656	84.3385	-7.0	104.7696	105.8375	111.2478	-7.0	101.4238	96.9389	108.4477	-7.0	98.9322	104.6747	110.7732	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	86.9488	83.5792	88.4437	-7.0	104.6984	108.5189	104.0103	-8.0	103.3933	109.3494	108.5663	-8.0	107.4036	108.7087	111.3902	-8.0	107.6646	108.7325	108.6138	-8.0	107.4273	109.3257	102.0645	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	105.0544	102.5391	102.3492	-7.5	105.4341	104.5561	106.9765	-9.0	104.7459	104.9832	109.3969	-9.0	104.3188	109.0172	102.8475	-9.0	105.4103	111.9359	108.9460	-9.0	103.1086	109.4206	111.9359	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	106.2646	104.3425	105.4815	-8.5	112.3868	110.7257	97.6271	-10.0	105.2442	103.7730	110.1562	-10.0	105.2205	113.6207	110.6308	-10.0	105.5290	109.1596	112.7665	-10.0	94.7321	106.3358	101.9933	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		TTN009 4-23-08		Positive Control		TTN009 4-23-08		5.0		TTN009 4-23-08		16.0		TTN009 4-23-08		17.0		TTN009 4-23-08		22.0		TTN009 4-23-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values		"bad initial values"		Best-fit values			
13	1	BOTTOM		-1.014		BOTTOM		1.665		BOTTOM		-0.2946		BOTTOM		-2.558		BOTTOM		13.56		BOTTOM		83.07		BOTTOM			
14	2	TOP		106.8		TOP		109.6		TOP		107.7		TOP		107.8		TOP		107.2		TOP		109.4		TOP			
15	3	LOGIC50		-8.808		LOGIC50		-6.328		LOGIC50		-6.4		LOGIC50		-5.121		LOGIC50		-5.378		LOGIC50		n/a		LOGIC50			
16	1	HILLSLOPE		-1.08		HILLSLOPE		-1.511		HILLSLOPE		-0.9702		HILLSLOPE		-2.348		HILLSLOPE		-2.001		HILLSLOPE		n/a		HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.17		BOTTOM		2.747		BOTTOM		1.26		BOTTOM		1.447		BOTTOM		2.658		BOTTOM				BOTTOM			
19	1	TOP		1.364		TOP		2.441		TOP		0.9522		TOP		1.001		TOP		1.306		TOP				TOP			
20	2	LOGIC50		0.01624		LOGIC50		0.02923		LOGIC50		0.02387		LOGIC50		0.04924		LOGIC50		0.0583		LOGIC50				LOGIC50			
21	3	HILLSLOPE		0.05527		HILLSLOPE		0.174		HILLSLOPE		0.05022		HILLSLOPE		0.9295		HILLSLOPE		0.2483		HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.2

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Estradiol
Run ID: TTN008
Assay start date: 4/23/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-7.0	-1.5
1	1	2	-7.0	0.7
1	1	3	-7.0	5.5
1	2	1	-8.0	9.5
1	2	2	-8.0	10.2
1	2	3	-8.0	13.6
1	3	1	-8.5	28.2
1	3	2	-8.5	31.3
1	3	3	-8.5	29.6
1	4	1	-9.0	65.2
1	4	2	-9.0	62.1
1	4	3	-9.0	65.9
1	5	1	-9.5	86.9
1	5	2	-9.5	83.6
1	5	3	-9.5	88.4
1	6	1	-10.0	105.1
1	6	2	-10.0	102.5
1	6	3	-10.0	102.3
1	7	1	-11.0	106.3
1	7	2	-11.0	104.3
1	7	3	-11.0	105.5
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.7

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Norethynodrel
Run ID: TTN009
Assay start date: 4/23/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-4.0	39.0
1	1	2	-4.0	49.2
1	1	3	-4.0	45.6
1	2	1	-4.5	-0.6
1	2	2	-4.5	-1.2
1	2	3	-4.5	-0.3
1	3	1	-5.5	24.9
1	3	2	-5.5	29.3
1	3	3	-5.5	27.6
1	4	1	-6.0	8.9
1	4	2	-6.0	6.6
1	4	3	-6.0	7.2
1	5	1	-6.5	62.0
1	5	2	-6.5	58.4
1	5	3	-6.5	61.3
1	6	1	-7.0	104.7
1	6	2	-7.0	108.5
1	6	3	-7.0	104.0
1	7	1	-7.5	105.4
1	7	2	-7.5	104.6
1	7	3	-7.5	107.0
1	8	1	-8.5	112.4
1	8	2	-8.5	110.7
1	8	3	-8.5	97.6
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.9

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID:	Chem Code 5
Run ID:	TTN009
Assay start date:	4/23/08
Technician ID:	TTN

run	xgroup	rep	x	y
1	1	1	-3.0	31.2
1	1	2	-3.0	40.5
1	1	3	-3.0	40.9
1	2	1	-4.0	-1.3
1	2	2	-4.0	-1.0
1	2	3	-4.0	0.9
1	3	1	-5.0	3.6
1	3	2	-5.0	4.1
1	3	3	-5.0	5.1
1	4	1	-6.0	26.6
1	4	2	-6.0	28.7
1	4	3	-6.0	29.7
1	5	1	-7.0	81.0
1	5	2	-7.0	80.6
1	5	3	-7.0	84.3
1	6	1	-8.0	103.4
1	6	2	-8.0	109.3
1	6	3	-8.0	108.6
1	7	1	-9.0	104.7
1	7	2	-9.0	105.0
1	7	3	-9.0	109.4
1	8	1	-10.0	105.2
1	8	2	-10.0	103.8
1	8	3	-10.0	110.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.3

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Chem Code 16
Run ID: TTN009
Assay start date: 4/23/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	-1.1
1	1	2	-3.0	1.9
1	1	3	-3.0	-1.6
1	2	1	-4.0	-5.3
1	2	2	-4.0	-4.3
1	2	3	-4.0	-4.4
1	3	1	-5.0	30.9
1	3	2	-5.0	30.8
1	3	3	-5.0	37.0
1	4	1	-6.0	100.6
1	4	2	-6.0	110.2
1	4	3	-6.0	107.9
1	5	1	-7.0	104.8
1	5	2	-7.0	105.8
1	5	3	-7.0	111.2
1	6	1	-8.0	107.4
1	6	2	-8.0	108.7
1	6	3	-8.0	111.4
1	7	1	-9.0	104.3
1	7	2	-9.0	109.0
1	7	3	-9.0	102.8
1	8	1	-10.0	105.2
1	8	2	-10.0	113.6
1	8	3	-10.0	110.6
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.1

Provide information in all blue cells.
Laboratory Code: TRL B
Compound ID: Chem Code 17
Run ID: TTN009
Assay start date: 4/23/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	167.9
1	1	2	-3.0	184.2
1	1	3		
1	2	1	-4.0	12.1
1	2	2	-4.0	14.3
1	2	3	-4.0	14.7
1	3	1	-5.0	17.3
1	3	2	-5.0	24.9
1	3	3	-5.0	26.6
1	4	1	-6.0	93.5
1	4	2	-6.0	99.0
1	4	3	-6.0	106.4
1	5	1	-7.0	101.4
1	5	2	-7.0	96.9
1	5	3	-7.0	108.4
1	6	1	-8.0	107.7
1	6	2	-8.0	108.7
1	6	3	-8.0	108.6
1	7	1	-9.0	105.4
1	7	2	-9.0	111.9
1	7	3	-9.0	108.9
1	8	1	-10.0	105.5
1	8	2	-10.0	109.2
1	8	3	-10.0	112.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 10.1

Provide information in all blue cells.

Laboratory Code: TRL B

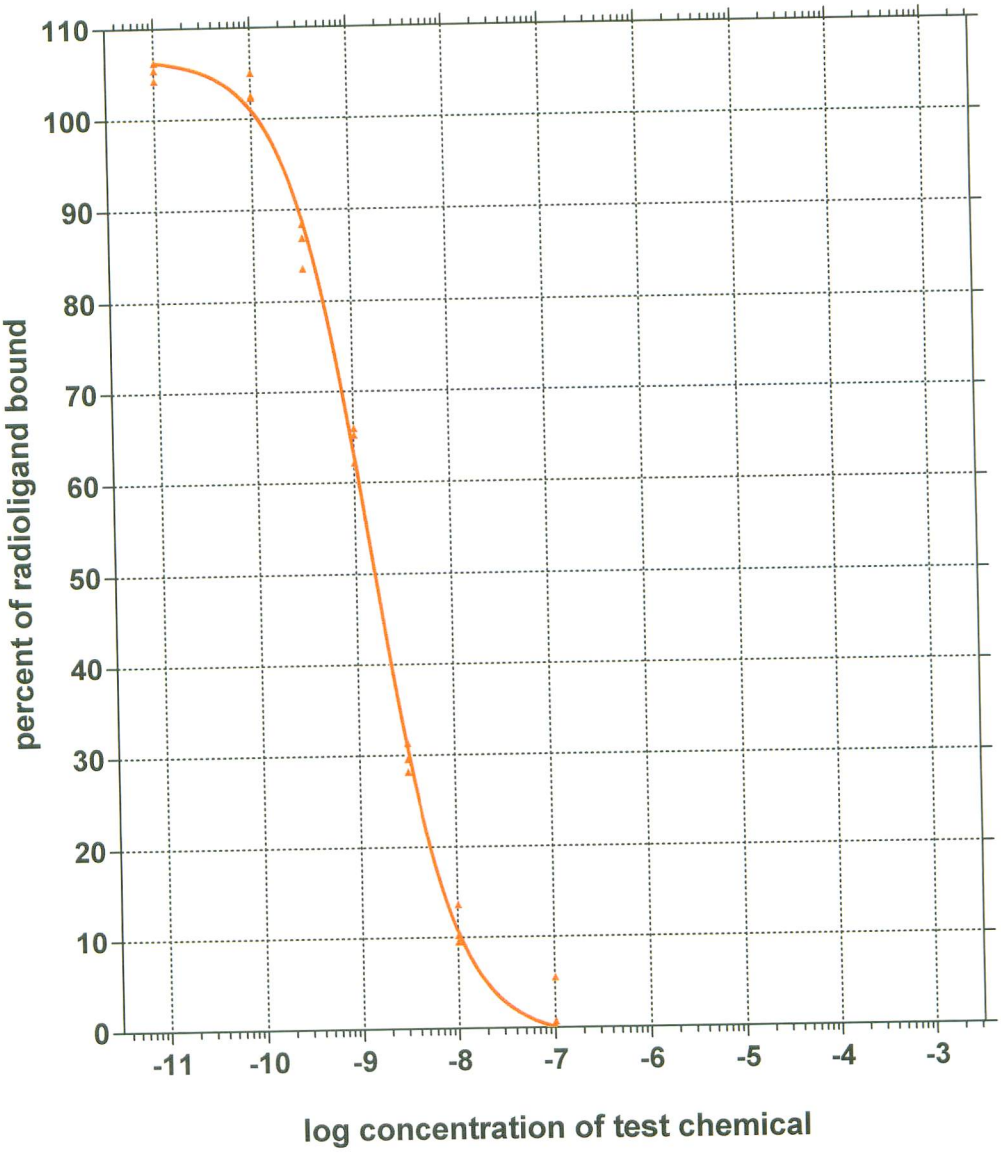
Compound ID: Chem Code 22
Run ID: TTN009
Assay start date: 4/23/08
Technician ID: TTN

run	xgroup	rep	x	y
1	1	1	-3.0	88.8
1	1	2	-3.0	90.2
1	1	3	-3.0	70.1
1	2	1	-4.0	101.9
1	2	2	-4.0	104.7
1	2	3	-4.0	62.6
1	3	1	-5.0	106.8
1	3	2	-5.0	111.2
1	3	3	-5.0	110.2
1	4	1	-6.0	104.9
1	4	2	-6.0	97.2
1	4	3	-6.0	106.0
1	5	1	-7.0	98.9
1	5	2	-7.0	104.7
1	5	3	-7.0	110.8
1	6	1	-8.0	107.4
1	6	2	-8.0	109.3
1	6	3	-8.0	102.1
1	7	1	-9.0	103.1
1	7	2	-9.0	109.4
1	7	3	-9.0	111.9
1	8	1	-10.0	94.7
1	8	2	-10.0	106.3
1	8	3	-10.0	102.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

ble format: XY		X	A		
		log concentration	run TTN009 4-23-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-1.5147	0.6921	5.4855
2	Title	-7.0	-2.5826	-1.1351	-0.9452
3	Title	-8.0	9.5195	10.2076	13.6247
4	Title	-8.5	28.1946	31.3269	29.5946
5	Title	-9.0	65.1888	62.1277	65.9245
6	Title	-9.5	86.9488	83.5792	88.4437
7	Title	-10.0	105.0544	102.5391	102.3492
8	Title	-11.0	106.2646	104.3425	105.4815

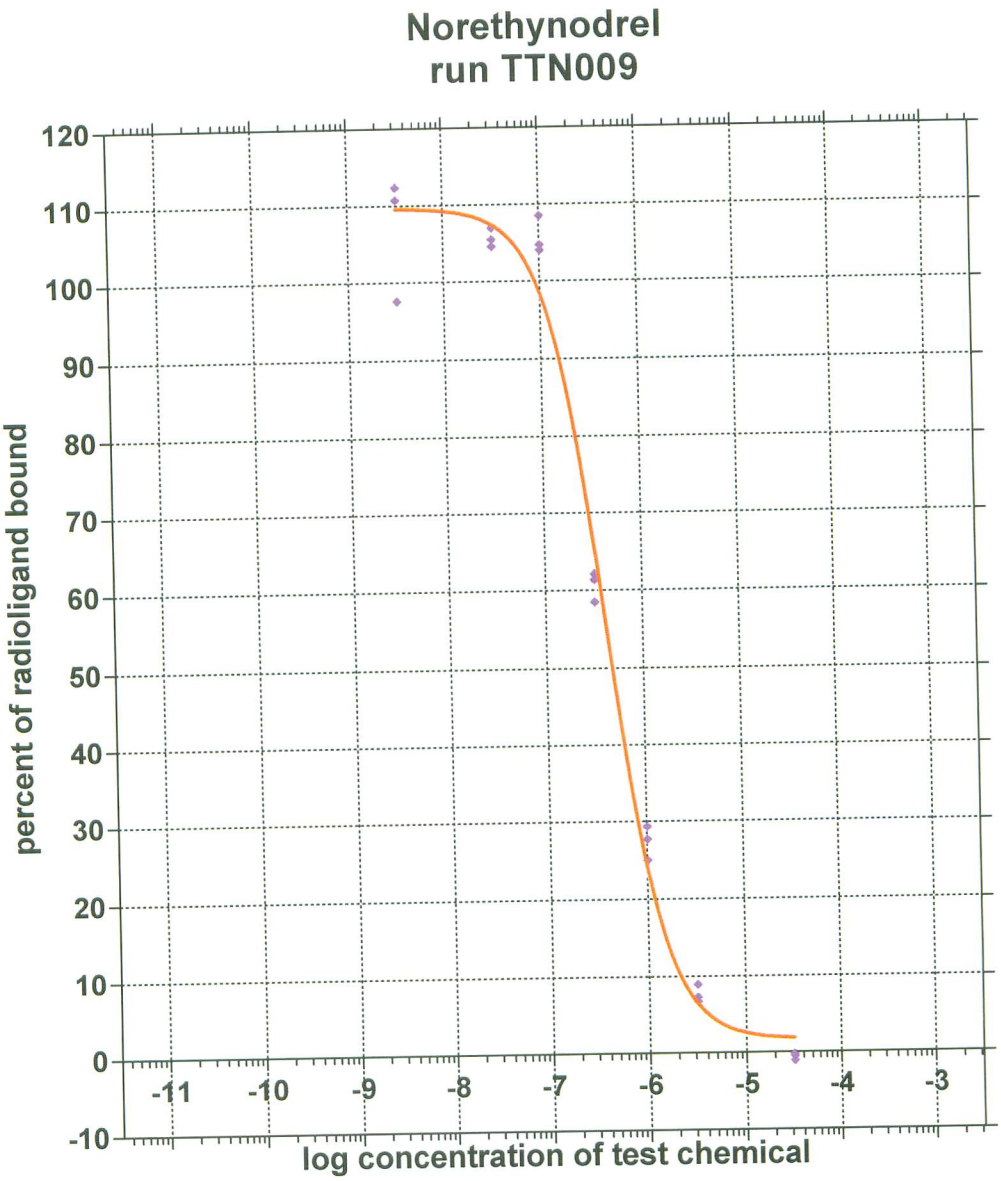
	Nonlin fit Table of results	A run TTN009 4-23-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.014
4	TOP	106.8
5	LOGIC50	-8.808
6	HILLSLOPE	-1.080
7	Std. Error	
8	BOTTOM	1.170
9	TOP	1.364
10	LOGIC50	0.01624
11	HILLSLOPE	0.05527
12	95% Confidence Intervals	
13	BOTTOM	-3.455 to 1.427
14	TOP	103.9 to 109.6
15	LOGIC50	-8.842 to -8.774
16	HILLSLOPE	-1.195 to -0.9643
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9969
20	Absolute Sum of Squares	134.9
21	Sy.x	2.597
22	Number of points	
23	Analyzed	24

Estradiol reference standard
run TTN009



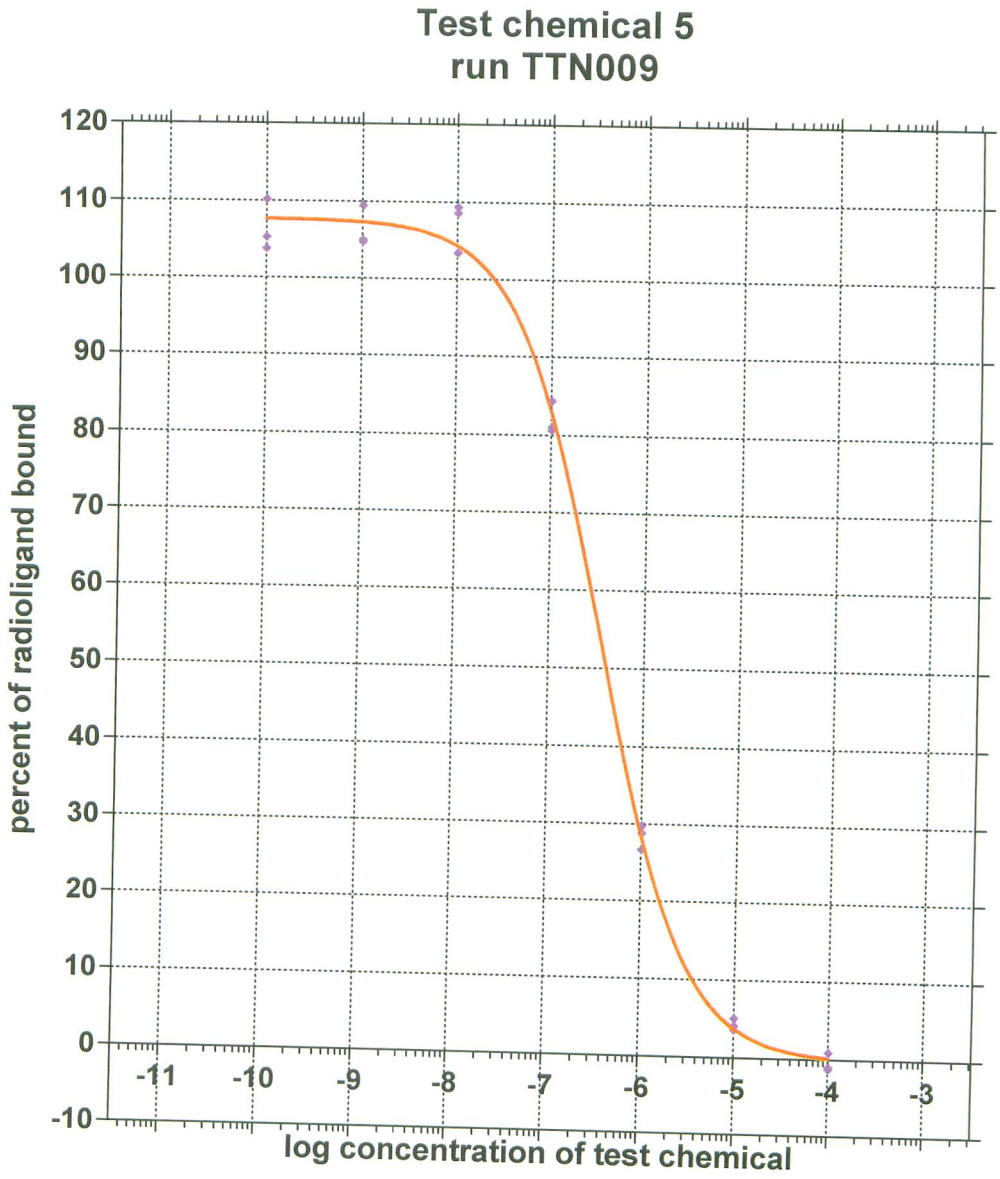
ble format: XY		X	A		
		log concentration	run TTN009 4-23-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	-0.5656	-1.1588	-0.3283
3	Title	-5.5	8.8788	6.6482	7.2177
4	Title	-6.0	24.9199	29.2861	27.6013
5	Title	-6.5	62.0328	58.4259	61.2972
6	Title	-7.0	104.6984	108.5189	104.0103
7	Title	-7.5	105.4341	104.5561	106.9765
8	Title	-8.5	112.3868	110.7257	97.6271

	Nonlin fit Table of results	A run TTN009 4-23-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.665
4	TOP	109.6
5	LOGIC50	-6.328
6	HILLSLOPE	-1.511
7	Std. Error	
8	BOTTOM	2.747
9	TOP	2.441
10	LOGIC50	0.02923
11	HILLSLOPE	0.1740
12	95% Confidence Intervals	
13	BOTTOM	-4.132 to 7.462
14	TOP	104.5 to 114.8
15	LOGIC50	-6.390 to -6.267
16	HILLSLOPE	-1.878 to -1.144
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9877
20	Absolute Sum of Squares	515.1
21	Sy.x	5.504
22	Number of points	
23	Analyzed	21


Norethynodrel
run TTN009
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

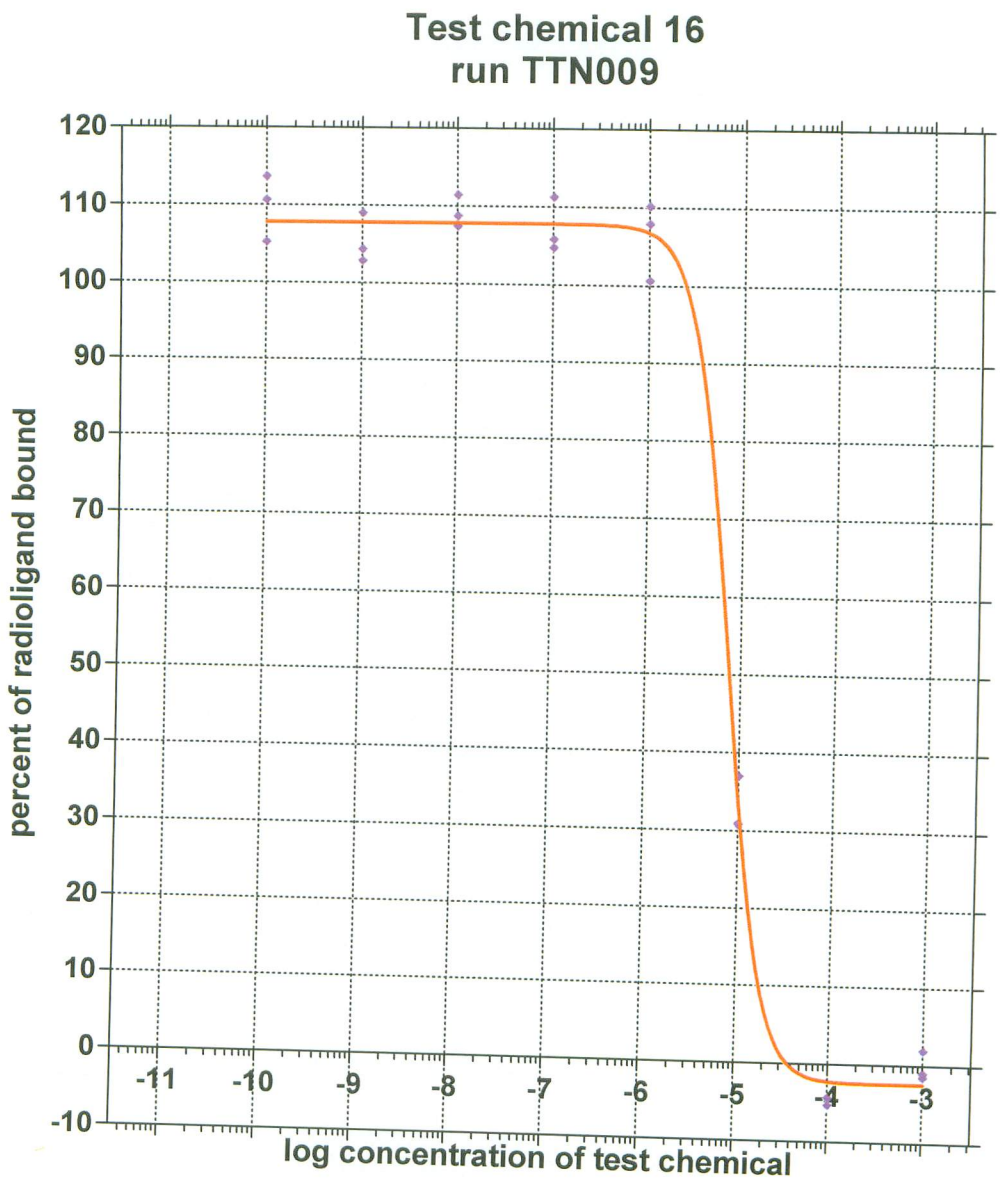
ɔle format: XY		X	A		
		log concentration	Test chemical 5 run TTN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-1.2537	-1.0164	0.8819
3	Title	-5.0	3.5634	4.1092	5.0583
4	Title	-6.0	26.5810	28.7404	29.7133
5	Title	-7.0	80.9927	80.5656	84.3385
6	Title	-8.0	103.3933	109.3494	108.5663
7	Title	-9.0	104.7459	104.9832	109.3969
8	Title	-10.0	105.2442	103.7730	110.1562

	Nonlin fit Table of results	A Test chemical 5 run TTN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.2946
4	TOP	107.7
5	LOGIC50	-6.400
6	HILLSLOPE	-0.9702
7	Std. Error	
8	BOTTOM	1.260
9	TOP	0.9522
10	LOGIC50	0.02387
11	HILLSLOPE	0.05022
12	95% Confidence Intervals	
13	BOTTOM	-2.953 to 2.364
14	TOP	105.7 to 109.7
15	LOGIC50	-6.450 to -6.349
16	HILLSLOPE	-1.076 to -0.8643
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9975
20	Absolute Sum of Squares	109.3
21	Sy.x	2.535
22	Number of points	
23	Analyzed	21


Test chemical 5
run TTN009
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

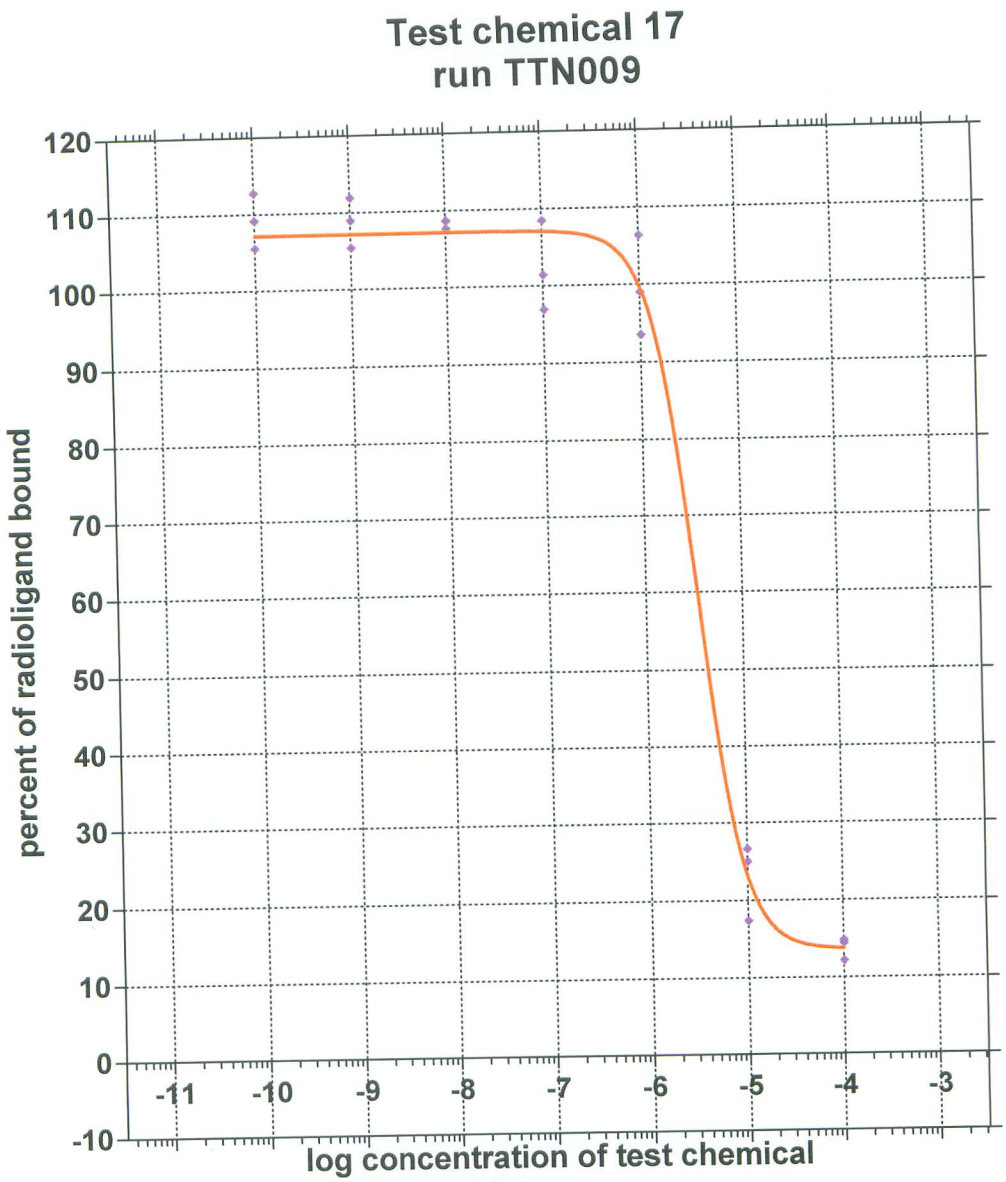
ble format: XY		X	A		
		log concentration	Test chemical 16 run TTN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	-1.0639	1.8786	-1.5622
2	Title	-4.0	-5.2877	-4.3148	-4.3860
3	Title	-5.0	30.8997	30.7574	36.9508
4	Title	-6.0	100.6407	110.1562	107.9019
5	Title	-7.0	104.7696	105.8375	111.2478
6	Title	-8.0	107.4036	108.7087	111.3902
7	Title	-9.0	104.3188	109.0172	102.8475
8	Title	-10.0	105.2205	113.6207	110.6308

	Nonlin fit Table of results	A Test chemical 16 run TTN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.558
4	TOP	107.8
5	LOGIC50	-5.121
6	HILLSLOPE	-2.348
7	Std. Error	
8	BOTTOM	1.447
9	TOP	1.001
10	LOGIC50	0.04924
11	HILLSLOPE	0.9295
12	95% Confidence Intervals	
13	BOTTOM	-5.577 to 0.4614
14	TOP	105.7 to 109.9
15	LOGIC50	-5.224 to -5.019
16	HILLSLOPE	-4.287 to -0.4086
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9958
20	Absolute Sum of Squares	240.6
21	Sy.x	3.469
22	Number of points	
23	Analyzed	24


Test chemical 16
run TTN009
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

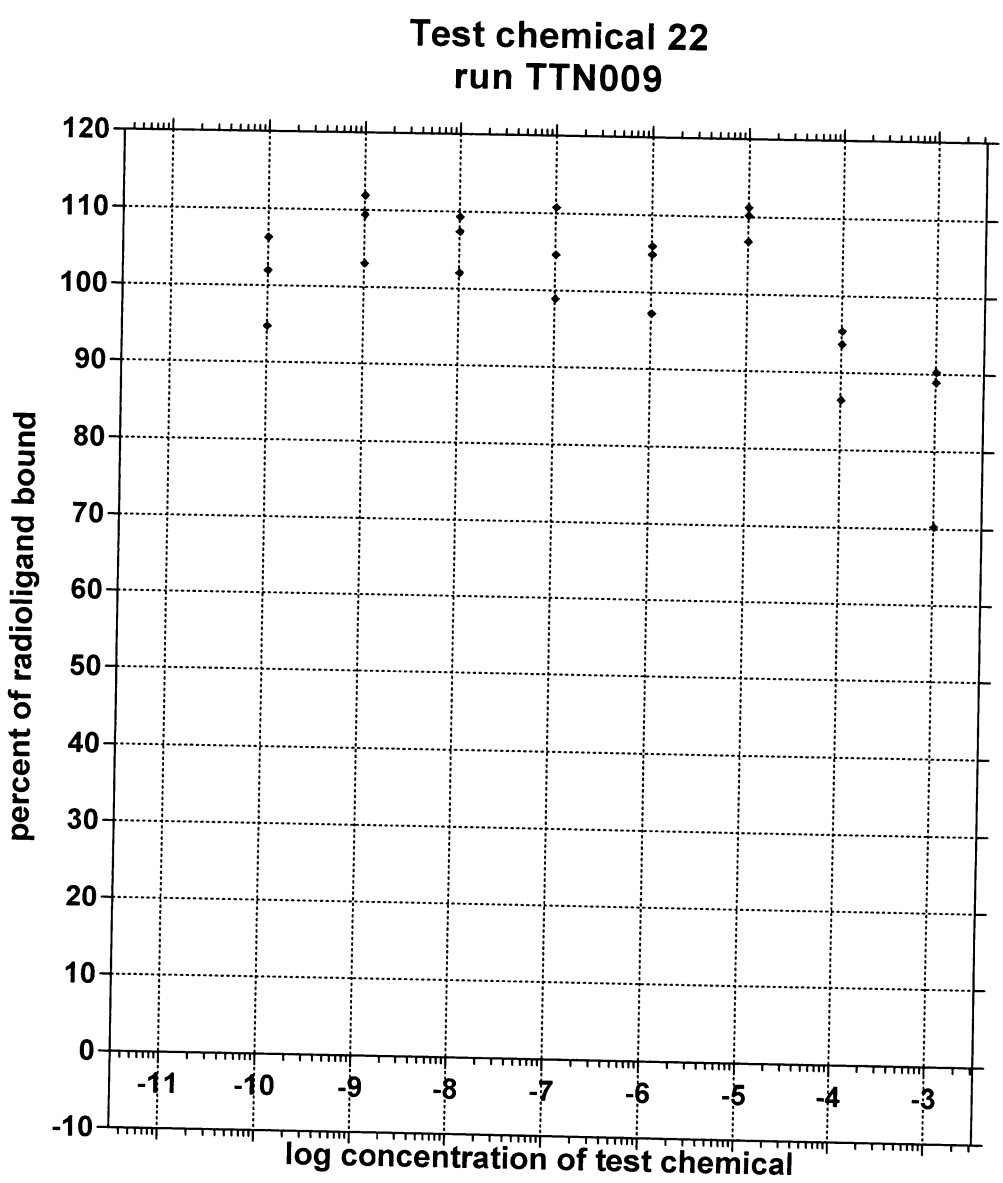
ole format: XY		X	A		
		log concentration	Test chemical 17 run TTN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	12.0585	14.3366	14.7162
3	Title	-5.0	17.3027	24.9199	26.5572
4	Title	-6.0	93.5456	98.9796	106.3595
5	Title	-7.0	101.4238	96.9389	108.4477
6	Title	-8.0	107.6646	108.7325	108.6138
7	Title	-9.0	105.4103	111.9359	108.9460
8	Title	-10.0	105.5290	109.1596	112.7665

	Nonlin fit Table of results	A Test chemical 17 run TTN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	13.56
4	TOP	107.2
5	LOGIC50	-5.378
6	HILLSLOPE	-2.001
7	Std. Error	
8	BOTTOM	2.658
9	TOP	1.306
10	LOGIC50	0.05830
11	HILLSLOPE	0.2483
12	95% Confidence Intervals	
13	BOTTOM	7.953 to 19.17
14	TOP	104.4 to 109.9
15	LOGIC50	-5.501 to -5.255
16	HILLSLOPE	-2.525 to -1.478
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9897
20	Absolute Sum of Squares	344.3
21	Sy.x	4.500
22	Number of points	
23	Analyzed	21


Test chemical 17
run TTN009
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

ble format: XY		X	A		
		log concentration	Test chemical 22 run TTN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	88.8471	90.2235	70.1483
2	Title	-4.0	93.6729	95.3859	86.3787
3	Title	-5.0	106.8104	111.2478	110.2037
4	Title	-6.0	104.9357	97.2237	106.0036
5	Title	-7.0	98.9322	104.6747	110.7732
6	Title	-8.0	107.4273	109.3257	102.0645
7	Title	-9.0	103.1086	109.4206	111.9359
8	Title	-10.0	94.7321	106.3358	101.9933

	Nonlin fit Table of results	A Test chemical 22 run TTN009
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	83.07
4	TOP	109.4
5	LOGIC50	-6.889
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	24


Test chemical 22
run TTN009
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

APPENDIX L

Excel and Prism Files of the Run Identifier TTN010

ER Competitive Assay - Four Unknowns

183 assay tubes

Laboratory Code:	TRL B
Run identification:	TTN010
Assay start date:	4/28/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	109.02 Ci/mmole
Cytosol vial or lot identification:	RUC 02-22-08
Protein (cytosol):	50 microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.14 M
Weak Positive, Max Concentration:	5.00E-03 M
log(IC50), norethynodrel (Weak Positive):	-5.17 M
RBA, norethynodrel (Weak Positive):	1.084E-03 relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03 M
log(IC50), Unknown 1:	-8.54 4.0
RBA, Unknown 1:	2.553E+00 relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03 M
log(IC50), Unknown 2:	-6.05 9.0
RBA, Unknown 2:	n/a relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03 M
log(IC50), Unknown 3:	-5.64 14.0
RBA, Unknown 3:	n/a relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03 M
log(IC50), Unknown 4:	-4.85 17.0
RBA, Unknown 4:	n/a relative to E2=1.0
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software. and the maximum concentrations for each of the unknowns.

Check the 10% rule: 1.05%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	1389.5	78.84	Total Binding, solvent control, tubes
Hot	6	132278.3	1197.21	Total hot E2 added to each tube
NSB	5	391.2	23.89	Nonspecific Binding
Specific EtOH	6	998.3	78.84	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.01	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.28	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	1.4
R1881	#N/A	not applicable
norethynodrel	-4.5	3.7
Unknown 1	-5.0	-7.4
Unknown 2	-3.0	55.7
Unknown 3	-5.0	71.9
Unknown 4	-4.0	38.7

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	983.00	1474.50	TRUE			—	108.5	1474.5	1083.3	131195.0	108.5	99.8	108.5
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	928.00	1392.00	TRUE			—	100.3	1392	1000.8	131277.5	100.3		100.3
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	865.00	1297.50	TRUE			—	90.8	1297.5	906.3	131372.0	90.8		90.8
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	262.00	393.00	TRUE		cold E2	-7.0	0.2	393	1.8	132276.5	0.2	1.4	0.2
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	268.00	402.00	TRUE		cold E2	-7.0	1.1	402	10.8	132267.5	1.1		1.1
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	281.00	421.50	TRUE		cold E2	-7.0	3.0	421.5	30.3	132248.0	3.0		3.0
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	498.00	747.00	TRUE		cold E2	-8.0	35.6	747	355.8	131922.5	35.6	41.6	35.6
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	575.00	862.50	TRUE		cold E2	-8.0	47.2	862.5	471.3	131807.0	47.2		47.2
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	539.00	808.50	TRUE		cold E2	-8.0	41.8	808.5	417.3	131861.0	41.8		41.8
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	716.00	1074.00	TRUE		cold E2	-8.5	68.4	1074	682.8	131595.5	68.4	73.3	68.4
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	753.00	1129.50	TRUE		cold E2	-8.5	74.0	1129.5	738.3	131540.0	74.0		74.0
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	776.00	1164.00	TRUE		cold E2	-8.5	77.4	1164	772.8	131505.5	77.4		77.4
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	894.00	1341.00	TRUE		cold E2	-9.0	95.1	1341	949.8	131328.5	95.1	105.3	95.1
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1001.00	1501.50	TRUE		cold E2	-9.0	111.2	1501.5	1110.3	131168.0	111.2		111.2
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	989.00	1483.50	TRUE		cold E2	-9.0	109.4	1483.5	1092.3	131186.0	109.4		109.4
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	995.00	1492.50	TRUE		cold E2	-9.5	110.3	1492.5	1101.3	131177.0	110.3	109.6	110.3
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	1051.00	1576.50	TRUE		cold E2	-9.5	118.7	1576.5	1185.3	131093.0	118.7		118.7
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	924.00	1386.00	TRUE		cold E2	-9.5	99.6	1386	994.8	131283.5	99.6		99.6
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1191.00	1786.50	TRUE		cold E2	-10.0	139.8	1786.5	1395.3	130883.0	139.8	129.0	139.8
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1087.00	1630.50	TRUE		cold E2	-10.0	124.1	1630.5	1239.3	131039.0	124.1		124.1
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1080.00	1620.00	TRUE		cold E2	-10.0	123.1	1620	1228.8	131049.5	123.1		123.1
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1030.00	1545.00	TRUE		cold E2	-11.0	115.6	1545	1153.8	131124.5	115.6	122.2	115.6
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1085.00	1627.50	TRUE		cold E2	-11.0	123.8	1627.5	1236.3	131042.0	123.8		123.8
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1107.00	1660.50	TRUE		cold E2	-11.0	127.1	1660.5	1269.3	131009.0	127.1		127.1
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32		Competitive Assay Tube Layout - Four Test Chemicals														Check the 10% rule:	1.05%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
68		35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A		
69		36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A		
70		37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A		
71		38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A		
72		39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A		
73		40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A		
74		41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A		
75		42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A		
76		43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A		
77		44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
78		45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
79		46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A		
80		47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
81		48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
82		49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2397.00	3595.50	TRUE		norethynodrel	-4.0	321.0	3595.5	3204.3	129074.0	321.0	362.6			
83		50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2637.00	3955.50	TRUE		norethynodrel	-4.0	357.0	3955.5	3564.3	128714.0	357.0				
84		51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2989.00	4483.50	TRUE		norethynodrel	-4.0	409.9	4483.5	4092.3	128186.0	409.9				
85		52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	310.00	465.00	TRUE		norethynodrel	-4.5	7.4	465	73.8	132204.5	7.4	3.7	7.4		
86		53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	270.00	405.00	TRUE		norethynodrel	-4.5	1.4	405	13.8	132264.5	1.4		1.4		
87		54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	276.00	414.00	TRUE		norethynodrel	-4.5	2.3	414	22.8	132255.5	2.3		2.3		
88		55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	745.00	1117.50	TRUE		norethynodrel	-5.5	72.8	1117.5	726.3	131552.0	72.8	76.7	72.8		
89		56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	758.00	1137.00	TRUE		norethynodrel	-5.5	74.7	1137	745.8	131532.5	74.7		74.7		
90		57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	811.00	1216.50	TRUE		norethynodrel	-5.5	82.7	1216.5	825.3	131453.0	82.7		82.7		
91		58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	915.00	1372.50	TRUE		norethynodrel	-6.0	98.3	1372.5	981.3	131297.0	98.3	102.7	98.3		
92		59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	980.00	1470.00	TRUE		norethynodrel	-6.0	108.1	1470	1078.8	131199.5	108.1		108.1		
93		60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	937.00	1405.50	TRUE		norethynodrel	-6.0	101.6	1405.5	1014.3	131264.0	101.6		101.6		
94		61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	1031.00	1546.50	TRUE		norethynodrel	-6.5	115.7	1546.5	1155.3	131123.0	115.7	123.3	115.7		
95		62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	1081.00	1621.50	TRUE		norethynodrel	-6.5	123.2	1621.5	1230.3	131048.0	123.2		123.2		
96		63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	1133.00	1699.50	TRUE		norethynodrel	-6.5	131.1	1699.5	1308.3	130970.0	131.1		131.1		
97		64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	1079.00	1618.50	TRUE		norethynodrel	-7.0	122.9	1618.5	1227.3	131051.0	122.9	130.5	122.9		
98		65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	1107.00	1660.50	TRUE		norethynodrel	-7.0	127.1	1660.5	1269.3	131009.0	127.1		127.1		
99		66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	1201.00	1801.50	TRUE		norethynodrel	-7.0	141.3	1801.5	1410.3	130868.0	141.3		141.3		
100		67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	1074.00	1611.00	TRUE		norethynodrel	-7.5	122.2	1611	1219.8	131058.5	122.2	128.0	122.2		
101		68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	1132.00	1698.00	TRUE		norethynodrel	-7.5	130.9	1698	1306.8	130971.5	130.9		130.9		
102		69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	1133.00	1699.50	TRUE		norethynodrel	-7.5	131.1	1699.5	1308.3	130970.0	131.1		131.1		
103		70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	1108.00	1662.00	TRUE		norethynodrel	-8.5	127.3	1662	1270.8	131007.5	127.3	131.6	127.3		
104		71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	1176.00	1764.00	TRUE		norethynodrel	-8.5	137.5	1764	1372.8	130905.5	137.5		137.5		
105		72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	1126.00	1689.00	TRUE		norethynodrel	-8.5	130.0	1689	1297.8	130980.5	130.0		130.0		
106		73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1521.00	2281.50	TRUE		Unknown 1	-3.0	189.4	2281.5	1890.3	130388.0	189.4	164.6			
107		74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1240.00	1860.00	TRUE		Unknown 1	-3.0	147.1	1860	1468.8	130809.5	147.1				
108		75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1307.00	1960.50	TRUE		Unknown 1	-3.0	157.2	1960.5	1569.3	130709.0	157.2				
109		76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	675.00	1012.50	TRUE		Unknown 1	-4.0	62.2	1012.5	621.3	131657.0	62.2	78.5			
110		77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	866.00	1299.00	TRUE		Unknown 1	-4.0	90.9	1299	907.8	131370.5	90.9				
111		78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	809.00	1213.50	TRUE		Unknown 1	-4.0	82.4	1213.5	822.3	131456.0	82.4				
112		79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	210.00	315.00	TRUE		Unknown 1	-5.0	-7.6	315	-76.2	132354.5	-7.6	-7.4	-7.6		
113		80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	223.00	334.50	TRUE		Unknown 1	-5.0	-5.7	334.5	-56.7	132335.0	-5.7		-5.7		
114		81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	201.00	301.50	TRUE		Unknown 1	-5.0	-9.0	301.5	-89.7	132368.0	-9.0		-9.0		
115		82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	221.00	331.50	TRUE		Unknown 1	-6.0	-6.0	331.5	-59.7	132338.0	-6.0	-5.8	-6.0		
116		83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	217.00	325.50	TRUE		Unknown 1	-6.0	-6.6	325.5	-65.7	132344.0	-6.6		-6.6		
117		84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	229.00	343.50	TRUE		Unknown 1	-6.0	-4.8	343.5	-47.7	132326.0	-4.8		-4.8		
118		85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	235.00	352.50	TRUE		Unknown 1	-7.0	-3.9	352.5	-38.7	132317.0	-3.9	-2.9	-3.9		
119		86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	253.00	379.50	TRUE		Unknown 1	-7.0	-1.2	379.5	-11.7	132290.0	-1.2		-1.2		
120		87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	236.00	354.00	TRUE		Unknown 1	-7.0	-3.7	354	-37.2	132315.5	-3.7		-3.7		
121		88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	368.00	552.00	TRUE		Unknown 1	-8.0	16.1	552	160.8	132117.5	16.1	15.4	16.1		
122		89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	357.00	535.50	TRUE		Unknown 1	-8.0	14.5	535.5	144.3	132134.0	14.5		14.5		
123		90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	364.00	546.00	TRUE		Unknown 1	-8.0	15.5	546	154.8	132123.5	15.5		15.5		
124		91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	788.00	1182.00	TRUE		Unknown 1	-9.0	79.2	1182	790.8	131487.5	79.2	74.0	79.2		
125		92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	628.00	942.00	TRUE		Unknown 1	-9.0	55.2	942	550.8	131727.5	55.2		55.2		
126		93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	843.00	1264.50	TRUE		Unknown 1	-9.0	87.5	1264.5	873.3	131405.0	87.5		87.5		
127		94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	979.00	1468.50	TRUE		Unknown 1	-10.0	107.9	1468.5	1077.3	131201.0	107.9	86.7	107.9		
128		95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	868.00	1302.00	TRUE		Unknown 1	-10.0	91.2	1302	910.8	131367.5	91.2		91.2		
129		96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	667.00	1000.50	TRUE		Unknown 1	-10.0	61.0	1000.5	609.3	131669.0	61.0		61.0		
130		97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	637.00	955.50	TRUE		Unknown 2	-3.0	56.5	955.5	564.3	131714.0	56.5	55.7	56.5		
131		98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	651.00	976.50	TRUE		Unknown 2	-3.0	58.6	976.5	585.3	131693.0	58.6		58.6		
132		99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	606.00	909.00	TRUE		Unknown 2	-3.0	51.9	909	517.8	131760.5	51.9		51.9		
133		100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	661.00	991.50	TRUE		Unknown 2	-4.0	60.1	991.5	600.3	131678.0	60.1	63.6	60.1		
134		101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	704.00	1056.00	TRUE		Unknown 2	-4.0	66.6	1056	664.8	131613.5	66.6		66.6		
135		102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	688.00	1032.00	TRUE		Unknown 2	-4.0	64.2	1032	640.8	131637.5	64.2		64.2		
136		103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	675.00	1012.50	TRUE		Unknown 2	-5.0	62.2	1012.5	621.3	131657.0	62.2	66.0	62.2		
137		104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	725.00	1087.50	TRUE		Unknown 2	-5.0	69.7	1087.5	696.3	131582.0	69.7		69.7		
138		105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	700.00	1050.00	TRUE		Unknown 2	-5.0	66.0	1050	658.8	131619.5	66.0		66.0		
139		106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	861.00	1291.50	TRUE		Unknown 2	-6.0	90.2	1291.5	900.3	131378.0	90.2	92.0	90.2		
140		107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	885.00	1327.50	TRUE		Unknown 2	-6.0	93.8	1327.5	936.3	131342.0	93.8		93.8		
141		108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	873.00	1309.50	TRUE		Unknown 2	-6.0	92.0	1309.5	918.3	131360.0	92.0		92.0		
142		109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1065.00	1597.50	TRUE		Unknown 2	-7.0	120.8	1597.5	1206.3	131072.0	120.8	121.0	120.8		
143		110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1075.00	1612.50	TRUE		Unknown 2	-7.0	122.3	1612.5	1221.3	131057.0	122.3		122.3		
144		111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1058.00	1587.00	TRUE		Unknown 2	-7.0	119.8	1587	1195.8	131082.5	119.8		119.8		
145		112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1091.00	1636.50	TRUE		Unknown 2	-8.0	124.7	1636.5	1245.3	131033.0	124.7	129.2	124.7		
146		113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1140.00	1710.00	TRUE		Unknown 2	-8.0	132.1	1710	1318.8	130959.5	132.1		132.1		
147		114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1132.00	1698.00	TRUE		Unknown 2	-8.0	130.9	1698	1306.8	130971.5	130.9		130.9		
148		115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1013.00	1519.50	TRUE		Unknown 2	-9.0	113.0	1519.5	1128.3	131150.0	113.0	127.6	113.0		
149		116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1181.00	1771.50	TRUE		Unknown 2	-9.0	138.3	1771.5	1380.3	130898.0	138.3		138.3		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	1.05%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.						
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
150		117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1136.00	1704.00	TRUE		Unknown 2	-9.0	131.5	1704	1312.8	130965.5	131.5		131.5		
151		118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1084.00	1626.00	TRUE		Unknown 2	-10.0	123.7	1626	1234.8	131043.5	123.7	131.3	123.7		
152		119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1168.00	1752.00	TRUE		Unknown 2	-10.0	136.3	1752	1360.8	130917.5	136.3		136.3		
153		120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1152.00	1728.00	TRUE		Unknown 2	-10.0	133.9	1728	1336.8	130941.5	133.9		133.9		
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	36111.00	54166.50	TRUE		Unknown 3	-3.0	5386.7	54166.5	53775.3	78503.0	5386.7	6100.6			
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	42598.00	63897.00	TRUE		Unknown 3	-3.0	6361.4	63897	63505.8	68772.5	6361.4				
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	43877.00	65815.50	TRUE		Unknown 3	-3.0	6553.6	65815.5	65424.3	66854.0	6553.6				
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	10076.00	15114.00	TRUE		Unknown 3	-4.0	1474.8	15114	14722.8	117555.5	1474.8	1715.8			
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	13054.00	19581.00	TRUE		Unknown 3	-4.0	1922.2	19581	19189.8	113088.5	1922.2				
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	11910.00	17865.00	TRUE		Unknown 3	-4.0	1750.4	17865	17473.8	114804.5	1750.4				
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	721.00	1081.50	TRUE		Unknown 3	-5.0	69.1	1081.5	690.3	131588.0	69.1	71.9	69.1		
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	749.00	1123.50	TRUE		Unknown 3	-5.0	73.4	1123.5	732.3	131546.0	73.4		73.4		
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	747.00	1120.50	TRUE		Unknown 3	-5.0	73.1	1120.5	729.3	131549.0	73.1		73.1		
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1035.00	1552.50	TRUE		Unknown 3	-6.0	116.3	1552.5	1161.3	131117.0	116.3	122.8	116.3		
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1100.00	1650.00	TRUE		Unknown 3	-6.0	126.1	1650	1258.8	131019.5	126.1		126.1		
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1099.00	1648.50	TRUE		Unknown 3	-6.0	125.9	1648.5	1257.3	131021.0	125.9		125.9		
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1062.00	1593.00	TRUE		Unknown 3	-7.0	120.4	1593	1201.8	131076.5	120.4	137.4	120.4		
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1344.00	2016.00	TRUE		Unknown 3	-7.0	162.8	2016	1624.8	130653.5	162.8		162.8		
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1119.00	1678.50	TRUE		Unknown 3	-7.0	128.9	1678.5	1287.3	130991.0	128.9		128.9		
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1036.00	1554.00	TRUE		Unknown 3	-8.0	116.5	1554	1162.8	131115.5	116.5	130.2	116.5		
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1154.00	1731.00	TRUE		Unknown 3	-8.0	134.2	1731	1339.8	130938.5	134.2		134.2		
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1191.00	1786.50	TRUE		Unknown 3	-8.0	139.8	1786.5	1395.3	130883.0	139.8		139.8		
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1099.00	1648.50	TRUE		Unknown 3	-9.0	125.9	1648.5	1257.3	131021.0	125.9	132.6	125.9		
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1171.00	1756.50	TRUE		Unknown 3	-9.0	136.8	1756.5	1365.3	130913.0	136.8		136.8		
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1160.00	1740.00	TRUE		Unknown 3	-9.0	135.1	1740	1348.8	130929.5	135.1		135.1		
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1021.00	1531.50	TRUE		Unknown 3	-10.0	114.2	1531.5	1140.3	131138.0	114.2	125.8	114.2		
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1147.00	1720.50	TRUE		Unknown 3	-10.0	133.2	1720.5	1329.3	130949.0	133.2		133.2		
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1127.00	1690.50	TRUE		Unknown 3	-10.0	130.2	1690.5	1299.3	130979.0	130.2		130.2		
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4507.00	6760.50	TRUE		Unknown 4	-3.0	638.0	6760.5	6369.3	125909.0	638.0	551.5			
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4447.00	6670.50	TRUE		Unknown 4	-3.0	629.0	6670.5	6279.3	125999.0	629.0				
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2840.00	4260.00	TRUE		Unknown 4	-3.0	387.5	4260	3868.8	128409.5	387.5				
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	486.00	729.00	TRUE		Unknown 4	-4.0	33.8	729	337.8	131940.5	33.8	38.7	33.8		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	528.00	792.00	TRUE		Unknown 4	-4.0	40.1	792	400.8	131877.5	40.1		40.1		
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	542.00	813.00	TRUE		Unknown 4	-4.0	42.3	813	421.8	131856.5	42.3		42.3		
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	564.00	846.00	TRUE		Unknown 4	-5.0	45.6	846	454.8	131823.5	45.6	56.4	45.6		
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	661.00	991.50	TRUE		Unknown 4	-5.0	60.1	991.5	600.3	131678.0	60.1		60.1		
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	683.00	1024.50	TRUE		Unknown 4	-5.0	63.4	1024.5	633.3	131645.0	63.4		63.4		
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1006.00	1509.00	TRUE		Unknown 4	-6.0	112.0	1509	1117.8	131160.5	112.0	117.3	112.0		
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1066.00	1599.00	TRUE		Unknown 4	-6.0	121.0	1599	1207.8	131070.5	121.0		121.0		
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1053.00	1579.50	TRUE		Unknown 4	-6.0	119.0	1579.5	1188.3	131090.0	119.0		119.0		
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1032.00	1548.00	TRUE		Unknown 4	-7.0	115.9	1548	1156.8	131121.5	115.9	125.7	115.9		
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1097.00	1645.50	TRUE		Unknown 4	-7.0	125.6	1645.5	1254.3	131024.0	125.6		125.6		
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1164.00	1746.00	TRUE		Unknown 4	-7.0	135.7	1746	1354.8	130923.5	135.7		135.7		
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1068.00	1602.00	TRUE		Unknown 4	-8.0	121.3	1602	1210.8	131067.5	121.3	127.8	121.3		
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1139.00	1708.50	TRUE		Unknown 4	-8.0	132.0	1708.5	1317.3	130961.0	132.0		132.0		
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1127.00	1690.50	TRUE		Unknown 4	-8.0	130.2	1690.5	1299.3	130979.0	130.2		130.2		
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1094.00	1641.00	TRUE		Unknown 4	-9.0	125.2	1641	1249.8	131028.5	125.2	131.2	125.2		
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1172.00	1758.00	TRUE		Unknown 4	-9.0	136.9	1758	1366.8	130911.5	136.9		136.9		
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1135.00	1702.50	TRUE		Unknown 4	-9.0	131.4	1702.5	1311.3	130967.0	131.4		131.4		
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1060.00	1590.00	TRUE		Unknown 4	-10.0	120.1	1590	1198.8	131079.5	120.1	124.6	120.1		
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1136.00	1704.00	TRUE		Unknown 4	-10.0	131.5	1704	1312.8	130965.5	131.5		131.5		
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1074.00	1611.00	TRUE		Unknown 4	-10.0	122.2	1611	1219.8	131058.5	122.2		122.2		
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	434.00	651.00	FALSE	mispipet	cold E2	-7.0							-2.1		
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	238.00	357.00	TRUE		cold E2	-7.0	-3.4	357	-34.2	132312.5	-3.4		-3.4		
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	255.00	382.50	TRUE		cold E2	-7.0	-0.9	382.5	-8.7	132287.0	-0.9		-0.9		
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	908.00	1362.00	TRUE			—	97.2	1362	970.8	131307.5	97.2	100.2	97.2		
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	881.00	1321.50	TRUE			—	93.2	1321.5	930.3	131348.0	93.2		93.2		
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	993.00	1489.50	TRUE			—	110.0	1489.5	1098.3	131180.0	110.0		110.0		
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average			
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
211		178	1	none	Hot		—	—	—	10	—	—	—	—	89591.00	134386.50	TRUE					134386.5	133995.3						
212		179	2	none	Hot		—	—	—	10	—	—	—	—	87250.00	130875.00	TRUE					130875	130483.8						
213		180	3	none	Hot		—	—	—	10	—	—	—	—	87639.00	131458.50	TRUE					131458.5	131067.3						
214		181	1	none	Hot		—	—	—	10	—	—	—	—	88061.00	132091.50	TRUE					132091.5	131700.3						
215		182	2	none	Hot		—	—	—	10	—	—	—	—	88295.00	132442.50	TRUE					132442.5	132051.3						
216		183	3	none	Hot		—	—	—	10	—	—	—	—	88277.00	132415.50	TRUE					132415.5	132024.3						

	B	C	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31			Prism data, excluding data points in the right leg of U-shaped curves																											
32			*standard curve*				*weak positive*				*4.0*				*9.0*				*14.0*				*17.0*				*negative control*			
33	*Position*	*Replicate*	*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1	-7.0	0.1803	1.0818	3.0352	-4.0				-3.0				-3.0	56.5261	58.6297	51.8682	-3.0				-3.0				-3.0	#N/A	#N/A	#N/A
35	2	2	-7.0		-3.4258	-0.8715	-4.5	7.3926	1.3823	2.2839	-4.0				-4.0	60.1322	66.5932	64.1891	-4.0				-4.0	33.8375	40.1483	42.2518	-4.0	#N/A	#N/A	#N/A
36	3	3	-8.0	35.6406	47.2103	41.8011	-5.5	72.7537	74.7070	82.6705	-5.0	-7.6330	-5.6797	-8.9853	-5.0	62.2358	69.7486	65.9922	-5.0	69.1476	73.3547	73.0542	-5.0	45.5574	60.1322	63.4378	-5.0	#N/A	#N/A	#N/A
37	4	1	-8.5	68.3963	73.9557	77.4116	-6.0	98.2971	108.0637	101.6027	-6.0	-5.9802	-6.5812	-4.7781	-6.0	90.1833	93.7894	91.9864	-6.0	116.3278	126.0944	125.9441	-6.0	111.9703	120.8657	119.0324	-6.0	#N/A	#N/A	#N/A
38	5	2	-9.0	95.1417	111.2191	109.4160	-6.5	115.7357	123.2395	131.0528	-7.0	-3.8766	-1.1720	-3.7263	-7.0	120.8354	122.3380	119.7836	-7.0	120.3647	162.7567	128.9492	-7.0	115.8770	126.8436	135.7107	-7.0	#N/A	#N/A	#N/A
39	6	3	-9.5	110.3175	118.7318	99.6494	-7.0	122.9390	127.1461	141.2702	-8.0	16.1074	14.4545	15.5064	-8.0	124.7421	132.1046	130.9025	-8.0	116.4780	134.2082	139.7676	-8.0	121.2862	131.9543	130.1513	-8.0	#N/A	#N/A	#N/A
40	7	1	-10.0	139.7676	124.1410	123.0893	-7.5	122.1877	130.9025	131.0528	-9.0	79.2147	55.1738	87.4787	-9.0	113.0221	138.2651	131.5036	-9.0	125.9441	136.7625	135.1097	-9.0	125.1928	136.9128	131.3533	-9.0	#N/A	#N/A	#N/A
41	8	2	-11.0	115.6765	123.8405	127.1461	-8.5	127.2964	137.5138	130.0010	-10.0	107.9135	91.2351	61.0338	-10.0	123.6903	136.3117	133.9076	-10.0	114.2242	133.1564	130.1513	-10.0	120.0841	131.5036	122.1872	-10.0	#N/A	#N/A	#N/A
42	9	3																												
43	10	1	Standard Curve		TTN010 04-28-08		Positive Control		TTN010 04-28-08		4.0		TTN010 04-28-08		9.0		TTN010 04-28-08		14.0		TTN010 04-28-08		17.0		TTN010 04-28-08		Negative Control		Prism run ID & date	
44	11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values		bad initial values		Best-fit values		bad initial values		Best-fit values				Best-fit values			
46	13	1	BOTTOM		-9.274		BOTTOM		-27.67		BOTTOM		-5.96		BOTTOM		55.67		BOTTOM		71.85		BOTTOM		37.95		BOTTOM			
47	14	2	TOP		125.5		TOP		132.1		TOP		87.72		TOP		131.3		TOP		137.4		TOP		127.4		TOP			
48	15	3	LOGIC50		-8.136		LOGIC50		-5.171		LOGIC50		-8.543		LOGIC50		-6.052		LOGIC50		-5.643		LOGIC50		-4.849		LOGIC50			
49	16	1	HILLSLOPE		-0.8994		HILLSLOPE		-0.8737		HILLSLOPE		-1.28		HILLSLOPE		-1		HILLSLOPE		-1		HILLSLOPE		-1.475		HILLSLOPE			
50	17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3	BOTTOM		6.92		BOTTOM		21.54		BOTTOM		3.908		BOTTOM				BOTTOM				BOTTOM		4.19		BOTTOM			
52	19	1	TOP		3.637		TOP		2.704		TOP		7.322		TOP				TOP				TOP		1.944		TOP			
53	20	2	LOGIC50		0.05161		LOGIC50		0.06447		LOGIC50		0.1288		LOGIC50		na		LOGIC50		na		LOGIC50		0.09133		LOGIC50			
54	21	3	HILLSLOPE		0.145		HILLSLOPE		0.1787		HILLSLOPE		0.3696		HILLSLOPE				HILLSLOPE				HILLSLOPE		0.2499		HILLSLOPE			
55	22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2	Data				Data				Data				Data				Data				Data				Data			
66	33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
70	37	1																												

Entered by: _____
Verified by: _____
Read and reviewed by _____:

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.05

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	0.2
1	1	2	-7.0	1.1
1	1	3	-7.0	3.0
1	2	1	-8.0	35.6
1	2	2	-8.0	47.2
1	2	3	-8.0	41.8
1	3	1	-8.5	68.4
1	3	2	-8.5	74.0
1	3	3	-8.5	77.4
1	4	1	-9.0	95.1
1	4	2	-9.0	111.2
1	4	3	-9.0	109.4
1	5	1	-9.5	110.3
1	5	2	-9.5	118.7
1	5	3	-9.5	99.6
1	6	1	-10.0	139.8
1	6	2	-10.0	124.1
1	6	3	-10.0	123.1
1	7	1	-11.0	115.6
1	7	2	-11.0	123.8
1	7	3	-11.0	127.1
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.18

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Norethynodrel
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1		
1	1	2		
1	1	3		
1	2	1	-4.5	7.4
1	2	2	-4.5	1.4
1	2	3	-4.5	2.3
1	3	1	-5.5	72.8
1	3	2	-5.5	74.7
1	3	3	-5.5	82.7
1	4	1	-6.0	98.3
1	4	2	-6.0	108.1
1	4	3	-6.0	101.6
1	5	1	-6.5	115.7
1	5	2	-6.5	123.2
1	5	3	-6.5	131.1
1	6	1	-7.0	122.9
1	6	2	-7.0	127.1
1	6	3	-7.0	141.3
1	7	1	-7.5	122.2
1	7	2	-7.5	130.9
1	7	3	-7.5	131.1
1	8	1	-8.5	127.3
1	8	2	-8.5	137.5
1	8	3	-8.5	130.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 13.95

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 4
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	189.4
1	1	2	-3.0	147.1
1	1	3	-3.0	157.2
1	2	1	-4.0	62.2
1	2	2	-4.0	90.9
1	2	3	-4.0	82.4
1	3	1	-5.0	-7.6
1	3	2	-5.0	-5.7
1	3	3	-5.0	-9.0
1	4	1	-6.0	-6.0
1	4	2	-6.0	-6.6
1	4	3	-6.0	-4.8
1	5	1	-7.0	-3.9
1	5	2	-7.0	-1.2
1	5	3	-7.0	-3.7
1	6	1	-8.0	16.1
1	6	2	-8.0	14.5
1	6	3	-8.0	15.5
1	7	1	-9.0	79.2
1	7	2	-9.0	55.2
1	7	3	-9.0	87.5
1	8	1	-10.0	107.9
1	8	2	-10.0	91.2
1	8	3	-10.0	61.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.84

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 9
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	56.5
1	1	2	-3.0	58.6
1	1	3	-3.0	51.9
1	2	1	-4.0	60.1
1	2	2	-4.0	66.6
1	2	3	-4.0	64.2
1	3	1	-5.0	62.2
1	3	2	-5.0	69.7
1	3	3	-5.0	66.0
1	4	1	-6.0	90.2
1	4	2	-6.0	93.8
1	4	3	-6.0	92.0
1	5	1	-7.0	120.8
1	5	2	-7.0	122.3
1	5	3	-7.0	119.8
1	6	1	-8.0	124.7
1	6	2	-8.0	132.1
1	6	3	-8.0	130.9
1	7	1	-9.0	113.0
1	7	2	-9.0	138.3
1	7	3	-9.0	131.5
1	8	1	-10.0	123.7
1	8	2	-10.0	136.3
1	8	3	-10.0	133.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 10.15

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 14
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	
1	2	2	-4.0	
1	2	3	-4.0	
1	3	1	-5.0	69.1
1	3	2	-5.0	73.4
1	3	3	-5.0	73.1
1	4	1	-6.0	116.3
1	4	2	-6.0	126.1
1	4	3	-6.0	125.9
1	5	1	-7.0	120.4
1	5	2	-7.0	162.8
1	5	3	-7.0	128.9
1	6	1	-8.0	116.5
1	6	2	-8.0	134.2
1	6	3	-8.0	139.8
1	7	1	-9.0	125.9
1	7	2	-9.0	136.8
1	7	3	-9.0	135.1
1	8	1	-10.0	114.2
1	8	2	-10.0	133.2
1	8	3	-10.0	130.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.47

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 17
Run ID: TTN 010
Assay start date: 4/28/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	33.8
1	2	2	-4.0	40.1
1	2	3	-4.0	42.3
1	3	1	-5.0	45.6
1	3	2	-5.0	60.1
1	3	3	-5.0	63.4
1	4	1	-6.0	112.0
1	4	2	-6.0	121.0
1	4	3	-6.0	119.0
1	5	1	-7.0	115.9
1	5	2	-7.0	125.6
1	5	3	-7.0	135.7
1	6	1	-8.0	121.3
1	6	2	-8.0	132.0
1	6	3	-8.0	130.2
1	7	1	-9.0	125.2
1	7	2	-9.0	136.9
1	7	3	-9.0	131.4
1	8	1	-10.0	120.1
1	8	2	-10.0	131.5
1	8	3	-10.0	122.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run TTN010 04-28-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.1803	1.0818	3.0352
2	Title	-7.0		-3.4258	-0.8715
3	Title	-8.0	35.6406	47.2103	41.8011
4	Title	-8.5	68.3963	73.9557	77.4116
5	Title	-9.0	95.1417	111.2191	109.4160
6	Title	-9.5	110.3175	118.7318	99.6494
7	Title	-10.0	139.7676	124.1410	123.0893
8	Title	-11.0	115.5765	123.8405	127.1461

Nonlin fit Table of results		A run TTN010 04-28-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-9.274
4	TOP	125.5
5	LOGIC50	-8.136
6	HILLSLOPE	-0.8994
7	Std. Error	
8	BOTTOM	6.920
9	TOP	3.637
10	LOGIC50	0.05161
11	HILLSLOPE	0.1450
12	95% Confidence Intervals	
13	BOTTOM	-23.76 to 5.208
14	TOP	117.9 to 133.1
15	LOGIC50	-8.244 to -8.028
16	HILLSLOPE	-1.203 to -0.5960
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9804
20	Absolute Sum of Squares	1058
21	Sy.x	7.462
22	Number of points	
23	Analyzed	23

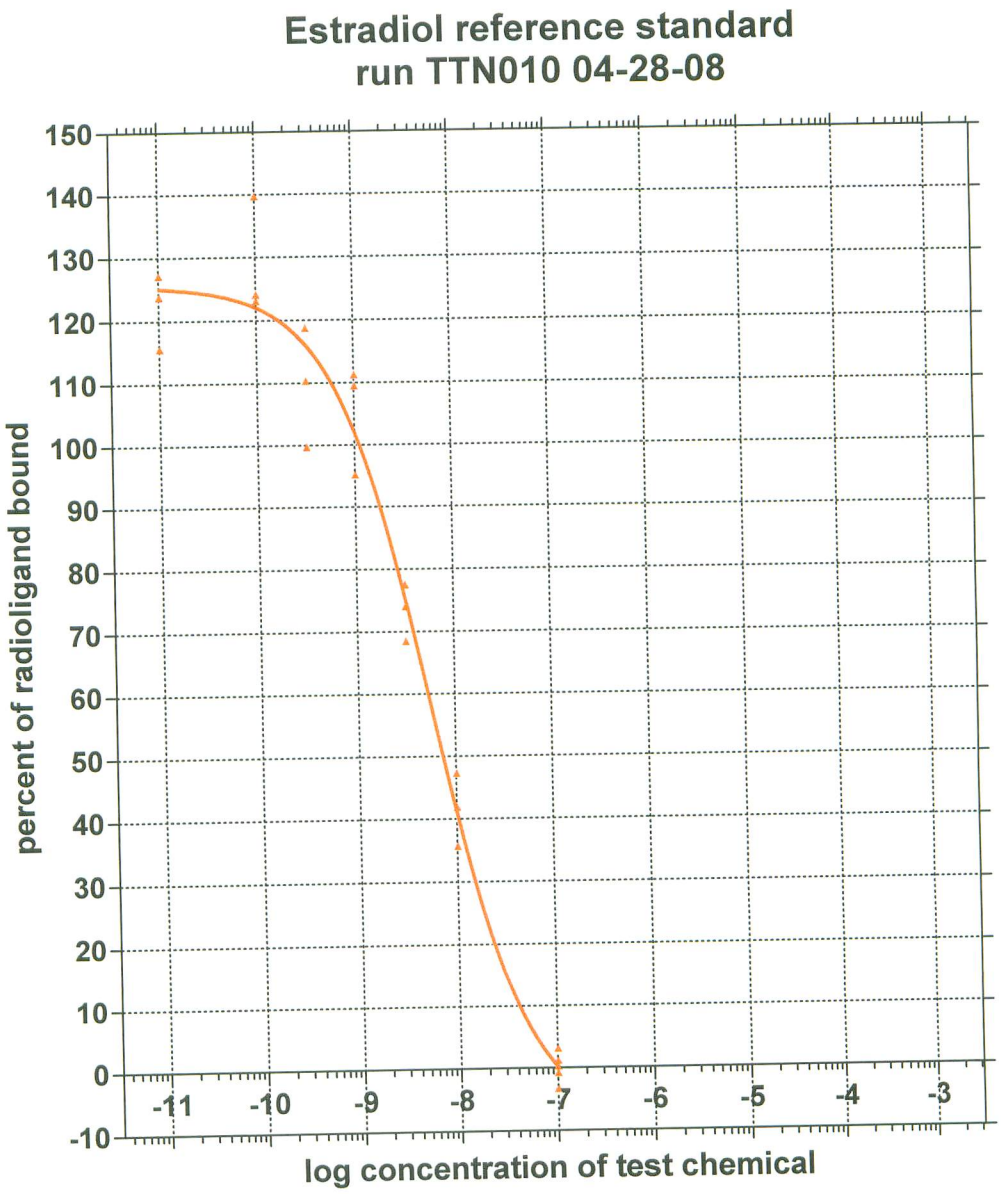

Estradiol reference standard
run TTN010 04-28-08
percent of radioligand bound
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run TTN010 04-28-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	7.3926	1.3823	2.2839
3	Title	-5.5	72.7537	74.7070	82.6705
4	Title	-6.0	98.2971	108.0637	101.6027
5	Title	-6.5	115.7267	123.2395	131.0528
6	Title	-7.0	122.9390	127.1461	141.2702
7	Title	-7.5	122.1877	130.9025	131.0528
8	Title	-8.5	127.2964	137.5138	130.0010

	Nonlin fit Table of results	A run TTN010 04-28-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-27.67
4	TOP	132.1
5	LOGIC50	-5.171
6	HILLSLOPE	-0.8737
7	Std. Error	
8	BOTTOM	21.54
9	TOP	2.704
10	LOGIC50	0.06447
11	HILLSLOPE	0.1787
12	95% Confidence Intervals	
13	BOTTOM	-73.11 to 17.77
14	TOP	126.4 to 137.8
15	LOGIC50	-5.307 to -5.035
16	HILLSLOPE	-1.251 to -0.4967
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9846
20	Absolute Sum of Squares	614.0
21	Sy.x	6.010
22	Number of points	
23	Analyzed	21

Norethynodrel
run TTN010 04-28-08

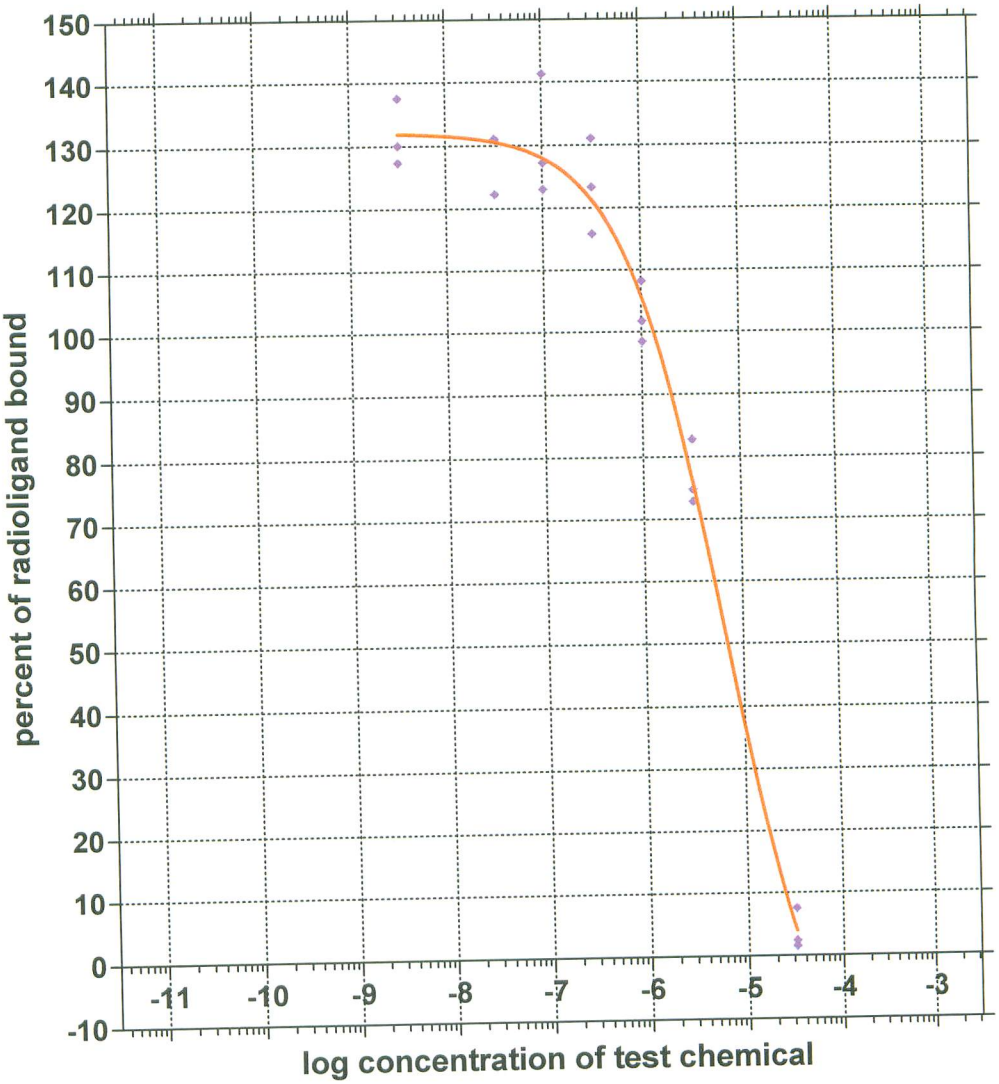


Table format: XY		X	A		
		log concentration	Test chemical		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	-7.6330	-5.6797	-8.9853
4	Title	-6.0	-5.9802	-6.5812	-4.7781
5	Title	-7.0	-3.8766	-1.1720	-3.7263
6	Title	-8.0	16.1074	14.4546	15.5064
7	Title	-9.0	79.2147	55.1738	87.4787
8	Title	-10.0	107.9135	91.2351	61.0338

	Nonlin fit Table of results	A Test chemical
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-5.960
4	TOP	87.72
5	LOGIC50	-8.543
6	HILLSLOPE	-1.280
7	Std. Error	
8	BOTTOM	3.908
9	TOP	7.322
10	LOGIC50	0.1288
11	HILLSLOPE	0.3696
12	95% Confidence Intervals	
13	BOTTOM	-14.34 to 2.423
14	TOP	72.02 to 103.4
15	LOGIC50	-8.819 to -8.267
16	HILLSLOPE	-2.072 to -0.4868
17	Goodness of Fit	
18	Degrees of Freedom	14
19	R^2	0.9405
20	Absolute Sum of Squares	1720
21	Sy.x	11.09
22	Number of points	
23	Analyzed	18

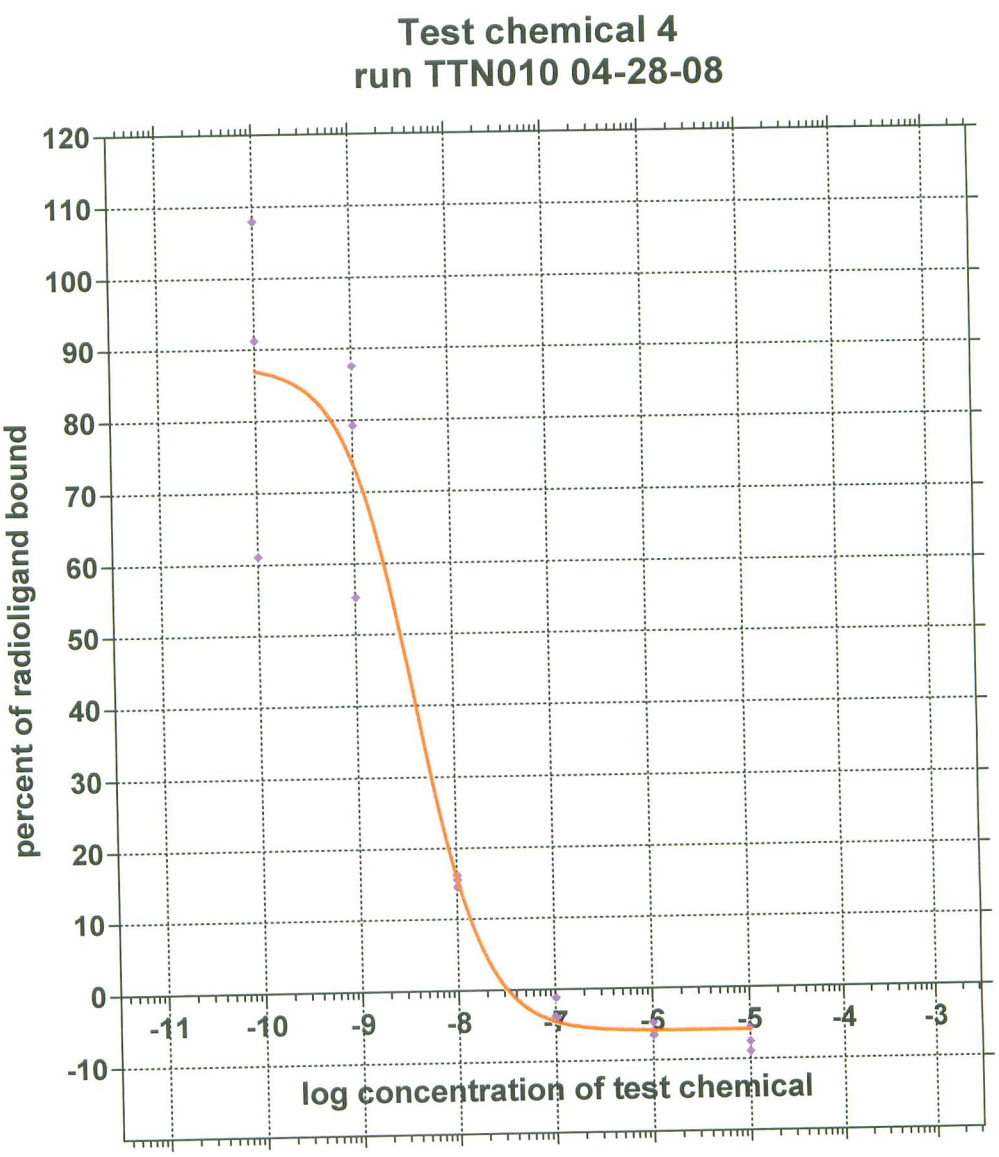

Test chemical 4
run TTN010 04-28-08
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Data Set-A		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	56.5261	58.6297	51.8682
2	Title	-4.0	60.1322	66.5932	64.1891
3	Title	-5.0	62.2358	69.7486	65.9922
4	Title	-6.0	90.1833	93.7894	91.9864
5	Title	-7.0	120.8354	122.3380	119.7836
6	Title	-8.0	124.7421	132.1046	130.9025
7	Title	-9.0	113.0221	138.2651	131.5036
8	Title	-10.0	123.6903	136.3117	133.9076

	Nonlin fit Table of results	A Data Set-A
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	55.67
4	TOP	131.3
5	LOGIC50	-6.052
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	24

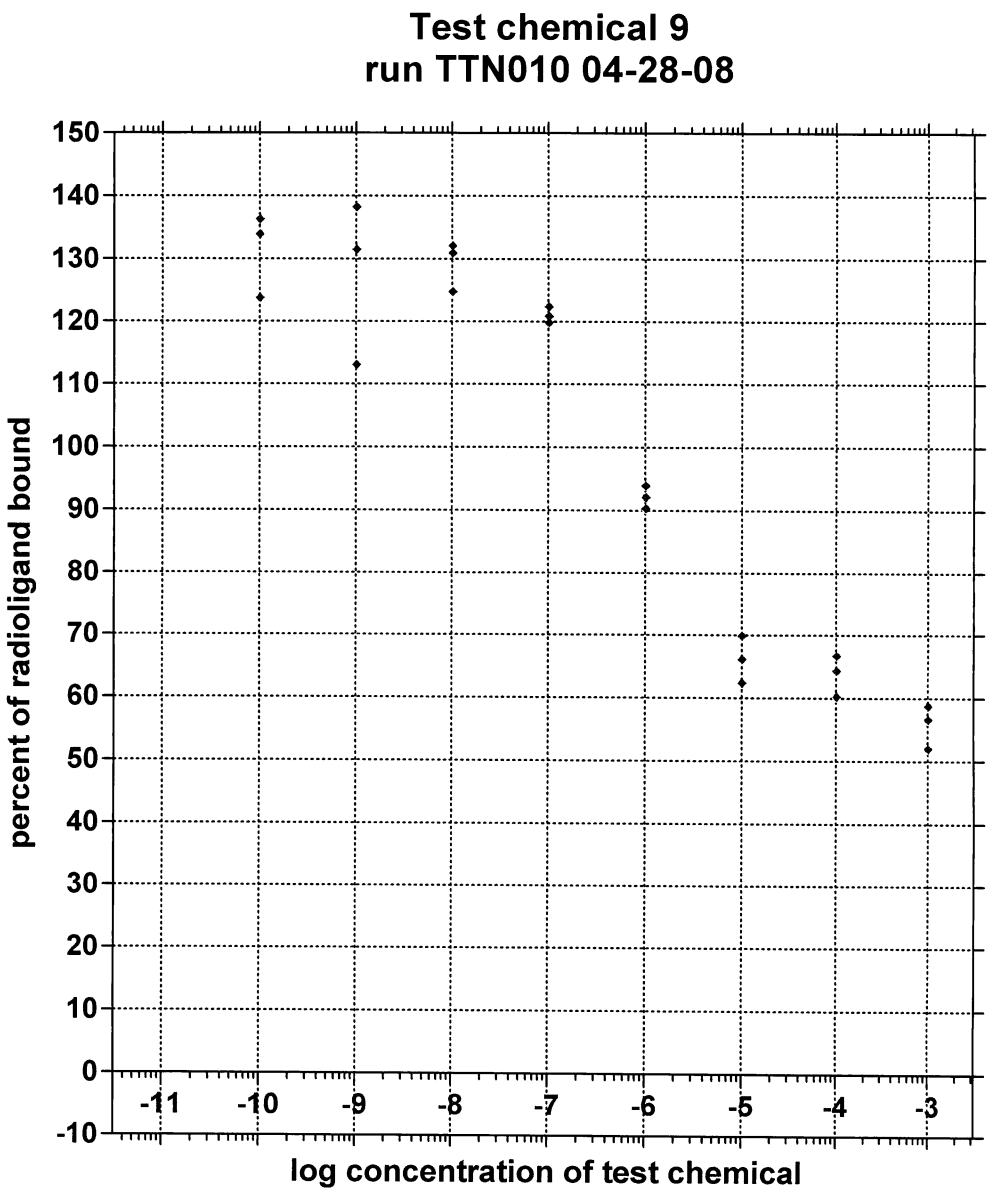

Test chemical 9
run TTN010 04-28-08
percent of radioligand bound
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A			B		
		log concentration	Data Set-A			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	69.1476	73.3547	73.0542			
4	Title	-6.0	116.3278	126.0944	125.9441			
5	Title	-7.0	120.3847	162.7567	128.9492			
6	Title	-8.0	116.4780	134.2082	139.7676			
7	Title	-9.0	125.9441	136.7625	135.1097			
8	Title	-10.0	114.2242	133.1564	130.1513			

	Nonlin fit Table of results	A Data Set-A	B Data Set-B
		Y	Y
1	logIC50	Bad initial values	
2	Best-fit values		
3	BOTTOM	71.85	
4	TOP	137.4	
5	LOGIC50	-5.643	
6	HILLSLOPE	-1.000	
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	18	

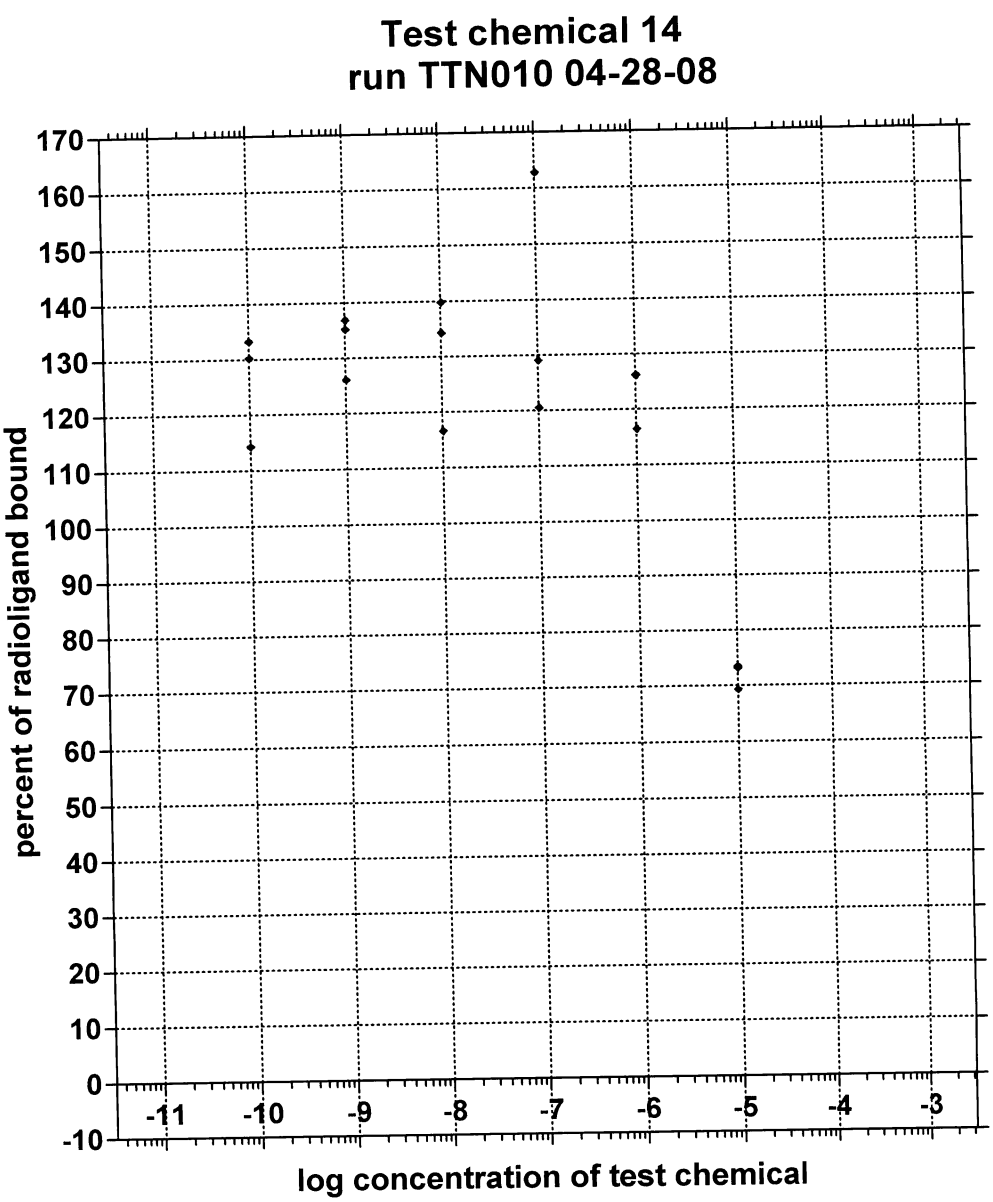
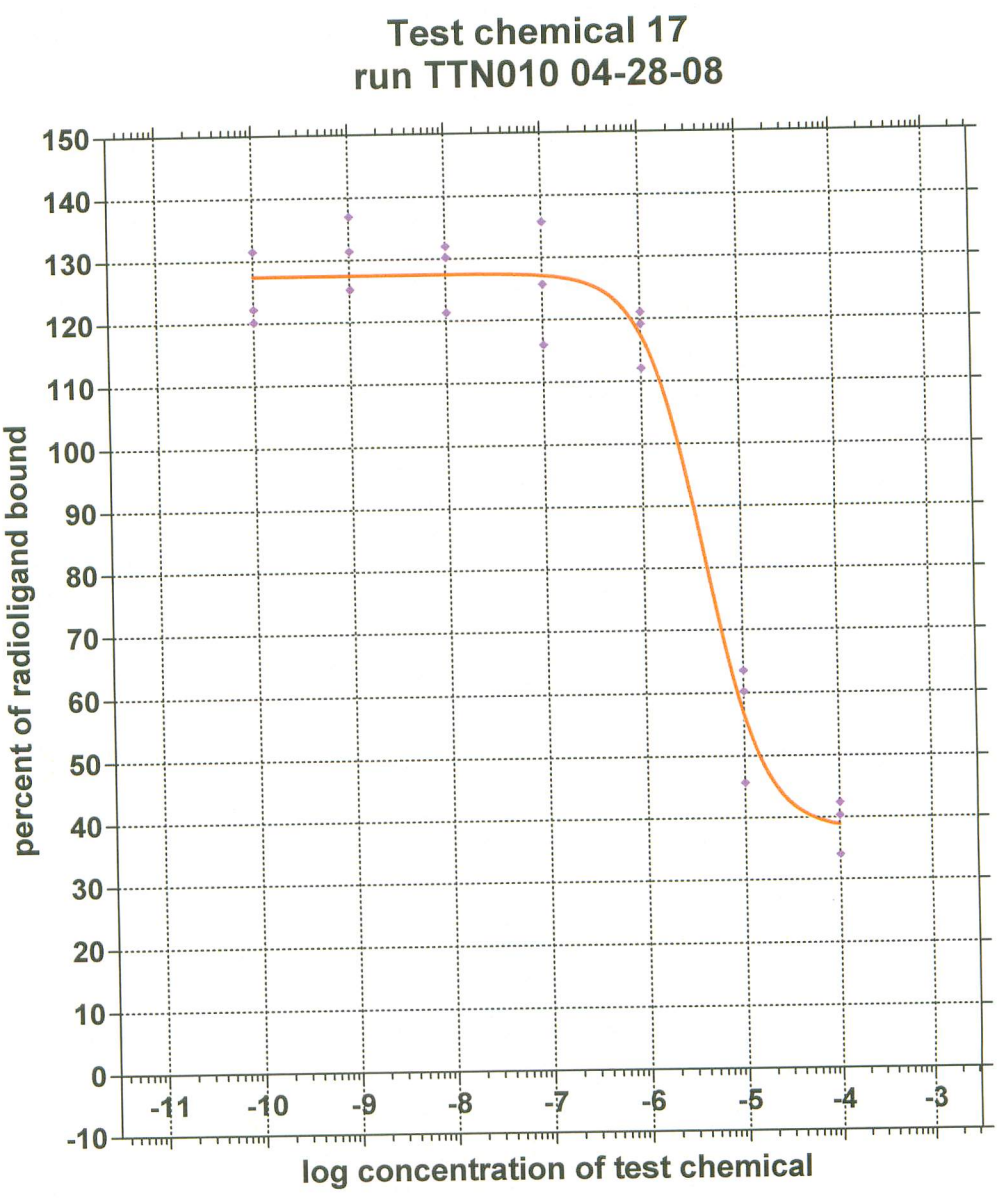

Test chemical 14
run TTN010 04-28-08
percent of radioligand bound
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Data Set-A		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	33.8375	40.1483	42.2518
3	Title	-5.0	45.5574	60.1322	63.4378
4	Title	-6.0	111.9703	120.9857	119.0324
5	Title	-7.0	115.8770	125.6436	135.7107
6	Title	-8.0	121.2862	131.9543	130.1513
7	Title	-9.0	125.1928	136.9128	131.3533
8	Title	-10.0	120.0841	131.5036	122.1877

	Nonlin fit Table of results	A Data Set-A
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	37.95
4	TOP	127.4
5	LOGIC50	-4.849
6	HILLSLOPE	-1.475
7	Std. Error	
8	BOTTOM	4.190
9	TOP	1.944
10	LOGIC50	0.09133
11	HILLSLOPE	0.2499
12	95% Confidence Intervals	
13	BOTTOM	29.11 to 46.79
14	TOP	123.3 to 131.5
15	LOGIC50	-5.042 to -4.656
16	HILLSLOPE	-2.002 to -0.9473
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9730
20	Absolute Sum of Squares	740.2
21	Sy.x	6.599
22	Number of points	
23	Analyzed	21


Test chemical 17
run TTN010 04-28-08
percent of radioligand bound
150
140
130
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

APPENDIX M

Excel and Prism Files of the Run Identifier TTN011

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B
Run identification:	TTN011
Assay start date:	4/29/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	109.01 Ci/mmole
Cytosol vial or lot identification:	RUC 02-22-08
Protein (cytosol):	50 microgram per tube

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

log(IC50), Cold E2 (Standard Curve):	-8.04	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-8.04	M
RBA, norethynodrel (Weak Positive):	1.000E+00	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-8.34	4.0
RBA, Unknown 1:	2.009E+00	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	n/a	9.0
RBA, Unknown 2:	n/a	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	n/a	14.0
RBA, Unknown 3:	n/a	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	n/a	20.0
RBA, Unknown 4:	n/a	relative to E2=1.0

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 0.81%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	1084.5	24.07	Total Binding, solvent control, tubes
Hot	6	134488.3	6772.36	Total hot E2 added to each tube
NSB	6	462.0	30.01	Nonspecific Binding
Specific EtOH	6	622.5	24.07	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.01	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.43	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	-1.4
R1881	#N/A	not applicable
norethynodrel	-4.0	2.9
Unknown 1	-5.0	-15.7
Unknown 2	-3.0	58.6
Unknown 3	-5.0	69.8
Unknown 4	-5.0	96.1

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	714.00	1071.00	TRUE		—	97.8	1071	609.0	133879.3	97.8	98.0	97.8
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	698.00	1047.00	TRUE		—	94.0	1047	585.0	133903.3	94.0		94.0
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	732.00	1098.00	TRUE		—	102.2	1098	636.0	133852.3	102.2		102.2
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	284.00	426.00	TRUE		cold E2	-7.0	-5.8	426	-36.0	134524.3	-5.8	-1.4	-5.8
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	321.00	481.50	TRUE		cold E2	-7.0	3.1	481.5	19.5	134468.8	3.1		3.1
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	302.00	453.00	TRUE		cold E2	-7.0	-1.4	453	-9.0	134497.3	-1.4		-1.4
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	501.00	751.50	TRUE		cold E2	-8.0	46.5	751.5	289.5	134198.8	46.5	45.1	46.5
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	489.00	733.50	TRUE		cold E2	-8.0	43.6	733.5	271.5	134216.8	43.6		43.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	580.00	870.00	FALSE	mispipet	cold E2	-8.0							
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	667.00	1000.50	TRUE		cold E2	-8.5	86.5	1000.5	538.5	133949.8	86.5	93.1	86.5
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	716.00	1074.00	TRUE		cold E2	-8.5	98.3	1074	612.0	133876.3	98.3		98.3
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	700.00	1050.00	TRUE		cold E2	-8.5	94.5	1050	588.0	133900.3	94.5		94.5
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	787.00	1180.50	TRUE		cold E2	-9.0	115.4	1180.5	718.5	133769.8	115.4	117.1	115.4
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	795.00	1192.50	TRUE		cold E2	-9.0	117.3	1192.5	730.5	133757.8	117.3		117.3
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	800.00	1200.00	TRUE		cold E2	-9.0	118.6	1200	738.0	133750.3	118.6		118.6
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	795.00	1192.50	FALSE	mispipet	cold E2	-9.5						134.3	
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	862.00	1293.00	TRUE		cold E2	-9.5	133.5	1293	831.0	133657.3	133.5		133.5
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	869.00	1303.50	TRUE		cold E2	-9.5	135.2	1303.5	841.5	133646.8	135.2		135.2
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	954.00	1431.00	TRUE		cold E2	-10.0	155.7	1431	969.0	133519.3	155.7	146.7	155.7
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	895.00	1342.50	TRUE		cold E2	-10.0	141.4	1342.5	880.5	133607.8	141.4		141.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	901.00	1351.50	TRUE		cold E2	-10.0	142.9	1351.5	889.5	133598.8	142.9		142.9
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	979.00	1468.50	FALSE	mispipet	cold E2	-11.0						137.0	
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	866.00	1299.00	TRUE		cold E2	-11.0	134.5	1299	837.0	133651.3	134.5		134.5
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	887.00	1330.50	TRUE		cold E2	-11.0	139.5	1330.5	868.5	133619.8	139.5		139.5
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 0.81% — If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	312.00	468.00	TRUE		norethynodrel	-4.0	1.0	468	6.0	134482.3	1.0	2.9	1.0
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	312.00	468.00	TRUE		norethynodrel	-4.0	1.0	468	6.0	134482.3	1.0		1.0
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	336.00	504.00	TRUE		norethynodrel	-4.0	6.7	504	42.0	134446.3	6.7		6.7
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	344.00	516.00	TRUE		norethynodrel	-4.5	8.7	516	54.0	134434.3	8.7	10.8	8.7
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	361.00	541.50	TRUE		norethynodrel	-4.5	12.8	541.5	79.5	134408.8	12.8		12.8
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	353.00	529.50	TRUE		norethynodrel	-4.5	10.8	529.5	67.5	134420.8	10.8		10.8
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	629.00	943.50	TRUE		norethynodrel	-5.5	77.3	943.5	481.5	134006.8	77.3	84.4	77.3
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	688.00	1032.00	TRUE		norethynodrel	-5.5	91.6	1032	570.0	133918.3	91.6		91.6
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	658.00	987.00	TRUE		norethynodrel	-5.5	84.3	987	525.0	133963.3	84.3		84.3
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	742.00	1113.00	TRUE		norethynodrel	-6.0	104.6	1113	651.0	133837.3	104.6	109.0	104.6
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	747.00	1120.50	TRUE		norethynodrel	-6.0	105.8	1120.5	658.5	133829.8	105.8		105.8
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	792.00	1188.00	TRUE		norethynodrel	-6.0	116.6	1188	726.0	133762.3	116.6		116.6
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	797.00	1195.50	TRUE		norethynodrel	-6.5	117.8	1195.5	733.5	133754.8	117.8	118.6	117.8
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	819.00	1228.50	TRUE		norethynodrel	-6.5	123.1	1228.5	766.5	133721.8	123.1		123.1
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	784.00	1176.00	TRUE		norethynodrel	-6.5	114.7	1176	714.0	133774.3	114.7		114.7
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	868.00	1302.00	TRUE		norethynodrel	-7.0	134.9	1302	840.0	133648.3	134.9	126.3	134.9
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	803.00	1204.50	TRUE		norethynodrel	-7.0	119.3	1204.5	742.5	133745.8	119.3		119.3
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	826.00	1239.00	TRUE		norethynodrel	-7.0	124.6	1239	777.0	133711.3	124.6		124.6
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	814.00	1221.00	TRUE		norethynodrel	-7.5	121.9	1221	759.0	133729.3	121.9	130.0	121.9
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	837.00	1255.50	TRUE		norethynodrel	-7.5	127.5	1255.5	793.5	133694.8	127.5		127.5
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	891.00	1336.50	TRUE		norethynodrel	-7.5	140.5	1336.5	874.5	133613.8	140.5		140.5
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	848.00	1272.00	TRUE		norethynodrel	-8.5	130.1	1272	810.0	133678.3	130.1	134.5	130.1
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	847.00	1270.50	TRUE		norethynodrel	-8.5	129.9	1270.5	808.5	133679.8	129.9		129.9
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	903.00	1354.50	TRUE		norethynodrel	-8.5	143.4	1354.5	892.5	133595.8	143.4		143.4
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1145.00	1717.50	TRUE		Unknown 1	-3.0	201.7	1717.5	1255.5	133232.8	201.7	232.9	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	963.00	1444.50	TRUE		Unknown 1	-3.0	157.8	1444.5	982.5	133505.8	157.8		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1716.00	2574.00	TRUE		Unknown 1	-3.0	339.3	2574	2112.0	132376.3	339.3		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	503.00	754.50	TRUE		Unknown 1	-4.0	47.0	754.5	292.5	134195.8	47.0	71.7	
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	588.00	882.00	TRUE		Unknown 1	-4.0	67.5	882	420.0	134068.3	67.5		
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	726.00	1089.00	TRUE		Unknown 1	-4.0	100.7	1089	627.0	133861.3	100.7		
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	229.00	343.50	TRUE		Unknown 1	-5.0	-19.0	343.5	-118.5	134606.8	-19.0	-15.7	-19.0
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	250.00	375.00	TRUE		Unknown 1	-5.0	-14.0	375	-87.0	134575.3	-14.0		-14.0
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	249.00	373.50	TRUE		Unknown 1	-5.0	-14.2	373.5	-88.5	134576.8	-14.2		-14.2
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	266.00	399.00	TRUE		Unknown 1	-6.0	-10.1	399	-63.0	134551.3	-10.1	-9.9	-10.1
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	264.00	396.00	TRUE		Unknown 1	-6.0	-10.6	396	-66.0	134554.3	-10.6		-10.6
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	271.00	406.50	TRUE		Unknown 1	-6.0	-8.9	406.5	-55.5	134543.8	-8.9		-8.9
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	290.00	435.00	TRUE		Unknown 1	-7.0	-4.3	435	-27.0	134515.3	-4.3	-3.8	-4.3
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	315.00	472.50	TRUE		Unknown 1	-7.0	1.7	472.5	10.5	134477.8	1.7		1.7
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	272.00	408.00	TRUE		Unknown 1	-7.0	-8.7	408	-54.0	134542.3	-8.7		-8.7
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	409.00	613.50	TRUE		Unknown 1	-8.0	24.3	613.5	151.5	134336.8	24.3	26.4	24.3
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	444.00	666.00	TRUE		Unknown 1	-8.0	32.8	666	204.0	134284.3	32.8		32.8
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	400.00	600.00	TRUE		Unknown 1	-8.0	22.2	600	138.0	134350.3	22.2		22.2
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	654.00	981.00	TRUE		Unknown 1	-9.0	83.4	981	519.0	133969.3	83.4	96.5	83.4
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	733.00	1099.50	TRUE		Unknown 1	-9.0	102.4	1099.5	637.5	133850.8	102.4		102.4
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	739.00	1108.50	TRUE		Unknown 1	-9.0	103.9	1108.5	646.5	133841.8	103.9		103.9
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	807.00	1210.50	TRUE		Unknown 1	-10.0	120.2	1210.5	748.5	133739.8	120.2	127.7	120.2
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	846.00	1269.00	TRUE		Unknown 1	-10.0	129.6	1269	807.0	133681.3	129.6		129.6
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	861.00	1291.50	TRUE		Unknown 1	-10.0	133.3	1291.5	829.5	133658.8	133.3		133.3
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	508.00	762.00	TRUE		Unknown 2	-3.0	48.2	762	300.0	134188.3	48.2	58.6	48.2
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	516.00	774.00	TRUE		Unknown 2	-3.0	50.1	774	312.0	134176.3	50.1		50.1
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	630.00	945.00	TRUE		Unknown 2	-3.0	77.6	945	483.0	134005.3	77.6		77.6
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	536.00	804.00	TRUE		Unknown 2	-4.0	54.9	804	342.0	134146.3	54.9	66.7	54.9
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	631.00	946.50	TRUE		Unknown 2	-4.0	77.8	946.5	484.5	134003.8	77.8		77.8
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	588.00	882.00	TRUE		Unknown 2	-4.0	67.5	882	420.0	134068.3	67.5		67.5
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	629.00	943.50	TRUE		Unknown 2	-5.0	77.3	943.5	481.5	134006.8	77.3	84.0	77.3
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	681.00	1021.50	TRUE		Unknown 2	-5.0	89.9	1021.5	559.5	133928.8	89.9		89.9
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	660.00	990.00	TRUE		Unknown 2	-5.0	84.8	990	528.0	133960.3	84.8		84.8
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	801.00	1201.50	TRUE		Unknown 2	-6.0	118.8	1201.5	739.5	133748.8	118.8	117.4	118.8
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	793.00	1189.50	TRUE		Unknown 2	-6.0	116.9	1189.5	727.5	133760.8	116.9		116.9
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	792.00	1188.00	TRUE		Unknown 2	-6.0	116.6	1188	726.0	133762.3	116.6		116.6
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	805.00	1207.50	TRUE		Unknown 2	-7.0	119.8	1207.5	745.5	133742.8	119.8	127.6	119.8
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	871.00	1306.50	TRUE		Unknown 2	-7.0	135.7	1306.5	844.5	133643.8	135.7		135.7
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	836.00	1254.00	TRUE		Unknown 2	-7.0	127.2	1254	792.0	133696.3	127.2		127.2
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	814.00	1221.00	TRUE		Unknown 2	-8.0	121.9	1221	759.0	133729.3	121.9	131.6	121.9
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	882.00	1323.00	TRUE		Unknown 2	-8.0	138.3	1323	861.0	133627.3	138.3		138.3
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	866.00	1299.00	TRUE		Unknown 2	-8.0	134.5	1299	837.0	133651.3	134.5		134.5
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	799.00	1198.50	TRUE		Unknown 2	-9.0	118.3	1198.5	736.5	133751.8	118.3	124.3	118.3
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	857.00	1285.50	TRUE		Unknown 2	-9.0	132.3	1285.5	823.5	133664.8	132.3		132.3
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	816.00	1224.00	TRUE		Unknown 2	-9.0	122.4	1224	762.0	133726.3	122.4		122.4
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	771.00	1156.50	TRUE		Unknown 2	-10.0	111.6	1156.5	694.5	133793.8	111.6	123.4	111.6
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	842.00	1263.00	TRUE		Unknown 2	-10.0	128.7	1263	801.0	133687.3	128.7		128.7
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	847.00	1270.50	TRUE		Unknown 2	-10.0	129.9	1270.5	808.5	133679.8	129.9		129.9

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	0.81%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.						
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	40059.00	60088.50	TRUE		Unknown 3	-3.0	9578.6	60088.5	59626.5	74861.8	9578.6	8151.2			
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	15042.00	22563.00	TRUE		Unknown 3	-3.0	3550.4	22563	22101.0	112387.3	3550.4				
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	47305.00	70957.50	TRUE		Unknown 3	-3.0	11324.6	70957.5	70495.5	63992.8	11324.6				
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	8883.00	13324.50	TRUE		Unknown 3	-4.0	2066.3	13324.5	12862.5	121625.8	2066.3	2185.0			
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	9680.00	14520.00	TRUE		Unknown 3	-4.0	2258.3	14520	14058.0	120430.3	2258.3				
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	9564.00	14346.00	TRUE		Unknown 3	-4.0	2230.4	14346	13884.0	120604.3	2230.4				
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	402.00	603.00	TRUE		Unknown 3	-5.0	22.7	603	141.0	134347.3	22.7	69.8	22.7		
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	752.00	1128.00	TRUE		Unknown 3	-5.0	107.0	1128	666.0	133822.3	107.0		107.0		
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	639.00	958.50	TRUE		Unknown 3	-5.0	79.8	958.5	496.5	133991.8	79.8		79.8		
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	803.00	1204.50	TRUE		Unknown 3	-6.0	119.3	1204.5	742.5	133745.8	119.3	129.2	119.3		
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	861.00	1291.50	TRUE		Unknown 3	-6.0	133.3	1291.5	829.5	133658.8	133.3		133.3		
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	869.00	1303.50	TRUE		Unknown 3	-6.0	135.2	1303.5	841.5	133646.8	135.2		135.2		
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	768.00	1152.00	TRUE		Unknown 3	-7.0	110.8	1152	690.0	133798.3	110.8	116.4	110.8		
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	809.00	1213.50	TRUE		Unknown 3	-7.0	120.7	1213.5	751.5	133736.8	120.7		120.7		
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	796.00	1194.00	TRUE		Unknown 3	-7.0	117.6	1194	732.0	133756.3	117.6		117.6		
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	799.00	1198.50	TRUE		Unknown 3	-8.0	118.3	1198.5	736.5	133751.8	118.3	128.4	118.3		
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	867.00	1300.50	TRUE		Unknown 3	-8.0	134.7	1300.5	838.5	133649.8	134.7		134.7		
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	857.00	1285.50	TRUE		Unknown 3	-8.0	132.3	1285.5	823.5	133664.8	132.3		132.3		
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	819.00	1228.50	TRUE		Unknown 3	-9.0	123.1	1228.5	766.5	133721.8	123.1	125.1	123.1		
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	828.00	1242.00	TRUE		Unknown 3	-9.0	125.3	1242	780.0	133708.3	125.3		125.3		
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	834.00	1251.00	TRUE		Unknown 3	-9.0	126.7	1251	789.0	133699.3	126.7		126.7		
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	784.00	1176.00	TRUE		Unknown 3	-10.0	114.7	1176	714.0	133774.3	114.7	127.6	114.7		
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	877.00	1315.50	TRUE		Unknown 3	-10.0	137.1	1315.5	853.5	133634.8	137.1		137.1		
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	851.00	1276.50	TRUE		Unknown 3	-10.0	130.8	1276.5	814.5	133673.8	130.8		130.8		
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6354.00	9531.00	TRUE		Unknown 4	-3.0	1456.9	9531	9069.0	125419.3	1456.9	1751.2			
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	8588.00	12882.00	TRUE		Unknown 4	-3.0	1995.2	12882	12420.0	122068.3	1995.2				
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7785.00	11677.50	TRUE		Unknown 4	-3.0	1801.7	11677.5	11215.5	123272.8	1801.7				
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2824.00	4236.00	TRUE		Unknown 4	-4.0	606.3	4236	3774.0	130714.3	606.3	597.9	93.7		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2807.00	4210.50	TRUE		Unknown 4	-4.0	602.2	4210.5	3748.5	130739.8	602.2		95.4		
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2737.00	4105.50	TRUE		Unknown 4	-4.0	585.3	4105.5	3643.5	130844.8	585.3		86.4		
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	673.00	1009.50	TRUE		Unknown 4	-5.0	88.0	1009.5	547.5	133940.8	88.0	96.1	88.0		
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	707.00	1060.50	TRUE		Unknown 4	-5.0	96.1	1060.5	598.5	133889.8	96.1		96.1		
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	740.00	1110.00	TRUE		Unknown 4	-5.0	104.1	1110	648.0	133840.3	104.1		104.1		
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	777.00	1165.50	TRUE		Unknown 4	-6.0	113.0	1165.5	703.5	133784.8	113.0	117.8	113.0		
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	829.00	1243.50	TRUE		Unknown 4	-6.0	125.5	1243.5	781.5	133706.8	125.5		125.5		
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	785.00	1177.50	TRUE		Unknown 4	-6.0	114.9	1177.5	715.5	133772.8	114.9		114.9		
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	760.00	1140.00	TRUE		Unknown 4	-7.0	108.9	1140	678.0	133810.3	108.9	116.5	108.9		
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	818.00	1227.00	TRUE		Unknown 4	-7.0	122.9	1227	765.0	133723.3	122.9		122.9		
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	797.00	1195.50	TRUE		Unknown 4	-7.0	117.8	1195.5	733.5	133754.8	117.8		117.8		
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	730.00	1095.00	TRUE		Unknown 4	-8.0	101.7	1095	633.0	133855.3	101.7	113.6	101.7		
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	761.00	1141.50	TRUE		Unknown 4	-8.0	109.2	1141.5	679.5	133808.8	109.2		109.2		
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	847.00	1270.50	TRUE		Unknown 4	-8.0	129.9	1270.5	808.5	133679.8	129.9		129.9		
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	732.00	1098.00	TRUE		Unknown 4	-9.0	102.2	1098	636.0	133852.3	102.2	118.0	102.2		
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	818.00	1227.00	TRUE		Unknown 4	-9.0	122.9	1227	765.0	133723.3	122.9		122.9		
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	843.00	1264.50	TRUE		Unknown 4	-9.0	128.9	1264.5	802.5	133685.8	128.9		128.9		
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	799.00	1198.50	TRUE		Unknown 4	-10.0	118.3	1198.5	736.5	133751.8	118.3	125.3	118.3		
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	841.00	1261.50	TRUE		Unknown 4	-10.0	128.4	1261.5	799.5	133688.8	128.4		128.4		
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	844.00	1266.00	TRUE		Unknown 4	-10.0	129.2	1266	804.0	133684.3	129.2		129.2		
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	294.00	441.00	TRUE		cold E2	-7.0	-3.4	441	-21.0	134509.3	-3.4	1.4	-3.4		
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	307.00	460.50	TRUE		cold E2	-7.0	-0.2	460.5	-1.5	134489.8	-0.2		-0.2		
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	340.00	510.00	TRUE		cold E2	-7.0	7.7	510	48.0	134440.3	7.7		7.7		
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	722.00	1083.00	TRUE			—	99.8	1083	621.0	133867.3	99.8	102.0	99.8		
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	727.00	1090.50	TRUE			—	101.0	1090.5	628.5	133859.8	101.0		101.0		
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	745.00	1117.50	TRUE			—	105.3	1117.5	655.5	133832.8	105.3		105.3		
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average			
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
211		178	1	none	Hot		—	—	—	10	—	—	—	—	88970.00	133455.00	TRUE					133455	132993.0						
212		179	2	none	Hot		—	—	—	10	—	—	—	—	81397.00	122095.50	TRUE					122095.5	121633.5						
213		180	3	none	Hot		—	—	—	10	—	—	—	—	91303.00	136954.50	TRUE					136954.5	136492.5						
214		181	1	none	Hot		—	—	—	10	—	—	—	—	94201.00	141301.50	TRUE					141301.5	140839.5						
215		182	2	none	Hot		—	—	—	10	—	—	—	—	92778.00	139167.00	TRUE					139167	138705.0						
216		183	3	none	Hot		—	—	—	10	—	—	—	—	89304.00	133956.00	TRUE					133956	133494.0						
217																													

Prism data, excluding data points in the right leg of U-shaped curves

	B	C	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31				Prism data, excluding data points in the right leg of U-shaped curves																											
32				*standard curve*				*weak positive*				*4.0*				*9.0*				*14.0*				*20.0*				*negative control*			
33	*Position*	*Replicate*		*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1		-7.0	-5.7831	3.1325	-1.4458	-4.0	0.9639	0.9639	6.7470	-3.0				-3.0	48.1928	50.1205	77.5904	-3.0				-3.0				-3.0	#N/A	#N/A	#N/A
35	2	2		-7.0	-3.3735	-0.2410	7.7108	-4.5	8.6747	12.7711	10.8434	-4.0				-4.0	54.9398	77.8313	67.4699	-4.0				-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
36	3	3		-8.0	46.5060	43.6145		-5.5	77.3494	91.5663	84.3373	-5.0	-19.0361	-13.9759	-14.2169	-5.0	77.3494	89.8795	84.8193	-5.0	22.6506	106.9880	79.7590	-5.0	87.9518	96.1446	104.0964	-5.0	#N/A	#N/A	#N/A
37	4	1		-8.5	86.5060	98.3133	94.4578	-6.0	104.5783	105.7831	116.6265	-6.0	-10.1205	-10.6024	-8.9157	-6.0	118.7952	116.8675	116.6265	-6.0	119.2771	133.2530	135.1807	-6.0	113.0120	125.5422	114.9396	-6.0	#N/A	#N/A	#N/A
38	5	2		-9.0	115.4217	117.3494	118.5542	-6.5	117.8313	123.1325	114.6988	-7.0	-4.3373	1.6867	-8.6747	-7.0	119.7590	135.6627	127.2289	-7.0	110.8434	120.7229	117.5904	-7.0	108.9157	122.8916	117.8313	-7.0	#N/A	#N/A	#N/A
39	6	3		-9.5		133.4940	135.1807	-7.0	134.9398	119.2771	124.8193	-8.0	24.3373	32.7711	22.1687	-8.0	121.9277	138.3133	134.4578	-8.0	118.3133	134.6988	132.2892	-8.0	101.6867	109.1566	129.8795	-8.0	#N/A	#N/A	#N/A
40	7	1		-10.0	155.6627	141.4458	142.8916	-7.5	121.9277	127.4699	140.4819	-9.0	83.3735	102.4096	103.8554	-9.0	116.3133	132.2892	122.4096	-9.0	123.1325	125.3012	126.7470	-9.0	102.1687	122.8916	128.9157	-9.0	#N/A	#N/A	#N/A
41	8	2		-11.0		134.4578	139.5181	-8.5	130.1205	129.8795	143.3735	-10.0	120.2410	129.6386	133.2530	-10.0	111.5663	126.6747	129.8795	-10.0	114.6988	137.1084	130.8434	-10.0	118.3133	128.4337	129.1566	-10.0	#N/A	#N/A	#N/A
42	9	3																													
43	10	1		Standard Curve		TTN011 04-29-08		Positive Control		TTN011 04-29-08		4.0		TTN011 04-29-08		9.0		TTN011 04-29-08		14.0		TTN011 04-29-08		20.0		TTN011 04-29-08		Negative Control		Prism run ID & date	
44	11	2		logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3		Best-fit values				Best-fit values				Best-fit values				Best-fit values		bad initial values		Best-fit values		bad initial values		Best-fit values		bad initial values		Best-fit values			
46	13	1		BOTTOM		-8.199		BOTTOM		-8.199		BOTTOM		-12.79		BOTTOM		58.63		BOTTOM		69.8		BOTTOM		91.81		BOTTOM			
47	14	2		TOP		142.9		TOP		142.9		TOP		134.8		TOP		131.6		TOP		129.2		TOP		125.3		TOP			
48	15	3		LOGIC50		-8.035		LOGIC50		-8.035		LOGIC50		-8.338		LOGIC50		n/a		LOGIC50		n/a		LOGIC50		n/a		LOGIC50			
49	16	1		HILLSLOPE		-1.011		HILLSLOPE		-1.011		HILLSLOPE		-0.8706		HILLSLOPE		n/a		HILLSLOPE		n/a		HILLSLOPE		n/a		HILLSLOPE			
50	17	2		Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3		BOTTOM		4.786		BOTTOM		7.599		BOTTOM		2.501		BOTTOM				BOTTOM				BOTTOM				BOTTOM			
52	19	1		TOP		2.959		TOP		2.445		TOP		5.782		TOP				TOP				TOP				TOP			
53	20	2		LOGIC50		0.04421		LOGIC50		0.06298		LOGIC50		0.0465		LOGIC50				LOGIC50				LOGIC50				LOGIC50			
54	21	3		HILLSLOPE		0.1236		HILLSLOPE		0.1487		HILLSLOPE		0.09981		HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
55	22	1		95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2		BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3		TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1		LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2		HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3		Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1		Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2		R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3		Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1		Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2		Data				Data				Data				Data				Data				Data				Data			
66	33	3		Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1		Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2		Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3		Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
70	37	1																													

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.84

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	-5.8
1	1	2	-7.0	3.1
1	1	3	-7.0	-1.4
1	2	1	-8.0	46.5
1	2	2	-8.0	43.6
1	2	3		
1	3	1	-8.5	86.5
1	3	2	-8.5	98.3
1	3	3	-8.5	94.5
1	4	1	-9.0	115.4
1	4	2	-9.0	117.3
1	4	3	-9.0	118.6
1	5	1		
1	5	2	-9.5	133.5
1	5	3	-9.5	135.2
1	6	1	-10.0	155.7
1	6	2	-10.0	141.4
1	6	3	-10.0	142.9
1	7	1		
1	7	2	-11.0	134.5
1	7	3	-11.0	139.5
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.51

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Norethynodrel
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.0	1.0
1	1	2	-4.0	1.0
1	1	3	-4.0	6.7
1	2	1	-4.5	8.7
1	2	2	-4.5	12.8
1	2	3	-4.5	10.8
1	3	1	-5.5	77.3
1	3	2	-5.5	91.6
1	3	3	-5.5	84.3
1	4	1	-6.0	104.6
1	4	2	-6.0	105.8
1	4	3	-6.0	116.6
1	5	1	-6.5	117.8
1	5	2	-6.5	123.1
1	5	3	-6.5	114.7
1	6	1	-7.0	134.9
1	6	2	-7.0	119.3
1	6	3	-7.0	124.8
1	7	1	-7.5	121.9
1	7	2	-7.5	127.5
1	7	3	-7.5	140.5
1	8	1	-8.5	130.1
1	8	2	-8.5	129.9
1	8	3	-8.5	143.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.37

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 4
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	-19.0
1	1	2	-5.0	-14.0
1	1	3	-5.0	-14.2
1	2	1	-6.0	-10.1
1	2	2	-6.0	-10.6
1	2	3	-6.0	-8.9
1	3	1	-7.0	-4.3
1	3	2	-7.0	1.7
1	3	3	-7.0	-8.7
1	4	1	-8.0	24.3
1	4	2	-8.0	32.8
1	4	3	-8.0	22.2
1	5	1	-9.0	83.4
1	5	2	-9.0	102.4
1	5	3	-9.0	103.9
1	6	1	-10.0	120.2
1	6	2	-10.0	129.6
1	6	3	-10.0	133.3
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.59

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 9
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	48.2
1	1	2	-3.0	50.1
1	1	3	-3.0	77.6
1	2	1	-4.0	54.9
1	2	2	-4.0	77.8
1	2	3	-4.0	67.5
1	3	1	-5.0	77.3
1	3	2	-5.0	89.9
1	3	3	-5.0	84.8
1	4	1	-6.0	118.8
1	4	2	-6.0	116.9
1	4	3	-6.0	116.6
1	5	1	-7.0	119.8
1	5	2	-7.0	135.7
1	5	3	-7.0	127.2
1	6	1	-8.0	121.9
1	6	2	-8.0	138.3
1	6	3	-8.0	134.5
1	7	1	-9.0	118.3
1	7	2	-9.0	132.3
1	7	3	-9.0	122.4
1	8	1	-10.0	111.6
1	8	2	-10.0	128.7
1	8	3	-10.0	129.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 19.01

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 14
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	22.7
1	1	2	-5.0	107.0
1	1	3	-5.0	79.8
1	2	1	-6.0	119.3
1	2	2	-6.0	133.3
1	2	3	-6.0	135.2
1	3	1	-7.0	110.8
1	3	2	-7.0	120.7
1	3	3	-7.0	117.6
1	4	1	-8.0	118.3
1	4	2	-8.0	134.7
1	4	3	-8.0	132.3
1	5	1	-9.0	123.1
1	5	2	-9.0	125.3
1	5	3	-9.0	126.7
1	6	1	-10.0	114.7
1	6	2	-10.0	137.1
1	6	3	-10.0	130.8
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 10.06

Provide information in all blue cells.

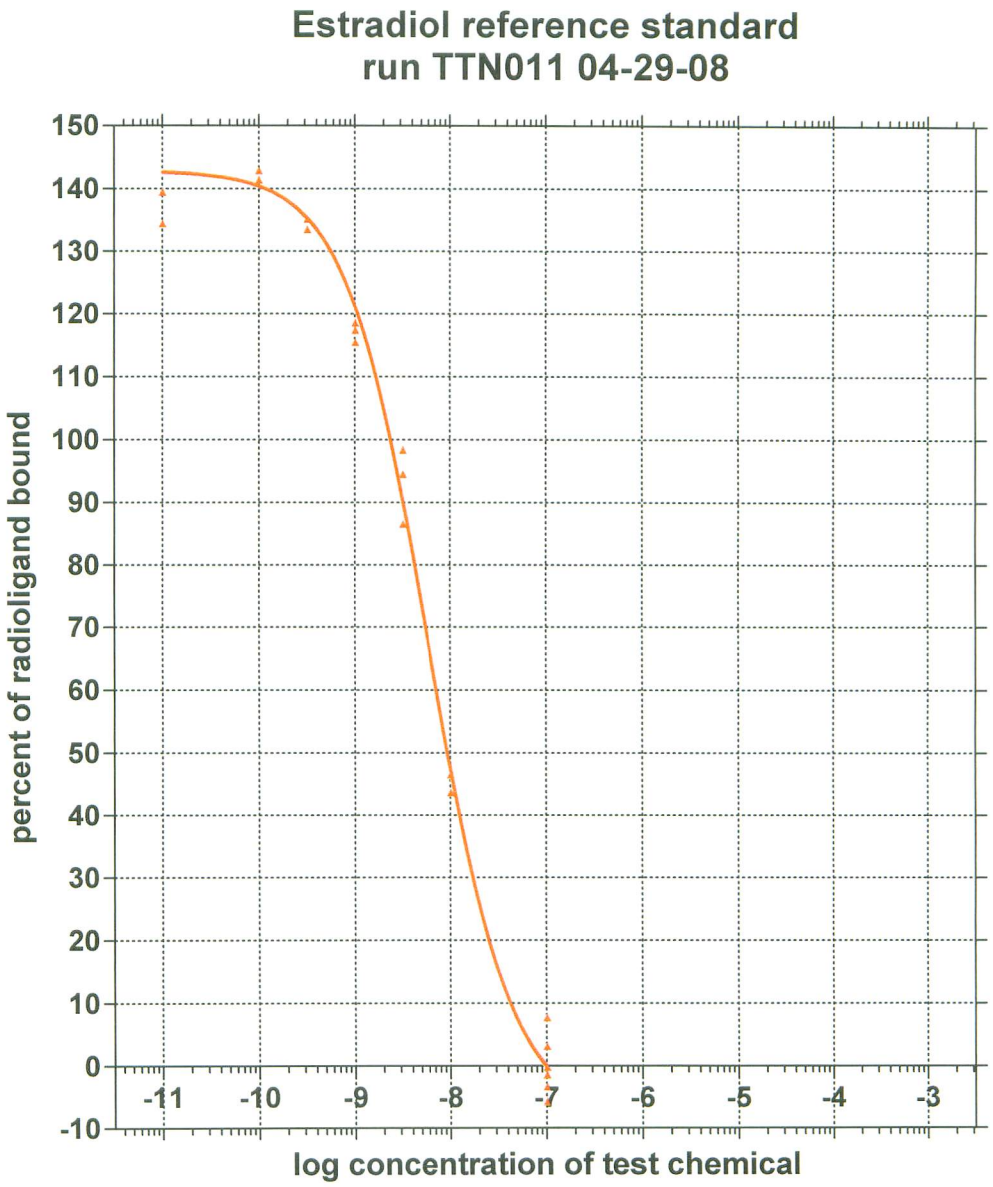
Laboratory Code: B

Compound ID: Chem Code 20
Run ID: TTN 011
Assay start date: 4/29/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	88.0
1	1	2	-5.0	96.1
1	1	3	-5.0	104.1
1	2	1	-6.0	113.0
1	2	2	-6.0	125.5
1	2	3	-6.0	114.9
1	3	1	-7.0	108.9
1	3	2	-7.0	122.9
1	3	3	-7.0	117.8
1	4	1	-8.0	101.7
1	4	2	-8.0	109.2
1	4	3	-8.0	129.9
1	5	1	-9.0	102.2
1	5	2	-9.0	122.9
1	5	3	-9.0	128.9
1	6	1	-10.0	118.3
1	6	2	-10.0	128.4
1	6	3	-10.0	129.2
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

ole format: XY		X	A		
		log concentration	run TTN011 04-29-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-5.7831	3.1325	-1.4458
2	Title	-7.0	-3.3735	-0.2410	7.7108
3	Title	-8.0	46.5060	43.6145	
4	Title	-8.5	86.5060	98.3133	94.4578
5	Title	-9.0	115.4217	117.3494	118.5542
6	Title	-9.5		133.4940	135.1807
7	Title	-10.0	155.6627	141.4458	142.8916
8	Title	-11.0		134.4578	139.5181

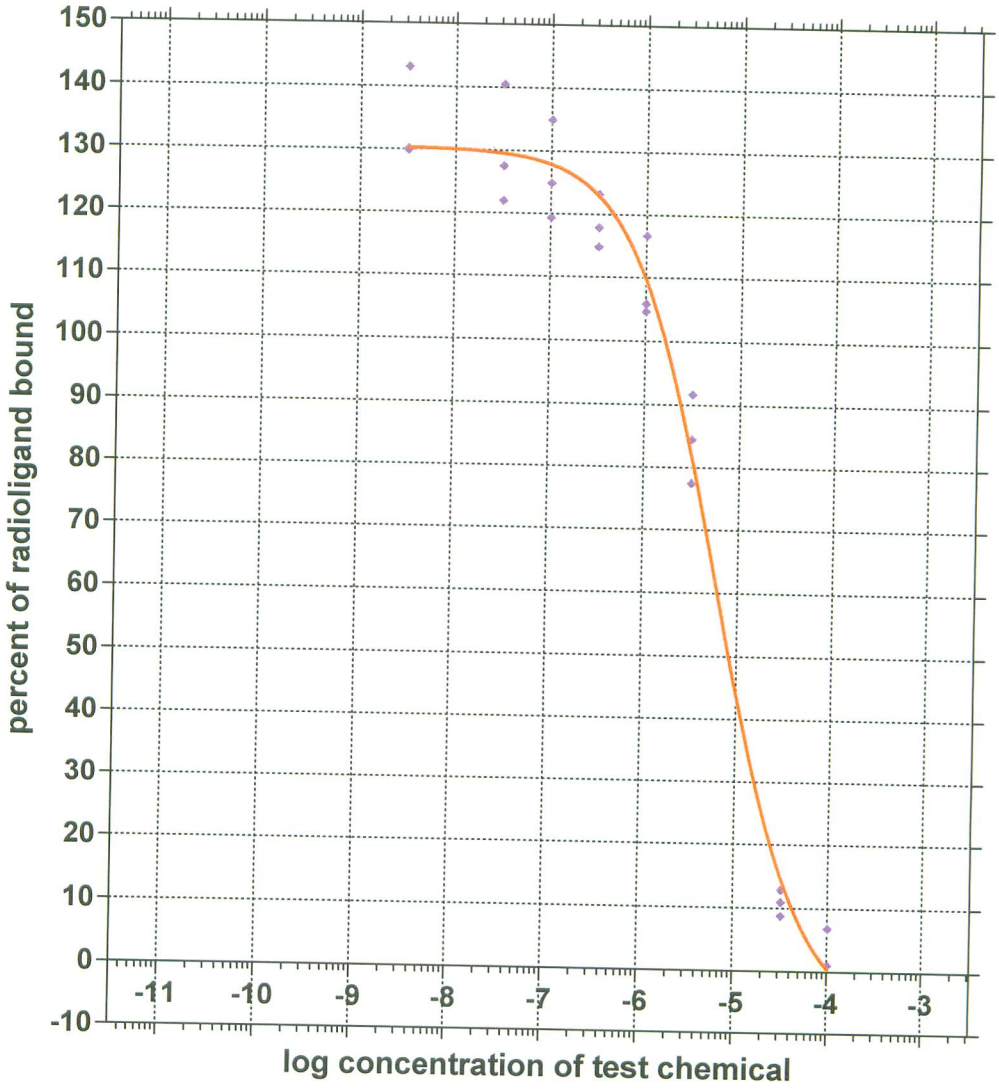
	Nonlin fit Table of results	A run TTN011 04-29-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-8.199
4	TOP	142.9
5	LOGIC50	-8.035
6	HILLSLOPE	-1.011
7	Std. Error	
8	BOTTOM	4.786
9	TOP	2.959
10	LOGIC50	0.04421
11	HILLSLOPE	0.1236
12	95% Confidence Intervals	
13	BOTTOM	-18.30 to 1.899
14	TOP	136.6 to 149.1
15	LOGIC50	-8.128 to -7.941
16	HILLSLOPE	-1.272 to -0.7499
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9916
20	Absolute Sum of Squares	603.5
21	Sy.x	5.958
22	Number of points	
23	Analyzed	21


Estradiol reference standard
run TTN011 04-29-08
percent of radioligand bound
150
140
130
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

ıble format: XY		X	A		
		log concentration	run TTN011 04-29-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0	0.9639	0.9639	6.7470
2	Title	-4.5	8.6747	12.7711	10.8434
3	Title	-5.5	77.3494	91.5663	84.3373
4	Title	-6.0	104.5783	105.7831	116.6265
5	Title	-6.5	117.8313	123.1325	114.6988
6	Title	-7.0	134.9398	119.2771	124.8193
7	Title	-7.5	121.9277	127.4699	140.4819
8	Title	-8.5	130.1205	129.8795	143.1325

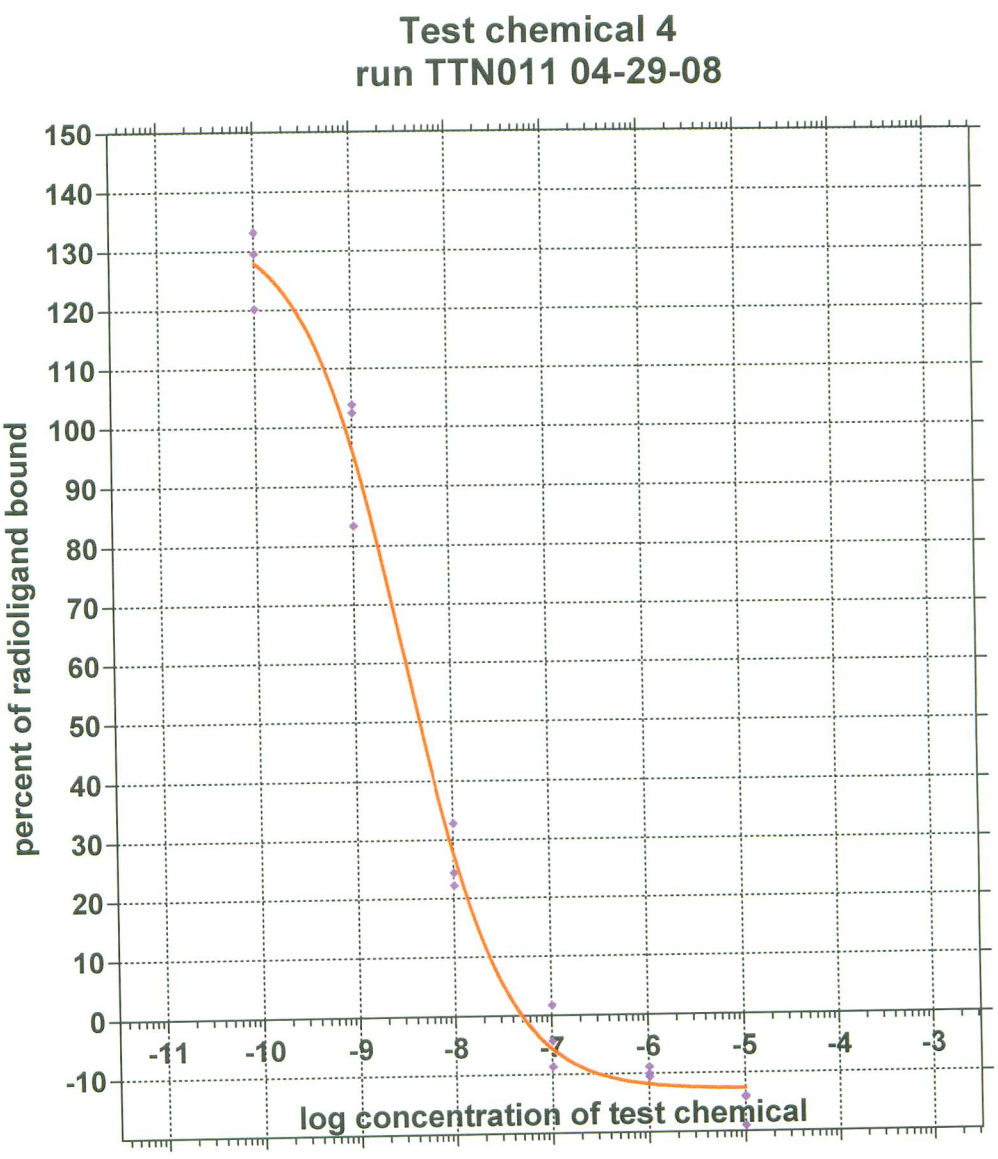
	Nonlin fit Table of results	A run TTN011 04-29-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-7.615
4	TOP	130.3
5	LOGIC50	-5.087
6	HILLSLOPE	-0.9819
7	Std. Error	
8	BOTTOM	7.599
9	TOP	2.445
10	LOGIC50	0.06298
11	HILLSLOPE	0.1487
12	95% Confidence Intervals	
13	BOTTOM	-23.47 to 8.236
14	TOP	125.2 to 135.4
15	LOGIC50	-5.219 to -4.956
16	HILLSLOPE	-1.292 to -0.6717
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9855
20	Absolute Sum of Squares	879.4
21	Sy.x	6.631
22	Number of points	
23	Analyzed	24

Norethynodrel
run TTN011 04-29-08



ole format: XY		X	A		
		log concentration	Test chemical 4		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	-19.0361	-13.9759	-14.2169
4	Title	-6.0	-10.1205	-10.6024	-8.9157
5	Title	-7.0	-4.3373	1.6867	-8.6747
6	Title	-8.0	24.3373	32.7711	22.1687
7	Title	-9.0	83.3735	102.4096	103.8554
8	Title	-10.0	120.2410	129.6386	133.2530

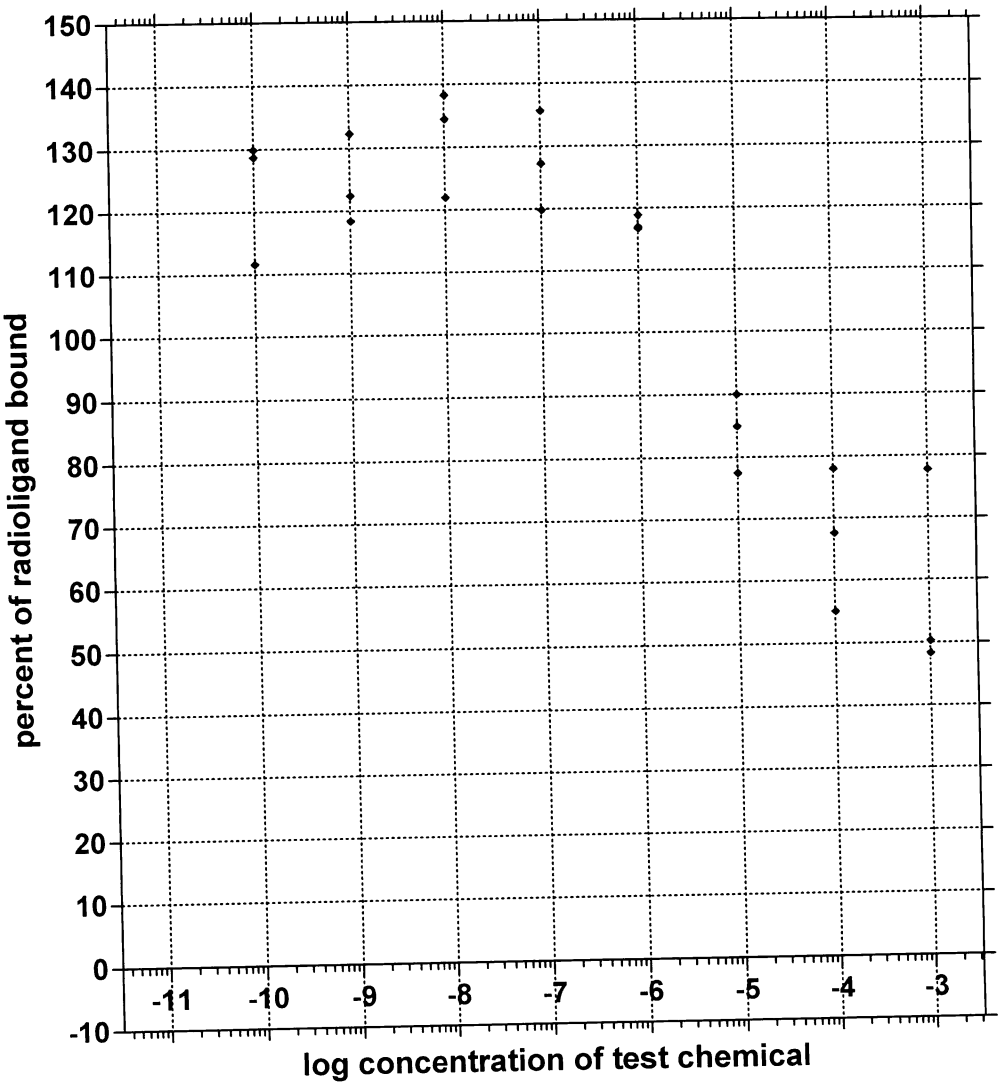
	Nonlin fit Table of results	A Test chemical 4
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-12.79
4	TOP	134.8
5	LOGIC50	-8.338
6	HILLSLOPE	-0.8706
7	Std. Error	
8	BOTTOM	2.501
9	TOP	5.782
10	LOGIC50	0.04650
11	HILLSLOPE	0.09981
12	95% Confidence Intervals	
13	BOTTOM	-18.15 to -7.425
14	TOP	122.4 to 147.2
15	LOGIC50	-8.438 to -8.239
16	HILLSLOPE	-1.085 to -0.6565
17	Goodness of Fit	
18	Degrees of Freedom	14
19	R^2	0.9903
20	Absolute Sum of Squares	542.0
21	Sy.x	6.222
22	Number of points	
23	Analyzed	18


Test chemical 4
run TTN011 04-29-08
percent of radioligand bound
150
140
130
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

ble format: XY		X	A		
		log concentration	Test chemical 9		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	48.1928	50.1205	77.5904
2	Title	-4.0	54.9398	77.8313	67.4699
3	Title	-5.0	77.3494	89.8795	84.8193
4	Title	-6.0	118.7952	116.8675	116.6265
5	Title	-7.0	119.7590	135.6627	127.2289
6	Title	-8.0	121.9277	138.3133	134.4578
7	Title	-9.0	118.3133	132.2892	122.4096
8	Title	-10.0	111.5663	128.6747	129.8795

	Nonlin fit Table of results	A Test chemical 9
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	58.63
4	TOP	131.6
5	LOGIC50	-5.332
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	24

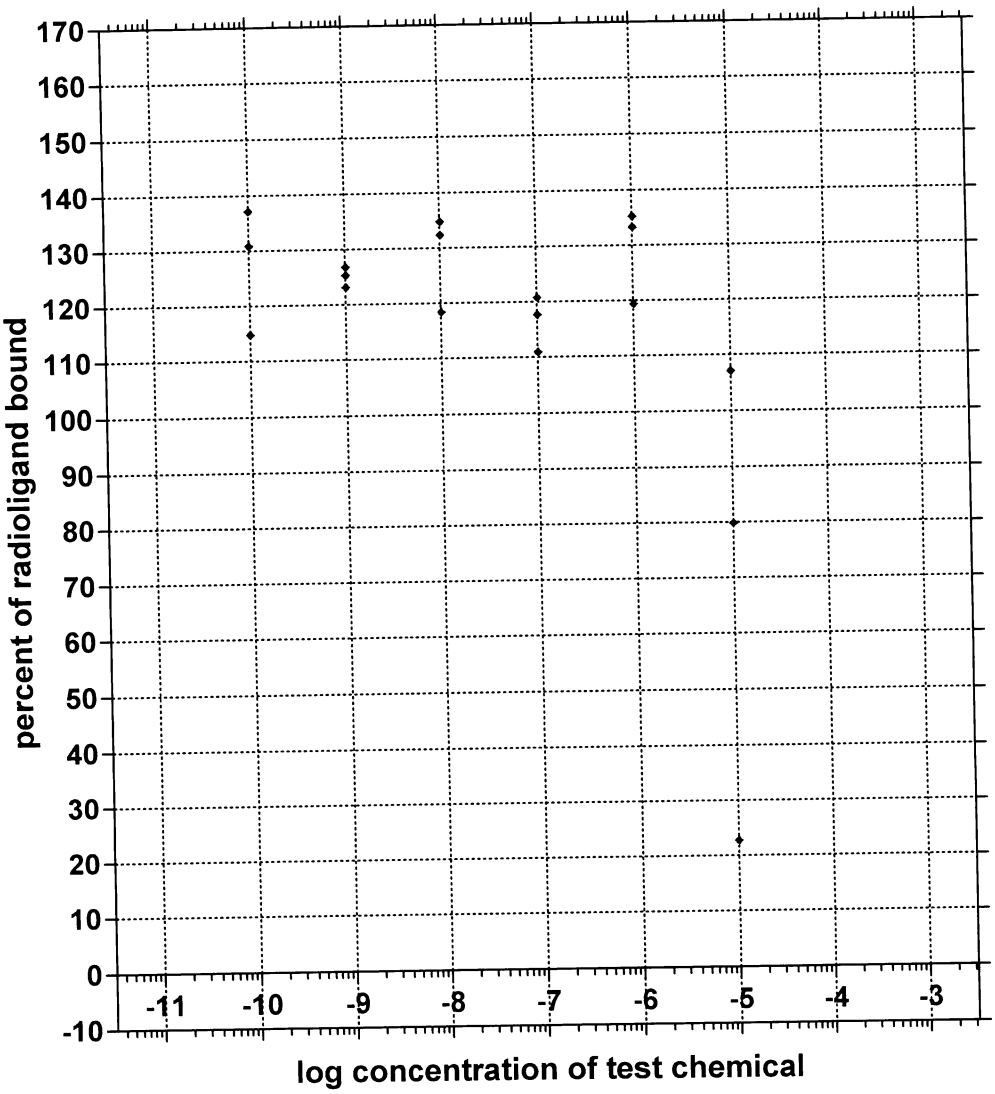
Test chemical 9
run TTN011 04-29-08



ole format: XY		X	A			B		
		log concentration	Test chemical 14			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	22.6506	106.9880	79.7590			
4	Title	-6.0	119.2771	133.2530	135.1807			
5	Title	-7.0	110.8434	120.7229	117.5904			
6	Title	-8.0	118.3133	134.6988	132.2892			
7	Title	-9.0	123.1325	125.3012	126.7470			
8	Title	-10.0	114.6988	137.1084	130.8434			

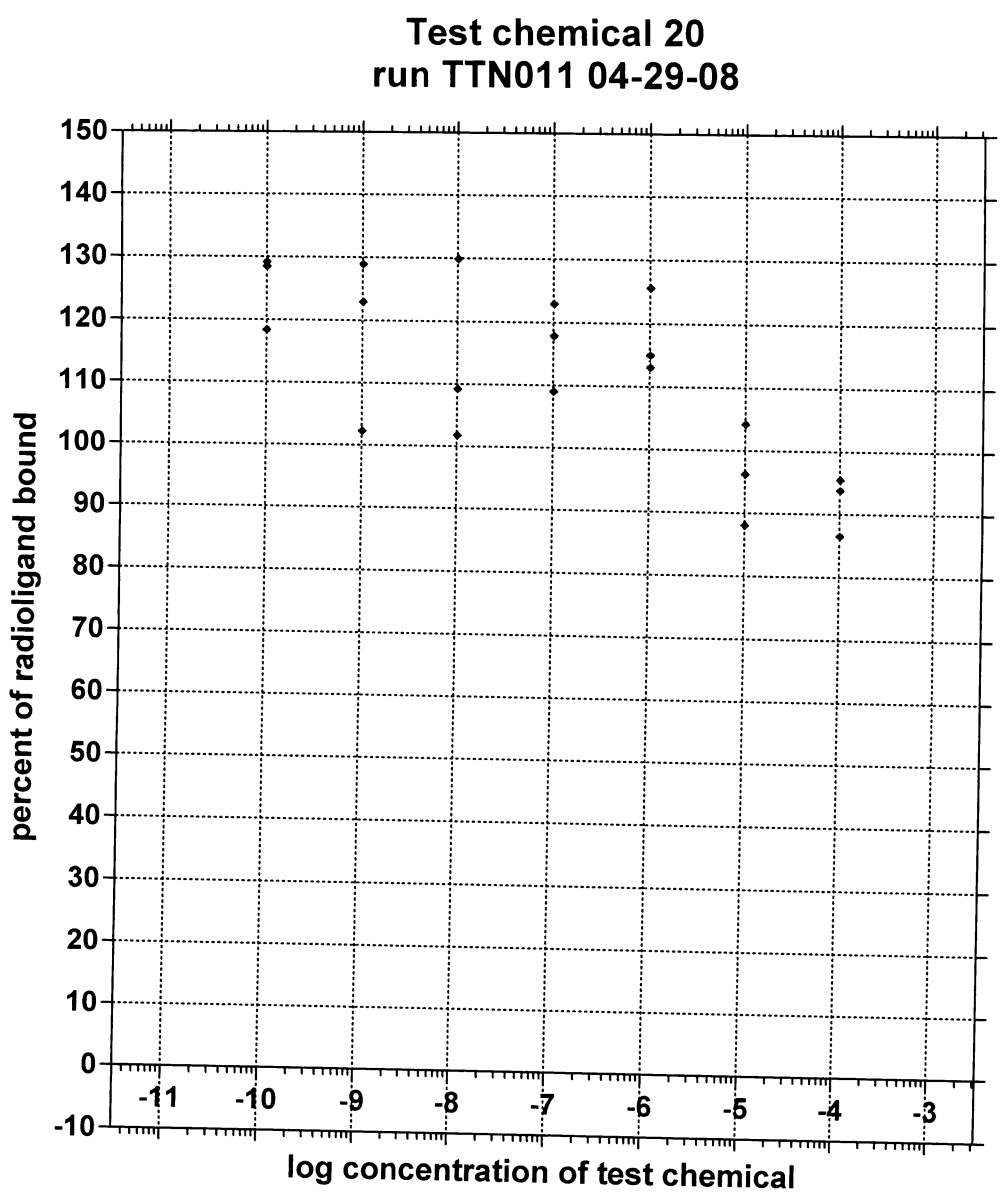
	Nonlin fit Table of results	A Test chemical 14	B Data Set-B
		Y	Y
1	logIC50	Bad initial values	
2	Best-fit values		
3	BOTTOM	69.80	
4	TOP	129.2	
5	LOGIC50	-6.276	
6	HILLSLOPE	-1.000	
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	18	

Test chemical 14
run TTN011 04-29-08



ble format: XY		X	A		
		log concentration	Test chemical 20		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	93.6729	95.3859	86.3787
3	Title	-5.0	87.9518	96.1446	104.0964
4	Title	-6.0	113.0120	125.5422	114.9398
5	Title	-7.0	108.9157	122.8916	117.8313
6	Title	-8.0	101.6867	109.1566	129.8795
7	Title	-9.0	102.1687	122.8916	128.9157
8	Title	-10.0	118.3133	128.4337	129.1566

	Nonlin fit Table of results	A Test chemical 20
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	91.81
4	TOP	125.3
5	LOGIC50	-7.140
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	21


Test chemical 20
run TTN011 04-29-08
percent of radioligand bound
150
140
130
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

APPENDIX N

Excel and Prism Files of the Run Identifier TTN013

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B
Run identification:	TTN013
Assay start date:	5/5/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	108.91 Ci/mmole
Cytosol vial or lot identification:	RUC3-03-08 A,M
Protein (cytosol):	50 microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.42 M
Weak Positive, Max Concentration:	5.00E-03 M
log(IC50), norethynodrel (Weak Positive):	-5.60 M
RBA, norethynodrel (Weak Positive):	1.517E-03 relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03 M
log(IC50), Unknown 1:	-8.95 4.0
RBA, Unknown 1:	3.396E+00 relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03 M
log(IC50), Unknown 2:	-4.60 9.0
RBA, Unknown 2:	1.510E-04 relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03 M
log(IC50), Unknown 3:	-5.09 14.0
RBA, Unknown 3:	4.613E-04 relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03 M
log(IC50), Unknown 4:	n/a 17.0
RBA, Unknown 4:	n/a relative to E2=1.0
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Check the 10% rule: 3.13% — If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	3665.3	425.44	Total Binding, solvent control, tubes
Hot	6	116971.3	5172.35	Total hot E2 added to each tube
NSB	6	264.0	15.18	Nonspecific Binding
Specific EtOH	6	3401.3	425.44	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.03	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.07	around 0.25 ?	

log conc'n at min hot binding	min hot (%)
cold E2	-7.0	-0.2
R1881	#N/A	not applicable
norethynodrel	-4.5	2.9
Unknown 1	-6.0	-3.1
Unknown 2	-4.0	23.4
Unknown 3	-5.0	37.3
Unknown 4	-5.0	13.9

Values for analysis by nonlinear regression: Standard Curve; Negative Control; Weak Positive; Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	concentration (logM)	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2458.00	3687.00	TRUE		—	100.6	3687	3423.0	113548.3	100.6	92.2	100.6
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2277.00	3415.50	TRUE		—	92.7	3415.5	3151.5	113819.8	92.7		92.7
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2065.00	3097.50	TRUE		—	83.3	3097.5	2833.5	114137.8	83.3		83.3
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	185.00	277.50	TRUE		cold E2	-7.0	0.4	277.5	13.5	116957.8	0.4	-0.2	0.4
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	161.00	241.50	TRUE		cold E2	-7.0	-0.7	241.5	-22.5	116993.8	-0.7		-0.7
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	170.00	255.00	TRUE		cold E2	-7.0	-0.3	255	-9.0	116980.3	-0.3		-0.3
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	631.00	946.50	TRUE		cold E2	-8.0	20.1	946.5	682.5	116288.8	20.1	21.6	20.1
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	708.00	1062.00	TRUE		cold E2	-8.0	23.5	1062	798.0	116173.3	23.5		23.5
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	661.00	991.50	TRUE		cold E2	-8.0	21.4	991.5	727.5	116243.8	21.4		21.4
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1451.00	2176.50	TRUE		cold E2	-8.5	56.2	2176.5	1912.5	115058.8	56.2	56.4	56.2
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1448.00	2172.00	TRUE		cold E2	-8.5	56.1	2172	1908.0	115063.3	56.1		56.1
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1463.00	2194.50	TRUE		cold E2	-8.5	56.8	2194.5	1930.5	115040.8	56.8		56.8
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2664.00	3996.00	TRUE		cold E2	-9.0	109.7	3996	3732.0	113239.3	109.7	114.3	109.7
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2861.00	4291.50	TRUE		cold E2	-9.0	118.4	4291.5	4027.5	112943.8	118.4		118.4
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2779.00	4168.50	TRUE		cold E2	-9.0	114.8	4168.5	3904.5	113066.8	114.8		114.8
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3700.00	5550.00	TRUE		cold E2	-9.5	155.4	5550	5286.0	111685.3	155.4	159.3	155.4
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3851.00	5776.50	TRUE		cold E2	-9.5	162.1	5776.5	5512.5	111458.8	162.1		162.1
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3814.00	5721.00	TRUE		cold E2	-9.5	160.4	5721	5457.0	111514.3	160.4		160.4
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4410.00	6615.00	TRUE		cold E2	-10.0	186.7	6615	6351.0	110620.3	186.7	186.5	186.7
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4449.00	6673.50	TRUE		cold E2	-10.0	188.4	6673.5	6409.5	110561.8	188.4		188.4
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4354.00	6531.00	TRUE		cold E2	-10.0	184.3	6531	6267.0	110704.3	184.3		184.3
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4608.00	6912.00	TRUE		cold E2	-11.0	195.5	6912	6648.0	110323.3	195.5	196.8	195.5
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4576.00	6864.00	TRUE		cold E2	-11.0	194.0	6864	6600.0	110371.3	194.0		194.0
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4730.00	7095.00	TRUE		cold E2	-11.0	200.8	7095	6831.0	110140.3	200.8		200.8
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	3.13%	If the ratio of EtOH / Rot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
75		42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A		
76		43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A		
77		44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
78		45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
79		46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A		
80		47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
81		48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
82		49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	264.00	396.00	TRUE		norethynodrel	-4.0	3.9	396	132.0	116839.3	3.9	6.1	3.9		
83		50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	277.00	415.50	TRUE		norethynodrel	-4.0	4.5	415.5	151.5	116819.8	4.5		4.5		
84		51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	401.00	601.50	TRUE		norethynodrel	-4.0	9.9	601.5	337.5	116633.8	9.9		9.9		
85		52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	228.00	342.00	TRUE		norethynodrel	-4.5	2.3	342	78.0	116893.3	2.3	2.9	2.3		
86		53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	245.00	367.50	TRUE		norethynodrel	-4.5	3.0	367.5	103.5	116867.8	3.0		3.0		
87		54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	253.00	379.50	TRUE		norethynodrel	-4.5	3.4	379.5	115.5	116855.8	3.4		3.4		
88		55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1126.00	1689.00	TRUE		norethynodrel	-5.5	41.9	1689	1425.0	115546.3	41.9	43.7	41.9		
89		56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1179.00	1768.50	TRUE		norethynodrel	-5.5	44.2	1768.5	1504.5	115466.8	44.2		44.2		
90		57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1197.00	1795.50	TRUE		norethynodrel	-5.5	45.0	1795.5	1531.5	115439.8	45.0		45.0		
91		58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2097.00	3145.50	TRUE		norethynodrel	-6.0	84.7	3145.5	2881.5	114089.8	84.7	86.6	84.7		
92		59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2189.00	3283.50	TRUE		norethynodrel	-6.0	88.8	3283.5	3019.5	113951.8	88.8		88.8		
93		60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2134.00	3201.00	TRUE		norethynodrel	-6.0	86.4	3201	2937.0	114034.3	86.4		86.4		
94		61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3500.00	5250.00	TRUE		norethynodrel	-6.5	146.6	5250	4986.0	111985.3	146.6	144.4	146.6		
95		62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3418.00	5127.00	TRUE		norethynodrel	-6.5	143.0	5127	4863.0	112108.3	143.0		143.0		
96		63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3430.00	5145.00	TRUE		norethynodrel	-6.5	143.5	5145	4881.0	112090.3	143.5		143.5		
97		64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3923.00	5884.50	TRUE		norethynodrel	-7.0	165.2	5884.5	5620.5	111350.8	165.2	171.9	165.2		
98		65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4205.00	6307.50	TRUE		norethynodrel	-7.0	177.7	6307.5	6043.5	110927.8	177.7		177.7		
99		66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4094.00	6141.00	TRUE		norethynodrel	-7.0	172.8	6141	5877.0	111094.3	172.8		172.8		
100		67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4223.00	6334.50	TRUE		norethynodrel	-7.5	178.5	6334.5	6070.5	110900.8	178.5	188.5	178.5		
101		68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4670.00	7005.00	TRUE		norethynodrel	-7.5	198.2	7005	6741.0	110230.3	198.2		198.2		
102		69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4461.00	6691.50	TRUE		norethynodrel	-7.5	189.0	6691.5	6427.5	110543.8	189.0		189.0		
103		70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4501.00	6751.50	TRUE		norethynodrel	-8.5	190.7	6751.5	6487.5	110483.8	190.7	197.1	190.7		
104		71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4689.00	7033.50	TRUE		norethynodrel	-8.5	199.0	7033.5	6769.5	110201.8	199.0		199.0		
105		72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4748.00	7122.00	TRUE		norethynodrel	-8.5	201.6	7122	6858.0	110113.3	201.6		201.6		
106		73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3238.00	4857.00	TRUE		Unknown 1	-3.0	135.0	4857	4593.0	112378.3	135.0	103.7			
107		74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2458.00	3687.00	TRUE		Unknown 1	-3.0	100.6	3687	3423.0	113548.3	100.6				
108		75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1884.00	2826.00	TRUE		Unknown 1	-3.0	75.3	2826	2562.0	114409.3	75.3				
109		76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	716.00	1074.00	TRUE		Unknown 1	-4.0	23.8	1074	810.0	116161.3	23.8	22.9			
110		77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	657.00	985.50	TRUE		Unknown 1	-4.0	21.2	985.5	721.5	116249.8	21.2				
111		78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	710.00	1065.00	TRUE		Unknown 1	-4.0	23.6	1065	801.0	116170.3	23.6				
112		79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	111.00	166.50	TRUE		Unknown 1	-5.0	-2.9	166.5	-97.5	117068.8	-2.9	-3.0	-2.9		
113		80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	100.00	150.00	TRUE		Unknown 1	-5.0	-3.4	150	-114.0	117085.3	-3.4		-3.4		
114		81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	114.00	171.00	TRUE		Unknown 1	-5.0	-2.7	171	-93.0	117064.3	-2.7		-2.7		
115		82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	105.00	157.50	TRUE		Unknown 1	-6.0	-3.1	157.5	-106.5	117077.8	-3.1	-3.1	-3.1		
116		83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	103.00	154.50	TRUE		Unknown 1	-6.0	-3.2	154.5	-109.5	117080.8	-3.2		-3.2		
117		84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	109.00	163.50	TRUE		Unknown 1	-6.0	-3.0	163.5	-100.5	117071.8	-3.0		-3.0		
118		85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	154.00	231.00	TRUE		Unknown 1	-7.0	-1.0	231	-33.0	117004.3	-1.0	-1.3	-1.0		
119		86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	155.00	232.50	TRUE		Unknown 1	-7.0	-0.9	232.5	-31.5	117002.8	-0.9		-0.9		
120		87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	131.00	196.50	TRUE		Unknown 1	-7.0	-2.0	196.5	-67.5	117038.8	-2.0		-2.0		
121		88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	269.00	403.50	TRUE		Unknown 1	-8.0	4.1	403.5	139.5	116831.8	4.1	4.9	4.1		
122		89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	288.00	432.00	TRUE		Unknown 1	-8.0	4.9	432	168.0	116803.3	4.9		4.9		
123		90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	305.00	457.50	TRUE		Unknown 1	-8.0	5.7	457.5	193.5	116777.8	5.7		5.7		
124		91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1265.00	1897.50	TRUE		Unknown 1	-9.0	48.0	1897.5	1633.5	115337.8	48.0	54.2	48.0		
125		92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1426.00	2139.00	TRUE		Unknown 1	-9.0	55.1	2139	1875.0	115096.3	55.1		55.1		
126		93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1523.00	2284.50	TRUE		Unknown 1	-9.0	59.4	2284.5	2020.5	114950.8	59.4		59.4		
127		94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3528.00	5292.00	TRUE		Unknown 1	-10.0	147.8	5292	5028.0	111943.3	147.8	147.2	147.8		
128		95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1148.00	1722.00	FALSE	mispipet	Unknown 1	-10.0									
129		96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3499.00	5248.50	TRUE		Unknown 1	-10.0	146.5	5248.5	4984.5	111986.8	146.5		146.5		
130		97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2148.00	3222.00	TRUE		Unknown 2	-3.0	87.0	3222	2958.0	114013.3	87.0	89.7			
131		98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2279.00	3418.50	TRUE		Unknown 2	-3.0	92.7	3418.5	3154.5	113816.8	92.7				
132		99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2203.00	3304.50	TRUE		Unknown 2	-3.0	89.4	3304.5	3040.5	113930.8	89.4				
133		100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	694.00	1041.00	TRUE		Unknown 2	-4.0	22.8	1041	777.0	116194.3	22.8	23.4	22.8		
134		101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	799.00	1198.50	TRUE		Unknown 2	-4.0	27.5	1198.5	934.5	116036.8	27.5		27.5		
135		102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	628.00	942.00	TRUE		Unknown 2	-4.0	19.9	942	678.0	116293.3	19.9		19.9		
136		103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1267.00	1900.50	TRUE		Unknown 2	-5.0	48.1	1900.5	1636.5	115334.8	48.1	79.2	48.1		
137		104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2125.00	3187.50	TRUE		Unknown 2	-5.0	86.0	3187.5	2923.5	114047.8	86.0		86.0		
138		105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2525.00	3787.50	TRUE		Unknown 2	-5.0	103.6	3787.5	3523.5	113447.8	103.6		103.6		
139		106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	874.00	1311.00	TRUE		Unknown 2	-6.0	30.8	1311	1047.0	115924.3	30.8	79.6	30.8		
140		107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1548.00	2322.00	TRUE		Unknown 2	-6.0	60.5	2322	2058.0	114913.3	60.5		60.5		
141		108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3521.00	5281.50	TRUE		Unknown 2	-6.0	147.5	5281.5	5017.5	111953.8	147.5		147.5		
142		109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1249.00	1873.50	TRUE		Unknown 2	-7.0	47.3	1873.5	1609.5	115361.8	47.3	121.6	47.3		
143		110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3360.00	5040.00	TRUE		Unknown 2	-7.0	140.4	5040	4776.0	112195.3	140.4		140.4		
144		111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4191.00	6286.50	TRUE		Unknown 2	-7.0	177.1	6286.5	6022.5	110948.8	177.1		177.1		
145		112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2067.00	3100.50	TRUE		Unknown 2	-8.0	83.4	3100.5	2836.5	114134.8	83.4	135.9	83.4		
146		113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3990.00	5985.00	TRUE		Unknown 2	-8.0	168.2	5985	5721.0	111250.3	168.2		168.2		
147		114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3716.00	5574.00	TRUE		Unknown 2	-8.0	156.1	5574	5310.0	111661.3	156.1		156.1		
148		115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4087.00	6130.50	TRUE		Unknown 2	-9.0	172.5	6130.5	5866.5	111104.8	172.5	162.9	172.5		
149		116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4002.00	6003.00	TRUE		Unknown 2	-9.0	168.7	6003	5739.0	111232.3	168.7		168.7		
150		117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3518.00	5277.00	TRUE		Unknown 2	-9.0	147.4	5277	5013.0	111958.3	147.4		147.4		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32	Competitive Assay Tube Layout - Four Test Chemicals															Check the 10% rule:	3.13%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown		% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
151		118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4153.00	6229.50	TRUE		Unknown 2	-10.0	175.4	6229.5	5965.5	111005.8	175.4	178.4	175.4		
152		119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4211.00	6316.50	TRUE		Unknown 2	-10.0	177.9	6316.5	6052.5	110918.8	177.9		177.9		
153		120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4301.00	6451.50	TRUE		Unknown 2	-10.0	181.9	6451.5	6187.5	110783.8	181.9		181.9		
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	26042.00	39063.00	TRUE		Unknown 3	-3.0	1140.7	39063	38799.0	78172.3	1140.7	941.0			
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	20734.00	31101.00	TRUE		Unknown 3	-3.0	906.6	31101	30837.0	86134.3	906.6				
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	17764.00	26646.00	TRUE		Unknown 3	-3.0	775.7	26646	26382.0	90589.3	775.7				
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	8230.00	12345.00	TRUE		Unknown 3	-4.0	355.2	12345	12081.0	104890.3	355.2	351.0			
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	8579.00	12868.50	TRUE		Unknown 3	-4.0	370.6	12868.5	12604.5	104366.8	370.6				
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	7597.00	11395.50	TRUE		Unknown 3	-4.0	327.3	11395.5	11131.5	105839.8	327.3				
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	984.00	1476.00	TRUE		Unknown 3	-5.0	35.6	1476	1212.0	115759.3	35.6	37.3	35.6		
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1002.00	1503.00	TRUE		Unknown 3	-5.0	36.4	1503	1239.0	115732.3	36.4		36.4		
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1077.00	1615.50	TRUE		Unknown 3	-5.0	39.7	1615.5	1351.5	115619.8	39.7		39.7		
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3132.00	4698.00	TRUE		Unknown 3	-6.0	130.4	4698	4434.0	112537.3	130.4	133.9	130.4		
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3183.00	4774.50	TRUE		Unknown 3	-6.0	132.6	4774.5	4510.5	112460.8	132.6		132.6		
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3320.00	4980.00	TRUE		Unknown 3	-6.0	138.7	4980	4716.0	112255.3	138.7		138.7		
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3316.00	4974.00	TRUE		Unknown 3	-7.0	138.5	4974	4710.0	112261.3	138.5	113.9	138.5		
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2916.00	4374.00	TRUE		Unknown 3	-7.0	120.8	4374	4110.0	112861.3	120.8		120.8		
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2047.00	3070.50	TRUE		Unknown 3	-7.0	82.5	3070.5	2806.5	114164.8	82.5		82.5		
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3416.00	5124.00	TRUE		Unknown 3	-8.0	142.9	5124	4860.0	112111.3	142.9	146.8	142.9		
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2914.00	4371.00	TRUE		Unknown 3	-8.0	120.7	4371	4107.0	112864.3	120.7		120.7		
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4181.00	6271.50	TRUE		Unknown 3	-8.0	176.6	6271.5	6007.5	110963.8	176.6		176.6		
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3659.00	5488.50	TRUE		Unknown 3	-9.0	153.6	5488.5	5224.5	111746.8	153.6	142.3	153.6		
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3960.00	5940.00	TRUE		Unknown 3	-9.0	166.9	5940	5676.0	111295.3	166.9		166.9		
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2588.00	3882.00	TRUE		Unknown 3	-9.0	106.4	3882	3618.0	113353.3	106.4		106.4		
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3465.00	5197.50	TRUE		Unknown 3	-10.0	145.0	5197.5	4933.5	112037.8	145.0	159.6	145.0		
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4210.00	6315.00	TRUE		Unknown 3	-10.0	177.9	6315	6051.0	110920.3	177.9		177.9		
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3708.00	5562.00	TRUE		Unknown 3	-10.0	155.8	5562	5298.0	111673.3	155.8		155.8		
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2046.00	3069.00	TRUE		Unknown 4	-3.0	82.5	3069	2805.0	114166.3	82.5	129.6			
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4271.00	6406.50	TRUE		Unknown 4	-3.0	180.6	6406.5	6142.5	110828.8	180.6				
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3024.00	4536.00	TRUE		Unknown 4	-3.0	125.6	4536	4272.0	112699.3	125.6				
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	625.00	937.50	TRUE		Unknown 4	-4.0	19.8	937.5	673.5	116297.8	19.8	19.9	93.7		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	639.00	958.50	TRUE		Unknown 4	-4.0	20.4	958.5	694.5	116276.8	20.4		95.4		
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	615.00	922.50	TRUE		Unknown 4	-4.0	19.4	922.5	658.5	116312.8	19.4		86.4		
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	421.00	631.50	TRUE		Unknown 4	-5.0	10.8	631.5	367.5	116603.8	10.8	13.9	10.8		
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	525.00	787.50	TRUE		Unknown 4	-5.0	15.4	787.5	523.5	116447.8	15.4		15.4		
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	528.00	792.00	TRUE		Unknown 4	-5.0	15.5	792	528.0	116443.3	15.5		15.5		
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2276.00	3414.00	TRUE		Unknown 4	-6.0	92.6	3414	3150.0	113821.3	92.6	113.5	92.6		
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2790.00	4185.00	TRUE		Unknown 4	-6.0	115.3	4185	3921.0	113050.3	115.3		115.3		
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3180.00	4770.00	TRUE		Unknown 4	-6.0	132.5	4770	4506.0	112465.3	132.5		132.5		
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3814.00	5721.00	TRUE		Unknown 4	-7.0	160.4	5721	5457.0	111514.3	160.4	147.6	160.4		
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2654.00	3981.00	TRUE		Unknown 4	-7.0	109.3	3981	3717.0	113254.3	109.3		109.3		
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4103.00	6154.50	TRUE		Unknown 4	-7.0	173.2	6154.5	5890.5	111080.8	173.2		173.2		
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4176.00	6264.00	TRUE		Unknown 4	-8.0	176.4	6264	6000.0	110971.3	176.4	164.5	176.4		
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3747.00	5620.50	TRUE		Unknown 4	-8.0	157.5	5620.5	5356.5	111614.8	157.5		157.5		
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3793.00	5689.50	TRUE		Unknown 4	-8.0	159.5	5689.5	5425.5	111545.8	159.5		159.5		
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1665.00	2497.50	TRUE		Unknown 4	-9.0	65.7	2497.5	2233.5	114737.8	65.7	104.7	65.7		
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1968.00	2952.00	TRUE		Unknown 4	-9.0	79.0	2952	2688.0	114283.3	79.0		79.0		
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4015.00	6022.50	TRUE		Unknown 4	-9.0	169.3	6022.5	5758.5	111212.8	169.3		169.3		
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1924.00	2886.00	TRUE		Unknown 4	-10.0	77.1	2886	2622.0	114349.3	77.1	66.6	77.1		
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1657.00	2485.50	TRUE		Unknown 4	-10.0	65.3	2485.5	2221.5	114749.8	65.3		65.3		
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1477.00	2215.50	TRUE		Unknown 4	-10.0	57.4	2215.5	1951.5	115019.8	57.4		57.4		
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	176.00	264.00	TRUE		cold E2	-7.0	0.0	264	0.0	116971.3	0.0	0.2	0.0		
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	175.00	262.50	TRUE		cold E2	-7.0	0.0	262.5	-1.5	116972.8	0.0		0.0		
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	189.00	283.50	TRUE		cold E2	-7.0	0.6	283.5	19.5	116951.8	0.6		0.6		
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2421.00	3631.50	TRUE			—	99.0	3631.5	3367.5	113603.8	99.0	107.8	99.0		
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2917.00	4375.50	TRUE			—	120.9	4375.5	4111.5	112859.8	120.9		120.9		
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2523.00	3784.50	TRUE			—	103.5	3784.5	3520.5	113450.8	103.5		103.5		
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average			
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
211		178	1	none	Hot		—	—	—	10	—	—	—	—	76822.00	115233.00	TRUE					115233	114969.0							
212		179	2	none	Hot		—	—	—	10	—	—	—	—	84813.00	127219.50	TRUE					127219.5	126955.5							
213		180	3	none	Hot		—	—	—	10	—	—	—	—	77577.00	116365.50	TRUE					116365.5	116101.5							
214		181	1	none	Hot		—	—	—	10	—	—	—	—	75110.00	112665.00	TRUE					112665	112401.0							
215		182	2	none	Hot		—	—	—	10	—	—	—	—	76509.00	114763.50	TRUE					114763.5	114499.5							
216		183	3	none	Hot		—	—	—	10	—	—	—	—	77054.00	115581.00	TRUE					115581	115317.0							

	B	C	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31			Prism data, excluding data points in the right leg of U-shaped curves																											
32			***standard curve***				***weak positive***				***4.0***				***9.0***				***14.0***				***17.0***				***negative control***			
33	*Position*	*Replicate*	*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1	-7.0	0.3969	-0.6615	-0.2646	-4.0	3.8809	4.4542	9.9228	-3.0				-3.0				-3.0				-3.0				-3.0	#N/A	#N/A	#N/A
35	2	2	-7.0	0.0000	-0.0441	0.5733	-4.5	2.2933	3.0430	3.3958	-4.0				-4.0	22.8445	27.4752	19.9338	-4.0				-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
36	3	3	-8.0	20.0662	23.4620	21.3892	-5.5	41.8964	44.2337	45.0276	-5.0	-2.8666	-3.3517	-2.7343	-5.0	48.1147	85.9537	103.5943	-5.0	35.6340	36.4278	39.7354	-5.0	10.8049	15.3914	15.5237	-5.0	#N/A	#N/A	#N/A
37	4	1	-8.5	56.2293	56.0970	56.7585	-6.0	84.7189	88.7762	86.3506	-6.0	-3.1312	-3.2194	-2.9548	-6.0	30.7828	60.5072	147.5193	-6.0	130.3638	132.6130		-6.0	92.6130	115.2811	132.4807	-6.0	#N/A	#N/A	#N/A
38	5	2	-9.0	109.7244	118.4123	114.7960	-6.5	146.5932	142.9768	143.5061	-7.0	-0.9702	-0.9261	-1.9846	-7.0	47.3208	140.4190	177.0673	-7.0	138.4785	120.8379	82.5138	-7.0	160.4410	109.2834	173.1863	-7.0	#N/A	#N/A	#N/A
39	6	3	-9.5	155.4135	162.0728	160.4410	-7.0	165.2481	177.6847	172.7894	-8.0	4.1014	4.9394	5.6891	-8.0	83.3958	168.2029	156.1191	-8.0	142.8886	120.7497	176.6262	-8.0	176.4057	157.4862	159.5149	-8.0	#N/A	#N/A	#N/A
40	7	1	-10.0	186.7255	188.4454	184.2558	-7.5	178.4785	198.1918	188.9746	-9.0	48.0265	55.1268	59.4046	-9.0	172.4807	168.7321	147.3870	-9.0	153.6053	166.8798	106.3727	-9.0	65.6670	79.0298	169.3054	-9.0	#N/A	#N/A	#N/A
41	8	2	-11.0	195.4576	194.0463	200.8379	-8.5	190.7387	199.0298	201.6318	-10.0	147.8280		146.5491	-10.0	175.3914	177.9493	181.9184	-10.0	145.0496	177.9052	155.7663	-10.0	77.0893	65.3142	57.3760	-10.0	#N/A	#N/A	#N/A
42	9	3																												
43	10	1	Standard Curve		TTN013 5-05-08		Positive Control		TTN013 5-05-08		4.0		TTN013 5-05-08		9.0		TTN013 5-05-08		14.0		TTN013 5-05-08		17.0		TTN013 5-05-08		Negative Control		Prism run ID & date	
44	11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values		ambiguous		Best-fit values				Best-fit values		Not converged		Best-fit values			
46	13	1	BOTTOM		-2.478		BOTTOM		1.398		BOTTOM		-2.803		BOTTOM		-2048		BOTTOM		-488700000		BOTTOM		n/a		BOTTOM			
47	14	2	TOP		198.7		TOP		196.7		TOP		180.1		TOP		285.7		TOP		146.2		TOP		n/a		TOP			
48	15	3	LOGIC50		-8.422		LOGIC50		-5.603		LOGIC50		-8.953		LOGIC50		-4.601		LOGIC50		-5.086		LOGIC50		n/a		LOGIC50			
49	16	1	HILLSLOPE		-1.009		HILLSLOPE		-0.9816		HILLSLOPE		-1.003		HILLSLOPE		~ -0.06720		HILLSLOPE		-0.4941		HILLSLOPE		n/a		HILLSLOPE			
50	17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3	BOTTOM		1.174		BOTTOM		2.531		BOTTOM		0.8507		BOTTOM		224606		BOTTOM		1.812E+13		BOTTOM				BOTTOM			
52	19	1	TOP		1.392		TOP		2.58		TOP		11.14		TOP		1447		TOP		10.36		TOP				TOP			
53	20	2	LOGIC50		0.01209		LOGIC50		0.02845		LOGIC50		0.0148		LOGIC50		0.4629		LOGIC50		0.2265		LOGIC50				LOGIC50			
54	21	3	HILLSLOPE		0.02712		HILLSLOPE		0.05653		HILLSLOPE		0.103		HILLSLOPE		~ 1.063		HILLSLOPE		0.2465		HILLSLOPE				HILLSLOPE			
55	22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2	Data				Data				Data				Data				Data				Data				Data			
66	33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
70	37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.72

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 013
Assay start date: 5/5/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	0.4
1	1	2	-7.0	-0.7
1	1	3	-7.0	-0.3
1	2	1	-8.0	20.1
1	2	2	-8.0	23.5
1	2	3	-8.0	21.4
1	3	1	-8.5	56.2
1	3	2	-8.5	56.1
1	3	3	-8.5	56.8
1	4	1	-9.0	109.7
1	4	2	-9.0	118.4
1	4	3	-9.0	114.8
1	5	1	-9.5	155.4
1	5	2	-9.5	162.1
1	5	3	-9.5	160.4
1	6	1	-10.0	186.7
1	6	2	-10.0	188.4
1	6	3	-10.0	184.3
1	7	1	-11.0	195.5
1	7	2	-11.0	194.0
1	7	3	-11.0	200.8
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 40.13

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 9
Run ID: TTN 013
Assay start date: 5/5/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.0	22.8
1	1	2	-4.0	27.5
1	1	3	-4.0	19.9
1	2	1	-5.0	48.1
1	2	2	-5.0	86.0
1	2	3	-5.0	103.6
1	3	1	-6.0	30.8
1	3	2	-6.0	60.5
1	3	3	-6.0	147.5
1	4	1	-7.0	47.3
1	4	2	-7.0	140.4
1	4	3	-7.0	177.1
1	5	1	-8.0	83.4
1	5	2	-8.0	168.2
1	5	3	-8.0	156.1
1	6	1	-9.0	172.5
1	6	2	-9.0	168.7
1	6	3	-9.0	147.4
1	7	1	-10.0	175.4
1	7	2	-10.0	177.9
1	7	3	-10.0	181.9
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.51

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 4
Run ID: TTN 013
Assay start date: 5/5/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	-2.9
1	1	2	-5.0	-3.4
1	1	3	-5.0	-2.7
1	2	1	-6.0	-3.1
1	2	2	-6.0	-3.2
1	2	3	-6.0	-3.0
1	3	1	-7.0	-1.0
1	3	2	-7.0	-0.9
1	3	3	-7.0	-2.0
1	4	1	-8.0	4.1
1	4	2	-8.0	4.9
1	4	3	-8.0	5.7
1	5	1	-9.0	48.0
1	5	2	-9.0	55.1
1	5	3	-9.0	59.4
1	6	1	-10.0	147.8
1	6	2		
1	6	3	-10.0	146.5
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 22.08

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 14
Run ID: TTN 013
Assay start date: 5/5/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	35.6
1	1	2	-5.0	36.4
1	1	3	-5.0	39.7
1	2	1	-6.0	130.4
1	2	2	-6.0	132.6
1	2	3	-6.0	138.7
1	3	1	-7.0	138.5
1	3	2	-7.0	120.8
1	3	3	-7.0	82.5
1	4	1	-8.0	142.9
1	4	2	-8.0	120.7
1	4	3	-8.0	176.6
1	5	1	-9.0	153.6
1	5	2	-9.0	166.9
1	5	3	-9.0	106.4
1	6	1	-10.0	145.0
1	6	2	-10.0	177.9
1	6	3	-10.0	155.8
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 26.55

Provide information in all blue cells.

Laboratory Code: B

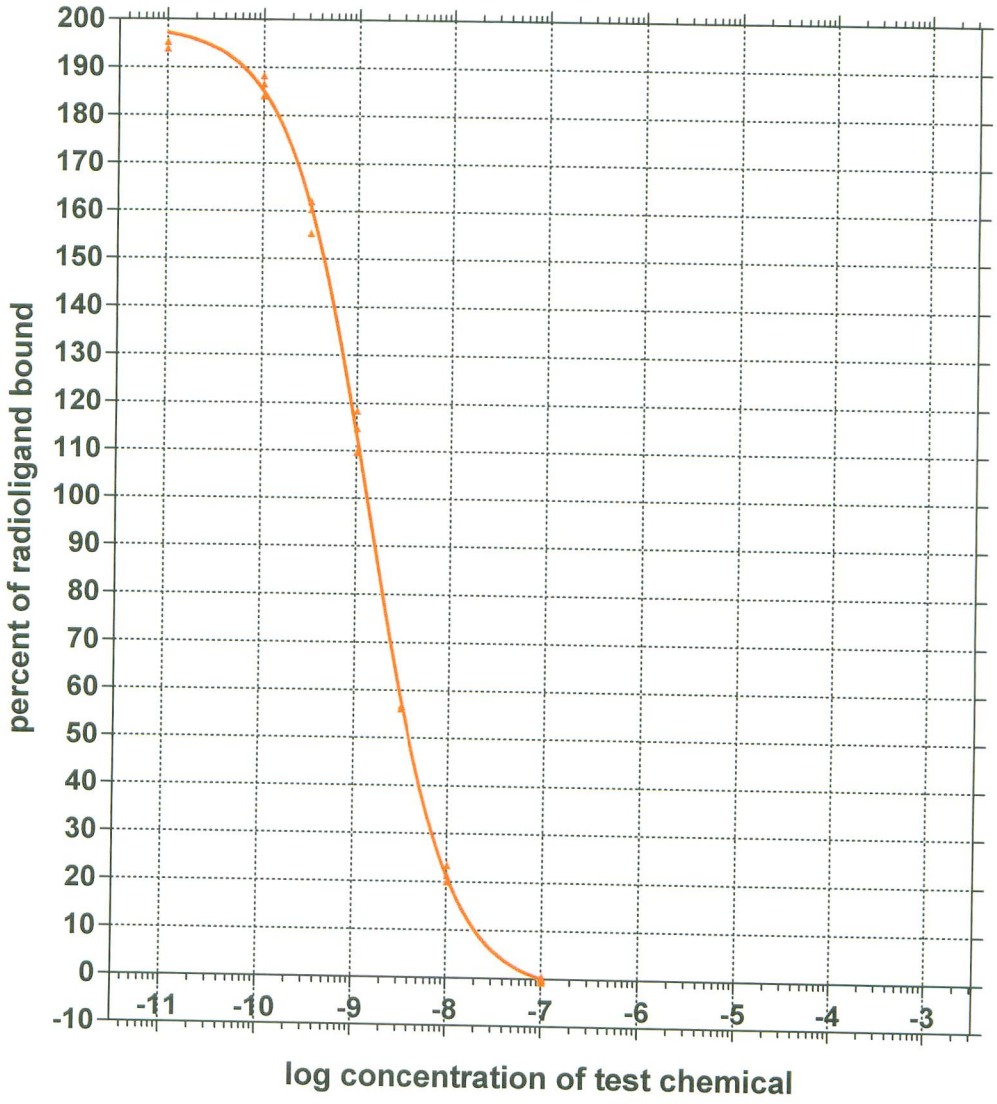
Compound ID: Chem Code 17
Run ID: TTN 013
Assay start date: 5/5/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.0	19.8
1	1	2	-4.0	20.4
1	1	3	-4.0	19.4
1	2	1	-5.0	10.8
1	2	2	-5.0	15.4
1	2	3	-5.0	15.5
1	3	1	-6.0	92.6
1	3	2	-6.0	115.3
1	3	3	-6.0	132.5
1	4	1	-7.0	160.4
1	4	2	-7.0	109.3
1	4	3	-7.0	173.2
1	5	1	-8.0	176.4
1	5	2	-8.0	157.5
1	5	3	-8.0	159.5
1	6	1	-9.0	65.7
1	6	2	-9.0	79.0
1	6	3	-9.0	169.3
1	7	1	-10.0	77.1
1	7	2	-10.0	65.3
1	7	3	-10.0	57.4
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

ole format: XY		X	A		
		log concentration	run TTN013-05-05-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.3969	-0.6615	-0.2646
2	Title	-7.0	0.0000	-0.0441	0.5733
3	Title	-8.0	20.0662	23.4620	21.3892
4	Title	-8.5	56.2293	56.0970	56.7585
5	Title	-9.0	109.7244	118.4123	114.7960
6	Title	-9.5	155.4135	162.0728	160.4410
7	Title	-10.0	186.7255	188.4454	184.2558
8	Title	-11.0	195.4576	194.0463	200.8379

	Nonlin fit Table of results	A run TTN013-05-05-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.478
4	TOP	198.7
5	LOGIC50	-8.422
6	HILLSLOPE	-1.009
7	Std. Error	
8	BOTTOM	1.174
9	TOP	1.392
10	LOGIC50	0.01209
11	HILLSLOPE	0.02712
12	95% Confidence Intervals	
13	BOTTOM	-4.927 to -0.02937
14	TOP	195.8 to 201.6
15	LOGIC50	-8.448 to -8.397
16	HILLSLOPE	-1.066 to -0.9526
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9991
20	Absolute Sum of Squares	125.5
21	Sy.x	2.505
22	Number of points	
23	Analyzed	24

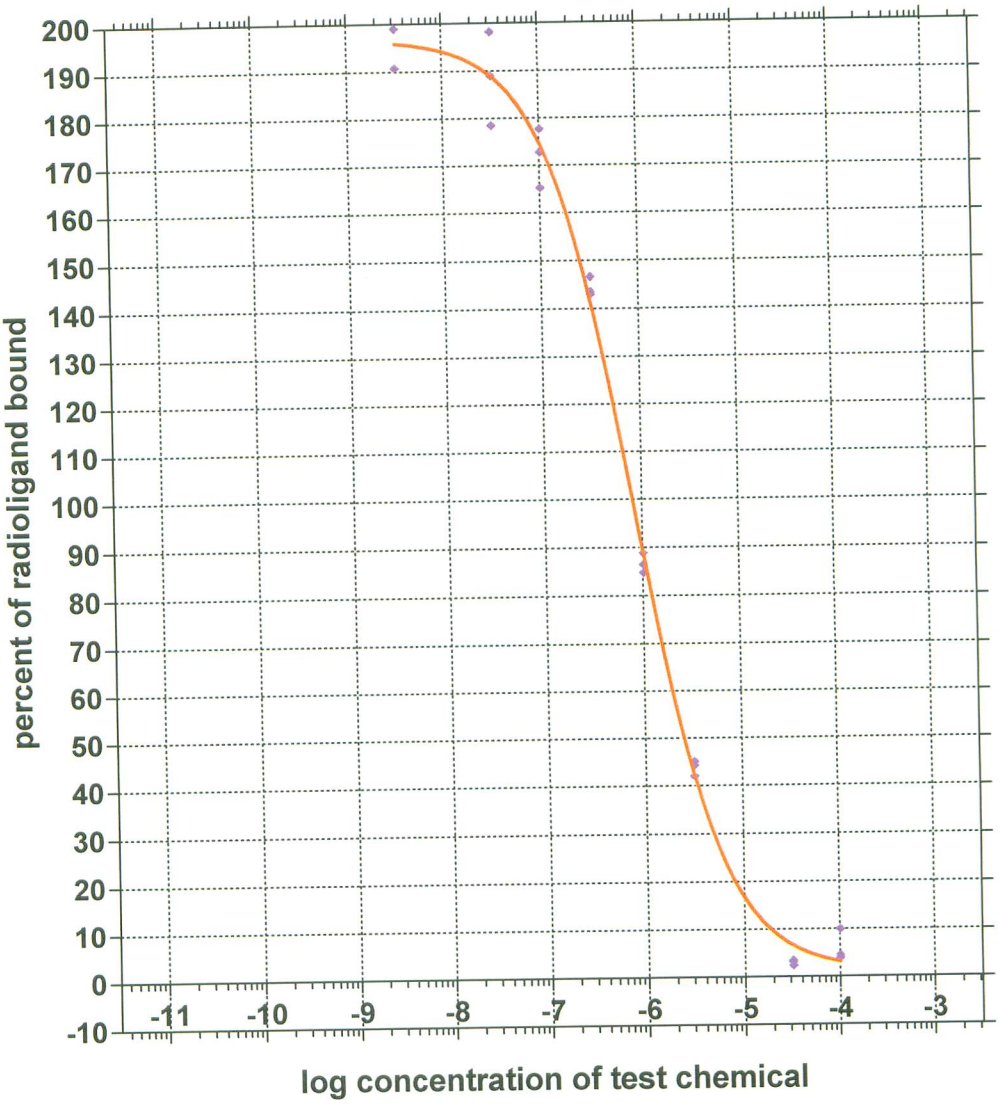
Estradiol reference standard
run TTN013



ole format: XY		X	A		
		log concentration	run TTN013-05-05-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0	3.8809	4.4542	9.9228
2	Title	-4.5	2.2933	3.0430	3.3958
3	Title	-5.5	41.8964	44.2337	45.0276
4	Title	-6.0	84.7189	88.7762	86.3506
5	Title	-6.5	146.5932	142.9768	143.5061
6	Title	-7.0	165.2481	177.6847	172.7894
7	Title	-7.5	178.4785	198.1918	188.9746
8	Title	-8.5	190.7387	199.0298	201.6318

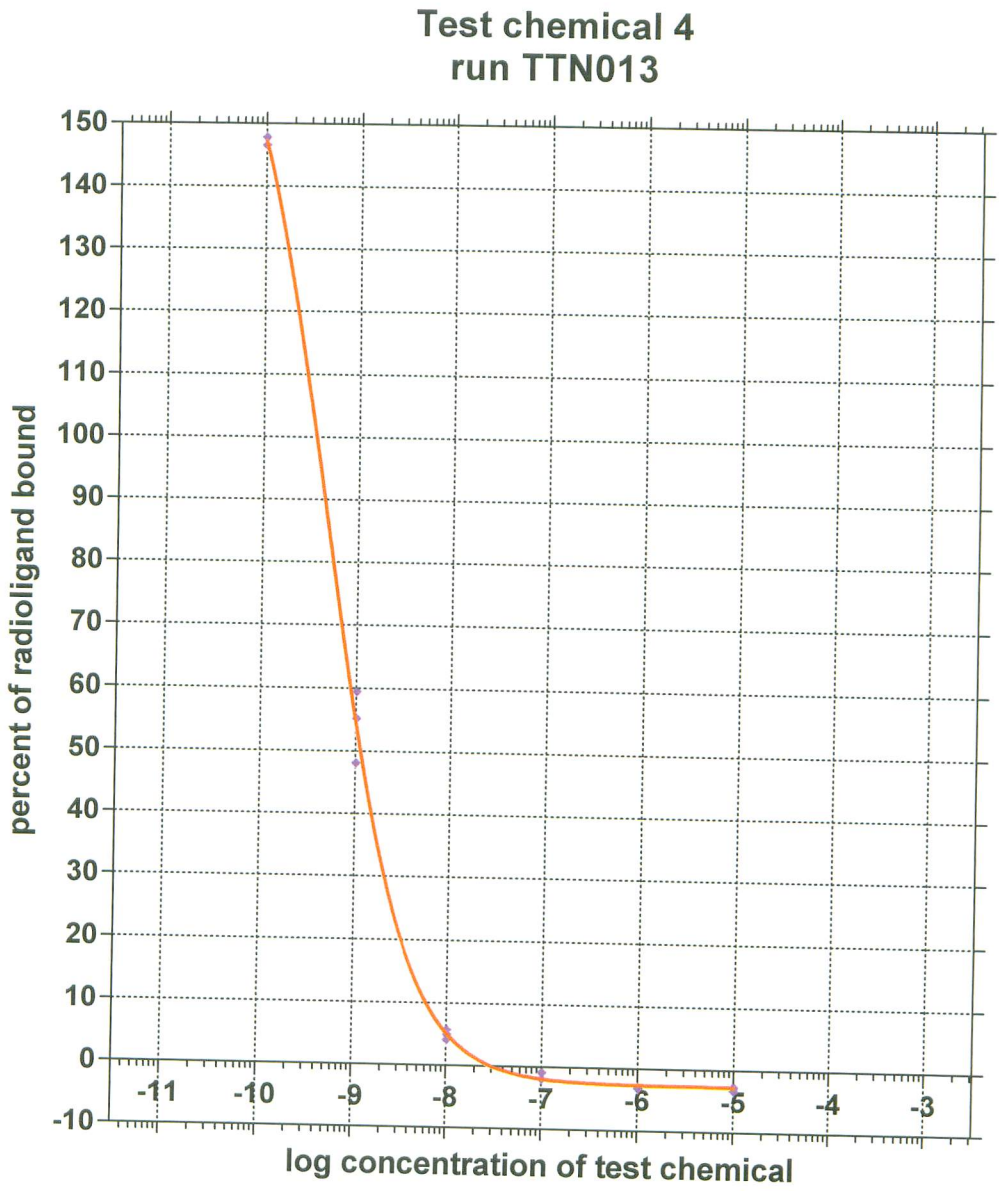
	Nonlin fit Table of results	A run TTN013-05-05-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.398
4	TOP	196.7
5	LOGIC50	-5.603
6	HILLSLOPE	-0.9816
7	Std. Error	
8	BOTTOM	2.531
9	TOP	2.580
10	LOGIC50	0.02845
11	HILLSLOPE	0.05653
12	95% Confidence Intervals	
13	BOTTOM	-3.882 to 6.678
14	TOP	191.4 to 202.1
15	LOGIC50	-5.662 to -5.544
16	HILLSLOPE	-1.100 to -0.8637
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9961
20	Absolute Sum of Squares	530.7
21	Sy.x	5.151
22	Number of points	
23	Analyzed	24

Norethynodrel
run TTN013



ole format: XY		X	A			B		
		log concentration	Test chemical			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	-2.8666	-3.3517	-2.7343			
4	Title	-6.0	-3.1312	-3.2194	-2.9548			
5	Title	-7.0	-0.9702	-0.9261	-1.9846			
6	Title	-8.0	4.1014	4.9394	5.6891			
7	Title	-9.0	48.0265	55.1268	59.4046			
8	Title	-10.0	147.8280		146.5491			

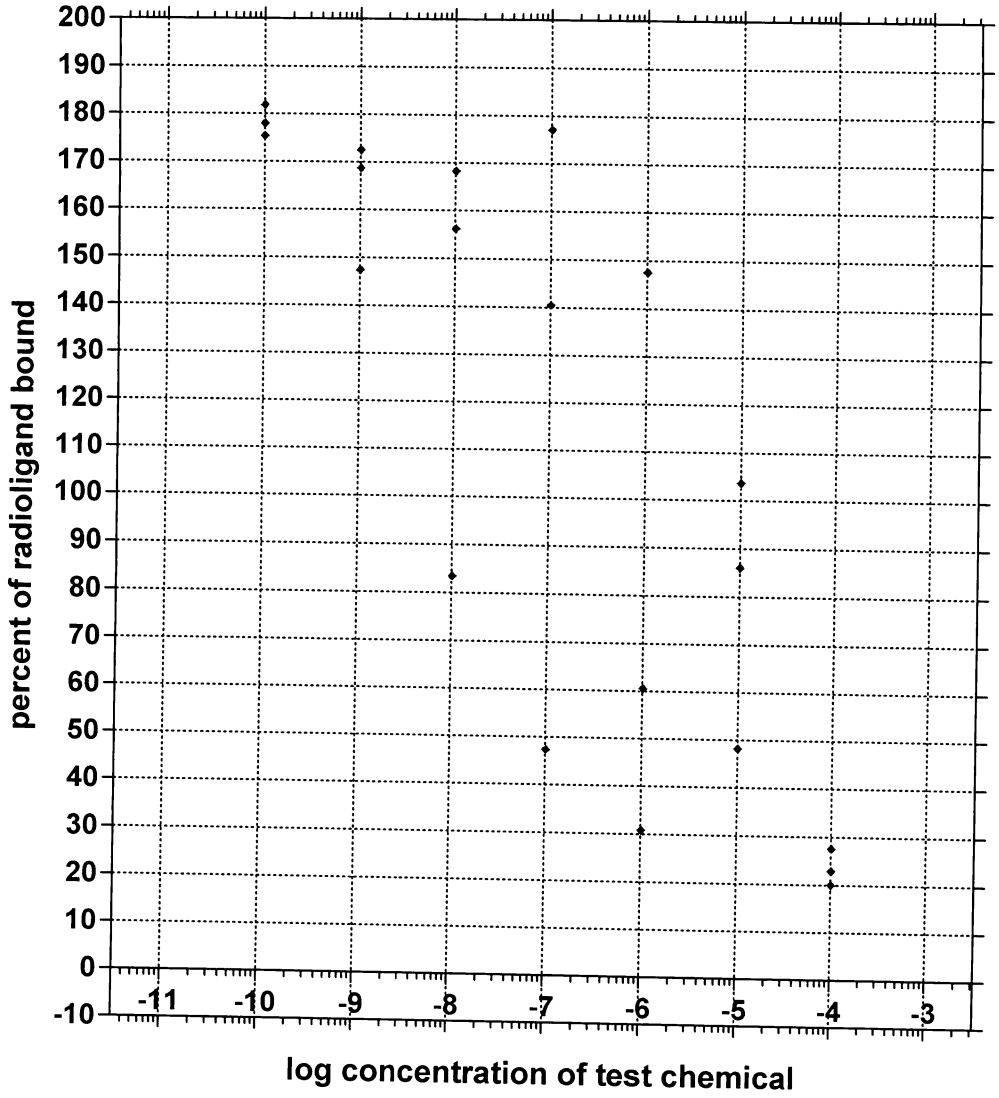
	Nonlin fit Table of results	A Test chemical	B Data Set-B
		Y	Y
1	logIC50		
2	Best-fit values		
3	BOTTOM	-2.803	
4	TOP	180.1	
5	LOGIC50	-8.953	
6	HILLSLOPE	-1.003	
7	Std. Error		
8	BOTTOM	0.8507	
9	TOP	11.14	
10	LOGIC50	0.01480	
11	HILLSLOPE	0.1030	
12	95% Confidence Intervals		
13	BOTTOM	-4.641 to -0.9655	
14	TOP	156.0 to 204.1	
15	LOGIC50	-8.985 to -8.921	
16	HILLSLOPE	-1.226 to -0.7811	
17	Goodness of Fit		
18	Degrees of Freedom	13	
19	R^2	0.9982	
20	Absolute Sum of Squares	71.17	
21	Sy.x	2.340	
22	Number of points		
23	Analyzed	17	


Test chemical 4
run TTN013
percent of radioligand bound
150
140
130
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

ple format: XY		X	A		
		log concentration	Data Set-A		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	22.8445	27.4752	19.9338
3	Title	-5.0	48.1147	85.9537	103.5943
4	Title	-6.0	30.7828	60.5072	147.5193
5	Title	-7.0	47.3208	140.4190	177.0673
6	Title	-8.0	83.3958	168.2029	156.1191
7	Title	-9.0	172.4807	168.7321	147.3870
8	Title	-10.0	175.3914	177.9493	181.9184

	Nonlin fit Table of results	A Data Set-A
		Y
1	logIC50	Ambiguous
2	Best-fit values	
3	BOTTOM	-2048
4	TOP	285.7
5	LOGIC50	-4.601
6	HILLSLOPE	~ -0.06720
7	Std. Error	
8	BOTTOM	224606
9	TOP	1447
10	LOGIC50	0.4629
11	HILLSLOPE	~ 1.063
12	95% Confidence Intervals	
13	BOTTOM	-476000 to 471870
14	TOP	-2767 to 3338
15	LOGIC50	-5.578 to -3.625
16	HILLSLOPE	(Very wide)
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.6810
20	Absolute Sum of Squares	24064
21	Sy.x	37.62
22	Number of points	
23	Analyzed	21

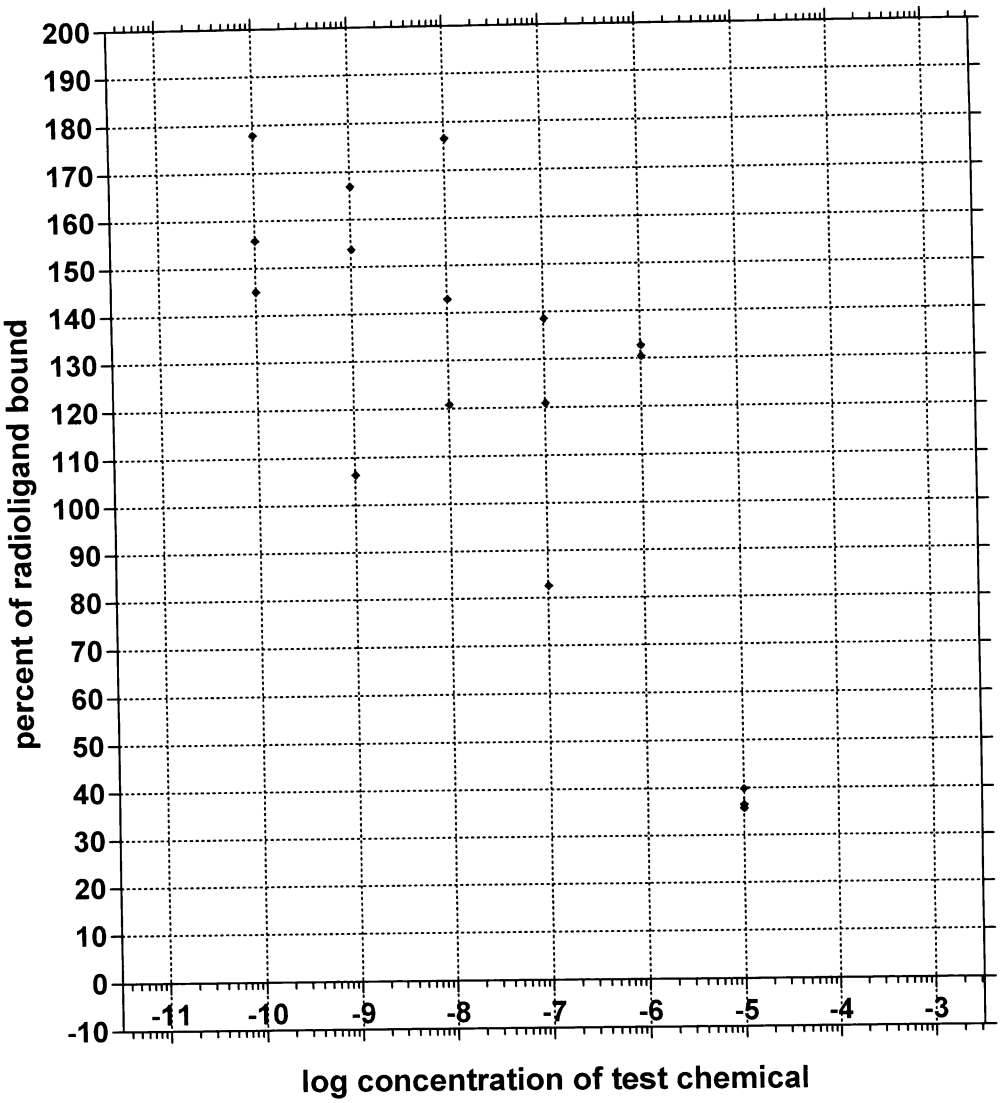
Test chemical 9
run TTN013



ɔle format: XY		X	A			B		
		log concentration	Data Set-A			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	35.6340	36.4278	39.7354			
4	Title	-6.0	130.3638	132.6130				
5	Title	-7.0	138.4785	120.8379	82.5138			
6	Title	-8.0	142.8886	120.7497	176.6262			
7	Title	-9.0	153.6053	166.8798	106.3727			
8	Title	-10.0	145.0496	177.9052	155.7663			

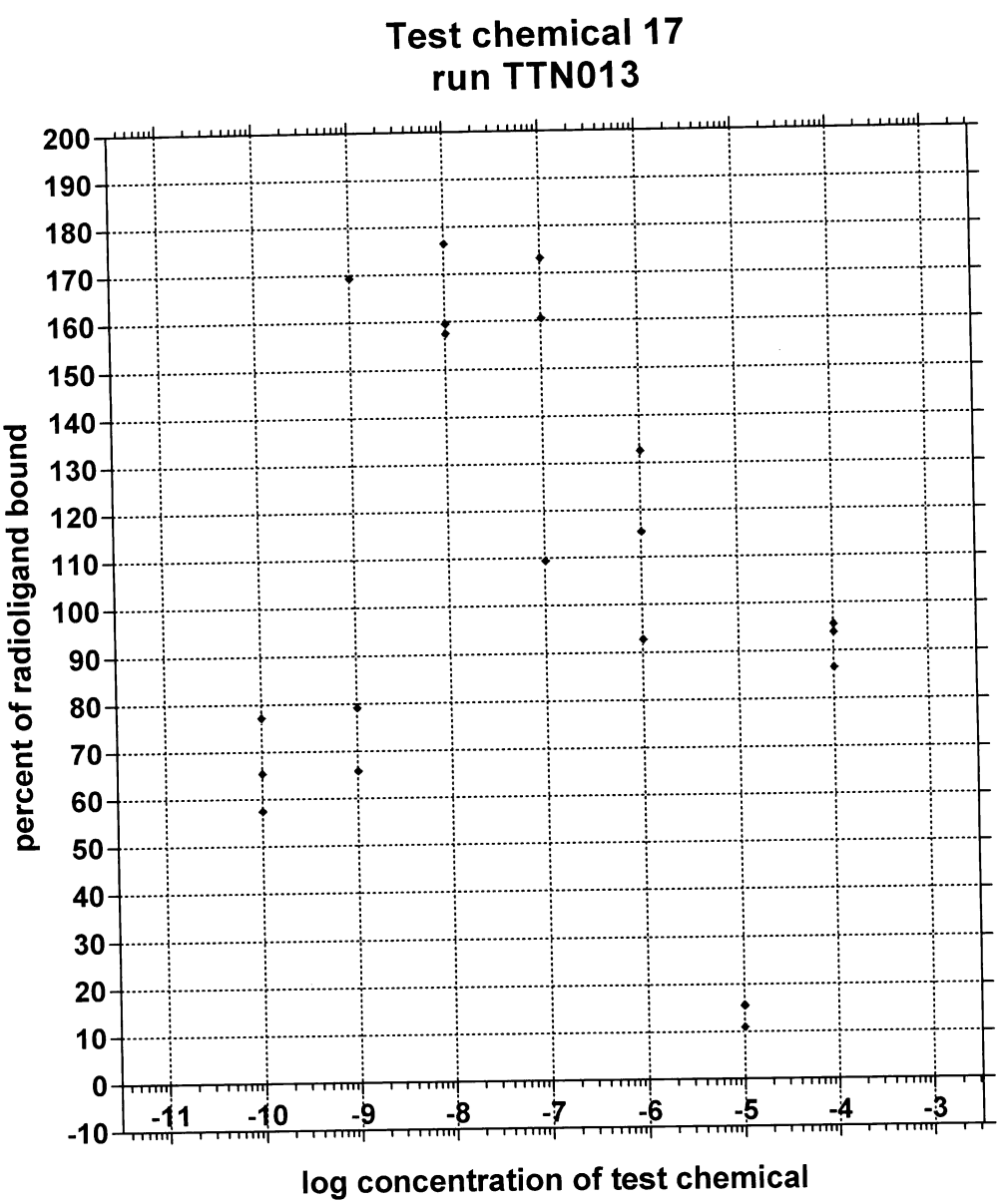
	Nonlin fit Table of results	A Data Set-A	B Data Set-B
		Y	Y
1	logIC50		
2	Best-fit values		
3	BOTTOM	-4.887e+008	
4	TOP	146.2	
5	LOGIC50	-5.086	
6	HILLSLOPE	-0.4941	
7	Std. Error		
8	BOTTOM	1.812e+013	
9	TOP	10.36	
10	LOGIC50	0.2265	
11	HILLSLOPE	0.2465	
12	95% Confidence Intervals		
13	BOTTOM	-3.913e+013 to 3.913e+013	
14	TOP	123.8 to 168.6	
15	LOGIC50	-5.575 to -4.597	
16	HILLSLOPE	-1.026 to 0.03827	
17	Goodness of Fit		
18	Degrees of Freedom	13	
19	R^2	0.7547	
20	Absolute Sum of Squares	8592	
21	Sy.x	25.71	
22	Number of points		
23	Analyzed	17	

Test chemical 14
run TTN013



ɔle format: XY		X	A			B		
		log concentration	Data Set-A			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0	93.6729	95.3859	86.3787			
3	Title	-5.0	10.8049	15.3914	15.5237			
4	Title	-6.0	92.6130	115.2811	132.4807			
5	Title	-7.0	160.4410	109.2834	173.1863			
6	Title	-8.0	176.4057	157.4862	159.5149			
7	Title	-9.0	65.6670	79.0298	169.3054			
8	Title	-10.0	77.0893	65.3142	57.3760			

	Nonlin fit Table of results	A Data Set-A	B Data Set-B
		Y	Y
1	logIC50	Not converged	
2	Best-fit values		
3	BOTTOM		
4	TOP		
5	LOGIC50		
6	HILLSLOPE		
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	21	


Test chemical 17
run TTN013
percent of radioligand bound
200
190
180
170
160
150
140
130
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

APPENDIX O

Excel and Prism Files of the Run Identifier TTN014

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R;
the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	TTN014	
Assay start date:	5/7/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.87	Ci/mmole
Cytosol vial or lot identification:	RUC3-03-08 A,M	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.42	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.50	M
RBA, norethynodrel (Weak Positive):	1.197E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-8.91	4.0
RBA, Unknown 1:	3.126E+00	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	n/a	9.0
RBA, Unknown 2:	n/a	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-6.60	14.0
RBA, Unknown 3:	1.503E-02	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	n/a	20.0
RBA, Unknown 4:	n/a	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 1.69%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values	n	Mean	SD	
EtOH	5	2115.9	53.19	Total Binding, solvent control, tubes
Hot	6	125098.8	11184.81	Total hot E2 added to each tube
NSB	6	231.5	25.17	Nonspecific Binding
Specific EtOH	5	1884.4	53.19	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values			
EtOH / Hot	0.02	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.11	around 0.25 ?	

Values for analysis by nonlinear regression
Standard Curve
Negative Control
Weak Positive
Unknown

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	-0.8
R1881	#N/A	not applicable
norethynodrel	-4.5	9.4
Unknown 1	-5.0	-1.6
Unknown 2	-4.0	16.3
Unknown 3	-5.0	23.8
Unknown 4	-5.0	51.8

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1365.00	2047.50	TRUE			—	96.4	2047.5	1816.0	123282.8	96.4	97.8	96.4
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	398.00	597.00	FALSE	mispipet		—							
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1400.00	2100.00	TRUE			—	99.2	2100	1868.5	123230.3	99.2		99.2
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	135.00	202.50	TRUE		cold E2	-7.0	-1.5	202.5	-29.0	125127.8	-1.5	-0.8	-1.5
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	149.00	223.50	TRUE		cold E2	-7.0	-0.4	223.5	-8.0	125106.8	-0.4		-0.4
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	148.00	222.00	TRUE		cold E2	-7.0	-0.5	222	-9.5	125108.3	-0.5		-0.5
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	510.00	765.00	TRUE		cold E2	-8.0	28.3	765	533.5	124565.3	28.3	26.5	28.3
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	463.00	694.50	TRUE		cold E2	-8.0	24.6	694.5	463.0	124635.8	24.6		24.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	487.00	730.50	TRUE		cold E2	-8.0	26.5	730.5	499.0	124599.8	26.5		26.5
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	881.00	1321.50	TRUE		cold E2	-8.5	57.8	1321.5	1090.0	124008.8	57.8	56.5	57.8
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	800.00	1200.00	TRUE		cold E2	-8.5	51.4	1200	968.5	124130.3	51.4		51.4
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	912.00	1368.00	TRUE		cold E2	-8.5	60.3	1368	1136.5	123962.3	60.3		60.3
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1186.00	1779.00	TRUE		cold E2	-9.0	82.1	1779	1547.5	123551.3	82.1	84.0	82.1
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1171.00	1756.50	TRUE		cold E2	-9.0	80.9	1756.5	1525.0	123573.8	80.9		80.9
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1271.00	1906.50	TRUE		cold E2	-9.0	88.9	1906.5	1675.0	123423.8	88.9		88.9
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	1414.00	2121.00	TRUE		cold E2	-9.5	100.3	2121	1889.5	123209.3	100.3	93.4	100.3
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	1296.00	1944.00	TRUE		cold E2	-9.5	90.9	1944	1712.5	123386.3	90.9		90.9
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	1274.00	1911.00	TRUE		cold E2	-9.5	89.1	1911	1679.5	123419.3	89.1		89.1
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1677.00	2515.50	TRUE		cold E2	-10.0	121.2	2515.5	2284.0	122614.8	121.2	119.8	121.2
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1711.00	2566.50	TRUE		cold E2	-10.0	123.9	2566.5	2335.0	122763.8	123.9		123.9
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1589.00	2383.50	TRUE		cold E2	-10.0	114.2	2383.5	2152.0	122946.8	114.2		114.2
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1528.00	2292.00	FALSE	mispipet	cold E2	-11.0						123.8	
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1700.00	2550.00	TRUE		cold E2	-11.0	123.0	2550	2318.5	122780.3	123.0		123.0
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	1719.00	2578.50	TRUE		cold E2	-11.0	124.5	2578.5	2347.0	122751.8	124.5		124.5
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	1.69%	If the ratio of EtOH / Hot is > 10% th there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.					
33	Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	
74	41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A	
75	42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A	
76	43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A	
77	44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A	
78	45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A	
79	46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A	
80	47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A	
81	48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A	
82	49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	**2185.00**	3277.50	TRUE		norethynodrel	-4.0	161.6	3277.5	3046.0	122052.8	161.6	168.9		
83	50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	**2372.00**	3558.00	TRUE		norethynodrel	-4.0	178.5	3558	3326.5	121772.3	178.5			
84	51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	**2273.00**	3409.50	TRUE		norethynodrel	-4.0	168.6	3409.5	3178.0	121920.8	168.6			
85	52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	**340.00**	510.00	TRUE		norethynodrel	-4.5	14.8	510	278.5	124820.3	14.8	9.4		14.8
86	53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	**227.00**	340.50	TRUE		norethynodrel	-4.5	5.8	340.5	109.0	124989.8	5.8			5.8
87	54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	**251.00**	376.50	TRUE		norethynodrel	-4.5	7.7	376.5	145.0	124953.8	7.7			7.7
88	55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	**786.00**	1179.00	TRUE		norethynodrel	-5.5	50.3	1179	947.5	124151.3	50.3	49.3		50.3
89	56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	**739.00**	1108.50	TRUE		norethynodrel	-5.5	46.5	1108.5	877.0	124221.8	46.5			46.5
90	57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	**795.00**	1192.50	TRUE		norethynodrel	-5.5	51.0	1192.5	961.0	124137.8	51.0			51.0
91	58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1147.00**	1720.50	TRUE		norethynodrel	-6.0	79.0	1720.5	1489.0	123609.8	79.0	75.5		79.0
92	59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1114.00**	1671.00	TRUE		norethynodrel	-6.0	76.4	1671	1439.5	123659.3	76.4			76.4
93	60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1049.00**	1573.50	TRUE		norethynodrel	-6.0	71.2	1573.5	1342.0	123756.8	71.2			71.2
94	61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	**1238.00**	1857.00	TRUE		norethynodrel	-6.5	86.3	1857	1625.5	123473.3	86.3	94.9		86.3
95	62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	**1386.00**	2079.00	TRUE		norethynodrel	-6.5	98.0	2079	1847.5	123251.3	98.0			98.0
96	63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	**1417.00**	2125.50	TRUE		norethynodrel	-6.5	100.5	2125.5	1894.0	123204.8	100.5			100.5
97	64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	**1530.00**	2295.00	TRUE		norethynodrel	-7.0	109.5	2295	2063.5	123035.3	109.5	97.5		109.5
98	65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	**1326.00**	1989.00	TRUE		norethynodrel	-7.0	93.3	1989	1757.5	123341.3	93.3			93.3
99	66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	**1281.00**	1921.50	TRUE		norethynodrel	-7.0	89.7	1921.5	1690.0	123408.8	89.7			89.7
100	67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	**1614.00**	2421.00	TRUE		norethynodrel	-7.5	116.2	2421	2189.5	122909.3	116.2	111.7		116.2
101	68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	**1501.00**	2251.50	TRUE		norethynodrel	-7.5	107.2	2251.5	2020.0	123078.8	107.2			107.2
102	69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	**1089.00**	1633.50	FALSE	mispipet	norethynodrel	-7.5								
103	70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	**1502.00**	2253.00	TRUE		norethynodrel	-8.5	107.3	2253	2021.5	123077.3	107.3	111.0		107.3
104	71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	**690.00**	1035.00	FALSE	mispipet	norethynodrel	-8.5								
105	72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	**1596.00**	2394.00	TRUE		norethynodrel	-8.5	114.8	2394	2162.5	122936.3	114.8			114.8
106	73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**2334.00**	3501.00	TRUE		Unknown 1	-3.0	173.5	3501	3269.5	121829.3	173.5	166.3		
107	74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**2226.00**	3339.00	TRUE		Unknown 1	-3.0	164.9	3339	3107.5	121991.3	164.9			
108	75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**2169.00**	3253.50	TRUE		Unknown 1	-3.0	160.4	3253.5	3022.0	122076.8	160.4			
109	76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**689.00**	1033.50	TRUE		Unknown 1	-4.0	42.6	1033.5	802.0	124296.8	42.6	49.1		
110	77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**786.00**	1179.00	TRUE		Unknown 1	-4.0	50.3	1179	947.5	124151.3	50.3			
111	78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**838.00**	1257.00	TRUE		Unknown 1	-4.0	54.4	1257	1025.5	124073.3	54.4			
112	79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**217.00**	325.50	TRUE		Unknown 1	-5.0	5.0	325.5	94.0	125004.8	5.0	-1.6		5.0
113	80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**97.00**	145.50	TRUE		Unknown 1	-5.0	-4.6	145.5	-86.0	125184.8	-4.6			-4.6
114	81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**88.00**	132.00	TRUE		Unknown 1	-5.0	-5.3	132	-99.5	125198.3	-5.3			-5.3
115	82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**238.00**	357.00	TRUE		Unknown 1	-6.0	6.7	357	125.5	124973.3	6.7	0.8		6.7
116	83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**137.00**	205.50	TRUE		Unknown 1	-6.0	-1.4	205.5	-26.0	125124.8	-1.4			-1.4
117	84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**120.00**	180.00	TRUE		Unknown 1	-6.0	-2.7	180	-51.5	125150.3	-2.7			-2.7
118	85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**123.00**	184.50	TRUE		Unknown 1	-7.0	-2.5	184.5	-47.0	125145.8	-2.5	-0.6		-2.5
119	86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**179.00**	268.50	TRUE		Unknown 1	-7.0	2.0	268.5	37.0	125061.8	2.0			2.0
120	87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**137.00**	205.50	TRUE		Unknown 1	-7.0	-1.4	205.5	-26.0	125124.8	-1.4			-1.4
121	88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**243.00**	364.50	TRUE		Unknown 1	-8.0	7.1	364.5	133.0	124965.8	7.1	8.8		7.1
122	89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**249.00**	373.50	TRUE		Unknown 1	-8.0	7.5	373.5	142.0	124956.8	7.5			7.5
123	90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**304.00**	456.00	TRUE		Unknown 1	-8.0	11.9	456	224.5	124874.3	11.9			11.9
124	91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	**843.00**	1264.50	TRUE		Unknown 1	-9.0	54.8	1264.5	1033.0	124065.8	54.8	52.9		54.8
125	92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	**979.00**	1468.50	TRUE		Unknown 1	-9.0	65.6	1468.5	1237.0	123861.8	65.6			65.6
126	93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	**636.00**	954.00	TRUE		Unknown 1	-9.0	38.3	954	722.5	124376.3	38.3			38.3
127	94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	**916.00**	1374.00	TRUE		Unknown 1	-10.0	60.6	1374	1142.5	123956.3	60.6	63.9		60.6
128	95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	**613.00**	919.50	TRUE		Unknown 1	-10.0	36.5	919.5	688.0	124410.8	36.5			36.5
129	96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	**1344.00**	2016.00	TRUE		Unknown 1	-10.0	94.7	2016	1784.5	123314.3	94.7			94.7
130	97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**578.00**	867.00	TRUE		Unknown 2	-3.0	33.7	867	635.5	124463.3	33.7	28.4		
131	98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**497.00**	745.50	TRUE		Unknown 2	-3.0	27.3	745.5	514.0	124584.8	27.3			
132	99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	**459.00**	688.50	TRUE		Unknown 2	-3.0	24.3	688.5	457.0	124641.8	24.3			
133	100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**669.00**	1003.50	TRUE		Unknown 2	-4.0	41.0	1003.5	772.0	124326.8	41.0	16.3		41.0
134	101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**217.00**	325.50	TRUE		Unknown 2	-4.0	5.0	325.5	94.0	125004.8	5.0			5.0
135	102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	**192.00**	288.00	TRUE		Unknown 2	-4.0	3.0	288	56.5	125042.3	3.0			3.0
136	103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**176.00**	264.00	TRUE		Unknown 2	-5.0	1.7	264	32.5	125066.3	1.7	33.5		1.7
137	104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**1262.00**	1893.00	TRUE		Unknown 2	-5.0	88.2	1893	1661.5	123437.3	88.2			88.2
138	105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	**286.00**	429.00	TRUE		Unknown 2	-5.0	10.5	429	197.5	124901.3	10.5			10.5
139	106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1293.00**	1939.50	TRUE		Unknown 2	-6.0	90.6	1939.5	1708.0	123390.8	90.6	90.2		90.6
140	107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1255.00**	1882.50	TRUE		Unknown 2	-6.0	87.6	1882.5	1651.0	123447.8	87.6			87.6
141	108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	**1313.00**	1969.50	TRUE		Unknown 2	-6.0	92.2	1969.5	1738.0	123360.8	92.2			92.2
142	109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**838.00**	1257.00	TRUE		Unknown 2	-7.0	54.4	1257	1025.5	124073.3	54.4	29.3		54.4
143	110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**332.00**	498.00	TRUE		Unknown 2	-7.0	14.1	498	266.5	124832.3	14.1			14.1
144	111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	**398.00**	597.00	TRUE		Unknown 2	-7.0	19.4	597	365.5	124733.3	19.4			19.4
145	112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**841.00**	1261.50	TRUE		Unknown 2	-8.0	54.7	1261.5	1030.0	124068.8	54.7	62.8		54.7
146	113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**1641.00**	2461.50	TRUE		Unknown 2	-8.0	118.3	2461.5	2230.0	122868.8	118.3			118.3
147	114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	**348.00**	522.00	TRUE		Unknown 2	-8.0	15.4	522	290.5	124808.3	15.4			15.4
148	115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	**1434.00**	2151.00	TRUE		Unknown 2	-9.0	101.9	2151	1919.5	123179.3	101.9	93.1		101.9
149	116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	**1071.00**	1606.50	TRUE		Unknown 2	-9.0	73.0	1606.5	1375.0	123723.8	73.0			73.0

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	1.68%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.					
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	
150		117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1465.00	2197.50	TRUE		Unknown 2	-9.0	104.3	2197.5	1966.0	123132.8	104.3		104.3	
151		118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	611.00	916.50	TRUE		Unknown 2	-10.0	36.4	916.5	685.0	124413.8	36.4	58.2	36.4	
152		119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1467.00	2200.50	TRUE		Unknown 2	-10.0	104.5	2200.5	1969.0	123129.8	104.5		104.5	
153		120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	578.00	867.00	TRUE		Unknown 2	-10.0	33.7	867	635.5	124463.3	33.7		33.7	
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	13243.00	19864.50	TRUE		Unknown 3	-3.0	1041.9	19864.5	19633.0	105465.8	1041.9	2024.1		
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	33408.00	50112.00	TRUE		Unknown 3	-3.0	2647.0	50112	49880.5	75218.3	2647.0			
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	30097.00	45145.50	TRUE		Unknown 3	-3.0	2383.5	45145.5	44914.0	80184.8	2383.5			
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	9391.00	14086.50	TRUE		Unknown 3	-4.0	735.2	14086.5	13855.0	111243.8	735.2	849.4		
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	11718.00	17577.00	TRUE		Unknown 3	-4.0	920.5	17577	17345.5	107753.3	920.5			
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	11365.00	17047.50	TRUE		Unknown 3	-4.0	892.4	17047.5	16816.0	108282.8	892.4			
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	476.00	714.00	TRUE		Unknown 3	-5.0	25.6	714	482.5	124616.3	25.6	23.8	25.6	
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	392.00	588.00	TRUE		Unknown 3	-5.0	18.9	588	356.5	124742.3	18.9		18.9	
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	492.00	738.00	TRUE		Unknown 3	-5.0	26.9	738	506.5	124592.3	26.9		26.9	
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	920.00	1380.00	TRUE		Unknown 3	-6.0	60.9	1380	1148.5	123950.3	60.9	28.4	60.9	
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	285.00	427.50	TRUE		Unknown 3	-6.0	10.4	427.5	196.0	124902.8	10.4		10.4	
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	329.00	493.50	TRUE		Unknown 3	-6.0	13.9	493.5	262.0	124836.8	13.9		13.9	
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	442.00	663.00	TRUE		Unknown 3	-7.0	22.9	663	431.5	124667.3	22.9	71.8	22.9	
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1395.00	2092.50	TRUE		Unknown 3	-7.0	98.8	2092.5	1861.0	123237.8	98.8		98.8	
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1332.00	1998.00	TRUE		Unknown 3	-7.0	93.7	1998	1766.5	123332.3	93.7		93.7	
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	247.00	370.50	TRUE		Unknown 3	-8.0	7.4	370.5	139.0	124959.8	7.4	69.6	7.4	
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1499.00	2248.50	TRUE		Unknown 3	-8.0	107.0	2248.5	2017.0	123081.8	107.0		107.0	
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1341.00	2011.50	TRUE		Unknown 3	-8.0	94.5	2011.5	1780.0	123318.8	94.5		94.5	
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1265.00	1897.50	TRUE		Unknown 3	-9.0	88.4	1897.5	1666.0	123432.8	88.4	98.6	88.4	
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1448.00	2172.00	TRUE		Unknown 3	-9.0	103.0	2172	1940.5	123158.3	103.0		103.0	
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1465.00	2197.50	TRUE		Unknown 3	-9.0	104.3	2197.5	1966.0	123132.8	104.3		104.3	
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1254.00	1881.00	TRUE		Unknown 3	-10.0	87.5	1881	1649.5	123449.3	87.5	104.3	87.5	
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1473.00	2209.50	TRUE		Unknown 3	-10.0	105.0	2209.5	1978.0	123120.8	105.0		105.0	
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1667.00	2500.50	TRUE		Unknown 3	-10.0	120.4	2500.5	2269.0	122829.8	120.4		120.4	
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	10798.00	16197.00	TRUE		Unknown 4	-3.0	847.2	16197	15965.5	109133.3	847.2	794.9		
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	9801.00	14701.50	TRUE		Unknown 4	-3.0	767.9	14701.5	14470.0	110628.8	767.9			
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	9823.00	14734.50	TRUE		Unknown 4	-3.0	769.6	14734.5	14503.0	110595.8	769.6			
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2816.00	4224.00	TRUE		Unknown 4	-4.0	211.9	4224	3992.5	121106.3	211.9	229.4		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2140.00	3210.00	TRUE		Unknown 4	-4.0	158.1	3210	2978.5	122120.3	158.1			
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4151.00	6226.50	TRUE		Unknown 4	-4.0	318.1	6226.5	5995.0	119103.8	318.1			
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	613.00	919.50	TRUE		Unknown 4	-5.0	36.5	919.5	688.0	124410.8	36.5	51.8	36.5	
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	489.00	733.50	TRUE		Unknown 4	-5.0	26.6	733.5	502.0	124596.8	26.6		26.6	
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1313.00	1969.50	TRUE		Unknown 4	-5.0	92.2	1969.5	1738.0	123360.8	92.2		92.2	
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	715.00	1072.50	TRUE		Unknown 4	-6.0	44.6	1072.5	841.0	124257.8	44.6	74.3	44.6	
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1324.00	1986.00	TRUE		Unknown 4	-6.0	93.1	1986	1754.5	123344.3	93.1		93.1	
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1225.00	1837.50	TRUE		Unknown 4	-6.0	85.2	1837.5	1606.0	123492.8	85.2		85.2	
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1342.00	2013.00	TRUE		Unknown 4	-7.0	94.5	2013	1781.5	123317.3	94.5	94.5	94.5	
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1215.00	1822.50	TRUE		Unknown 4	-7.0	84.4	1822.5	1591.0	123507.8	84.4		84.4	
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1466.00	2199.00	TRUE		Unknown 4	-7.0	104.4	2199	1967.5	123131.3	104.4		104.4	
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3249.00	4873.50	TRUE		Unknown 4	-8.0	246.3	4873.5	4642.0	120456.8	246.3	156.0	246.3	
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1713.00	2569.50	TRUE		Unknown 4	-8.0	124.1	2569.5	2338.0	122760.8	124.1		124.1	
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1380.00	2070.00	TRUE		Unknown 4	-8.0	97.6	2070	1838.5	123260.3	97.6		97.6	
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1255.00	1882.50	TRUE		Unknown 4	-9.0	87.6	1882.5	1651.0	123447.8	87.6	97.6	87.6	
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1420.00	2130.00	TRUE		Unknown 4	-9.0	100.7	2130	1898.5	123200.3	100.7		100.7	
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1468.00	2202.00	TRUE		Unknown 4	-9.0	104.6	2202	1970.5	123128.3	104.6		104.6	
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1389.00	2083.50	TRUE		Unknown 4	-10.0	98.3	2083.5	1852.0	123246.8	98.3	88.6	98.3	
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1265.00	1897.50	TRUE		Unknown 4	-10.0	88.4	1897.5	1666.0	123432.8	88.4		88.4	
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1149.00	1723.50	TRUE		Unknown 4	-10.0	79.2	1723.5	1492.0	123606.8	79.2		79.2	
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	151.00	226.50	TRUE		cold E2	-7.0	-0.3	226.5	-5.0	125103.8	-0.3	0.8	-0.3	
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	158.00	237.00	TRUE		cold E2	-7.0	0.3	237	5.5	125093.3	0.3		0.3	
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	185.00	277.50	TRUE		cold E2	-7.0	2.4	277.5	46.0	125052.8	2.4		2.4	
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1406.00	2109.00	TRUE			—	99.6	2109	1877.5	123221.3	99.6	101.5	99.6	
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1463.00	2194.50	TRUE			—	104.2	2194.5	1963.0	123135.8	104.2		104.2	
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	1419.00	2128.50	TRUE			—	100.7	2128.5	1897.0	123201.8	100.7		100.7	
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average		
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
211		178	1	none	Hot		—	—	—	10	—	—	—	—	86507.00	129760.50	TRUE					129760.5	129529.0					
212		179	2	none	Hot		—	—	—	10	—	—	—	—	83501.00	125251.50	TRUE					125251.5	125020.0					
213		180	3	none	Hot		—	—	—	10	—	—	—	—	74008.00	111012.00	TRUE					111012	110780.5					
214		181	1	none	Hot		—	—	—	10	—	—	—	—	76060.00	114090.00	TRUE					114090	113858.5					
215		182	2	none	Hot		—	—	—	10	—	—	—	—	94335.00	141502.50	TRUE					141502.5	141271.0					
216		183	3	none	Hot		—	—	—	10	—	—	—	—	85984.00	128976.00	TRUE					128976	128744.5					

	B	C	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31			Prism data, excluding data points in the right leg of U-shaped curves																											
32			***standard curve***				***weak positive***				***4.0***				***9.0***				***14.0***				***20.0***				***negative control***			
33	*Position*	*Replicate*	*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1	-7.0	-1.5390	-0.4245	-0.5041	-4.0				-3.0				-3.0				-3.0				-3.0				-3.0	#N/A	#N/A	#N/A
35	2	2	-7.0	-0.2653	0.2919	2.4411	-4.5	14.7792	5.7843	7.6948	-4.0				-4.0	40.9679	4.9883	2.9983	-4.0				-4.0				-4.0	#N/A	#N/A	#N/A
36	3	3	-8.0	28.3114	24.5702	26.4806	-5.5	50.2813	46.5400	50.9977	-5.0	4.9883	-4.5638	-5.2802	-5.0	1.7247	88.1713	10.4808	-5.0	25.6050	18.9185	26.8786	-5.0	36.5103	26.6398	92.2309	-5.0	#N/A	#N/A	#N/A
37	4	1	-8.5	57.8433	51.3957	60.3110	-6.0	79.0172	76.3904	71.2163	-6.0	6.6599	-1.3797	-2.7330	-6.0	90.6389	87.6141	92.2309	-6.0	60.9478	10.4012	13.9036	-6.0	44.6296	93.1066	85.2261	-6.0	#N/A	#N/A	#N/A
38	5	2	-9.0	82.1216	80.9276	88.8877	-6.5	86.2609	98.0418	100.5094	-7.0	-2.4942	1.9635	-1.3797	-7.0	54.4205	14.1424	19.3961	-7.0	22.8985	98.7582	93.7434	-7.0	94.5394	84.4301	104.4099	-7.0	#N/A	#N/A	#N/A
39	6	3	-9.5	100.2706	90.8777	89.1265	-7.0	109.5044	93.2658	89.6837	-8.0	7.0579	7.5356	11.9136	-8.0	54.6593	118.3401	15.4160	-8.0	7.3764	107.0367	94.4598	-8.0	246.3384	124.0713	97.5642	-8.0	#N/A	#N/A	#N/A
40	7	1	-10.0	121.2057	123.9121	114.2008	-7.5	116.1908	107.1959		-9.0	54.8185	65.6442	38.3411	-9.0	101.8627	72.9675	104.3303	-9.0	88.4101	102.9771	104.3303	-9.0	87.6141	100.7482	104.5691	-9.0	#N/A	#N/A	#N/A
41	8	2	-11.0		123.0365	124.5489	-8.5	107.2755		114.7580	-10.0	60.6294	36.5103	94.6986	-10.0	36.3511	104.4895	33.7243	-10.0	87.5345	104.9671	120.4097	-10.0	98.2806	88.4101	79.1764	-10.0	#N/A	#N/A	#N/A
42	9	3																												
43	10	1	Standard Curve		TTN014 05-07-08		Positive Control		TTN014 05-07-08		4.0		TTN014 05-07-08		9.0		TTN012 4-30-08		14.0		TTN014 05-07-08		20.0		TTN014 05-07-08		Negative Control		Prism run ID & date	
44	11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values		not converged		Best-fit values				Best-fit values		bad initial values		Best-fit values			
46	13	1	BOTTOM		-11.6		BOTTOM		-7.718		BOTTOM		-0.5954		BOTTOM		n/a		BOTTOM		-0.5798		BOTTOM		51.79		BOTTOM			
47	14	2	TOP		128.3		TOP		111.4		TOP		64.44		TOP		n/a		TOP		114.1		TOP		156		TOP			
48	15	3	LOGIC50		-8.419		LOGIC50		-5.497		LOGIC50		-8.914		LOGIC50		n/a		LOGIC50		-6.596		LOGIC50		n/a		LOGIC50			
49	16	1	HILLSLOPE		-0.6729		HILLSLOPE		-0.7547		HILLSLOPE		-1.441		HILLSLOPE		n/a		HILLSLOPE		-0.3343		HILLSLOPE		n/a		HILLSLOPE			
50	17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3	BOTTOM		5.542		BOTTOM		13.23		BOTTOM		4.35		BOTTOM				BOTTOM		109.7		BOTTOM				BOTTOM			
52	19	1	TOP		4.716		TOP		4.231		TOP		8.047		TOP				TOP		57.14		TOP				TOP			
53	20	2	LOGIC50		0.04036		LOGIC50		0.06627		LOGIC50		0.2185		LOGIC50				LOGIC50		0.6369		LOGIC50				LOGIC50			
54	21	3	HILLSLOPE		0.08577		HILLSLOPE		0.1715		HILLSLOPE		0.628		HILLSLOPE				HILLSLOPE		0.643		HILLSLOPE				HILLSLOPE			
55	22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2	Data				Data				Data				Data				Data				Data				Data			
66	33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.02

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	-1.5
1	1	2	-7.0	-0.4
1	1	3	-7.0	-0.5
1	2	1	-8.0	28.3
1	2	2	-8.0	24.6
1	2	3	-8.0	26.5
1	3	1	-8.5	57.8
1	3	2	-8.5	51.4
1	3	3	-8.5	60.3
1	4	1	-9.0	82.1
1	4	2	-9.0	80.9
1	4	3	-9.0	88.9
1	5	1	-9.5	100.3
1	5	2	-9.5	90.9
1	5	3	-9.5	89.1
1	6	1	-10.0	121.2
1	6	2	-10.0	123.9
1	6	3	-10.0	114.2
1	7	1		
1	7	2	-11.0	123.0
1	7	3	-11.0	124.5
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.42

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Norethynodrel
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.5	17.1
1	1	2	-4.5	6.7
1	1	3	-4.5	8.9
1	2	1	-5.5	58.1
1	2	2	-5.5	53.8
1	2	3	-5.5	58.9
1	3	1	-6.0	91.3
1	3	2	-6.0	88.2
1	3	3	-6.0	82.3
1	4	1	-6.5	99.6
1	4	2	-6.5	113.3
1	4	3	-6.5	116.1
1	5	1	-7.0	126.5
1	5	2	-7.0	107.7
1	5	3	-7.0	103.6
1	6	1	-7.5	134.2
1	6	2	-7.5	123.8
1	6	3		
1	7	1	-8.5	123.9
1	7	2		
1	7	3	-8.5	132.6
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 15.75

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 4
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	5.8
1	1	2	-5.0	-5.3
1	1	3	-5.0	-6.1
1	2	1	-6.0	7.7
1	2	2	-6.0	-1.6
1	2	3	-6.0	-3.2
1	3	1	-7.0	-2.9
1	3	2	-7.0	2.3
1	3	3	-7.0	-1.6
1	4	1	-8.0	8.2
1	4	2	-8.0	8.7
1	4	3	-8.0	13.8
1	5	1	-9.0	63.3
1	5	2	-9.0	75.8
1	5	3	-9.0	44.3
1	6	1	-10.0	70.0
1	6	2	-10.0	42.2
1	6	3	-10.0	109.4
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 38.67

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 9
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.0	47.3
1	1	2	-4.0	5.8
1	1	3	-4.0	3.5
1	2	1	-5.0	2.0
1	2	2	-5.0	102.4
1	2	3	-5.0	12.1
1	3	1	-6.0	104.7
1	3	2	-6.0	101.2
1	3	3	-6.0	106.5
1	4	1	-7.0	62.9
1	4	2	-7.0	16.3
1	4	3	-7.0	22.4
1	5	1	-8.0	63.1
1	5	2	-8.0	136.7
1	5	3	-8.0	17.8
1	6	1	-9.0	117.7
1	6	2	-9.0	84.3
1	6	3	-9.0	120.5
1	7	1	-10.0	42.0
1	7	2	-10.0	120.7
1	7	3	-10.0	39.0
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 36.25

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 14
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	29.6
1	1	2	-5.0	21.9
1	1	3	-5.0	31.0
1	2	1	-6.0	70.4
1	2	2	-6.0	12.0
1	2	3	-6.0	16.1
1	3	1	-7.0	26.5
1	3	2	-7.0	114.1
1	3	3	-7.0	108.3
1	4	1	-8.0	8.5
1	4	2	-8.0	123.6
1	4	3	-8.0	109.1
1	5	1	-9.0	102.1
1	5	2	-9.0	119.0
1	5	3	-9.0	120.5
1	6	1	-10.0	101.1
1	6	2	-10.0	121.3
1	6	3	-10.0	139.1
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 43.47

Provide information in all blue cells.

Laboratory Code: B

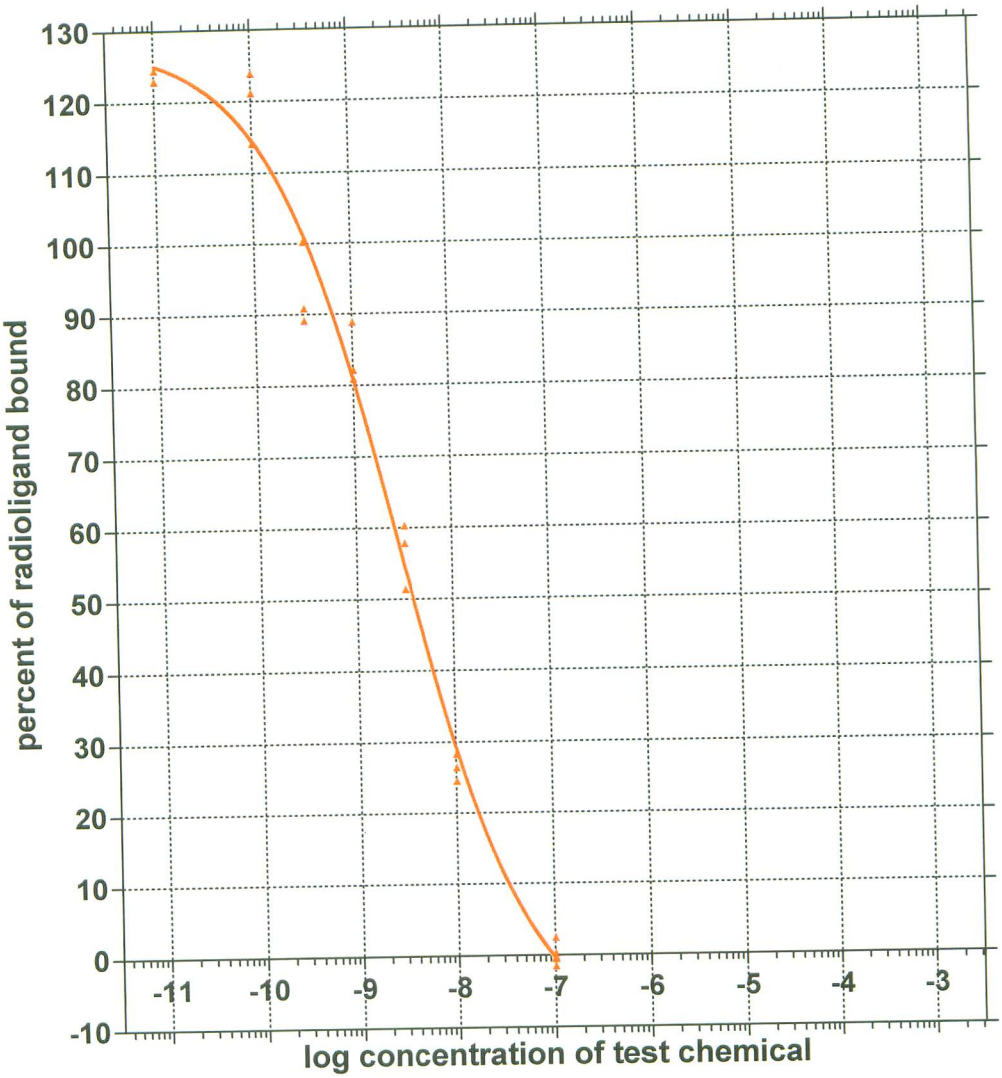
Compound ID: Chem Code 20
Run ID: TTN 014
Assay start date: 5/7/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	42.2
1	1	2	-5.0	30.8
1	1	3	-5.0	106.5
1	2	1	-6.0	51.6
1	2	2	-6.0	107.6
1	2	3	-6.0	98.5
1	3	1	-7.0	109.2
1	3	2	-7.0	97.5
1	3	3	-7.0	120.6
1	4	1	-8.0	284.6
1	4	2	-8.0	143.3
1	4	3	-8.0	112.7
1	5	1	-9.0	101.2
1	5	2	-9.0	116.4
1	5	3	-9.0	120.8
1	6	1	-10.0	113.5
1	6	2	-10.0	102.1
1	6	3	-10.0	91.5
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

ble format: XY		X	A		
		log concentration	run TTN014 05-07-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-1.5390	-0.4245	-0.5041
2	Title	-7.0	-0.2653	0.2919	2.4411
3	Title	-8.0	28.3114	24.5702	26.4806
4	Title	-8.5	57.8433	51.3957	60.3110
5	Title	-9.0	82.1216	80.9276	88.8877
6	Title	-9.5	100.2706	90.8777	89.1265
7	Title	-10.0	121.2057	123.9121	114.2008
8	Title	-11.0		123.0365	124.5489

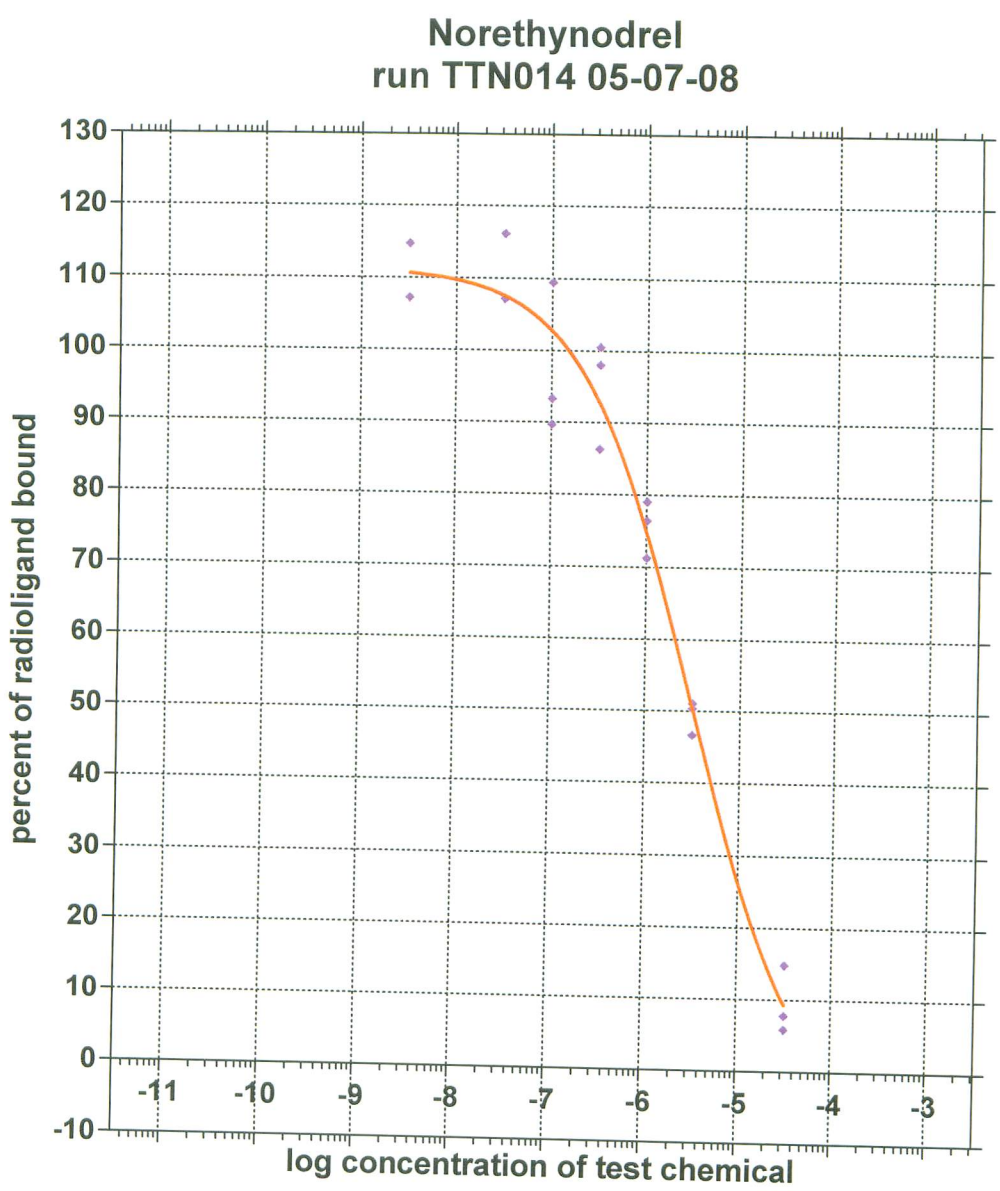
	Nonlin fit Table of results	A run TTN014 05-07-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-11.60
4	TOP	128.3
5	LOGIC50	-8.419
6	HILLSLOPE	-0.6729
7	Std. Error	
8	BOTTOM	5.542
9	TOP	4.716
10	LOGIC50	0.04036
11	HILLSLOPE	0.08577
12	95% Confidence Intervals	
13	BOTTOM	-23.20 to 0.003046
14	TOP	118.4 to 138.2
15	LOGIC50	-8.503 to -8.334
16	HILLSLOPE	-0.8524 to -0.4933
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9885
20	Absolute Sum of Squares	567.0
21	Sy.x	5.463
22	Number of points	
23	Analyzed	23

Estradiol reference standard
run TTN014 05-07-08



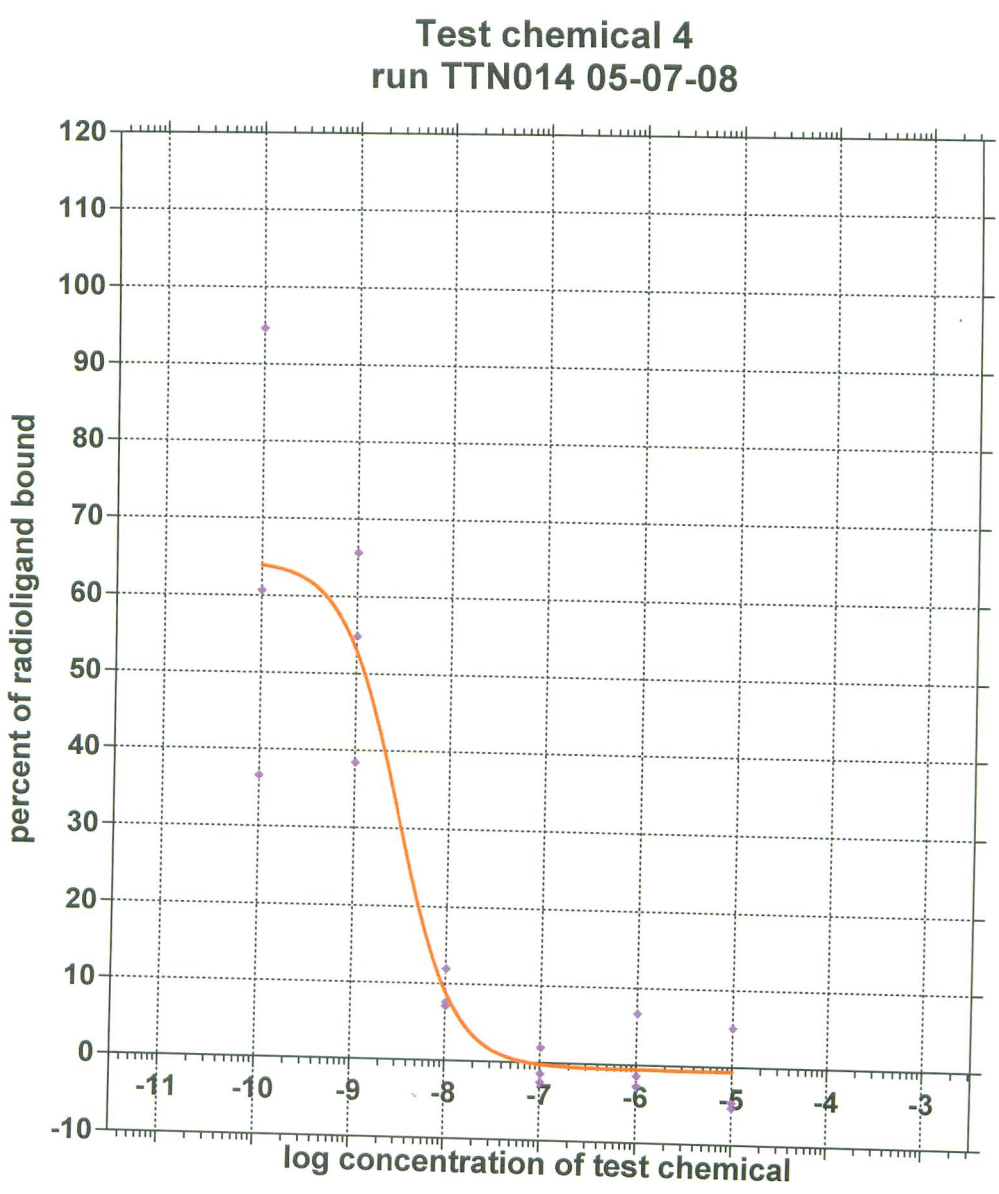
ole format: XY		X	A		
		log concentration	run TTN014 05-07-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	14.7792	5.7843	7.6948
3	Title	-5.5	50.2813	46.5400	50.9977
4	Title	-6.0	79.0172	76.3904	71.2163
5	Title	-6.5	86.2609	98.0418	100.5094
6	Title	-7.0	109.5044	93.2658	89.6837
7	Title	-7.5	116.1908	107.1959	
8	Title	-8.5	107.2755		114.7580

	Nonlin fit Table of results	A run TTN014 05-07-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-7.718
4	TOP	111.4
5	LOGIC50	-5.497
6	HILLSLOPE	-0.7547
7	Std. Error	
8	BOTTOM	13.23
9	TOP	4.231
10	LOGIC50	0.06627
11	HILLSLOPE	0.1715
12	95% Confidence Intervals	
13	BOTTOM	-35.92 to 20.48
14	TOP	102.4 to 120.4
15	LOGIC50	-5.639 to -5.356
16	HILLSLOPE	-1.120 to -0.3891
17	Goodness of Fit	
18	Degrees of Freedom	15
19	R^2	0.9730
20	Absolute Sum of Squares	631.7
21	Sy.x	6.490
22	Number of points	
23	Analyzed	19


Norethynodrel
run TTN014 05-07-08
percent of radioligand bound
130
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

ble format: XY		X	A		
		log concentration	Test chemical 4		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	4.9883	-4.5638	-5.2802
4	Title	-6.0	6.6599	-1.3797	-2.7330
5	Title	-7.0	-2.4942	1.9635	-1.3797
6	Title	-8.0	7.0579	7.5356	11.9136
7	Title	-9.0	54.8185	65.6442	38.3411
8	Title	-10.0	60.6294	36.5103	94.6986

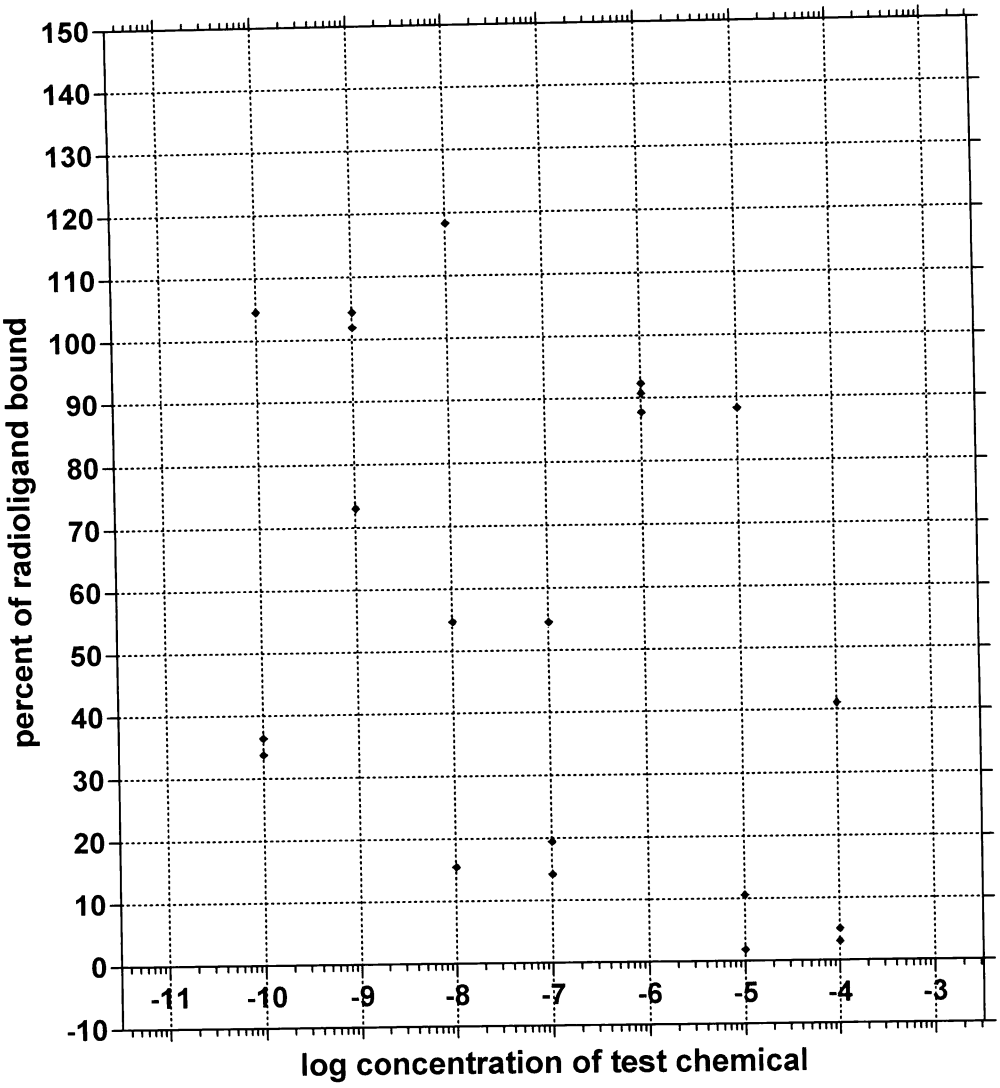
	Nonlin fit Table of results	A Test chemical 4
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.5954
4	TOP	64.44
5	LOGIC50	-8.914
6	HILLSLOPE	-1.441
7	Std. Error	
8	BOTTOM	4.350
9	TOP	8.047
10	LOGIC50	0.2185
11	HILLSLOPE	0.6280
12	95% Confidence Intervals	
13	BOTTOM	-9.925 to 8.735
14	TOP	47.18 to 81.70
15	LOGIC50	-9.383 to -8.445
16	HILLSLOPE	-2.788 to -0.09384
17	Goodness of Fit	
18	Degrees of Freedom	14
19	R^2	0.8548
20	Absolute Sum of Squares	2240
21	Sy.x	12.65
22	Number of points	
23	Analyzed	18


Test chemical 4
run TTN014 05-07-08
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

ble format: XY		X	A		
		log concentration	Test chemical 9		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	40.9679	4.9883	2.9983
3	Title	-5.0	1.7247	88.1713	10.4808
4	Title	-6.0	90.6389	87.6141	92.2309
5	Title	-7.0	54.4205	14.1424	19.3961
6	Title	-8.0	54.6593	118.3401	15.4160
7	Title	-9.0	101.8627	72.9675	104.3303
8	Title	-10.0	36.3511	104.4895	33.7243

	Nonlin fit Table of results	A Test chemical 9
		Y
1	logIC50	Not converged
2	Best-fit values	
3	BOTTOM	
4	TOP	
5	LOGIC50	
6	HILLSLOPE	
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	21

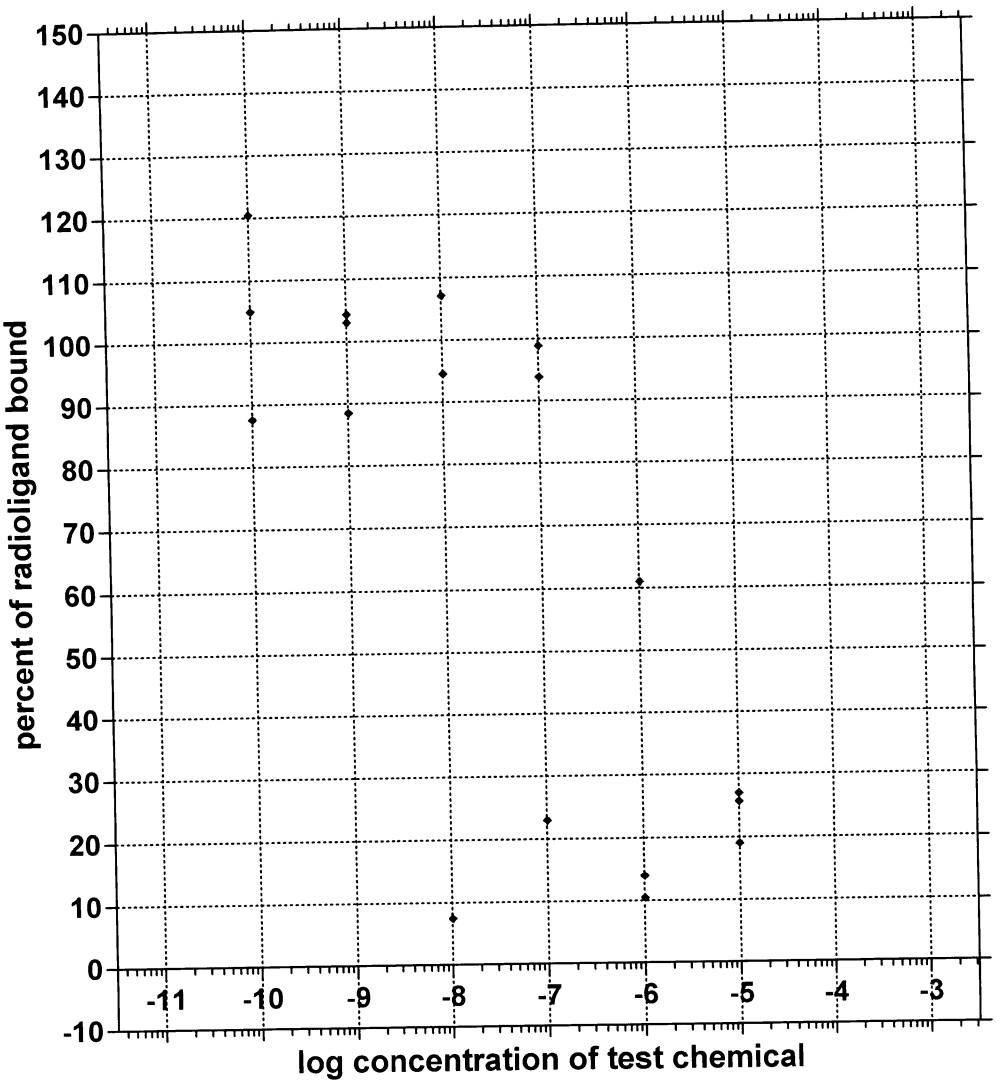
Test chemical 9
run TTN014 05-07-08



ble format: XY		X	A			B		
		log concentration	Test chemical 14			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	25.6050	18.9185	26.8786			
4	Title	-6.0	60.9478	10.4012	13.9036			
5	Title	-7.0	22.8985	98.7582	93.7434			
6	Title	-8.0	7.3764	107.0367	94.4598			
7	Title	-9.0	88.4101	102.9771	104.3303			
8	Title	-10.0	87.5345	104.9671	120.4097			

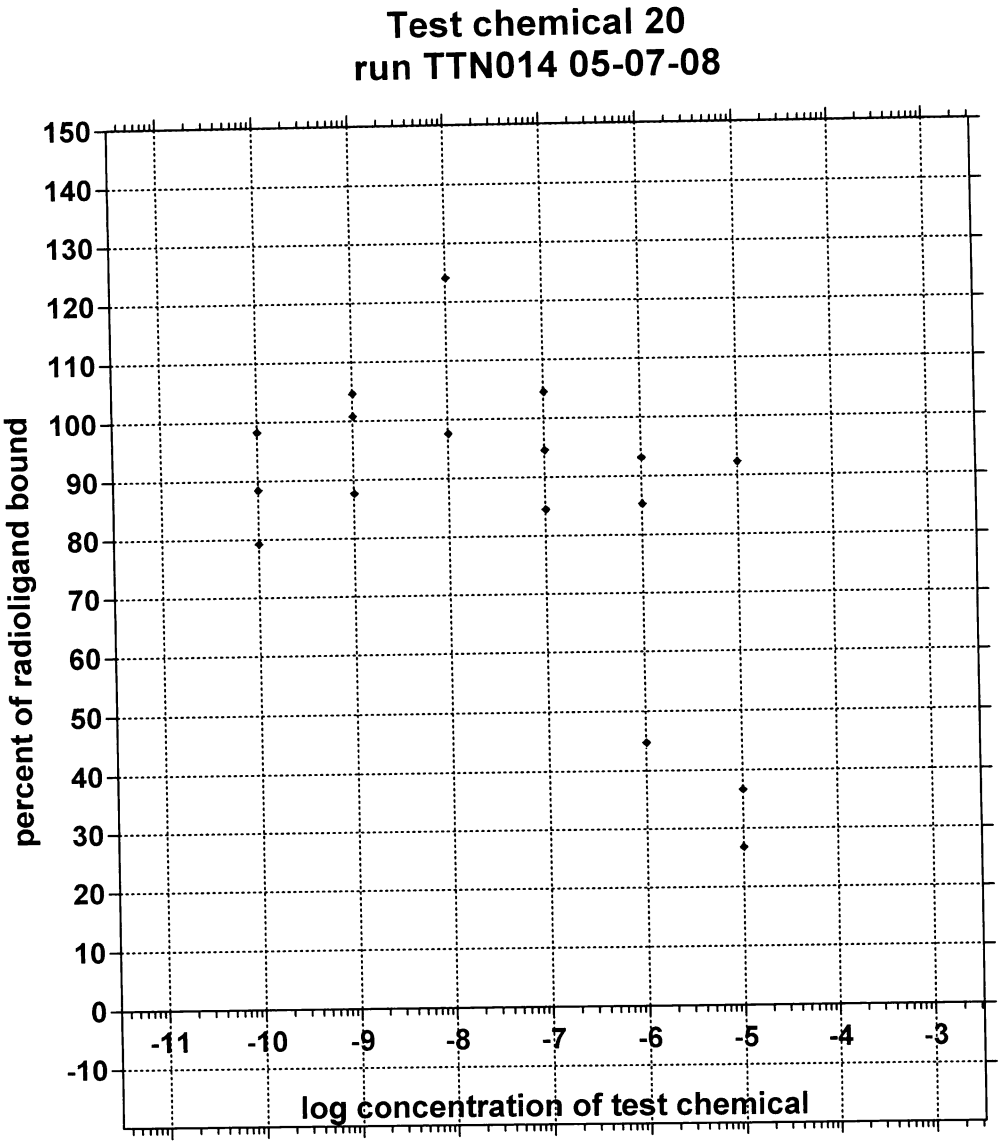
	Nonlin fit Table of results	A Test chemical 14 Y	B Data Set-B Y
1	logIC50		
2	Best-fit values		
3	BOTTOM	-0.5798	
4	TOP	114.1	
5	LOGIC50	-6.596	
6	HILLSLOPE	-0.3343	
7	Std. Error		
8	BOTTOM	109.7	
9	TOP	57.14	
10	LOGIC50	0.6369	
11	HILLSLOPE	0.6430	
12	95% Confidence Intervals		
13	BOTTOM	-235.8 to 234.6	
14	TOP	-8.424 to 236.7	
15	LOGIC50	-7.962 to -5.230	
16	HILLSLOPE	-1.714 to 1.045	
17	Goodness of Fit		
18	Degrees of Freedom	14	
19	R^2	0.5560	
20	Absolute Sum of Squares	12931	
21	Sy.x	30.39	
22	Number of points		
23	Analyzed	18	

Test chemical 14
run TTN014 05-07-08



ble format: XY		X	A		
		log concentration	Test chemical 20		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	36.5103	26.6398	92.2309
4	Title	-6.0	44.6296	93.1066	85.2261
5	Title	-7.0	94.5394	84.4301	104.4099
6	Title	-8.0	246.3384	124.0713	97.5642
7	Title	-9.0	87.6141	100.7482	104.5691
8	Title	-10.0	98.2806	88.4101	79.1764

	Nonlin fit Table of results	A Test chemical 20
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	51.79
4	TOP	156.0
5	LOGIC50	-8.893
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	18


Test chemical 20
run TTN014 05-07-08
percent of radioligand bound
150
140
130
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

APPENDIX P

Excel and Prism Files of the Run Identifier TTN015

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B
Run identification:	TTN015
Assay start date:	5/8/2008
Tracer lot number:	3589791/1
Specific activity on day of assay:	108.86 Ci/mmole
Cytosol vial or lot identification:	RUC3-03-08 A,M
Protein (cytosol):	50 microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.76 M
Weak Positive, Max Concentration:	5.00E-03 M
log(IC50), norethynodrel (Weak Positive):	-5.85 M
RBA, norethynodrel (Weak Positive):	1.245E-03 relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03 M
log(IC50), Unknown 1:	-9.94 1.0
RBA, Unknown 1:	1.524E+01 relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03 M
log(IC50), Unknown 2:	-9.46 3.0
RBA, Unknown 2:	5.058E+00 relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03 M
log(IC50), Unknown 3:	-6.99 20.0
RBA, Unknown 3:	1.698E-02 relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03 M
log(IC50), Unknown 4:	-7.15 23.0
RBA, Unknown 4:	2.472E-02 relative to E2=1.0
volume of ethanol counted:	1 mL
multiply DPM in sample by :	1.5

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.
Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software.
and the maximum concentrations for each of the unknowns.

Check the 10% rule: 3.27%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	5	5104.8	312.66	Total Binding, solvent control, tubes
Hot	6	156089.5	11713.47	Total hot E2 added to each tube
NSB	6	280.3	21.29	Nonspecific Binding
Specific EtOH	5	4824.6	312.66	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.03	less than 0.1?
R1881 / EtOH	#DIV/0!	close to 1.0 ? at 1.0 E-6 M only
NSB / EtOH	0.05	around 0.25 ?

log conc'n at min hot binding	min hot (%)
cold E2 -7.0	0.1
R1881 #N/A	not applicable
norethynodrel -4.5	1.5
Unknown 1 -5.0	-3.1
Unknown 2 -6.0	-1.8
Unknown 3 -6.0	23.3
Unknown 4 -4.0	10.8

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3467.00	5200.50	TRUE	
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3236.00	4854.00	TRUE	
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2693.00	4039.50	FALSE	MIS
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	193.00	289.50	TRUE	
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	200.00	300.00	TRUE	
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	176.00	264.00	TRUE	
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	740.00	1110.00	TRUE	
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	720.00	1080.00	TRUE	
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	581.00	871.50	TRUE	
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1436.00	2154.00	TRUE	
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	445.00	667.50	TRUE	
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1515.00	2272.50	TRUE	
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2407.00	3610.50	TRUE	
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2435.00	3652.50	TRUE	
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2424.00	3636.00	TRUE	
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3111.00	4666.50	TRUE	
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3295.00	4942.50	TRUE	
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3068.00	4602.00	TRUE	
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3596.00	5394.00	TRUE	
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3610.00	5415.00	TRUE	
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3634.00	5451.00	TRUE	
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3627.00	5440.50	TRUE	
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3693.00	5539.50	TRUE	
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3696.00	5544.00	TRUE	
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE	
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE	
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE	
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE	
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE	
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE	
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE	
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE	
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE	
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE	
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE	
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE	
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE	
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE	

	concentration (logM)	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
	—	102.0	5200.5	4920.3	151169.3	102.0	98.4	102.0
	—	94.8	4854	4573.8	151515.8	94.8		94.8
	—							
cold E2	-7.0	0.2	289.5	9.3	156080.3	0.2	0.1	0.2
cold E2	-7.0	0.4	300	19.8	156069.8	0.4		0.4
cold E2	-7.0	-0.3	264	-16.3	156105.8	-0.3		-0.3
cold E2	-8.0	17.2	1110	829.8	155259.8	17.2	15.3	17.2
cold E2	-8.0	16.6	1080	799.8	155289.8	16.6		16.6
cold E2	-8.0	12.3	871.5	591.3	155498.3	12.3		12.3
cold E2	-8.5	38.8	2154	1873.8	154215.8	38.8	29.4	38.8
cold E2	-8.5	8.0	667.5	387.3	155702.3	8.0		8.0
cold E2	-8.5	41.3	2272.5	1992.3	154097.3	41.3		41.3
cold E2	-9.0	69.0	3610.5	3330.3	152759.3	69.0	69.5	69.0
cold E2	-9.0	69.9	3652.5	3372.3	152717.3	69.9		69.9
cold E2	-9.0	69.6	3636	3355.8	152733.8	69.6		69.6
cold E2	-9.5	90.9	4666.5	4386.3	151703.3	90.9	92.4	90.9
cold E2	-9.5	96.6	4942.5	4662.3	151427.3	96.6		96.6
cold E2	-9.5	89.6	4602	4321.8	151767.8	89.6		89.6
cold E2	-10.0	106.0	5394	5113.8	150975.8	106.0	106.5	106.0
cold E2	-10.0	106.4	5415	5134.8	150954.8	106.4		106.4
cold E2	-10.0	107.2	5451	5170.8	150918.8	107.2		107.2
cold E2	-11.0	107.0	5440.5	5160.3	150929.3	107.0	108.4	107.0
cold E2	-11.0	109.0	5539.5	5259.3	150830.3	109.0		109.0
cold E2	-11.0	109.1	5544	5263.8	150825.8	109.1		109.1
R1881	-3.0						no average	#N/A
R1881	-3.0							#N/A
R1881	-3.0							#N/A
R1881	-4.0						no average	#N/A
R1881	-4.0							#N/A
R1881	-4.0							#N/A
R1881	-5.0						no average	#N/A
R1881	-5.0							#N/A
R1881	-5.0							#N/A
R1881	-6.0						no average	#N/A
R1881	-6.0							#N/A
R1881	-6.0							#N/A
R1881	-7.0						no average	#N/A
R1881	-7.0							#N/A
R1881	-7.0							#N/A
R1881	-8.0						no average	#N/A
R1881	-8.0							#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	3.27%	If the ratio of ETOH / Hot is > 10%, there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.						
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (Including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	
75		42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A	
76		43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A	
77		44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A	
78		45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A	
79		46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A	
80		47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A	
81		48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A	
82		49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2524.00	3786.00	TRUE		norethynodrel	-4.0	72.7	3786	3505.8	152583.8	72.7	25.9		
83		50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	284.00	426.00	TRUE		norethynodrel	-4.0	3.0	426	145.8	155943.8	3.0			
84		51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	253.00	379.50	TRUE		norethynodrel	-4.0	2.1	379.5	99.3	155990.3	2.1			
85		52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	193.00	289.50	TRUE		norethynodrel	-4.5	0.2	289.5	9.3	156080.3	0.2	1.5		
86		53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	256.00	384.00	TRUE		norethynodrel	-4.5	2.2	384	103.8	155985.8	2.2			
87		54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	256.00	384.00	TRUE		norethynodrel	-4.5	2.2	384	103.8	155985.8	2.2			
88		55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1054.00	1581.00	TRUE		norethynodrel	-5.5	27.0	1581	1300.8	154788.8	27.0	27.0		
89		56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	762.00	1143.00	TRUE		norethynodrel	-5.5	17.9	1143	862.8	155226.8	17.9			
90		57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1351.00	2026.50	TRUE		norethynodrel	-5.5	36.2	2026.5	1746.3	154343.3	36.2			
91		58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2264.00	3396.00	TRUE		norethynodrel	-6.0	64.6	3396	3115.8	152973.8	64.6	61.0		
92		59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2023.00	3034.50	TRUE		norethynodrel	-6.0	57.1	3034.5	2754.3	153335.3	57.1			
93		60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2161.00	3241.50	TRUE		norethynodrel	-6.0	61.4	3241.5	2961.3	153128.3	61.4			
94		61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3018.00	4527.00	TRUE		norethynodrel	-5.5	88.0	4527	4246.8	151842.8	88.0	88.3		
95		62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3025.00	4537.50	TRUE		norethynodrel	-5.5	88.2	4537.5	4257.3	151832.3	88.2			
96		63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3036.00	4554.00	TRUE		norethynodrel	-5.5	88.6	4554	4273.8	151815.8	88.6			
97		64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2347.00	5280.75	TRUE		norethynodrel	-7.0	103.6	5280.75	5000.5	151089.0	103.6	104.0		
98		65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2364.00	5319.00	TRUE		norethynodrel	-7.0	104.4	5319	5038.8	151050.8	104.4			
99		66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	977.00	1465.50	FALSE	mispipet	norethynodrel	-7.0								
100		67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3477.00	5215.50	TRUE		norethynodrel	-7.5	102.3	5215.5	4935.3	151154.3	102.3	101.7		
101		68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3438.00	5157.00	TRUE		norethynodrel	-7.5	101.1	5157	4876.8	151212.8	101.1			
102		69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4238.00	6357.00	FALSE	mispipet	norethynodrel	-7.5								
103		70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3508.00	5262.00	TRUE		norethynodrel	-8.5	103.3	5262	4981.8	151107.8	103.3	107.5		
104		71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3774.00	5661.00	TRUE		norethynodrel	-8.5	111.5	5661	5380.8	150708.8	111.5			
105		72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3653.00	5479.50	TRUE		norethynodrel	-8.5	107.8	5479.5	5199.3	150890.3	107.8			
106		73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	82934.00	124401.00	TRUE		Unknown 1	-3.0	2572.7	124401	124120.8	31968.8	2572.7	2646.2		
107		74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	86528.00	129792.00	TRUE		Unknown 1	-3.0	2684.4	129792	129511.8	26577.8	2684.4			
108		75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	86434.00	129651.00	TRUE		Unknown 1	-3.0	2681.5	129651	129370.8	26718.8	2681.5			
109		76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	14487.00	21730.50	TRUE		Unknown 1	-4.0	444.6	21730.5	21450.3	134639.3	444.6	441.7		
110		77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	11902.00	17853.00	TRUE		Unknown 1	-4.0	364.2	17853	17572.8	138516.8	364.2			
111		78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	16792.00	25188.00	TRUE		Unknown 1	-4.0	516.3	25188	24907.8	131181.8	516.3			
112		79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	82.00	123.00	TRUE		Unknown 1	-5.0	-3.3	123	-157.3	156246.8	-3.3	-3.1		
113		80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	78.00	117.00	TRUE		Unknown 1	-5.0	-3.4	117	-163.3	156252.8	-3.4			
114		81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	100.00	150.00	TRUE		Unknown 1	-5.0	-2.7	150	-130.3	156219.8	-2.7			
115		82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	103.00	154.50	TRUE		Unknown 1	-6.0	-2.6	154.5	-125.8	156215.3	-2.6	-2.6		
116		83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	101.00	151.50	TRUE		Unknown 1	-6.0	-2.7	151.5	-128.8	156218.3	-2.7			
117		84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	103.00	154.50	TRUE		Unknown 1	-6.0	-2.6	154.5	-125.8	156215.3	-2.6			
118		85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	183.00	274.50	TRUE		Unknown 1	-7.0	-0.1	274.5	-5.8	156095.3	-0.1	-0.8		
119		86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	150.00	225.00	TRUE		Unknown 1	-7.0	-1.1	225	-55.3	156144.8	-1.1			
120		87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	152.00	228.00	TRUE		Unknown 1	-7.0	-1.1	228	-52.3	156141.8	-1.1			
121		88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	305.00	457.50	TRUE		Unknown 1	-8.0	3.7	457.5	177.3	155912.3	3.7	4.6		
122		89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	399.00	598.50	TRUE		Unknown 1	-8.0	6.6	598.5	318.3	155771.3	6.6			
123		90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	301.00	451.50	TRUE		Unknown 1	-8.0	3.5	451.5	171.3	155918.3	3.5			
124		91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	862.00	1293.00	TRUE		Unknown 1	-9.0	21.0	1293	1012.8	155076.8	21.0	12.6		
125		92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	331.00	496.50	TRUE		Unknown 1	-9.0	4.5	496.5	216.3	155873.3	4.5			
126		93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	587.00	880.50	TRUE		Unknown 1	-9.0	12.4	880.5	600.3	155489.3	12.4			
127		94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1213.00	1819.50	TRUE		Unknown 1	-10.0	31.9	1819.5	1539.3	154550.3	31.9	55.0		
128		95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2251.00	3376.50	TRUE		Unknown 1	-10.0	64.2	3376.5	3096.3	152993.3	64.2			
129		96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2404.00	3606.00	TRUE		Unknown 1	-10.0	68.9	3606	3325.8	152763.8	68.9			
130		97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	742.00	1113.00	TRUE		Unknown 2	-3.0	17.3	1113	832.8	155256.8	17.3	18.4		
131		98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	706.00	1059.00	TRUE		Unknown 2	-3.0	16.1	1059	778.8	155310.8	16.1			
132		99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	885.00	1327.50	TRUE		Unknown 2	-3.0	21.7	1327.5	1047.3	155042.3	21.7			
133		100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	179.00	268.50	TRUE		Unknown 2	-4.0	-0.2	268.5	-11.8	156101.3	-0.2	0.6		
134		101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	241.00	361.50	TRUE		Unknown 2	-4.0	1.7	361.5	81.3	156008.3	1.7			
135		102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	198.00	297.00	TRUE		Unknown 2	-4.0	0.3	297	16.8	156072.8	0.3			
136		103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	155.00	232.50	TRUE		Unknown 2	-5.0	-1.0	232.5	-47.8	156137.3	-1.0	-1.6		
137		104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	126.00	189.00	TRUE		Unknown 2	-5.0	-1.9	189	-91.3	156180.8	-1.9			
138		105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	121.00	181.50	TRUE		Unknown 2	-5.0	-2.0	181.5	-98.8	156188.3	-2.0			
139		106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	134.00	201.00	TRUE		Unknown 2	-6.0	-1.6	201	-79.3	156168.8	-1.6	-1.8		
140		107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	125.00	187.50	TRUE		Unknown 2	-6.0	-1.9	187.5	-92.8	156182.3	-1.9			
141		108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	126.00	189.00	TRUE		Unknown 2	-6.0	-1.9	189	-91.3	156180.8	-1.9			
142		109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	129.00	193.50	TRUE		Unknown 2	-7.0	-1.8	193.5	-86.8	156176.3	-1.8	-1.6		
143		110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	139.00	208.50	TRUE		Unknown 2	-7.0	-1.5	208.5	-71.8	156161.3	-1.5			
144		111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	135.00	202.50	TRUE		Unknown 2	-7.0	-1.6	202.5	-77.8	156167.3	-1.6			
145		112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	245.00	367.50	TRUE		Unknown 2	-8.0	1.8	367.5	87.3	156002.3	1.8	1.5		
146		113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	211.00	316.50	TRUE		Unknown 2	-8.0	0.8	316.5	36.3	156053.3	0.8			
147		114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	247.00	370.50	TRUE		Unknown 2	-8.0	1.9	370.5	90.3	155999.3	1.9			
148		115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1014.00	1521.00	TRUE		Unknown 2	-9.0	25.7	1521	1240.8	154848.8	25.7	26.6		
149		116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1032.00	1548.00	TRUE		Unknown 2	-9.0	26.3	1548	1267.8	154821.8	26.3			
150		117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1077.00	1615.50	TRUE		Unknown 2	-9.0	27.7	1615.5	1335.3	154754.3	27.7			

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
32	Competitive Assay Tube Layout - Four Test Chemicals															Check the 10% rule:	3.27%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.						
33	Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	
151	118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2184.00	3276.00	TRUE		Unknown 2	-10.0	62.1	3276	2995.8	153093.8	62.1	62.5	62.1	
152	119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2271.00	3406.50	TRUE		Unknown 2	-10.0	64.8	3406.5	3126.3	152963.3	64.8		64.8	
153	120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2133.00	3199.50	TRUE		Unknown 2	-10.0	60.5	3199.5	2919.3	153170.3	60.5		60.5	
154	121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6854.00	10281.00	TRUE		Unknown 3	-3.0	207.3	10281	10000.8	146088.8	207.3	164.5		
155	122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7757.00	11635.50	TRUE		Unknown 3	-3.0	235.4	11635.5	11355.3	144734.3	235.4			
156	123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1825.00	2737.50	TRUE		Unknown 3	-3.0	50.9	2737.5	2457.3	153632.3	50.9			
157	124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4636.00	6954.00	TRUE		Unknown 3	-4.0	138.3	6954	6673.8	149415.8	138.3	102.4		
158	125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2090.00	3135.00	TRUE		Unknown 3	-4.0	59.2	3135	2854.8	153234.8	59.2			
159	126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3712.00	5568.00	TRUE		Unknown 3	-4.0	109.6	5568	5287.8	150801.8	109.6			
160	127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1012.00	1518.00	TRUE		Unknown 3	-5.0	25.7	1518	1237.8	154851.8	25.7	23.4	25.7	
161	128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	796.00	1194.00	TRUE		Unknown 3	-5.0	18.9	1194	913.8	155175.8	18.9		18.9	
162	129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1015.00	1522.50	TRUE		Unknown 3	-5.0	25.7	1522.5	1242.3	154847.3	25.7		25.7	
163	130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	560.00	840.00	TRUE		Unknown 3	-6.0	11.6	840	559.8	155529.8	11.6	23.3	11.6	
164	131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	356.00	534.00	TRUE		Unknown 3	-6.0	5.3	534	253.8	155835.8	5.3		5.3	
165	132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1897.00	2845.50	TRUE		Unknown 3	-6.0	53.2	2845.5	2565.3	153524.3	53.2		53.2	
166	133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2038.00	3057.00	TRUE		Unknown 3	-7.0	57.6	3057	2776.8	153312.8	57.6	50.9	57.6	
167	134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1758.00	2637.00	TRUE		Unknown 3	-7.0	48.8	2637	2356.8	153732.8	48.8		48.8	
168	135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1678.00	2517.00	TRUE		Unknown 3	-7.0	46.4	2517	2236.8	153852.8	46.4		46.4	
169	136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2882.00	4323.00	TRUE		Unknown 3	-8.0	83.8	4323	4042.8	152046.8	83.8	65.3	83.8	
170	137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1847.00	2770.50	TRUE		Unknown 3	-8.0	51.6	2770.5	2490.3	153599.3	51.6		51.6	
171	138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2133.00	3199.50	TRUE		Unknown 3	-8.0	60.5	3199.5	2919.3	153170.3	60.5		60.5	
172	139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2151.00	3226.50	TRUE		Unknown 3	-9.0	61.1	3226.5	2946.3	153143.3	61.1	66.4	61.1	
173	140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2365.00	3547.50	TRUE		Unknown 3	-9.0	67.7	3547.5	3267.3	152822.3	67.7		67.7	
174	141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2455.00	3682.50	TRUE		Unknown 3	-9.0	70.5	3682.5	3402.3	152687.3	70.5		70.5	
175	142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2443.00	3664.50	TRUE		Unknown 3	-10.0	70.1	3664.5	3384.3	152705.3	70.1	72.2	70.1	
176	143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2658.00	3987.00	TRUE		Unknown 3	-10.0	76.8	3987	3706.8	152382.8	76.8		76.8	
177	144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2426.00	3639.00	TRUE		Unknown 3	-10.0	69.6	3639	3358.8	152730.8	69.6		69.6	
178	145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	524.00	786.00	TRUE		Unknown 4	-3.0	10.5	786	505.8	155583.8	10.5	12.3	10.5	
179	146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	570.00	855.00	TRUE		Unknown 4	-3.0	11.9	855	574.8	155514.8	11.9		11.9	
180	147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	650.00	975.00	TRUE		Unknown 4	-3.0	14.4	975	694.8	155394.8	14.4		14.4	
181	148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	551.00	826.50	TRUE		Unknown 4	-4.0	11.3	826.5	546.3	155543.3	11.3	10.8	93.7	
182	149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	523.00	784.50	TRUE		Unknown 4	-4.0	10.5	784.5	504.3	155585.3	10.5		95.4	
183	150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	525.00	787.50	TRUE		Unknown 4	-4.0	10.5	787.5	507.3	155582.3	10.5		86.4	
184	151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1612.00	2418.00	TRUE		Unknown 4	-5.0	44.3	2418	2137.8	153951.8	44.3	42.5	44.3	
185	152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1402.00	2103.00	TRUE		Unknown 4	-5.0	37.8	2103	1822.8	154266.8	37.8		37.8	
186	153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1652.00	2478.00	TRUE		Unknown 4	-5.0	45.6	2478	2197.8	153891.8	45.6		45.6	
187	154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2222.00	3333.00	TRUE		Unknown 4	-6.0	63.3	3333	3052.8	153036.8	63.3	26.6	63.3	
188	155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	692.00	1038.00	TRUE		Unknown 4	-6.0	15.7	1038	757.8	155331.8	15.7		15.7	
189	156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	212.00	318.00	TRUE		Unknown 4	-6.0	0.8	318	37.8	156051.8	0.8		0.8	
190	157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1550.00	2325.00	TRUE		Unknown 4	-7.0	42.4	2325	2044.8	154044.8	42.4	43.6	42.4	
191	158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1856.00	2784.00	TRUE		Unknown 4	-7.0	51.9	2784	2503.8	153585.8	51.9		51.9	
192	159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1358.00	2037.00	TRUE		Unknown 4	-7.0	36.4	2037	1756.8	154332.8	36.4		36.4	
193	160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2483.00	3724.50	TRUE		Unknown 4	-8.0	71.4	3724.5	3444.3	152645.3	71.4	71.6	71.4	
194	161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2470.00	3705.00	TRUE		Unknown 4	-8.0	71.0	3705	3424.8	152664.8	71.0		71.0	
195	162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2512.00	3768.00	TRUE		Unknown 4	-8.0	72.3	3768	3487.8	152601.8	72.3		72.3	
196	163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2255.00	3382.50	TRUE		Unknown 4	-9.0	64.3	3382.5	3102.3	152987.3	64.3	70.7	64.3	
197	164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2406.00	3609.00	TRUE		Unknown 4	-9.0	69.0	3609	3328.8	152760.8	69.0		69.0	
198	165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2717.00	4075.50	TRUE		Unknown 4	-9.0	78.7	4075.5	3795.3	152294.3	78.7		78.7	
199	166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2398.00	3597.00	TRUE		Unknown 4	-10.0	68.7	3597	3316.8	152772.8	68.7	63.1	68.7	
200	167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2300.00	3450.00	TRUE		Unknown 4	-10.0	65.7	3450	3169.8	152919.8	65.7		65.7	
201	168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1948.00	2922.00	TRUE		Unknown 4	-10.0	54.8	2922	2641.8	153447.8	54.8		54.8	
202	169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	167.00	250.50	TRUE		cold E2	-7.0	-0.6	250.5	-29.8	156119.3	-0.6	-0.1	-0.6	
203	170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	203.00	304.50	TRUE		cold E2	-7.0	0.5	304.5	24.3	156065.3	0.5		0.5	
204	171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	182.00	273.00	TRUE		cold E2	-7.0	-0.2	273	-7.3	156096.8	-0.2		-0.2	
205	172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3712.00	5568.00	TRUE			—	109.6	5568	5287.8	150801.8	109.6	101.1	109.6	
206	173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3188.00	4782.00	TRUE			—	93.3	4782	4501.8	151587.8	93.3		93.3	
207	174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3413.00	5119.50	TRUE			—	100.3	5119.5	4839.3	151250.3	100.3		100.3	
208	175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average		
209	176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
210	177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0								
211	178	1	none	Hot		—	—	—	10	—	—	—	—	101942.00	152913.00	TRUE					152913	152632.8					
212	179	2	none	Hot		—	—	—	10	—	—	—	—	97942.00	146913.00	TRUE					146913	146632.8					
213	180	3	none	Hot		—	—	—	10	—	—	—	—	117275.00	175912.50	TRUE					175912.5	175632.3					
214	181	1	none	Hot		—	—	—	10	—	—	—	—	102119.00	153178.50	TRUE					153178.5	152898.3					
215	182	2	none	Hot		—	—	—	10	—	—	—	—	108863.00	163294.50	TRUE					163294.5	163014.3					
216	183	3	none	Hot		—	—	—	10	—	—	—	—	96217.00	144325.50	TRUE					144325.5	144045.3					

	B	C	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31			Prism data, excluding data points in the right leg of U-shaped curves																											
32			*standard curve*				*weak positive*				*1.0*				*3.0*				*20.0*				*23.0*				*negative control*			
33	*Position*	*Replicate*	*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1	-7.0	0.1917	0.4094	-0.3368	-4.0				-3.0				-3.0				-3.0				-3.0	10.4828	11.9130	14.4003	-3.0	#N/A	#N/A	#N/A
35	2	2	-7.0	-0.6166	0.5026	-0.1503	-4.5	0.1917	2.1505	2.1505	-4.0				-4.0	-0.2435	1.6841	0.3472	-4.0				-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
36	3	3	-8.0	17.1985	16.5767	12.2550	-5.5	26.9611	17.8825	36.1951	-5.0	-3.2594	-3.3837	-2.6997	-5.0	-0.9897	-1.8914	-2.0468	-5.0	25.6552	18.9396	25.7485	-5.0	44.3098	37.7807	45.5535	-5.0	#N/A	#N/A	#N/A
37	4	1	-8.5	38.8378	8.0267	41.2940	-6.0	64.5812	57.0882	61.3788	-6.0	-2.6065	-2.6686	-2.6065	-6.0	-1.6426	-1.9225	-1.8914	-6.0	11.6021	5.2596	53.1708	-6.0	63.2753	15.7061	0.7825	-6.0	#N/A	#N/A	#N/A
38	5	2	-9.0	69.0272	69.8977	69.5557	-6.5	88.0238	88.2414	88.5834	-7.0	-0.1192	-1.1452	-1.0830	-7.0	-1.7981	-1.4872	-1.6115	-7.0	57.5546	48.8491	46.3618	-7.0	42.3822	51.8960	36.4127	-7.0	#N/A	#N/A	#N/A
39	6	3	-9.5	90.9152	96.6360	89.5783	-7.0	103.6470	104.4398		-8.0	3.6739	6.5965	3.5496	-8.0	1.8085	0.7514	1.8706	-8.0	83.7954	51.6162	60.5082	-8.0	71.3901	70.9859	72.2917	-8.0	#N/A	#N/A	#N/A
40	7	1	-10.0	105.9943	106.4296	107.1756	-7.5	102.2945	101.0820		-9.0	20.9916	4.4823	12.4416	-9.0	25.7174	26.2771	27.6762	-9.0	61.0679	67.7213	70.5195	-9.0	64.3013	68.9961	78.6654	-9.0	#N/A	#N/A	#N/A
41	8	2	-11.0	106.9582	109.0102	109.1034	-8.5	103.2583	111.5285	107.7665	-10.0	31.9045	64.1770	68.9339	-10.0	62.0939	64.7988	60.5082	-10.0	70.1464	76.8310	69.6179	-10.0	68.7473	65.7004	54.7564	-10.0	#N/A	#N/A	#N/A
42	9	3																												
43	10	1	Standard Curve		TTN015 5-08-08		Positive Control		TTN015 5-08-08		1.0		TTN015 5-08-08		3.0		TTN015 5-08-08		20.0		TTN015 5-08-08		23.0		TTN015 5-08-08		Negative Control		Prism run ID & date	
44	11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3	Best-fit values				Best-fit values				Best-fit values		ambiguous		Best-fit values				Best-fit values				Best-fit values		ambiguous		Best-fit values			
46	13	1	BOTTOM		-0.003995		BOTTOM		-1.111		BOTTOM		-2.011		BOTTOM		-1.187		BOTTOM		22.49		BOTTOM		43.3		BOTTOM			
47	14	2	TOP		109.7		TOP		106.6		TOP		~ 2.778e+014		TOP		70.44		TOP		68.31		TOP		68.43		TOP			
48	15	3	LOGIC50		-8.755		LOGIC50		-5.85		LOGIC50		-9.938		LOGIC50		-9.459		LOGIC50		-6.985		LOGIC50		-7.148		LOGIC50			
49	16	1	HILLSLOPE		-1.14		HILLSLOPE		-1.16		HILLSLOPE		-0.5993		HILLSLOPE		-1.246		HILLSLOPE		-1.568		HILLSLOPE		~ -10.40		HILLSLOPE			
50	17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3	BOTTOM		2.88		BOTTOM		3.118		BOTTOM		3.039		BOTTOM		0.3934		BOTTOM		7.258		BOTTOM		7.494		BOTTOM			
52	19	1	TOP		3.271		TOP		1.937		TOP		~ 4.648e+015		TOP		2.55		TOP		4.469		TOP		10.6		TOP			
53	20	2	LOGIC50		0.03925		LOGIC50		0.02689		LOGIC50		0.2653		LOGIC50		0.0408		LOGIC50		0.1813		LOGIC50		~ 1.177e+006		LOGIC50			
54	21	3	HILLSLOPE		0.1434		HILLSLOPE		0.1123		HILLSLOPE		0.5448		HILLSLOPE		0.1561		HILLSLOPE		2.234		HILLSLOPE		~ 8.257e+007		HILLSLOPE			
55	22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2	Data				Data				Data				Data				Data				Data				Data			
66	33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
70	37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.96

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 015
Assay start date: 5/8/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	0.2
1	1	2	-7.0	0.4
1	1	3	-7.0	-0.3
1	2	1	-8.0	17.9
1	2	2	-8.0	17.2
1	2	3	-8.0	12.7
1	3	1	-8.5	40.3
1	3	2	-8.5	
1	3	3	-8.5	42.9
1	4	1	-9.0	71.7
1	4	2	-9.0	72.6
1	4	3	-9.0	72.2
1	5	1	-9.5	94.4
1	5	2	-9.5	100.3
1	5	3	-9.5	93.0
1	6	1	-10.0	110.0
1	6	2	-10.0	110.5
1	6	3	-10.0	111.3
1	7	1	-11.0	111.0
1	7	2	-11.0	113.2
1	7	3	-11.0	113.3
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.42

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Norethynodrel
Run ID: TTN 015
Assay start date: 5/8/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.5	0.2
1	1	2	-4.5	2.2
1	1	3	-4.5	2.2
1	2	1	-5.5	27.0
1	2	2	-5.5	17.9
1	2	3	-5.5	36.2
1	3	1	-6.0	64.6
1	3	2	-6.0	57.1
1	3	3	-6.0	61.4
1	4	1	-6.5	88.0
1	4	2	-6.5	88.2
1	4	3	-6.5	88.6
1	5	1	-7.0	103.6
1	5	2	-7.0	104.4
1	5	3		
1	6	1	-7.5	102.3
1	6	2	-7.5	101.1
1	6	3		
1	7	1	-8.5	103.3
1	7	2	-8.5	111.5
1	7	3	-8.5	107.8
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 8.92

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 1
Run ID: TTN 015
Assay start date: 5/8/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-5.0	-3.3
1	1	2	-5.0	-3.4
1	1	3	-5.0	-2.7
1	2	1	-6.0	-2.6
1	2	2	-6.0	-2.7
1	2	3	-6.0	-2.6
1	3	1	-7.0	-0.1
1	3	2	-7.0	-1.1
1	3	3	-7.0	-1.1
1	4	1	-8.0	3.7
1	4	2	-8.0	6.6
1	4	3	-8.0	3.5
1	5	1	-9.0	21.0
1	5	2	-9.0	4.5
1	5	3	-9.0	12.4
1	6	1	-10.0	31.9
1	6	2	-10.0	64.2
1	6	3	-10.0	68.9
1	7	1		
1	7	2		
1	7	3		
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.47

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 3
Run ID: TTN 015
Assay start date: 5/8/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	17.9
1	1	2	-3.0	16.8
1	1	3	-3.0	22.5
1	2	1	-4.0	-0.3
1	2	2	-4.0	1.7
1	2	3	-4.0	0.4
1	3	1	-5.0	-1.0
1	3	2	-5.0	-2.0
1	3	3	-5.0	-2.1
1	4	1	-6.0	-1.7
1	4	2	-6.0	-2.0
1	4	3	-6.0	-2.0
1	5	1	-7.0	-1.9
1	5	2	-7.0	-1.5
1	5	3	-7.0	-1.7
1	6	1	-8.0	1.9
1	6	2	-8.0	0.8
1	6	3	-8.0	1.9
1	7	1	-9.0	26.7
1	7	2	-9.0	27.3
1	7	3	-9.0	28.7
1	8	1	-10.0	64.5
1	8	2	-10.0	67.3
1	8	3	-10.0	62.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 10.37

Provide information in all blue cells.

Laboratory Code: B

Compound ID:	Chem Code 20
Run ID:	TTN 015
Assay start date:	5/8/2008
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	52.9
1	2	1	-4.0	
1	2	2	-4.0	61.4
1	2	3	-4.0	
1	3	1	-5.0	26.6
1	3	2	-5.0	19.7
1	3	3	-5.0	26.7
1	4	1	-6.0	12.0
1	4	2	-6.0	
1	4	3	-6.0	55.2
1	5	1	-7.0	59.8
1	5	2	-7.0	50.7
1	5	3	-7.0	48.1
1	6	1	-8.0	87.0
1	6	2	-8.0	53.6
1	6	3	-8.0	62.8
1	7	1	-9.0	63.4
1	7	2	-9.0	70.3
1	7	3	-9.0	73.2
1	8	1	-10.0	72.8
1	8	2	-10.0	79.8
1	8	3	-10.0	72.3
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 10.10

Provide information in all blue cells.

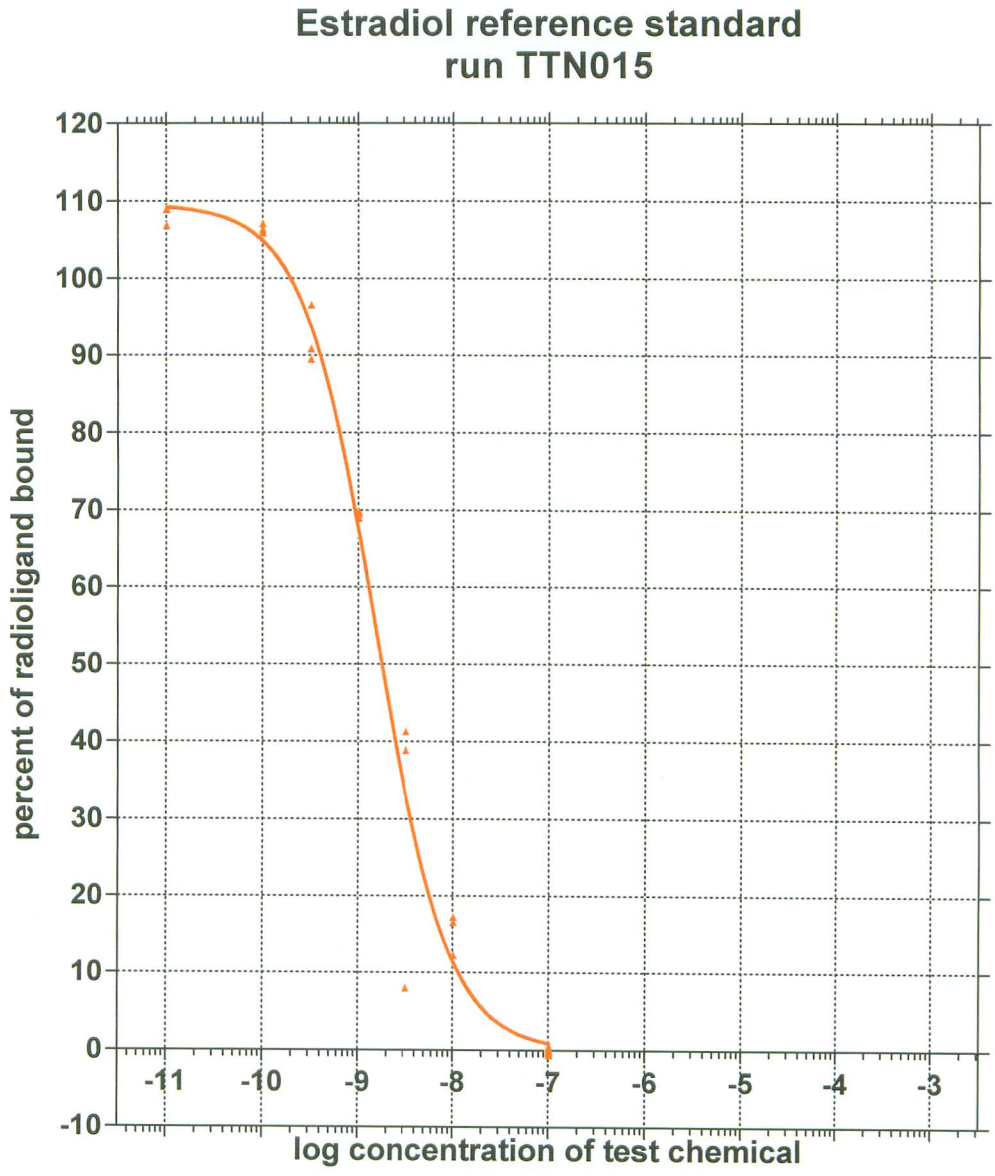
Laboratory Code: B

Compound ID: Chem Code 23
Run ID: TTN 015
Assay start date: 5/8/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	10.9
1	1	2	-3.0	12.4
1	1	3	-3.0	15.0
1	2	1	-4.0	11.8
1	2	2	-4.0	10.9
1	2	3	-4.0	10.9
1	3	1	-5.0	46.0
1	3	2	-5.0	39.2
1	3	3	-5.0	47.3
1	4	1	-6.0	65.7
1	4	2	-6.0	16.3
1	4	3	-6.0	
1	5	1	-7.0	44.0
1	5	2	-7.0	53.9
1	5	3	-7.0	37.8
1	6	1	-8.0	74.1
1	6	2	-8.0	73.7
1	6	3	-8.0	75.1
1	7	1	-9.0	66.8
1	7	2	-9.0	71.6
1	7	3	-9.0	81.7
1	8	1	-10.0	71.4
1	8	2	-10.0	68.2
1	8	3	-10.0	56.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

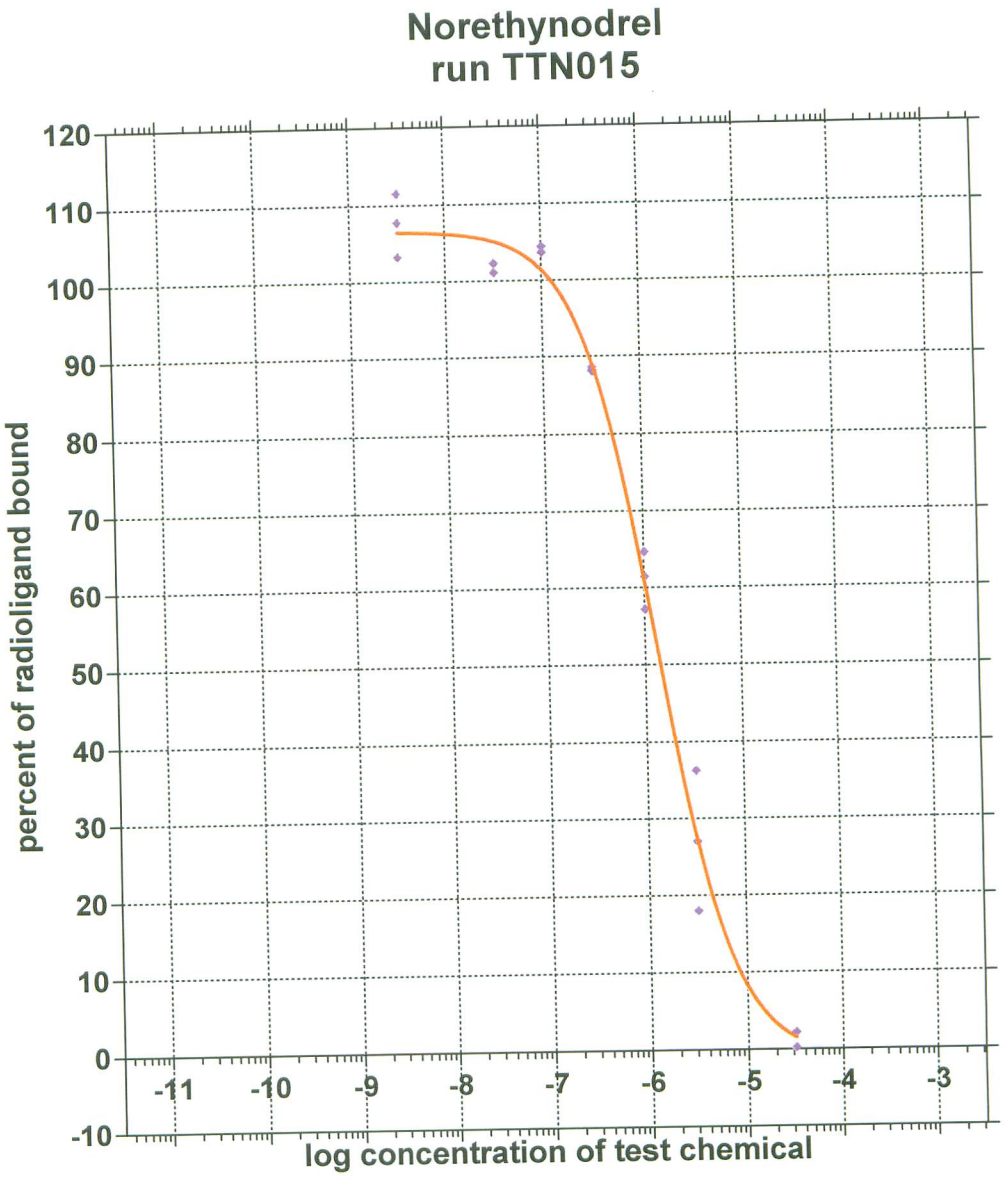
able format: XY		X	A		
		log concentration	run TTN015 05-08-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.1917	0.4094	-0.3368
2	Title	-7.0	-0.6166	0.5026	-0.1503
3	Title	-8.0	17.1985	16.5767	12.2550
4	Title	-8.5	38.8378	8.0267	41.2940
5	Title	-9.0	69.0272	69.8977	69.5557
6	Title	-9.5	90.9152	96.6360	89.5783
7	Title	-10.0	105.9943	106.4296	107.1758
8	Title	-11.0	106.9582	109.0102	109.1034

	Nonlin fit Table of results	A run TTN015 05-08-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.003995
4	TOP	109.7
5	LOGIC50	-8.755
6	HILLSLOPE	-1.140
7	Std. Error	
8	BOTTOM	2.880
9	TOP	3.271
10	LOGIC50	0.03925
11	HILLSLOPE	0.1434
12	95% Confidence Intervals	
13	BOTTOM	-6.012 to 6.004
14	TOP	102.8 to 116.5
15	LOGIC50	-8.837 to -8.673
16	HILLSLOPE	-1.440 to -0.8412
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9817
20	Absolute Sum of Squares	855.6
21	Sy.x	6.540
22	Number of points	
23	Analyzed	24


Estradiol reference standard
run TTN015
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

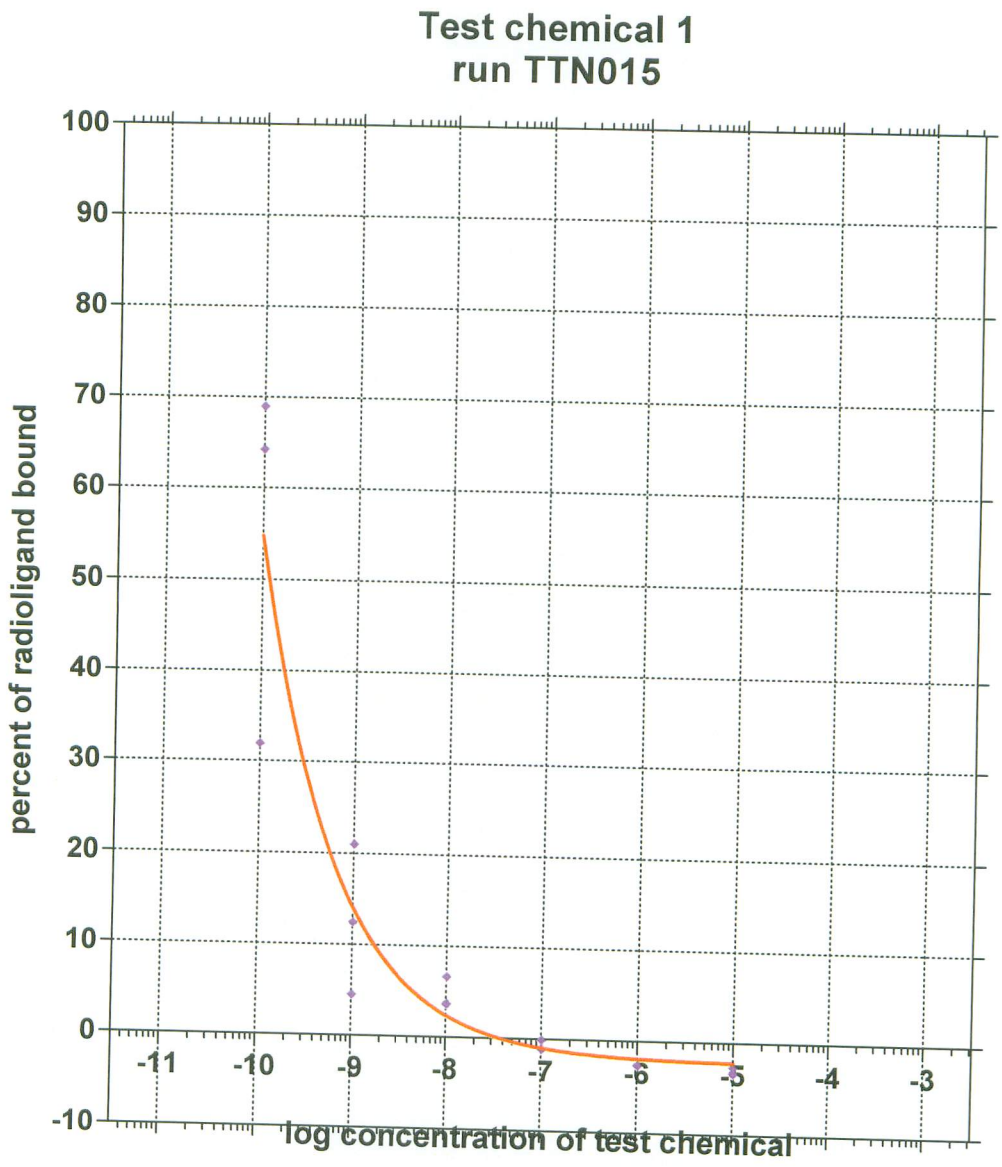
ible format: XY		X	A		
		log concentration	run TTN015 05-08-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	0.1917	2.1505	2.1505
3	Title	-5.5	26.9611	17.8825	36.1951
4	Title	-6.0	64.5812	57.0882	61.3788
5	Title	-6.5	88.0238	88.2414	88.5834
6	Title	-7.0	103.6470	104.4398	
7	Title	-7.5	102.2945	101.0820	
8	Title	-8.5	103.2583	111.5285	107.7665

	Nonlin fit Table of results	A run TTN015 05-08-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.111
4	TOP	106.6
5	LOGIC50	-5.850
6	HILLSLOPE	-1.160
7	Std. Error	
8	BOTTOM	3.118
9	TOP	1.937
10	LOGIC50	0.02689
11	HILLSLOPE	0.1123
12	95% Confidence Intervals	
13	BOTTOM	-7.755 to 5.534
14	TOP	102.5 to 110.7
15	LOGIC50	-5.907 to -5.792
16	HILLSLOPE	-1.399 to -0.9204
17	Goodness of Fit	
18	Degrees of Freedom	15
19	R^2	0.9906
20	Absolute Sum of Squares	277.8
21	Sy.x	4.303
22	Number of points	
23	Analyzed	19


Norethynodrel
run TTN015
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

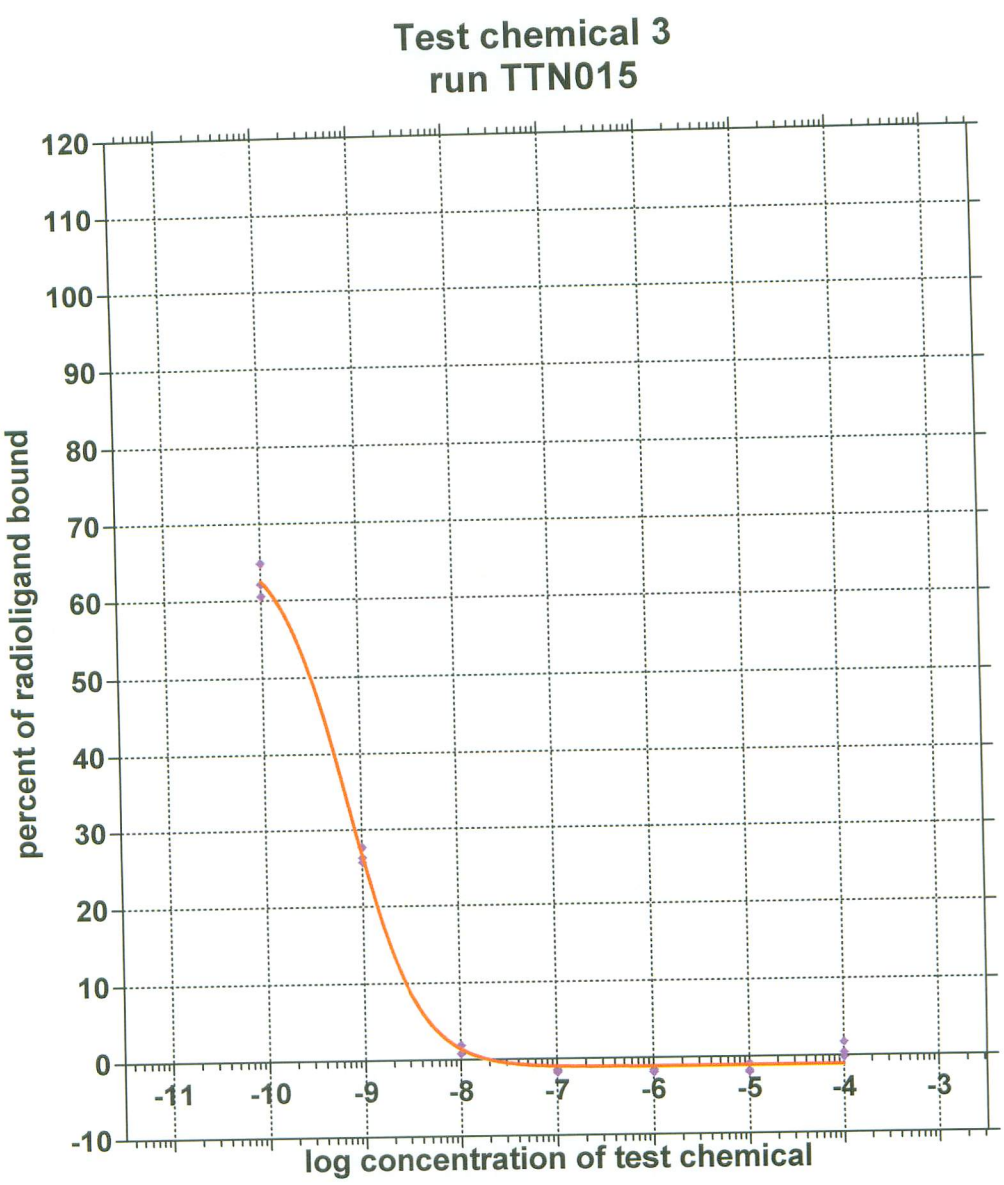
ıble format: XY		X	A			B		
		log concentration	Test chemical			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	-3.2594	-3.3837	-2.6997			
4	Title	-6.0	-2.6065	-2.6686	-2.6065			
5	Title	-7.0	-0.1192	-1.1452	-1.0830			
6	Title	-8.0	3.6739	6.5965	3.5496			
7	Title	-9.0	20.9916	4.4823	12.4416			
8	Title	-10.0	31.9045	64.1770	68.9339			

	Nonlin fit Table of results	A Test chemical	B Data Set-B
		Y	Y
1	logIC50		
2	Best-fit values		
3	BOTTOM	-2.342	
4	TOP	457936	
5	LOGIC50	-9.930	
6	HILLSLOPE	-0.5431	
7	Std. Error		
8	BOTTOM	4.129	
9	TOP	9.431e+009	
10	LOGIC50	0.09534	
11	HILLSLOPE	0.8023	
12	95% Confidence Intervals		
13	BOTTOM	-11.20 to 6.515	
14	TOP	-2.023e+010 to 2.023e+010	
15	LOGIC50	-10.13 to -9.726	
16	HILLSLOPE	-2.264 to 1.178	
17	Goodness of Fit		
18	Degrees of Freedom	14	
19	R^2	0.8842	
20	Absolute Sum of Squares	980.0	
21	Sy.x	8.367	
22	Number of points		
23	Analyzed	18	


Test chemical 1
run TTN015
percent of radioligand bound
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

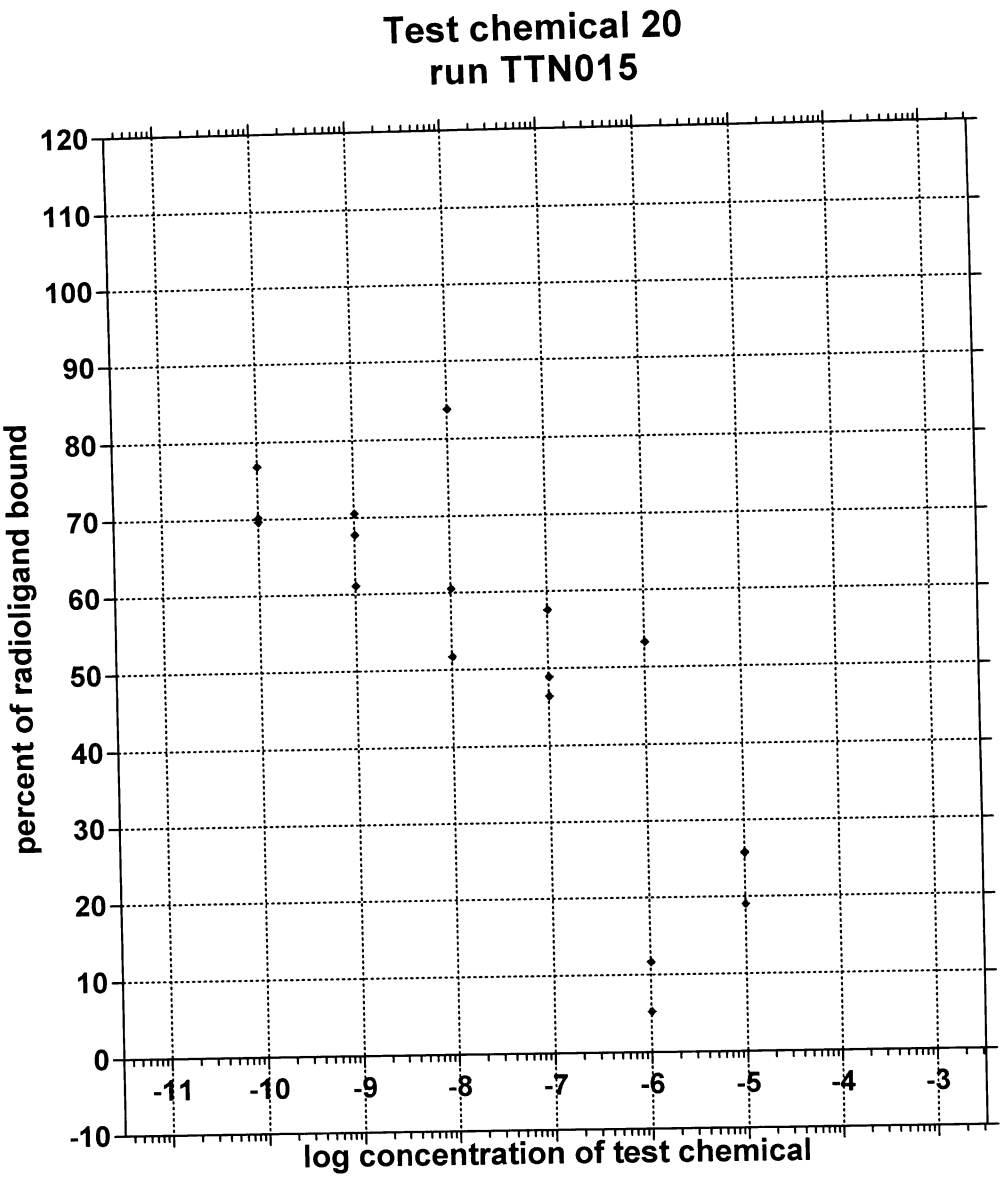
ıble format: XY		X	A		
		log concentration	Test chemical		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-0.2435	1.6841	0.3472
3	Title	-5.0	-0.9897	-1.8914	-2.0468
4	Title	-6.0	-1.6426	-1.9225	-1.8914
5	Title	-7.0	-1.7981	-1.4872	-1.6115
6	Title	-8.0	1.8085	0.7514	1.8706
7	Title	-9.0	25.7174	26.2771	27.6762
8	Title	-10.0	62.0939	64.7988	60.5082

	Nonlin fit Table of results	A Test chemical
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.144
4	TOP	67.85
5	LOGIC50	-9.506
6	HILLSLOPE	-1.246
7	Std. Error	
8	BOTTOM	0.3789
9	TOP	2.457
10	LOGIC50	0.04260
11	HILLSLOPE	0.1561
12	95% Confidence Intervals	
13	BOTTOM	-1.943 to -0.3440
14	TOP	62.66 to 73.03
15	LOGIC50	-9.596 to -9.416
16	HILLSLOPE	-1.575 to -0.9164
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9974
20	Absolute Sum of Squares	27.41
21	Sy.x	1.270
22	Number of points	
23	Analyzed	21


Test chemical 3
run TTN015
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

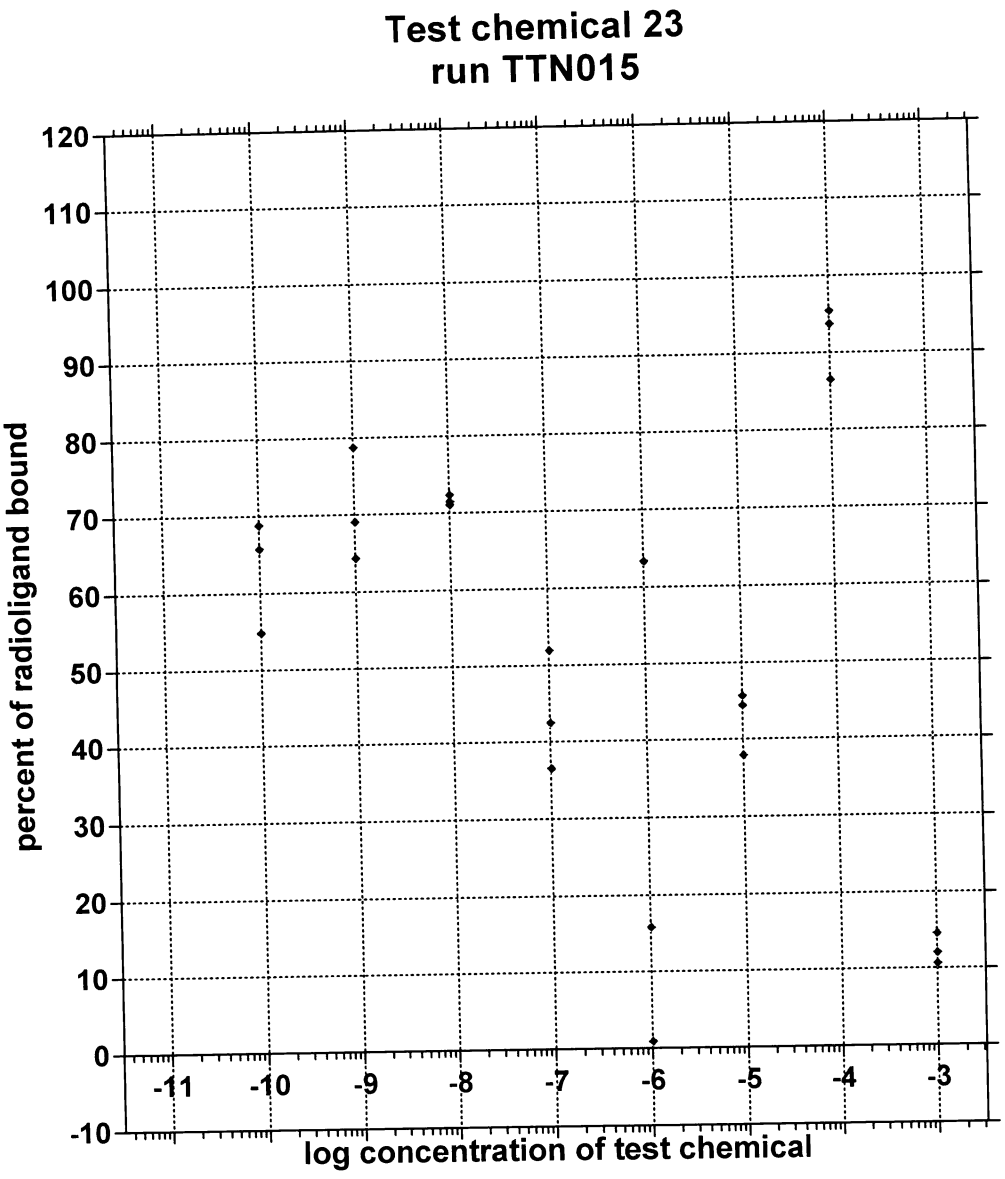
able format: XY		X	A			B		
		log concentration	Test chemical			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	25.6552	18.9396	25.7485			
4	Title	-6.0	11.6021	5.2596	53.1708			
5	Title	-7.0	57.5546	48.8491	46.3618			
6	Title	-8.0	83.7954	51.6162	60.5082			
7	Title	-9.0	61.0679	67.7213	70.5195			
8	Title	-10.0	70.1464	76.8310	69.6179			

	Nonlin fit Table of results	A Test chemical	B Data Set-B
		Y	Y
1	logIC50		
2	Best-fit values		
3	BOTTOM	22.49	
4	TOP	68.31	
5	LOGIC50	-6.985	
6	HILLSLOPE	-1.568	
7	Std. Error		
8	BOTTOM	7.258	
9	TOP	4.469	
10	LOGIC50	0.1813	
11	HILLSLOPE	2.234	
12	95% Confidence Intervals		
13	BOTTOM	6.924 to 38.06	
14	TOP	58.73 to 77.90	
15	LOGIC50	-7.374 to -6.596	
16	HILLSLOPE	-6.361 to 3.225	
17	Goodness of Fit		
18	Degrees of Freedom	14	
19	R^2	0.7681	
20	Absolute Sum of Squares	2163	
21	Sy.x	12.43	
22	Number of points		
23	Analyzed	18	


Test chemical 20
run TTN015
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

able format: XY		X	A			B		
		log concentration	Test chemical			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0	10.4828	11.9130	14.4003			
2	Title	-4.0	93.6729	95.3859	86.3787			
3	Title	-5.0	44.3098	37.7807	45.5535			
4	Title	-6.0	63.2753	15.7061	0.7825			
5	Title	-7.0	42.3822	51.8960	36.4127			
6	Title	-8.0	71.3901	70.9859	72.2917			
7	Title	-9.0	64.3013	68.9961	78.6654			
8	Title	-10.0	68.7473	65.7004	54.7564			

	Nonlin fit Table of results	A Test chemical	B Data Set-B
		Y	Y
1	logIC50	Ambiguous	
2	Best-fit values		
3	BOTTOM	43.30	
4	TOP	68.43	
5	LOGIC50	~ -7.148	
6	HILLSLOPE	~ -10.40	
7	Std. Error		
8	BOTTOM	7.494	
9	TOP	10.60	
10	LOGIC50	~ 1.177e+006	
11	HILLSLOPE	~ 8.257e+007	
12	95% Confidence Intervals		
13	BOTTOM	27.67 to 58.94	
14	TOP	46.32 to 90.53	
15	LOGIC50	(Very wide)	
16	HILLSLOPE	(Very wide)	
17	Goodness of Fit		
18	Degrees of Freedom	20	
19	R^2	0.2078	
20	Absolute Sum of Squares	13477	
21	Sy.x	25.96	
22	Number of points		
23	Analyzed	24	


Test chemical 23
run TTN015
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

APPENDIX Q

Excel and Prism Files of the Run Identifier TTN018

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B		
Run identification:	TTN018		
Assay start date:	6/12/2008		
Tracer lot number:	3589481/1		
Specific activity on day of assay:	108.27	Ci/mmole	
Cytosol vial or lot identification:	RUC06-06-08		
Protein (cytosol):	50	microgram per tube	
log(IC50), Cold E2 (Standard Curve):	-8.97	M	
Weak Positive, Max Concentration:	5.00E-03	M	
log(IC50), norethynodrel (Weak Positive):	-6.14	M	
RBA, norethynodrel (Weak Positive):	1.476E-03	relative to E2=1.0	
Max Concentration, Unknown 1:	1.00E-03	M	
log(IC50), Unknown 1:	n/a	1.0	
RBA, Unknown 1:	n/a	relative to E2=1.0	
Max Concentration, Unknown 2:	1.00E-03	M	
log(IC50), Unknown 2:	-9.93	3.0	
RBA, Unknown 2:	9.290E+00	relative to E2=1.0	
Max Concentration, Unknown 3:	1.00E-03	M	
log(IC50), Unknown 3:	-7.18	20.0	
RBA, Unknown 3:	1.622E-02	relative to E2=1.0	
Max Concentration, Unknown 4:	1.00E-03	M	
log(IC50), Unknown 4:	n/a	23.0	
RBA, Unknown 4:	n/a	relative to E2=1.0	
volume of ethanol counted:	1	mL	
multiply DPM in sample by :	1.5		

Check the 10% rule: 6.45% — If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	8376.3	849.88	Total Binding, solvent control, tubes
Hot	6	129772.5	5510.05	Total hot E2 added to each tube
NSB	6	1233.5	24.64	Nonspecific Binding
Specific EtOH	6	7142.8	849.88	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.06	less than 0.1?
R1881 / EtOH	#DIV/0!	close to 1.0 ? at 1.0 E-6 M only
NSB / EtOH	0.15	around 0.25 ?

log conc'n at min hot binding		min hot (%)
cold E2	-7.0	0.2
R1881	#N/A	not applicable
norethynodrel	-4.5	0.3
Unknown 1	-6.0	-3.8
Unknown 2	-5.0	-4.5
Unknown 3	-7.0	26.2
Unknown 4	-4.0	-2.6

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4881.00	7321.50	TRUE			—	85.2	7321.5	6088.0	123684.5	85.2	98.2	85.2
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5461.00	8191.50	TRUE			—	97.4	8191.5	6958.0	122814.5	97.4		97.4
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	6150.00	9225.00	TRUE			—	111.9	9225	7991.5	121781.0	111.9		111.9
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	836.00	1254.00	TRUE		cold E2	-7.0	0.3	1254	20.5	129752.0	0.3	0.2	0.3
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	832.00	1248.00	TRUE		cold E2	-7.0	0.2	1248	14.5	129758.0	0.2		0.2
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	822.00	1233.00	TRUE		cold E2	-7.0	0.0	1233	-0.5	129773.0	0.0		0.0
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1270.00	1905.00	TRUE		cold E2	-8.0	9.4	1905	671.5	129101.0	9.4	9.6	9.4
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1261.00	1891.50	TRUE		cold E2	-8.0	9.2	1891.5	658.0	129114.5	9.2		9.2
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	1310.00	1965.00	TRUE		cold E2	-8.0	10.2	1965	731.5	129041.0	10.2		10.2
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1855.00	2782.50	TRUE		cold E2	-8.5	21.7	2782.5	1549.0	128223.5	21.7	23.0	21.7
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2040.00	3060.00	TRUE		cold E2	-8.5	25.6	3060	1826.5	127946.0	25.6		25.6
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1854.00	2781.00	TRUE		cold E2	-8.5	21.7	2781	1547.5	128225.0	21.7		21.7
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3377.00	5065.50	TRUE		cold E2	-9.0	53.6	5065.5	3832.0	125940.5	53.6	51.5	53.6
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3253.00	4879.50	TRUE		cold E2	-9.0	51.0	4879.5	3646.0	126126.5	51.0		51.0
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	3196.00	4794.00	TRUE		cold E2	-9.0	49.8	4794	3560.5	126212.0	49.8		49.8
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4406.00	6609.00	TRUE		cold E2	-9.5	75.3	6609	5375.5	124397.0	75.3	82.0	75.3
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4895.00	7342.50	TRUE		cold E2	-9.5	85.5	7342.5	6109.0	123663.5	85.5		85.5
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	4881.00	7321.50	TRUE		cold E2	-9.5	85.2	7321.5	6088.0	123684.5	85.2		85.2
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3217.00	4825.50	FALSE	mispipet	cold E2	-10.0						87.9	
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4116.00	6174.00	FALSE	mispipet	cold E2	-10.0							
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	5006.00	7509.00	TRUE		cold E2	-10.0	87.9	7509	6275.5	123497.0	87.9		87.9
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5950.00	8925.00	TRUE		cold E2	-11.0	107.7	8925	7691.5	122081.0	107.7	103.2	107.7
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5309.00	7963.50	TRUE		cold E2	-11.0	94.2	7963.5	6730.0	123042.5	94.2		94.2
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	5948.00	8922.00	TRUE		cold E2	-11.0	107.6	8922	7688.5	122084.0	107.6		107.6
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32	Competitive Assay Tube Layout - Four Test Chemicals															Check the 10% rule:	6.45%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33	Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
75	42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A		
76	43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A		
77	44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
78	45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A		
79	46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A		
80	47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
81	48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A		
82	49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2388.00	3582.00	TRUE		norethynodrel	-4.0	32.9	3582	2348.5	127424.0	32.9	30.0			
83	50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2137.00	3205.50	TRUE		norethynodrel	-4.0	27.6	3205.5	1972.0	127800.5	27.6				
84	51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2231.00	3346.50	TRUE		norethynodrel	-4.0	29.6	3346.5	2113.0	127659.5	29.6				
85	52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	854.00	1281.00	TRUE		norethynodrel	-4.5	0.7	1281	47.5	129725.0	0.7	0.3			0.7
86	53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	822.00	1233.00	TRUE		norethynodrel	-4.5	0.0	1233	-0.5	129773.0	0.0				0.0
87	54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	830.00	1245.00	TRUE		norethynodrel	-4.5	0.2	1245	11.5	129761.0	0.2				0.2
88	55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1781.00	2671.50	TRUE		norethynodrel	-5.5	20.1	2671.5	1438.0	128334.5	20.1	20.4			20.1
89	56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	905.00	1357.50	FALSE	mispipet	norethynodrel	-5.5									
90	57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1807.00	2710.50	TRUE		norethynodrel	-5.5	20.7	2710.5	1477.0	128295.5	20.7				20.7
91	58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2611.00	3916.50	TRUE		norethynodrel	-4.0	37.6	3916.5	2683.0	127089.5	37.6	38.7			37.6
92	59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2561.00	3841.50	TRUE		norethynodrel	-4.0	36.5	3841.5	2608.0	127164.5	36.5				36.5
93	60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2818.00	4227.00	TRUE		norethynodrel	-4.0	41.9	4227	2993.5	126779.0	41.9				41.9
94	61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4686.00	7029.00	TRUE		norethynodrel	-4.5	81.1	7029	5795.5	123977.0	81.1	79.7			81.1
95	62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4637.00	6955.50	TRUE		norethynodrel	-4.5	80.1	6955.5	5722.0	124050.5	80.1				80.1
96	63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	4532.00	6798.00	TRUE		norethynodrel	-4.5	77.9	6798	5564.5	124208.0	77.9				77.9
97	64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4094.00	6141.00	TRUE		norethynodrel	-5.0	68.7	6141	4907.5	124865.0	68.7	64.6			68.7
98	65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	5479.00	8218.50	FALSE	mispipet	norethynodrel	-5.0									
99	66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3704.00	5556.00	TRUE		norethynodrel	-5.0	60.5	5556	4322.5	125450.0	60.5				60.5
100	67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5908.00	8862.00	TRUE		norethynodrel	-4.5	106.8	8862	7628.5	122144.0	106.8	106.6			106.8
101	68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5914.00	8871.00	TRUE		norethynodrel	-4.5	106.9	8871	7637.5	122135.0	106.9				106.9
102	69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	5873.00	8809.50	TRUE		norethynodrel	-4.5	106.1	8809.5	7576.0	122196.5	106.1				106.1
103	70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5270.00	7905.00	TRUE		norethynodrel	-5.5	93.4	7905	6671.5	123101.0	93.4	102.5			93.4
104	71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	6047.00	9070.50	TRUE		norethynodrel	-5.5	109.7	9070.5	7837.0	121935.5	109.7				109.7
105	72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	5799.00	8698.50	TRUE		norethynodrel	-5.5	104.5	8698.5	7465.0	122307.5	104.5				104.5
106	73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	47268.00	70902.00	TRUE		Unknown 1	-3.0	975.4	70902	69668.5	60104.0	975.4	1037.7			
107	74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	50547.00	75820.50	TRUE		Unknown 1	-3.0	1044.2	75820.5	74587.0	55185.5	1044.2				
108	75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	52894.00	79341.00	TRUE		Unknown 1	-3.0	1093.5	79341	78107.5	51665.0	1093.5				
109	76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2399.00	3598.50	TRUE		Unknown 1	-4.0	33.1	3598.5	2365.0	127407.5	33.1	38.8			
110	77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2348.00	3522.00	TRUE		Unknown 1	-4.0	32.0	3522	2288.5	127484.0	32.0				
111	78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3269.00	4903.50	TRUE		Unknown 1	-4.0	51.4	4903.5	3670.0	126102.5	51.4				
112	79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	657.00	985.50	TRUE		Unknown 1	-5.0	-3.5	985.5	-248.0	130020.5	-3.5	-3.8			-3.5
113	80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	644.00	966.00	TRUE		Unknown 1	-5.0	-3.7	966	-267.5	130040.0	-3.7				-3.7
114	81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	626.00	939.00	TRUE		Unknown 1	-5.0	-4.1	939	-294.5	130067.0	-4.1				-4.1
115	82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	618.00	927.00	TRUE		Unknown 1	-6.0	-4.3	927	-306.5	130079.0	-4.3	-3.8			-4.3
116	83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	631.00	946.50	TRUE		Unknown 1	-6.0	-4.0	946.5	-287.0	130059.5	-4.0				-4.0
117	84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	674.00	1011.00	TRUE		Unknown 1	-6.0	-3.1	1011	-222.5	129995.0	-3.1				-3.1
118	85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	680.00	1020.00	TRUE		Unknown 1	-7.0	-3.0	1020	-213.5	129986.0	-3.0	-2.3			-3.0
119	86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	724.00	1086.00	TRUE		Unknown 1	-7.0	-2.1	1086	-147.5	129920.0	-2.1				-2.1
120	87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	738.00	1107.00	TRUE		Unknown 1	-7.0	-1.8	1107	-126.5	129899.0	-1.8				-1.8
121	88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	766.00	1149.00	TRUE		Unknown 1	-8.0	-1.2	1149	-84.5	129857.0	-1.2	-0.4			-1.2
122	89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	956.00	1434.00	TRUE		Unknown 1	-8.0	2.8	1434	200.5	129572.0	2.8				2.8
123	90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	689.00	1033.50	TRUE		Unknown 1	-8.0	-2.8	1033.5	-200.0	129972.5	-2.8				-2.8
124	91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1127.00	1690.50	TRUE		Unknown 1	-9.0	6.4	1690.5	457.0	129315.5	6.4	6.7			6.4
125	92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1153.00	1729.50	TRUE		Unknown 1	-9.0	6.9	1729.5	496.0	129276.5	6.9				6.9
126	93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1692.00	2538.00	FALSE	mispipet	Unknown 1	-9.0									
127	94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1516.00	2274.00	TRUE		Unknown 1	-10.0	14.6	2274	1040.5	128732.0	14.6	10.9			14.6
128	95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1170.00	1755.00	TRUE		Unknown 1	-10.0	7.3	1755	521.5	129251.0	7.3				7.3
129	96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2632.00	3948.00	FALSE	mispipet	Unknown 1	-10.0									
130	97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1059.00	1588.50	TRUE		Unknown 2	-3.0	5.0	1588.5	355.0	129417.5	5.0	5.8			
131	98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1162.00	1743.00	TRUE		Unknown 2	-3.0	7.1	1743	509.5	129263.0	7.1				
132	99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1071.00	1606.50	TRUE		Unknown 2	-3.0	5.2	1606.5	373.0	129399.5	5.2				
133	100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	665.00	997.50	TRUE		Unknown 2	-4.0	-3.3	997.5	-236.0	130008.5	-3.3	-3.6			-3.3
134	101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	641.00	961.50	TRUE		Unknown 2	-4.0	-3.8	961.5	-272.0	130044.5	-3.8				-3.8
135	102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	644.00	966.00	TRUE		Unknown 2	-4.0	-3.7	966	-267.5	130040.0	-3.7				-3.7
136	103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	607.00	910.50	TRUE		Unknown 2	-5.0	-4.5	910.5	-323.0	130095.5	-4.5	-4.5			-4.5
137	104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	625.00	937.50	TRUE		Unknown 2	-5.0	-4.1	937.5	-296.0	130068.5	-4.1				-4.1
138	105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	597.00	895.50	TRUE		Unknown 2	-5.0	-4.7	895.5	-338.0	130110.5	-4.7				-4.7
139	106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	634.00	951.00	TRUE		Unknown 2	-6.0	-4.0	951	-282.5	130055.0	-4.0	-3.5			-4.0
140	107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	675.00	1012.50	TRUE		Unknown 2	-6.0	-3.1	1012.5	-221.0	129993.5	-3.1				-3.1
141	108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	653.00	979.50	TRUE		Unknown 2	-6.0	-3.6	979.5	-254.0	130026.5	-3.6				-3.6
142	109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	605.00	907.50	TRUE		Unknown 2	-7.0	-4.6	907.5	-326.0	130098.5	-4.6	-4.0			-4.6
143	110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	643.00	964.50	TRUE		Unknown 2	-7.0	-3.8	964.5	-269.0	130041.5	-3.8				-3.8
144	111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	648.00	972.00	TRUE		Unknown 2	-7.0	-3.7	972	-261.5	130034.0	-3.7				-3.7
145	112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	707.00	1060.50	TRUE		Unknown 2	-8.0	-2.4	1060.5	-173.0	129945.5	-2.4	-2.4			-2.4
146	113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	700.00	1050.00	TRUE		Unknown 2	-8.0	-2.6	1050	-183.5	129956.0	-2.6				-2.6
147	114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	714.00	1071.00	TRUE		Unknown 2	-8.0	-2.3	1071	-162.5	129935.0	-2.3				-2.3
148	115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1399.00	2098.50	TRUE		Unknown 2	-9.0	12.1	2098.5	865.0	128907.5	12.1	13.1			12.1
149	116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	983.00	1474.50	FALSE	mispipet	Unknown 2	-9.0									
150	117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1490.00	2235.00	TRUE		Unknown 2	-9.0	14.0	2235	1001.5	128771.0	14.0				14.0

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
32				Competitive Assay Tube Layout - Four Test Chemicals												Check the 10% rule:	6.45%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay		Unknown			% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.							
33		Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)		
151		118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3257.00	4885.50	TRUE		Unknown 2	-10.0	51.1	4885.5	3652.0	126120.5	51.1	52.9	51.1		
152		119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3271.00	4906.50	TRUE		Unknown 2	-10.0	51.4	4906.5	3673.0	126099.5	51.4		51.4		
153		120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3498.00	5247.00	TRUE		Unknown 2	-10.0	56.2	5247	4013.5	125759.0	56.2		56.2		
154		121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4379.00	6568.50	TRUE		Unknown 3	-3.0	74.7	6568.5	5335.0	124437.5	74.7	79.9			
155		122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4759.00	7138.50	TRUE		Unknown 3	-3.0	82.7	7138.5	5905.0	123867.5	82.7				
156		123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4737.00	7105.50	TRUE		Unknown 3	-3.0	82.2	7105.5	5872.0	123900.5	82.2				
157		124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3944.00	5916.00	TRUE		Unknown 3	-4.0	65.6	5916	4682.5	125090.0	65.6	68.2			
158		125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2790.00	4185.00	TRUE		Unknown 3	-4.0	41.3	4185	2951.5	126821.0	41.3				
159		126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	5474.00	8211.00	TRUE		Unknown 3	-4.0	97.7	8211	6977.5	122795.0	97.7				
160		127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1295.00	1942.50	FALSE	mispipet	Unknown 3	-5.0						28.7			
161		128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2294.00	3441.00	TRUE		Unknown 3	-5.0	30.9	3441	2207.5	127565.0	30.9		30.9		
162		129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2088.00	3132.00	TRUE		Unknown 3	-5.0	26.6	3132	1898.5	127874.0	26.6		26.6		
163		130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2767.00	4150.50	TRUE		Unknown 3	-6.0	40.8	4150.5	2917.0	126855.5	40.8	37.5	40.8		
164		131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2613.00	3919.50	TRUE		Unknown 3	-6.0	37.6	3919.5	2686.0	127086.5	37.6		37.6		
165		132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2439.00	3658.50	TRUE		Unknown 3	-6.0	34.0	3658.5	2425.0	127347.5	34.0		34.0		
166		133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2001.00	3001.50	TRUE		Unknown 3	-7.0	24.8	3001.5	1768.0	128004.5	24.8	26.2	24.8		
167		134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2989.00	4483.50	TRUE		Unknown 3	-7.0	45.5	4483.5	3250.0	126522.5	45.5		45.5		
168		135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1216.00	1824.00	TRUE		Unknown 3	-7.0	8.3	1824	590.5	129182.0	8.3		8.3		
169		136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	923.00	1384.50	FALSE	mispipet	Unknown 3	-8.0						65.7			
170		137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4087.00	6130.50	TRUE		Unknown 3	-8.0	68.6	6130.5	4897.0	124875.5	68.6		68.6		
171		138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3810.00	5715.00	TRUE		Unknown 3	-8.0	62.7	5715	4481.5	125291.0	62.7		62.7		
172		139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3302.00	4953.00	TRUE		Unknown 3	-9.0	52.1	4953	3719.5	126053.0	52.1	60.0	52.1		
173		140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4201.00	6301.50	TRUE		Unknown 3	-9.0	71.0	6301.5	5068.0	124704.5	71.0		71.0		
174		141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3536.00	5304.00	TRUE		Unknown 3	-9.0	57.0	5304	4070.5	125702.0	57.0		57.0		
175		142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3224.00	4836.00	TRUE		Unknown 3	-10.0	50.4	4836	3602.5	126170.0	50.4	59.7	50.4		
176		143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4149.00	6223.50	TRUE		Unknown 3	-10.0	69.9	6223.5	4990.0	124782.5	69.9		69.9		
177		144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3622.00	5433.00	TRUE		Unknown 3	-10.0	58.8	5433	4199.5	125573.0	58.8		58.8		
178		145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	832.00	1248.00	TRUE		Unknown 4	-3.0	0.2	1248	14.5	129758.0	0.2	0.7	0.2		
179		146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	862.00	1293.00	TRUE		Unknown 4	-3.0	0.8	1293	59.5	129713.0	0.8		0.8		
180		147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	876.00	1314.00	TRUE		Unknown 4	-3.0	1.1	1314	80.5	129692.0	1.1		1.1		
181		148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	693.00	1039.50	TRUE		Unknown 4	-4.0	-2.7	1039.5	-194.0	129966.5	-2.7	-2.6	93.7		
182		149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	715.00	1072.50	TRUE		Unknown 4	-4.0	-2.3	1072.5	-161.0	129933.5	-2.3		95.4		
183		150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	690.00	1035.00	TRUE		Unknown 4	-4.0	-2.8	1035	-198.5	129971.0	-2.8		86.4		
184		151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	721.00	1081.50	TRUE		Unknown 4	-5.0	-2.1	1081.5	-152.0	129924.5	-2.1	-2.5	-2.1		
185		152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	697.00	1045.50	TRUE		Unknown 4	-5.0	-2.6	1045.5	-188.0	129960.5	-2.6		-2.6		
186		153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	686.00	1029.00	TRUE		Unknown 4	-5.0	-2.9	1029	-204.5	129977.0	-2.9		-2.9		
187		154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1200.00	1800.00	TRUE		Unknown 4	-6.0	7.9	1800	566.5	129206.0	7.9	19.8	7.9		
188		155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2396.00	3594.00	TRUE		Unknown 4	-6.0	33.0	3594	2360.5	127412.0	33.0		33.0		
189		156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1699.00	2548.50	TRUE		Unknown 4	-6.0	18.4	2548.5	1315.0	128457.5	18.4		18.4		
190		157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1830.00	2745.00	TRUE		Unknown 4	-7.0	21.2	2745	1511.5	128261.0	21.2	35.6	21.2		
191		158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2642.00	3963.00	TRUE		Unknown 4	-7.0	38.2	3963	2729.5	127043.0	38.2		38.2		
192		159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3077.00	4615.50	TRUE		Unknown 4	-7.0	47.3	4615.5	3382.0	126390.5	47.3		47.3		
193		160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	1179.00	1768.50	TRUE		Unknown 4	-8.0	7.5	1768.5	535.0	129237.5	7.5	5.1	7.5		
194		161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	953.00	1429.50	TRUE		Unknown 4	-8.0	2.7	1429.5	196.0	129576.5	2.7		2.7		
195		162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2879.00	4318.50	FALSE	mispipet	Unknown 4	-8.0									
196		163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2465.00	3697.50	TRUE		Unknown 4	-9.0	34.5	3697.5	2464.0	127308.5	34.5	37.4	34.5		
197		164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2837.00	4255.50	TRUE		Unknown 4	-9.0	42.3	4255.5	3022.0	126750.5	42.3		42.3		
198		165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2511.00	3766.50	TRUE		Unknown 4	-9.0	35.5	3766.5	2533.0	127239.5	35.5		35.5		
199		166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1377.00	2065.50	TRUE		Unknown 4	-10.0	11.6	2065.5	832.0	128940.5	11.6	8.9	11.6		
200		167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1193.00	1789.50	TRUE		Unknown 4	-10.0	7.8	1789.5	556.0	129216.5	7.8		7.8		
201		168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	1175.00	1762.50	TRUE		Unknown 4	-10.0	7.4	1762.5	529.0	129243.5	7.4		7.4		
202		169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	801.00	1201.50	TRUE		cold E2	-7.0	-0.4	1201.5	-32.0	129804.5	-0.4	-0.2	-0.4		
203		170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	804.00	1206.00	TRUE		cold E2	-7.0	-0.4	1206	-27.5	129800.0	-0.4		-0.4		
204		171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	839.00	1258.50	TRUE		cold E2	-7.0	0.4	1258.5	25.0	129747.5	0.4		0.4		
205		172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5044.00	7566.00	TRUE			—	88.7	7566	6332.5	123440.0	88.7	101.8	88.7		
206		173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	5699.00	8548.50	TRUE			—	102.4	8548.5	7315.0	122457.5	102.4		102.4		
207		174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	6270.00	9405.00	TRUE			—	114.4	9405	8171.5	121601.0	114.4		114.4		
208		175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average			
209		176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
210		177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0									
211		178	1	none	Hot		—	—	—	10	—	—	—	—	87910.00	131865.00	TRUE					131865	130631.5						
212		179	2	none	Hot		—	—	—	10	—	—	—	—	83904.00	125856.00	TRUE					125856	124622.5						
213		180	3	none	Hot		—	—	—	10	—	—	—	—	93390.00	140085.00	TRUE					140085	138851.5						
214		181	1	none	Hot		—	—	—	10	—	—	—	—	84909.00	127363.50	TRUE					127363.5	126130.0						
215		182	2	none	Hot		—	—	—	10	—	—	—	—	85057.00	127585.50	TRUE					127585.5	126352.0						
216		183	3	none	Hot		—	—	—	10	—	—	—	—	83920.00	125880.00	TRUE					125880	124646.5						

	B	C	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
31			Prism data, excluding data points in the right leg of U-shaped curves																											
32			*standard curve*				*weak positive*				*1.0*				*3.0*				*20.0*				*23.0*				*negative control*			
33	*Position*	*Replicate*	*concentration (logM)*	Y1-SC	Y2-SC	Y3-SC	*concentration (logM)*	y1-PC	y2-PC	y3-PC	*concentration (logM)*	y1-U1	y2-U1	y3-U1	*concentration (logM)*	y1-U2	y2-U2	y3-U2	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	y1-U3	y2-U3	y3-U3	*concentration (logM)*	Y1-NC	Y2-NC	Y3-NC
34	1	1	-7.0	0.2670	0.2030	-0.0070	-4.0				-3.0				-3.0				-3.0				-3.0	0.2030	0.8330	1.1270	-3.0	#N/A	#N/A	#N/A
35	2	2	-7.0	-0.4480	-0.3650	0.3500	-4.5	0.6650	-0.0070	0.1610	-4.0				-4.0	-3.3040	-3.8081	-3.7451	-4.0				-4.0	93.6729	95.3859	86.3787	-4.0	#N/A	#N/A	#N/A
36	3	3	-8.0	9.4011	9.2121	10.2412	-5.5	20.1323		20.6783	-5.0	-3.4721	-3.7451	-4.1231	-5.0	-4.5221	-4.1441	-4.7321	-5.0		30.9055	26.5794	-5.0	-2.1280	-2.6320	-2.8630	-5.0	#N/A	#N/A	#N/A
37	4	1	-8.5	21.6863	25.5714	21.6653	-6.0	37.5626	36.5125	41.9096	-6.0	-4.2911	-4.0181	-3.1150	-6.0	-3.9551	-3.0940	-3.5561	-6.0	40.8386	37.6046	33.9505	-6.0	7.9311	33.0475	18.4103	-6.0	#N/A	#N/A	#N/A
38	5	2	-9.0	53.6488	51.0448	49.8477	-6.5	81.1382	80.1092	77.9042	-7.0	-2.9890	-2.0650	-1.7710	-7.0	-4.5641	-3.7661	-3.6611	-7.0	24.7524	45.5007	8.2671	-7.0	21.1613	38.2136	47.3487	-7.0	#N/A	#N/A	#N/A
39	6	3	-9.5	75.2581	85.5273	85.2333	-7.0	68.7060		60.5159	-8.0	-1.1830	2.8070	-2.8000	-8.0	-2.4220	-2.5690	-2.2750	-8.0		68.5590	62.7419	-8.0	7.4901	2.7440		-8.0	#N/A	#N/A	#N/A
40	7	1	-10.0			87.8583	-7.5	106.8006	106.9266	106.0656	-9.0	6.3981	6.9441		-9.0	12.1102		14.0212	-9.0	52.0738	70.9531	56.9879	-9.0	34.4965	42.3086	35.4625	-9.0	#N/A	#N/A	#N/A
41	8	2	-11.0	107.6826	94.2214	107.6406	-8.5	93.4024	109.7196	104.5116	-10.0	14.5672	7.3011		-10.0	51.1288	51.4228	56.1898	-10.0	50.4358	69.8610	58.7939	-10.0	11.6482	7.7841	7.4061	-10.0	#N/A	#N/A	#N/A
42	9	3																												
43	10	1	Standard Curve		TTN018 06-12-08		Positive Control		TTN018 06-12-08		1.0		TTN018 06-12-08		3.0		TTN018 06-12-08		20.0		TTN018 06-12-08		23.0		TTN018 06-12-08		Negative Control		Prism run ID & date	
44	11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
45	12	3	Best-fit values				Best-fit values				Best-fit values		Bad initial values		Best-fit values				Best-fit values		Ambiguous		Best-fit values		Not converged		Best-fit values			
46	13	1	BOTTOM		-0.4829		BOTTOM		-4.803		BOTTOM		-3.808		BOTTOM		-3.935		BOTTOM		33.99		BOTTOM		n/a		BOTTOM			
47	14	2	TOP		103		TOP		105.2		TOP		10.93		TOP		79.02		TOP		59.81		TOP		n/a		TOP			
48	15	3	LOGIC50		-8.966		LOGIC50		-6.135		LOGIC50		n/a		LOGIC50		-9.934		LOGIC50		-7.176		LOGIC50		n/a		LOGIC50			
49	16	1	HILLSLOPE		-1.055		HILLSLOPE		-0.8289		HILLSLOPE		n/a		HILLSLOPE		-1.039		HILLSLOPE		~ -30.48		HILLSLOPE		n/a		HILLSLOPE			
50	17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
51	18	3	BOTTOM		1.731		BOTTOM		9.856		BOTTOM				BOTTOM		0.677		BOTTOM		4.952		BOTTOM				BOTTOM			
52	19	1	TOP		2.357		TOP		6.894		TOP				TOP		46.29		TOP		118.9		TOP				TOP			
53	20	2	LOGIC50		0.02611		LOGIC50		0.08681		LOGIC50				LOGIC50		0.05067		LOGIC50		~ 6.671e+017		LOGIC50				LOGIC50			
54	21	3	HILLSLOPE		0.08688		HILLSLOPE		0.2415		HILLSLOPE				HILLSLOPE		0.4775		HILLSLOPE		~ 1.152e+020		HILLSLOPE				HILLSLOPE			
55	22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
56	23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
57	24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
58	25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
59	26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
60	27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
61	28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
62	29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
63	30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
64	31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
65	32	2	Data				Data				Data				Data				Data				Data				Data			
66	33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
67	34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
68	35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
69	36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.87

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Norethynodrel
Run ID: TTN 018
Assay start date: 6/12/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-4.0	32.9
1	1	2	-4.0	27.6
1	1	3	-4.0	29.6
1	2	1	-4.5	0.7
1	2	2	-4.5	0.0
1	2	3	-4.5	0.2
1	3	1	-5.5	20.1
1	3	2		
1	3	3	-5.5	20.7
1	4	1	-6.0	37.6
1	4	2	-6.0	36.5
1	4	3	-6.0	41.9
1	5	1	-6.5	81.1
1	5	2	-6.5	80.1
1	5	3	-6.5	77.9
1	6	1	-7.0	68.7
1	6	2		
1	6	3	-7.0	60.5
1	7	1	-7.5	106.8
1	7	2	-7.5	106.9
1	7	3	-7.5	106.1
1	8	1	-8.5	93.4
1	8	2	-8.5	109.7
1	8	3	-8.5	104.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.15

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Estradiol
Run ID: TTN 018
Assay start date: 6/12/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-7.0	0.3
1	1	2	-7.0	0.2
1	1	3	-7.0	0.0
1	2	1	-8.0	9.4
1	2	2	-8.0	9.2
1	2	3	-8.0	10.2
1	3	1	-8.5	21.7
1	3	2	-8.5	25.6
1	3	3	-8.5	21.7
1	4	1	-9.0	53.6
1	4	2	-9.0	51.0
1	4	3	-9.0	49.8
1	5	1	-9.5	75.3
1	5	2	-9.5	85.5
1	5	3	-9.5	85.2
1	6	1		
1	6	2		
1	6	3	-10.0	87.9
1	7	1	-11.0	107.7
1	7	2	-11.0	94.2
1	7	3	-11.0	107.6
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.12

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 1
Run ID: TTN 018
Assay start date: 6/12/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1		
1	1	2		
1	1	3		
1	2	1		
1	2	2		
1	2	3		
1	3	1	-5.0	-3.5
1	3	2	-5.0	-3.7
1	3	3	-5.0	-4.1
1	4	1	-6.0	-4.3
1	4	2	-6.0	-4.0
1	4	3	-6.0	-3.1
1	5	1	-7.0	-3.0
1	5	2	-7.0	-2.1
1	5	3	-7.0	-1.8
1	6	1	-8.0	-1.2
1	6	2	-8.0	2.8
1	6	3	-8.0	-2.8
1	7	1	-9.0	6.4
1	7	2	-9.0	6.9
1	7	3		
1	8	1	-10.0	14.6
1	8	2	-10.0	7.3
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.21

Provide information in all blue cells.

Laboratory Code: B

Compound ID: Chem Code 3
Run ID: TTN 018
Assay start date: 6/12/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	5.0
1	1	2	-3.0	7.1
1	1	3	-3.0	5.2
1	2	1	-4.0	-3.3
1	2	2	-4.0	-3.8
1	2	3	-4.0	-3.7
1	3	1	-5.0	-4.5
1	3	2	-5.0	-4.1
1	3	3	-5.0	-4.7
1	4	1	-6.0	-4.0
1	4	2	-6.0	-3.1
1	4	3	-6.0	-3.6
1	5	1	-7.0	-4.6
1	5	2	-7.0	-3.8
1	5	3	-7.0	-3.7
1	6	1	-8.0	-2.4
1	6	2	-8.0	-2.6
1	6	3	-8.0	-2.3
1	7	1	-9.0	12.1
1	7	2		
1	7	3	-9.0	14.0
1	8	1	-10.0	51.1
1	8	2	-10.0	51.4
1	8	3	-10.0	56.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 14.06

Provide information in all blue cells.

Laboratory Code: B

Compound ID:	Chem Code 20
Run ID:	TTN 015
Assay start date:	5/8/2008
Technician ID:	ttn

run	xgroup	rep	x	y
1	1	1	-3.0	74.7
1	1	2	-3.0	82.7
1	1	3	-3.0	82.2
1	2	1	-4.0	65.6
1	2	2	-4.0	41.3
1	2	3	-4.0	97.7
1	3	1		
1	3	2	-5.0	30.9
1	3	3	-5.0	26.6
1	4	1	-6.0	40.8
1	4	2	-6.0	37.6
1	4	3	-6.0	34.0
1	5	1	-7.0	24.8
1	5	2	-7.0	45.5
1	5	3	-7.0	8.3
1	6	1		
1	6	2	-8.0	68.6
1	6	3	-8.0	62.7
1	7	1	-9.0	52.1
1	7	2	-9.0	71.0
1	7	3	-9.0	57.0
1	8	1	-10.0	50.4
1	8	2	-10.0	69.9
1	8	3	-10.0	58.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.98

Provide information in all blue cells.

Laboratory Code: B

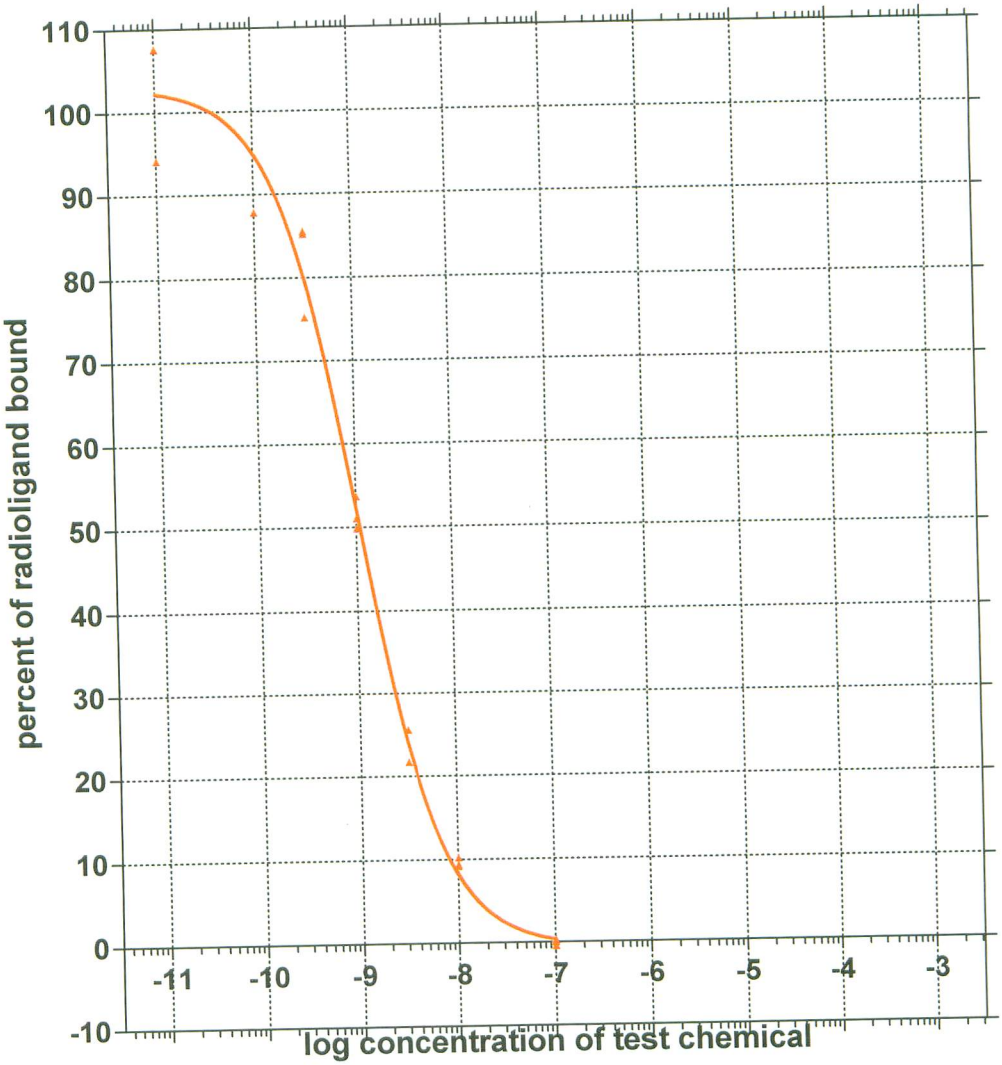
Compound ID: Chem Code 23
Run ID: TTN 018
Assay start date: 6/12/2008
Technician ID: ttn

run	xgroup	rep	x	y
1	1	1	-3.0	0.2
1	1	2	-3.0	0.8
1	1	3	-3.0	1.1
1	2	1	-4.0	-2.7
1	2	2	-4.0	-2.3
1	2	3	-4.0	-2.8
1	3	1	-5.0	-2.1
1	3	2	-5.0	-2.6
1	3	3	-5.0	-2.9
1	4	1	-6.0	7.9
1	4	2	-6.0	33.0
1	4	3	-6.0	18.4
1	5	1	-7.0	21.2
1	5	2	-7.0	38.2
1	5	3	-7.0	47.3
1	6	1	-8.0	7.5
1	6	2	-8.0	2.7
1	6	3		
1	7	1	-9.0	34.5
1	7	2	-9.0	42.3
1	7	3	-9.0	35.5
1	8	1	-10.0	11.6
1	8	2	-10.0	7.8
1	8	3	-10.0	7.4
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

able format: XY		X	A		
		log concentration	run TTN018 06-12-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.2870	0.2030	-0.0070
2	Title	-7.0	-0.4480	-0.3850	0.3500
3	Title	-8.0	9.4011	9.2121	10.2412
4	Title	-8.5	21.6863	25.5714	21.6653
5	Title	-9.0	53.6488	51.0448	49.8477
6	Title	-9.5	75.2581	85.5273	85.2333
7	Title	-10.0			87.8583
8	Title	-11.0	107.6826	94.2214	107.6406

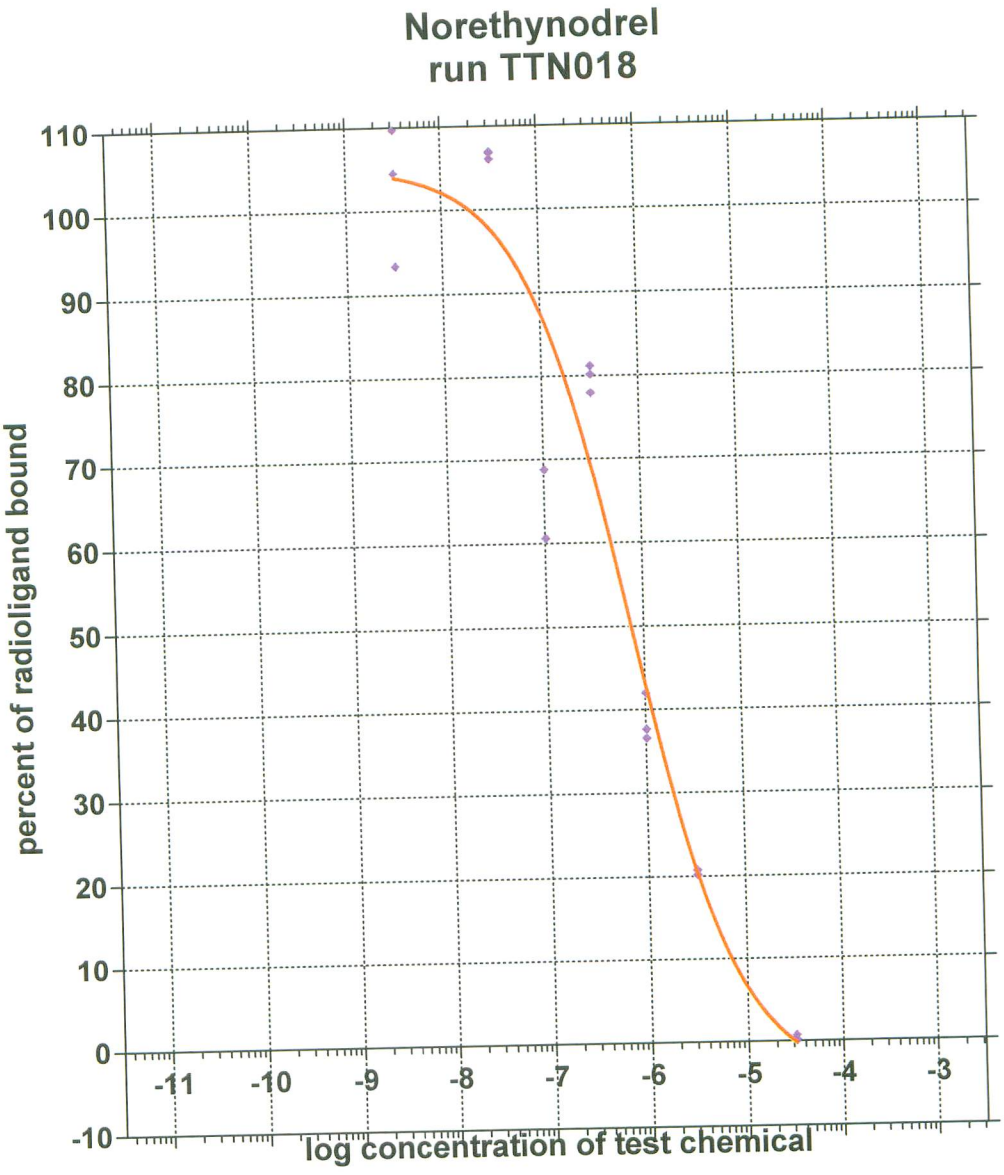
	Nonlin fit Table of results	A run TTN018 06-12-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.4829
4	TOP	103.0
5	LOGIC50	-8.966
6	HILLSLOPE	-1.055
7	Std. Error	
8	BOTTOM	1.731
9	TOP	2.357
10	LOGIC50	0.02611
11	HILLSLOPE	0.08688
12	95% Confidence Intervals	
13	BOTTOM	-4.120 to 3.154
14	TOP	98.06 to 108.0
15	LOGIC50	-9.021 to -8.911
16	HILLSLOPE	-1.237 to -0.8723
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9915
20	Absolute Sum of Squares	283.0
21	Sy.x	3.965
22	Number of points	
23	Analyzed	22

Estradiol reference standard
run TTN018



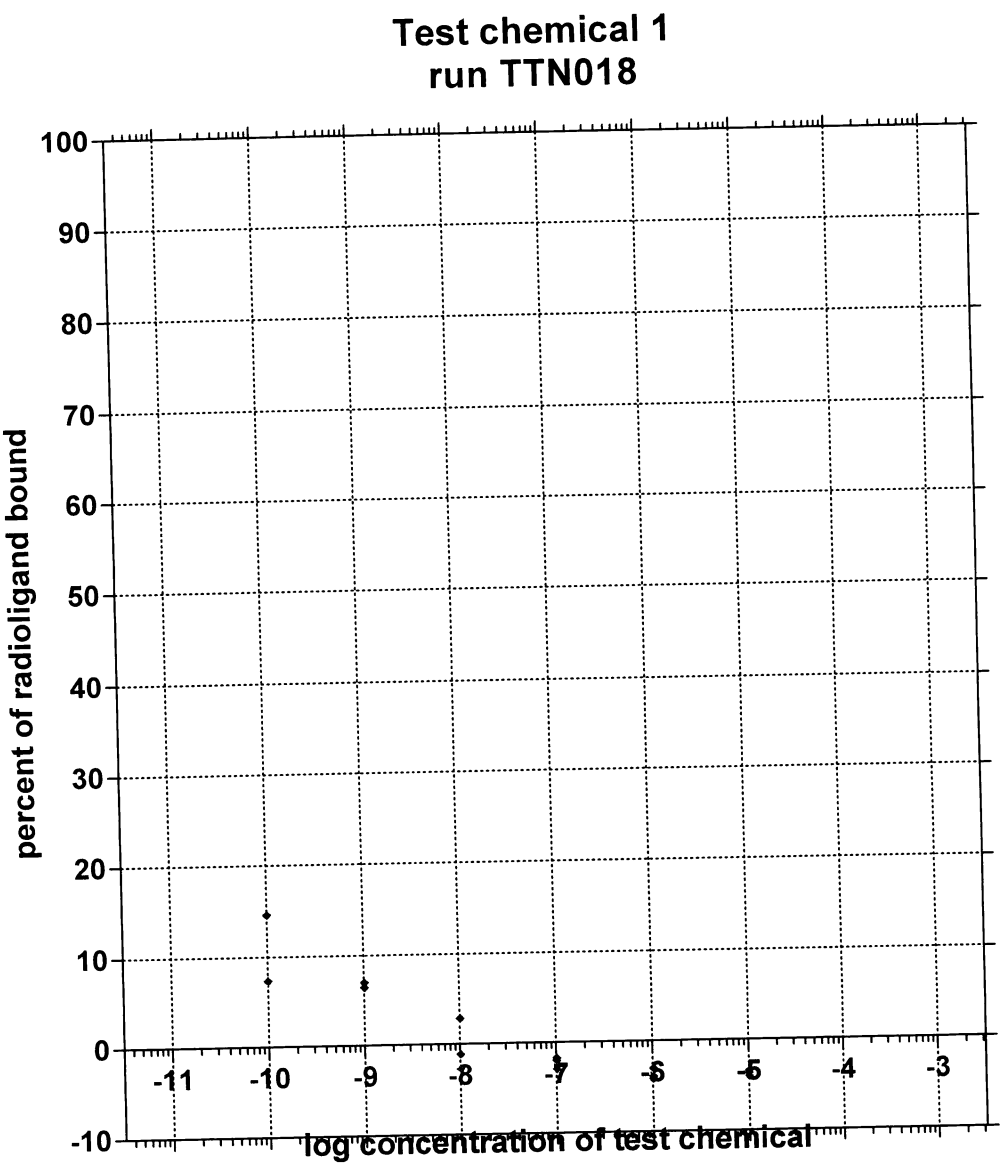
able format: XY		X	A		
		log concentration	run TTN018 06-12-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	0.6650	-0.0070	0.1610
3	Title	-5.5	20.1323		20.6783
4	Title	-6.0	37.5626	36.5125	41.9096
5	Title	-6.5	81.1382	80.1092	77.9042
6	Title	-7.0	68.7060		60.5159
7	Title	-7.5	106.8006	106.9266	106.0656
8	Title	-8.5	93.4024	109.7196	104.5116

	Nonlin fit Table of results	A run TTN018 06-12-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-4.803
4	TOP	105.2
5	LOGIC50	-6.135
6	HILLSLOPE	-0.8289
7	Std. Error	
8	BOTTOM	9.856
9	TOP	6.894
10	LOGIC50	0.08681
11	HILLSLOPE	0.2415
12	95% Confidence Intervals	
13	BOTTOM	-25.81 to 16.20
14	TOP	90.47 to 119.9
15	LOGIC50	-6.320 to -5.950
16	HILLSLOPE	-1.343 to -0.3142
17	Goodness of Fit	
18	Degrees of Freedom	15
19	R^2	0.9327
20	Absolute Sum of Squares	1920
21	Sy.x	11.31
22	Number of points	
23	Analyzed	19


Norethynodrel
run TTN018
percent of radioligand bound
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

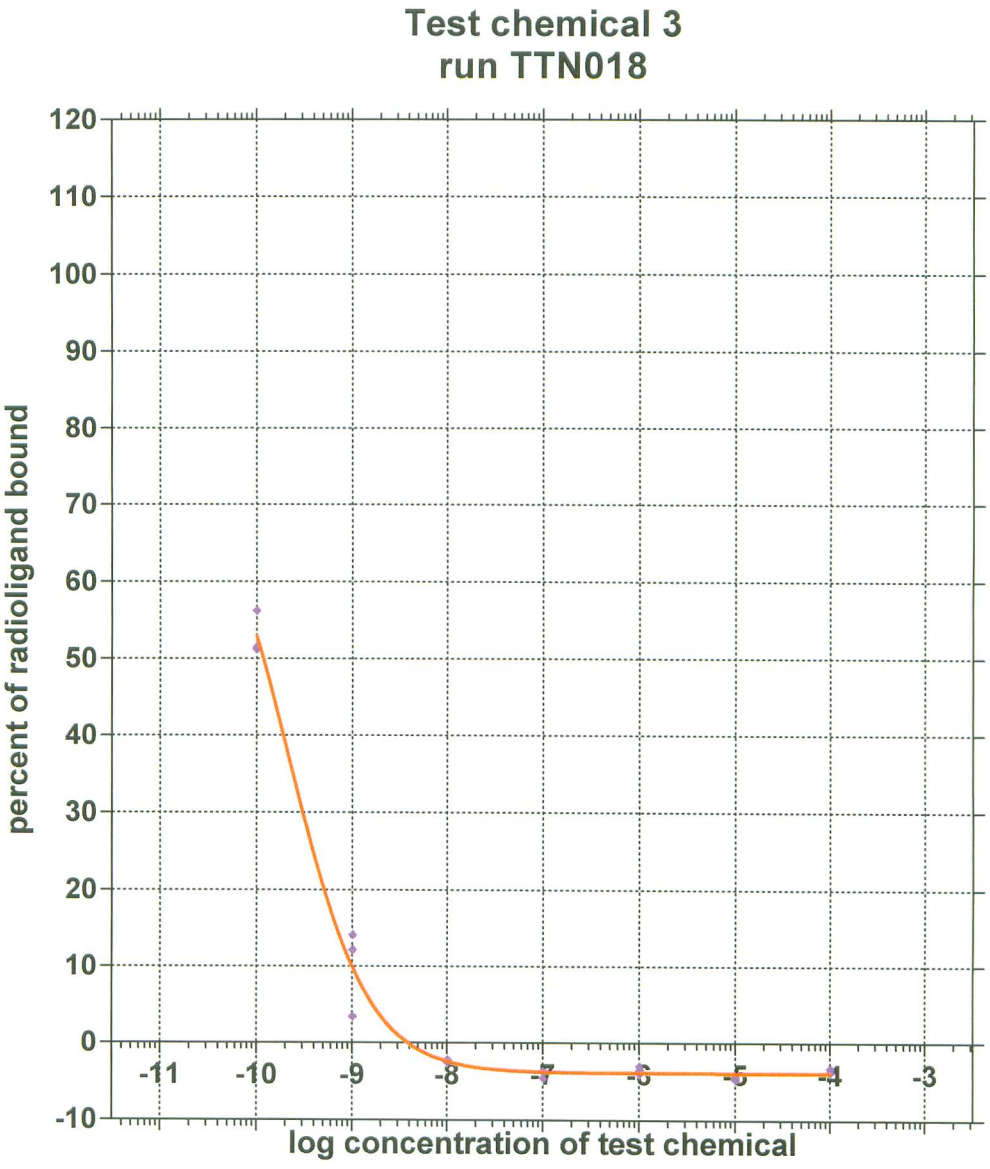
able format: XY		X	A			B		
		log concentration	Test chemical 1			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0	-3.4721	-3.7451	-4.1231			
4	Title	-6.0	-4.2911	-4.0181	-3.1150			
5	Title	-7.0	-2.9890	-2.0650	-1.7710			
6	Title	-8.0	-1.1830	2.8070	-2.8000			
7	Title	-9.0	6.3981	6.9441				
8	Title	-10.0	14.5672	7.3011				

	Nonlin fit Table of results	A Test chemical 1	B Data Set-B
		Y	Y
1	logIC50	Bad initial values	
2	Best-fit values		
3	BOTTOM	-3.808	
4	TOP	10.93	
5	LOGIC50	-8.560	
6	HILLSLOPE	-1.000	
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	16	


Test chemical 1
run TTN018
percent of radioligand bound
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

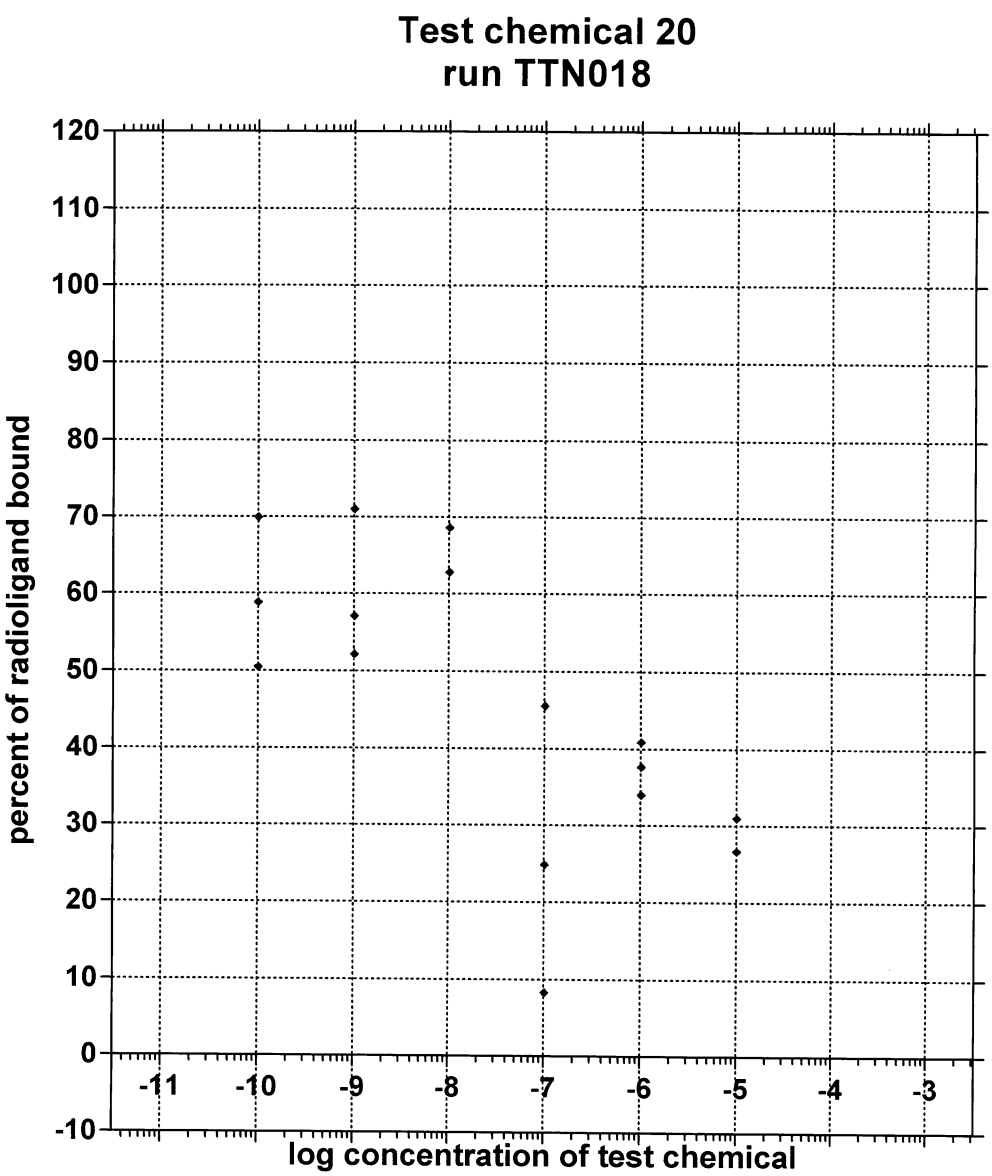
ble format: XY		X	A		
		log concentration	Test chemical 3		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-3.3040	-3.8081	-3.7451
3	Title	-5.0	-4.5221	-4.1441	-4.7321
4	Title	-6.0	-3.9551	-3.0940	-3.5561
5	Title	-7.0	-4.5641	-3.7661	-3.6611
6	Title	-8.0	-2.4220	-2.5690	-2.2750
7	Title	-9.0	12.1102	3.3741	14.0212
8	Title	-10.0	51.1288	51.4228	56.1898

	Nonlin fit Table of results	A Test chemical 3
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-3.935
4	TOP	79.02
5	LOGIC50	-9.934
6	HILLSLOPE	-1.039
7	Std. Error	
8	BOTTOM	0.6770
9	TOP	46.29
10	LOGIC50	0.05067
11	HILLSLOPE	0.4775
12	95% Confidence Intervals	
13	BOTTOM	-5.364 to -2.507
14	TOP	-18.64 to 176.7
15	LOGIC50	-10.04 to -9.827
16	HILLSLOPE	-2.047 to -0.03142
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9897
20	Absolute Sum of Squares	83.54
21	Sy.x	2.217
22	Number of points	
23	Analyzed	21


Test chemical 3
run TTN018
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

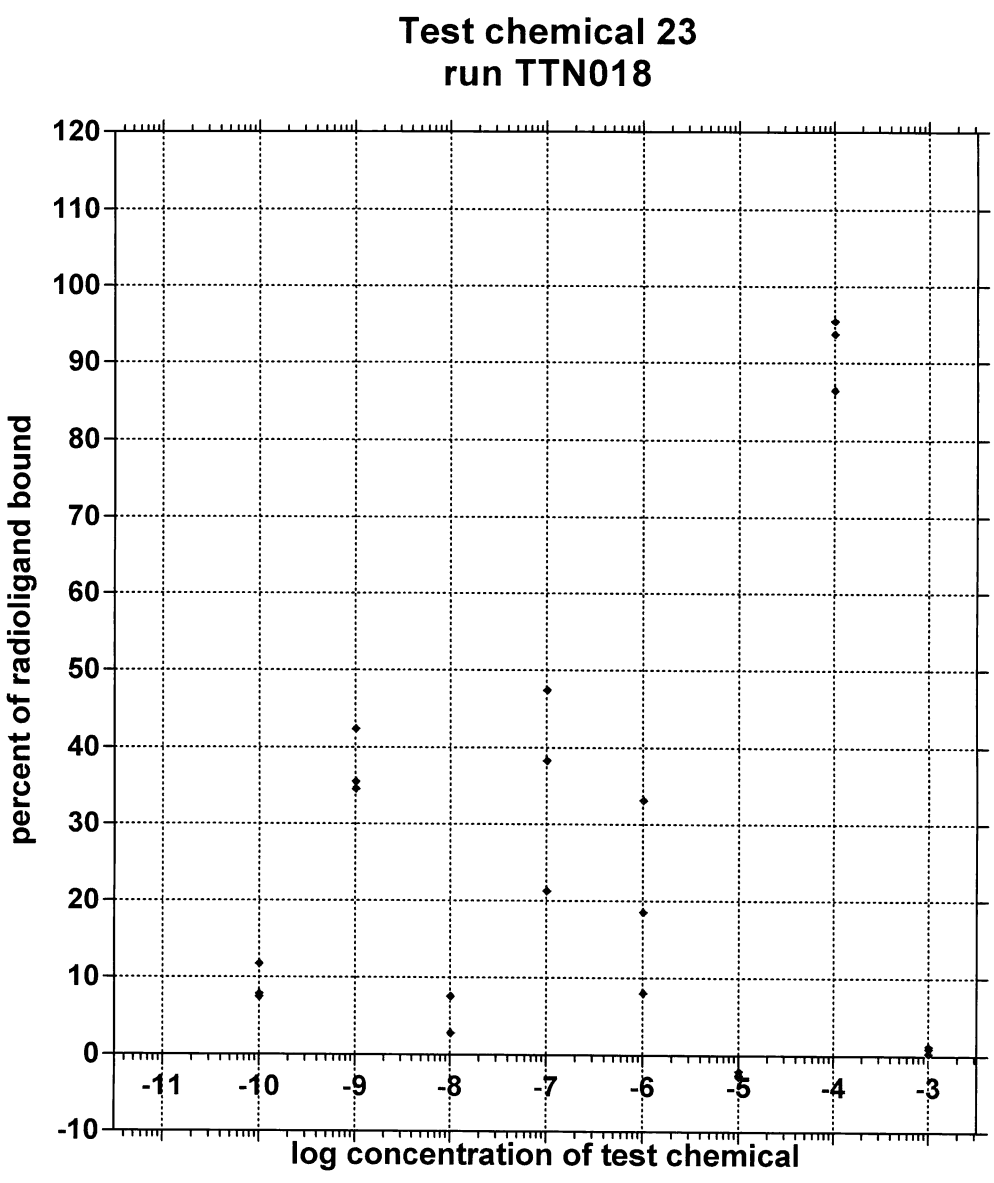
able format: XY		X	A			B		
		log concentration	Test chemical 20			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0						
2	Title	-4.0						
3	Title	-5.0		30.9055	26.5794			
4	Title	-6.0	40.8386	37.6046	33.9505			
5	Title	-7.0	24.7524	45.5007	8.2671			
6	Title	-8.0		68.5590	62.7419			
7	Title	-9.0	52.0738	70.9531	56.9879			
8	Title	-10.0	50.4358	69.8610	58.7939			

	Nonlin fit Table of results	A Test chemical 20	B Data Set-B
		Y	Y
1	logIC50	Ambiguous	
2	Best-fit values		
3	BOTTOM	33.99	
4	TOP	59.81	
5	LOGIC50	~ -7.176	
6	HILLSLOPE	~ -30.48	
7	Std. Error		
8	BOTTOM	4.952	
9	TOP	118.9	
10	LOGIC50	~ 6.671e+017	
11	HILLSLOPE	~ 1.152e+020	
12	95% Confidence Intervals		
13	BOTTOM	23.20 to 44.78	
14	TOP	-199.4 to 319.0	
15	LOGIC50	(Very wide)	
16	HILLSLOPE	(Very wide)	
17	Goodness of Fit		
18	Degrees of Freedom	12	
19	R^2	0.7084	
20	Absolute Sum of Squares	1471	
21	Sy.x	11.07	
22	Number of points		
23	Analyzed	16	


Test chemical 20
run TTN018
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

able format: XY		X	A			B		
		log concentration	Test chemical 23			Data Set-B		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	-3.0	0.2030	0.8330	1.1270			
2	Title	-4.0	93.6729	95.3859	86.3787			
3	Title	-5.0	-2.1280	-2.6320	-2.8630			
4	Title	-6.0	7.9311	33.0475	18.4103			
5	Title	-7.0	21.1613	38.2136	47.3487			
6	Title	-8.0	7.4901	2.7440				
7	Title	-9.0	34.4965	42.3086	35.4625			
8	Title	-10.0	11.6482	7.7841	7.4061			

	Nonlin fit Table of results	A Test chemical 23	B Data Set-B
		Y	Y
1	logIC50	Not converged	
2	Best-fit values		
3	BOTTOM		
4	TOP		
5	LOGIC50		
6	HILLSLOPE		
7	Std. Error		
8	BOTTOM		
9	TOP		
10	LOGIC50		
11	HILLSLOPE		
12	95% Confidence Intervals		
13	BOTTOM		
14	TOP		
15	LOGIC50		
16	HILLSLOPE		
17	Goodness of Fit		
18	Degrees of Freedom		
19	R^2		
20	Absolute Sum of Squares		
21	Sy.x		
22	Number of points		
23	Analyzed	23	


Test chemical 23
run TTN018
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

APPENDIX R

Excel and Prism Files of the Run Identifier RN004

ER Competitive Assay - Three Unknowns

159 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	RN004	
Assay start date:	4/24/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	109.09	Ci/mmole
Cytosol vial or lot identification:	RUC2-18-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.95	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-6.00	M
RBA, norethynodrel (Weak Positive):	1.125E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-5.90	6.0
RBA, Unknown 1:	8.810E-04	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-3.80	7.0
RBA, Unknown 2:	6.982E-06	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	n/a	18.0
RBA, Unknown 3:	n/a	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Provide information in all blue cells in column O, and cells AE43 : AU43 and AE48 : AU48.

If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AV contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 21 will contain output parameters from the nonlinear regression software. and the maximum concentrations for each of the unknowns.

Summary values	n	Mean	SD	
EtOH	6	6734.0	348.31	Total Binding, solvent control, tubes
Hot	6	96918.1	4540.47	Total hot E2 added to each tube
NSB	5	496.4	22.04	Nonspecific Binding
Specific EtOH	6	6237.5	348.31	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values			
EtOH / Hot	0.07	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.07	around 0.25 ?	

log conc'n at min hot binding	
cold E2	-7.0
R1881	#N/A
norethynodrel	-4.5
Unknown 1	-4.0
Unknown 2	-3.0
Unknown 3	-6.0

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

Check the 10% rule: 6.95%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red fo
(binding > 10% higher than at avg minimum) a

Competitive Assay Tube Layout - Three Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4072.10	6108.15	TRUE			—	90.0	6108.15	5611.7	91306.4	90.0
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4545.47	6818.21	TRUE			—	101.4	6818.205	6321.8	90596.3	101.4
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4645.26	6967.89	TRUE			—	103.8	6967.89	6471.5	90446.6	103.8
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	826.21	1239.32	FALSE	mispipet	cold E2	-7.0					
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	354.76	532.14	TRUE		cold E2	-7.0	0.6	532.14	35.7	96882.4	0.6
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	321.66	482.49	TRUE		cold E2	-7.0	-0.2	482.49	-13.9	96932.0	-0.2
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	821.86	1232.79	TRUE		cold E2	-8.0	11.8	1232.79	736.4	96181.7	11.8
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	771.01	1156.52	TRUE		cold E2	-8.0	10.6	1156.515	660.1	96258.0	10.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	715.52	1073.28	TRUE		cold E2	-8.0	9.2	1073.28	576.8	96341.2	9.2
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1425.29	2137.94	TRUE		cold E2	-8.5	26.3	2137.935	1641.5	95276.6	26.3
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1733.59	2600.39	TRUE		cold E2	-8.5	33.7	2600.385	2104.0	94814.1	33.7
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1417.47	2126.21	TRUE		cold E2	-8.5	26.1	2126.205	1629.8	95288.3	26.1
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2443.87	3665.81	TRUE		cold E2	-9.0	50.8	3665.805	3169.4	93748.7	50.8
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2568.86	3853.29	TRUE		cold E2	-9.0	53.8	3853.29	3356.9	93561.2	53.8
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2387.90	3581.85	TRUE		cold E2	-9.0	49.5	3581.85	3085.4	93832.7	49.5
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3493.60	5240.40	TRUE		cold E2	-9.5	76.1	5240.4	4744.0	92174.1	76.1
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3561.07	5341.61	TRUE		cold E2	-9.5	77.7	5341.605	4845.2	92072.9	77.7
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3461.14	5191.71	TRUE		cold E2	-9.5	75.3	5191.71	4695.3	92222.8	75.3
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	4056.28	6084.42	TRUE		cold E2	-10.0	89.6	6084.42	5588.0	91330.1	89.6
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3971.13	5956.70	TRUE		cold E2	-10.0	87.5	5956.695	5460.3	91457.8	87.5
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2462.67	3694.01	FALSE	mispipet	cold E2	-10.0					
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4264.55	6396.83	TRUE		cold E2	-11.0	94.6	6396.825	5900.4	91017.7	94.6
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4454.08	6681.12	TRUE		cold E2	-11.0	99.2	6681.12	6184.7	90733.4	99.2
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	4449.26	6673.89	TRUE		cold E2	-11.0	99.0	6673.89	6177.5	90740.6	99.0
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0					
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0					
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0					
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0					
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0					

Competitive Assay Tube Layout - Three Test Chemicals

Check the 10% rule: 6.5

e ratio of EtOH / Hot is > 10% then re are problems with the assay

Unknown

% binding >105 or < 0.9 (binding > 10% higher th

nted in red fo g minimum) a

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0					
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0					
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0					
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0					
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0					
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0					
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0					
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0					
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0					
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0					
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0					
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0					
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0					
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0					
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0					
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0					
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0					
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0					
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0					
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	3281.06	4921.59	TRUE		norethynodrel	-4.0	70.9	4921.59	4425.2	92492.9	70.9
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2758.83	4138.25	TRUE		norethynodrel	-4.0	58.4	4138.245	3641.8	93276.3	58.4
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	3045.84	4568.76	TRUE		norethynodrel	-4.0	65.3	4568.76	4072.3	92845.8	65.3
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	405.79	608.69	TRUE		norethynodrel	-4.5	1.8	608.685	112.3	96805.8	1.8
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	369.82	554.73	TRUE		norethynodrel	-4.5	0.9	554.73	58.3	96859.8	0.9
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	379.64	569.46	TRUE		norethynodrel	-4.5	1.2	569.46	73.0	96845.1	1.2
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1313.64	1970.46	TRUE		norethynodrel	-5.5	23.6	1970.46	1474.0	95444.1	23.6
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1365.82	2048.73	TRUE		norethynodrel	-5.5	24.9	2048.73	1552.3	95365.8	24.9
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1342.10	2013.15	TRUE		norethynodrel	-5.5	24.3	2013.15	1516.7	95401.4	24.3
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2368.43	3552.65	TRUE		norethynodrel	-6.0	49.0	3552.645	3056.2	93861.9	49.0
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2235.38	3353.07	TRUE		norethynodrel	-6.0	45.8	3353.07	2856.6	94061.4	45.8
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2224.65	3336.98	TRUE		norethynodrel	-6.0	45.5	3336.975	2840.5	94077.5	45.5
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3662.20	5493.30	TRUE		norethynodrel	-6.5	80.1	5493.3	4996.9	91921.2	80.1
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3520.98	5281.47	TRUE		norethynodrel	-6.5	76.7	5281.47	4785.0	92133.0	76.7
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3561.75	5342.63	TRUE		norethynodrel	-6.5	77.7	5342.625	4846.2	92071.9	77.7
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4117.79	6176.69	TRUE		norethynodrel	-7.0	91.1	6176.685	5680.3	91237.8	91.1
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4166.00	6249.00	TRUE		norethynodrel	-7.0	92.2	6249	5752.6	91165.5	92.2
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	4142.91	6214.37	TRUE		norethynodrel	-7.0	91.7	6214.365	5717.9	91200.1	91.7
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3962.54	5943.81	TRUE		norethynodrel	-7.5	87.3	5943.81	5447.4	91470.7	87.3
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4152.81	6229.22	TRUE		norethynodrel	-7.5	91.9	6229.215	5732.8	91185.3	91.9
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	4148.83	6223.25	TRUE		norethynodrel	-7.5	91.8	6223.245	5726.8	91191.3	91.8
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4322.47	6483.71	TRUE		norethynodrel	-8.5	96.0	6483.705	5987.3	90930.8	96.0
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4355.46	6533.19	TRUE		norethynodrel	-8.5	96.8	6533.19	6036.8	90881.3	96.8
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	4277.55	6416.33	TRUE		norethynodrel	-8.5	94.9	6416.325	5919.9	90998.2	94.9
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	16369.65	24554.48	FALSE	mispipet		Unknown 1	-3.0					
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	41414.88	62122.32	FALSE	mispipet		Unknown 1	-3.0					
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	10875.16	16312.74	FALSE	mispipet		Unknown 1	-3.0					
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2698.20	4047.30	FALSE	mispipet		Unknown 1	-4.0					
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1439.36	2159.04	FALSE	mispipet		Unknown 1	-4.0					
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	325.01	487.52	TRUE			Unknown 1	-4.0	-0.1	487.515	-8.9	96927.0	-0.1
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	796.19	1194.29	TRUE			Unknown 1	-5.0	11.2	1194.285	697.9	96220.2	11.2
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	772.08	1158.12	TRUE			Unknown 1	-5.0	10.6	1158.12	661.7	96256.4	10.6
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	753.62	1130.43	TRUE			Unknown 1	-5.0	10.2	1130.43	634.0	96284.1	10.2
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2628.20	3942.30	TRUE			Unknown 1	-6.0	55.2	3942.3	3445.9	93472.2	55.2
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2619.47	3929.21	TRUE			Unknown 1	-6.0	55.0	3929.205	3432.8	93485.3	55.0
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2705.00	4057.50	TRUE			Unknown 1	-6.0	57.1	4057.5	3561.1	93357.0	57.1
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4047.75	6071.63	TRUE			Unknown 1	-7.0	89.4	6071.625	5575.2	91342.9	89.4
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4057.37	6086.06	TRUE			Unknown 1	-7.0	89.6	6086.055	5589.6	91328.5	89.6
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4228.40	6342.60	TRUE			Unknown 1	-7.0	93.7	6342.6	5846.2	91071.9	93.7
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4587.72	6881.58	TRUE			Unknown 1	-8.0	102.4	6881.58	6385.1	90532.9	102.4
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4478.11	6717.17	TRUE			Unknown 1	-8.0	99.7	6717.165	6220.7	90697.3	99.7
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4375.06	6562.59	TRUE			Unknown 1	-8.0	97.3	6562.59	6066.2	90851.9	97.3
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4234.83	6352.25	TRUE			Unknown 1	-9.0	93.9	6352.245	5855.8	91062.3	93.9
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4248.29	6372.44	TRUE			Unknown 1	-9.0	94.2	6372.435	5876.0	91042.1	94.2
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4154.63	6231.95	TRUE			Unknown 1	-9.0	92.0	6231.945	5735.5	91182.6	92.0
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4315.94	6473.91	TRUE			Unknown 1	-10.0	95.8	6473.91	5977.5	90940.6	95.8
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4357.98	6536.97	TRUE			Unknown 1	-10.0	96.8	6536.97	6040.5	90877.5	96.8
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3954.92	5932.38	TRUE			Unknown 1	-10.0	87.1	5932.38	5435.9	91482.1	87.1
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	433.96	650.94	TRUE			Unknown 2	-3.0	2.5	650.94	154.5	96763.6	2.5
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	448.66	672.99	TRUE			Unknown 2	-3.0	2.8	672.99	176.6	96741.5	2.8
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1016.81	1525.22	FALSE	mispipet		Unknown 2	-3.0					
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2927.23	4390.85	TRUE			Unknown 2	-4.0	62.4	4390.845	3894.4	93023.7	62.4
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3025.54	4538.31	TRUE			Unknown 2	-4.0	64.8	4538.31	4041.9	92876.2	64.8
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3121.78	4682.67	TRUE			Unknown 2	-4.0	67.1	4682.67	4186.2	92731.8	67.1

Competitive Assay Tube Layout - Three Test Chemicals

Check the 10% rule: 6

...e ratio of EtOH / Hot is > 10% then ...ere are problems with the assay

Unknown

% binding >105 or < 0.9 (binding > 10% higher t...

...hted in red fo... ...g minimum) ...

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4229.71	6344.57	TRUE		Unknown 2	-5.0	93.8	6344.565	5848.1	91069.9	93.8
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4189.41	6284.12	TRUE		Unknown 2	-5.0	92.8	6284.115	5787.7	91130.4	92.8
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4305.65	6458.48	TRUE		Unknown 2	-5.0	95.6	6458.475	5962.0	90956.0	95.6
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4285.46	6428.19	TRUE		Unknown 2	-6.0	95.1	6428.19	5931.8	90986.3	95.1
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3584.37	5376.56	TRUE		Unknown 2	-6.0	78.2	5376.555	4880.1	92038.0	78.2
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4832.75	7249.13	TRUE		Unknown 2	-6.0	108.3	7249.125	6752.7	90165.4	108.3
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4186.00	6279.00	TRUE		Unknown 2	-7.0	92.7	6279	5782.6	91135.5	92.7
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4577.44	6866.16	TRUE		Unknown 2	-7.0	102.1	6866.16	6369.7	90548.4	102.1
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4336.80	6505.20	TRUE		Unknown 2	-7.0	96.3	6505.2	6008.8	90909.3	96.3
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4693.66	7040.49	TRUE		Unknown 2	-8.0	104.9	7040.49	6544.1	90374.0	104.9
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4449.27	6673.91	TRUE		Unknown 2	-8.0	99.0	6673.905	6177.5	90740.6	99.0
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4548.98	6823.47	TRUE		Unknown 2	-8.0	101.4	6823.47	6327.0	90591.0	101.4
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3253.51	4880.27	TRUE		Unknown 2	-9.0	70.3	4880.265	4383.8	92534.2	70.3
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4508.75	6763.13	TRUE		Unknown 2	-9.0	100.5	6763.125	6266.7	90651.4	100.5
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4130.63	6195.95	TRUE		Unknown 2	-9.0	91.4	6195.945	5699.5	91218.6	91.4
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4362.77	6544.16	TRUE		Unknown 2	-10.0	97.0	6544.155	6047.7	90870.4	97.0
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4428.93	6643.40	TRUE		Unknown 2	-10.0	98.5	6643.395	6147.0	90771.1	98.5
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4611.84	6917.76	TRUE		Unknown 2	-10.0	102.9	6917.76	6421.3	90496.8	102.9
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3503.23	5254.85	TRUE		Unknown 3	-3.0	76.3	5254.845	4758.4	92159.7	76.3
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3803.17	5704.76	TRUE		Unknown 3	-3.0	83.5	5704.755	5208.3	91709.8	83.5
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3765.05	5647.58	TRUE		Unknown 3	-3.0	82.6	5647.575	5151.1	91766.9	82.6
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4219.65	6329.48	TRUE		Unknown 3	-4.0	93.5	6329.475	5833.0	91085.0	93.5
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4344.25	6516.38	TRUE		Unknown 3	-4.0	96.5	6516.375	6019.9	90898.1	96.5
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	4313.34	6470.01	TRUE		Unknown 3	-4.0	95.8	6470.01	5973.6	90944.5	95.8
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4402.15	6603.23	TRUE		Unknown 3	-5.0	97.9	6603.225	6106.8	90811.3	97.9
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4384.52	6576.78	TRUE		Unknown 3	-5.0	97.5	6576.78	6080.3	90837.7	97.5
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	4332.75	6499.13	TRUE		Unknown 3	-5.0	96.2	6499.125	6002.7	90915.4	96.2
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3413.17	5119.76	TRUE		Unknown 3	-6.0	74.1	5119.755	4623.3	92294.8	74.1
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	4374.39	6561.59	FALSE	mispipet	Unknown 3	-6.0					
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3314.81	4972.22	TRUE		Unknown 3	-6.0	71.8	4972.215	4475.8	92442.3	71.8
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4489.12	6733.68	TRUE		Unknown 3	-7.0	100.0	6733.68	6237.2	90680.8	100.0
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1771.50	2657.25	FALSE	mispipet	Unknown 3	-7.0					
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	4420.85	6631.28	TRUE		Unknown 3	-7.0	98.4	6631.275	6134.8	90783.2	98.4
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4225.58	6338.37	TRUE		Unknown 3	-8.0	93.7	6338.37	5841.9	91076.1	93.7
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4349.41	6524.12	TRUE		Unknown 3	-8.0	96.6	6524.115	6027.7	90890.4	96.6
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	4291.81	6437.72	TRUE		Unknown 3	-8.0	95.3	6437.715	5941.3	90976.8	95.3
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4345.45	6518.18	TRUE		Unknown 3	-9.0	96.5	6518.175	6021.7	90896.3	96.5
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4613.84	6920.76	TRUE		Unknown 3	-9.0	103.0	6920.76	6424.3	90493.8	103.0
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	4188.00	6282.00	TRUE		Unknown 3	-9.0	92.8	6282	5785.6	91132.5	92.8
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4332.87	6499.31	TRUE		Unknown 3	-10.0	96.2	6499.305	6002.9	90915.2	96.2
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4403.31	6604.97	TRUE		Unknown 3	-10.0	97.9	6604.965	6108.5	90809.5	97.9
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	4101.94	6152.91	TRUE		Unknown 3	-10.0	90.7	6152.91	5656.5	91261.6	90.7
145	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	335.75	503.63	TRUE		cold E2	-7.0	0.1	503.625	7.2	96910.9	0.1
146	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	320.67	481.01	TRUE		cold E2	-7.0	-0.2	481.005	-15.4	96933.5	-0.2
147	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	321.93	482.90	TRUE		cold E2	-7.0	-0.2	482.895	-13.5	96931.6	-0.2
148	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4486.95	6730.43	TRUE			—	99.9	6730.425	6234.0	90684.1	99.9
149	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4745.58	7118.37	TRUE			—	106.2	7118.37	6621.9	90296.1	106.2
150	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	4440.55	6660.83	TRUE			—	98.8	6660.825	6164.4	90753.7	98.8
151	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0					
152	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0					
153	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0					
154	1	none	Hot		—	—	—	10	—	—	—	—	96766.64	96766.64	TRUE					96766.64	96270.2		
155	2	none	Hot		—	—	—	10	—	—	—	—	97684.70	97684.70	TRUE					97684.7	97188.3		
156	3	none	Hot		—	—	—	10	—	—	—	—	98097.50	98097.50	TRUE					98097.5	97601.1		
157	1	none	Hot		—	—	—	10	—	—	—	—	102977.50	102977.50	TRUE					102977.5	102481.1		
158	2	none	Hot		—	—	—	10	—	—	—	—	88897.28	88897.28	TRUE					88897.28	88400.8		
159	3	none	Hot		—	—	—	10	—	—	—	—	97084.86	97084.86	TRUE					97084.86	96588.4		

Prism data, ex[illegible] data points in the right leg of U-shaped curves

		standard curve			weak positive			6.0			7.0			18.0			negative control		
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0		0.5725	0.2230	-4.0				-3.0	0.0000	0.0000	0.0000	-3.0	2.4771	2.8306		-3.0	76.2866	83.4996	82.5829	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.1351	-0.2473	-0.2170	-4.5	1.7995	0.9346	1.1708	-4.0	-0.0000	0.0000	0.0000	-4.0	62.4350	64.7992	67.1136	-4.0	93.5150	96.5114	95.7681	-4.0	#N/A	#N/A	#N/A
3	3	-6.0	11.8053	10.5824	9.2480	-5.5	23.6316	24.8864	24.3160	-5.0	11.1880	10.6082	10.1642	-5.0	93.7570	92.7878	95.5832	-5.0	97.9038	97.4798	96.2349	-5.0	#N/A	#N/A	#N/A
4	1	-6.5	26.3165	33.7305	26.1284	-6.0	48.9971	45.7975	45.5394	-6.0	55.2440	55.0340	57.0909	-6.0	95.0976	78.2379	108.2588	-6.0	74.1209		71.7555	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	50.8112	53.8170	49.4653	-6.5	80.1095	76.7135	77.6939	-7.0	89.3812	89.8125	93.7255	-7.0	92.7058	102.1191	96.3323	-7.0	99.9952		98.3535	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	76.0550	77.6776	75.2745	-7.0	91.9655	92.2249	91.6696	-8.0	102.3664	99.7305	97.2523	-8.0	104.9140	99.0369	101.4347	-8.0	93.6576	96.6355	95.2503	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	89.5863	87.5387		-7.5	87.3321	91.9077	91.8120	-9.0	93.8801	94.2038	91.9514	-9.0	70.2814	100.4673	91.3743	-9.0	96.5403	102.9945	92.7539	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	94.5948	99.1526	99.0367	-8.5	95.9876	96.7810	94.9074	-10.0	95.8306	96.8416	87.1488	-10.0	96.9568	98.5478	102.9464	-10.0	96.2377	97.9317	90.6844	-10.0	#N/A	#N/A	#N/A
9	3																								
10	1	Standard Curve		RN004 4-24-08		Positive Control		RN004 4-24-08		6.0		RN004 4-24-08		7.0		RN004 4-24-08		18.0		RN004 4-24-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				Best-fit values				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				BOTTOM		72.94		Best-fit values			
13	1	BOTTOM		-1.909		BOTTOM		0.1788		BOTTOM		0.4869		BOTTOM		-7.978		TOP		99.17		BOTTOM			
14	2	TOP		98.93		TOP		95.29		TOP		95.65		TOP		95.97		LOGIC50		n/a		TOP			
15	3	LOGIC50		-8.952		LOGIC50		-6.003		LOGIC50		-5.897		LOGIC50		-3.796		HILLSLOPE		n/a		LOGIC50			
16	1	HILLSLOPE		-0.8988		HILLSLOPE		-1.138		HILLSLOPE		-1.104		HILLSLOPE		-1.311		Std. Error				HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				BOTTOM				Std. Error			
18	3	BOTTOM		1.157		BOTTOM		1.999		TOP		3.564		TOP		35.44		TOP				BOTTOM			
19	1	TOP		1.338		TOP		1.236		LOGIC50		1.17		LOGIC50		2.27		LOGIC50				TOP			
20	2	LOGIC50		0.01561		LOGIC50		0.01975		HILLSLOPE		0.03454		HILLSLOPE		0.1665		HILLSLOPE				LOGIC50			
21	3	HILLSLOPE		0.04322		HILLSLOPE		0.07997				0.1521				1.197						HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Dearees of Freedom				Dearees of Freedom				Dearees of Freedom				Dearees of Freedom				Dearees of Freedom				Dearees of Freedom			
29	2	R²				R²				R²				R²				R²				R²			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																								

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				6.0				7.0				18.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0		0.5735	-0.7235	-4.0				-3.0	0.0000	0.0000	0.0000	-3.0	2.4771	2.8306		-3.0	76.2865	83.4996	82.5829	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	0.1453	-0.5473	-0.2170	-4.5	1.7996	0.9348	1.1708	-4.0	0.0000	0.0000	0.0000	-4.0	62.4350	64.7992	67.1136	-4.0	93.5150	96.5114	95.7681	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	11.8053	10.5824	9.2480	-5.5	23.6316	24.8864	24.3160	-5.0	11.1880	10.6082	10.1642	-5.0	93.7570	92.7878	95.5832	-5.0	97.9038	97.4798	96.2349	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	26.3165	33.7305	26.1284	-6.0	48.9971	45.7975	45.5394	-6.0	55.2440	55.0340	57.0909	-6.0	95.0976	78.2379	108.2585	-6.0	74.1209		71.7555	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	50.8112	53.8170	49.4653	-6.5	80.1095	76.7135	77.6939	-7.0	89.3812	89.6125	93.7256	-7.0	92.7056	102.1191	96.3323	-7.0	99.9952		98.3535	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	76.0550	77.6776	75.2745	-7.0	91.0655	92.2249	91.6696	-8.0	102.3664	99.7305	97.2523	-8.0	104.9140	99.0369	101.4347	-8.0	93.6576	96.6355	95.2503	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	89.5863	87.5387		-7.5	87.3321	91.9077	91.8120	-9.0	93.8801	94.2038	91.9514	-9.0	70.2814	100.4673	91.3743	-9.0	96.5403	102.9945	92.7539	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	94.5948	99.1526	99.0367	-8.5	95.9876	96.7810	94.9074	-10.0	95.8306	96.8416	87.1488	-10.0	96.9568	98.5478	102.9464	-10.0	96.2377	97.9317	90.6844	-10.0	#N/A	#N/A	#N/A
9	3																								
10	1	Standard Curve	RN004 4-24-08			Positive Control	RN004 4-24-08			6.0	RN004 4-24-08			7.0	RN004 4-24-08			18.0	RN004 4-24-08			Negative Control	Prism run ID & date		
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values	Bad initial values			Best-fit values			
13	1	BOTTOM	-1.909			BOTTOM	0.1788			BOTTOM	0.4869			BOTTOM	-7.978			BOTTOM	72.94			BOTTOM			
14	2	TOP	98.93			TOP	95.29			TOP	95.65			TOP	95.97			TOP	99.17			TOP			
15	3	LOGIC50	-8.952			LOGIC50	-6.003			LOGIC50	-5.897			LOGIC50	-3.796			LOGIC50	n/a			LOGIC50			
16	1	HILLSLOPE	-0.8986			HILLSLOPE	-1.138			HILLSLOPE	-1.104			HILLSLOPE	-1.311			HILLSLOPE	n/a			HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM	1.157			BOTTOM	1.999			BOTTOM	3.564			BOTTOM	35.44			BOTTOM				BOTTOM			
19	1	TOP	1.338			TOP	1.238			TOP	1.17			TOP	2.27			TOP				TOP			
20	2	LOGIC50	0.01581			LOGIC50	0.01975			LOGIC50	0.03454			LOGIC50	0.1665			LOGIC50				LOGIC50			
21	3	HILLSLOPE	0.04322			HILLSLOPE	0.07997			HILLSLOPE	0.1521			HILLSLOPE	1.197			HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				95% Confidence Intervals			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Goodness of Fit			
29	2	R^2				R^2				R^2				R^2				R^2				Degrees of Freedom			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				R^2			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Absolute Sum of Squares			
32	2	Data				Data				Data				Data				Data				Sy.x			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Data			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of X values			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Number of Y replicates			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Total number of values			
37	1																					Number of missing values			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.4

Provide information in all blue cells.

			run	xgroup	rep	x	y
Laboratory Code:	TRL B						
Compound ID:	Estradiol		1	1	1		
Run ID:	RN004		1	1	2	-7.0	0.6
Assay start date:		4/24/08	1	1	3	-7.0	-0.2
Technician ID:	RN		1	2	1	-8.0	11.8
			1	2	2	-8.0	10.6
			1	2	3	-8.0	9.2
			1	3	1	-8.5	26.3
			1	3	2	-8.5	33.7
			1	3	3	-8.5	26.1
			1	4	1	-9.0	50.8
			1	4	2	-9.0	53.8
			1	4	3	-9.0	49.5
			1	5	1	-9.5	76.1
			1	5	2	-9.5	77.7
			1	5	3	-9.5	75.3
			1	6	1	-10.0	89.6
			1	6	2	-10.0	87.5
			1	6	3		
			1	7	1	-11.0	94.6
			1	7	2	-11.0	99.2
			1	7	3	-11.0	99.0
			1	8	1		
			1	8	2		
			1	8	3		
			1	9	1		
			1	9	2		
			1	9	3		
			1	10	1		
			1	10	2		
			1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.6

Provide information in all blue cells.

Laboratory Code:	TRL B
Compound ID:	Norethynodrel
Run ID:	RN004
Assay start date:	4/24/08
Technician ID:	RN

run	xgroup	rep	x	y
1	1	1	-4.0	70.9
1	1	2	-4.0	58.4
1	1	3	-4.0	65.3
1	2	1	-4.5	1.8
1	2	2	-4.5	0.9
1	2	3	-4.5	1.2
1	3	1	-5.5	23.6
1	3	2	-5.5	24.9
1	3	3	-5.5	24.3
1	4	1	-6.0	49.0
1	4	2	-6.0	45.8
1	4	3	-6.0	45.5
1	5	1	-6.5	80.1
1	5	2	-6.5	76.7
1	5	3	-6.5	77.7
1	6	1	-7.0	91.1
1	6	2	-7.0	92.2
1	6	3	-7.0	91.7
1	7	1	-7.5	87.3
1	7	2	-7.5	91.9
1	7	3	-7.5	91.8
1	8	1	-8.5	96.0
1	8	2	-8.5	96.8
1	8	3	-8.5	94.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.7

Provide information in all blue cells.
Laboratory Code: TRL B
Compound ID: Chem Code 6
Run ID: RN004
Assay start date: 4/24/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1		
1	1	2		
1	1	3		
1	2	1		
1	2	2		
1	2	3	-4.0	-0.1
1	3	1	-5.0	11.2
1	3	2	-5.0	10.6
1	3	3	-5.0	10.2
1	4	1	-6.0	55.2
1	4	2	-6.0	55.0
1	4	3	-6.0	57.1
1	5	1	-7.0	89.4
1	5	2	-7.0	89.6
1	5	3	-7.0	93.7
1	6	1	-8.0	102.4
1	6	2	-8.0	99.7
1	6	3	-8.0	97.3
1	7	1	-9.0	93.9
1	7	2	-9.0	94.2
1	7	3	-9.0	92.0
1	8	1	-10.0	95.8
1	8	2	-10.0	96.8
1	8	3	-10.0	87.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.0

Provide information in all blue cells.
Laboratory Code: TRL B

Compound ID: Chem Code 18
Run ID: RN004
Assay start date: 4/24/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	76.3
1	1	2	-3.0	83.5
1	1	3	-3.0	82.6
1	2	1	-4.0	93.5
1	2	2	-4.0	96.5
1	2	3	-4.0	95.8
1	3	1	-5.0	97.9
1	3	2	-5.0	97.5
1	3	3	-5.0	96.2
1	4	1	-6.0	74.1
1	4	2		
1	4	3	-6.0	71.8
1	5	1	-7.0	100.0
1	5	2		
1	5	3	-7.0	98.4
1	6	1	-8.0	93.7
1	6	2	-8.0	96.6
1	6	3	-8.0	95.3
1	7	1	-9.0	96.5
1	7	2	-9.0	103.0
1	7	3	-9.0	92.8
1	8	1	-10.0	96.2
1	8	2	-10.0	97.9
1	8	3	-10.0	90.7
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run RN004 4-24-08		
		X	A:Y1	A:Y2	A:Y3
	Title	-7.0		0.5725	-0.2235
2	Title	-7.0	0.1153	-0.2473	-0.2170
3	Title	-8.0	11.8053	10.5824	9.2480
4	Title	-8.5	26.3165	33.7305	26.1284
5	Title	-9.0	50.8112	53.8170	49.4653
6	Title	-9.5	76.0550	77.6776	75.2745
7	Title	-10.0	89.5863	87.5387	
8	Title	-11.0	94.5948	99.1526	99.0367

	Nonlin fit Table of results	A run RN004 4-24-08
		Y
	logIC50	
2	Best-fit values	
3	BOTTOM	-1.909
4	TOP	98.93
5	LOGIC50	-8.952
6	HILLSLOPE	-0.8986
7	Std. Error	
8	BOTTOM	1.157
9	TOP	1.338
10	LOGIC50	0.01581
11	HILLSLOPE	0.04322
12	95% Confidence Intervals	
13	BOTTOM	-4.340 to 0.5211
14	TOP	96.12 to 101.7
15	LOGIC50	-8.986 to -8.919
16	HILLSLOPE	-0.9894 to -0.8078
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9973
20	Absolute Sum of Squares	80.49
1	Sy.x	2.115
22	Number of points	
23	Analyzed	22

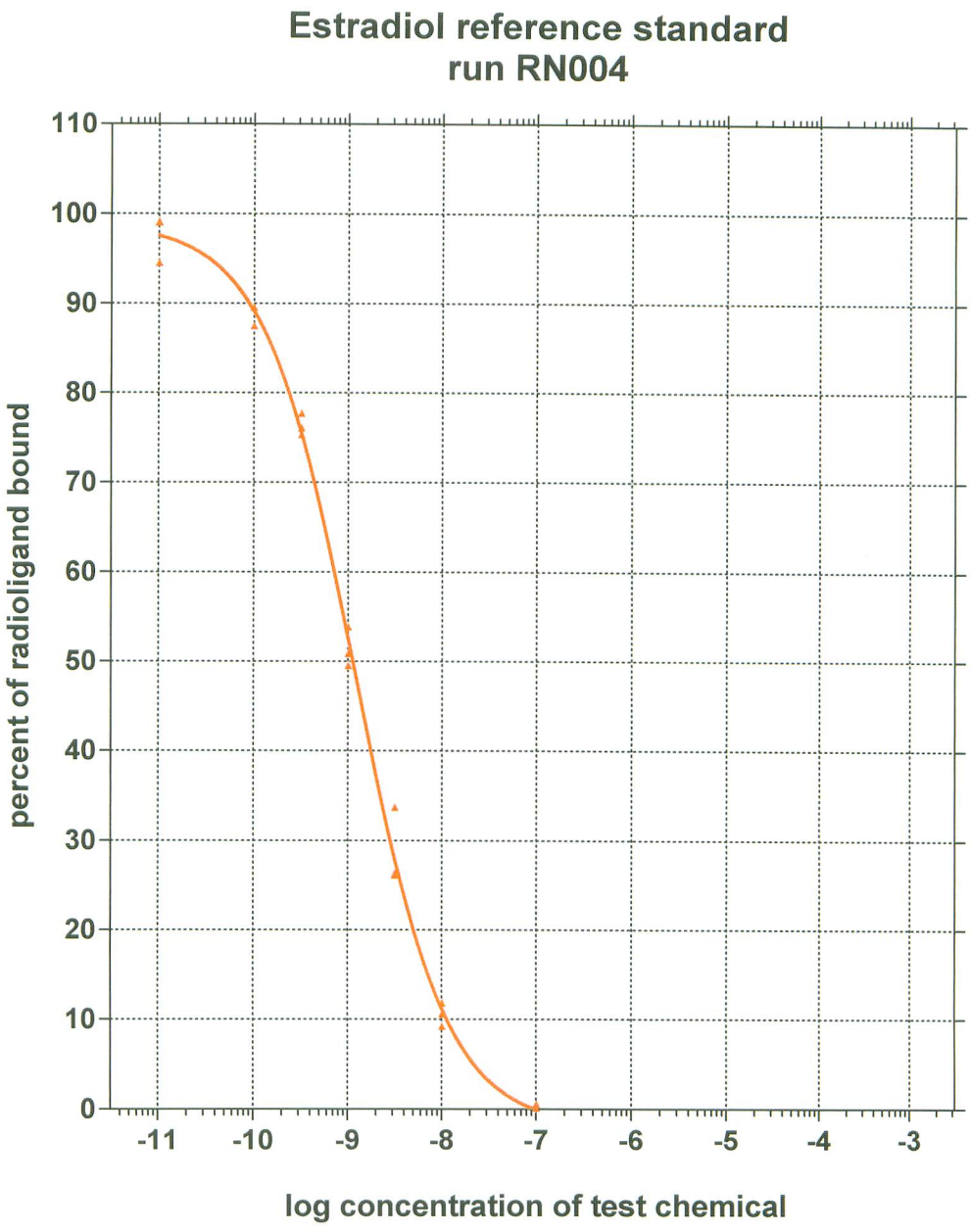

Estradiol reference standard
run RN004
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run RN004 4-24-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	1.7996	0.9346	1.1708
3	Title	-5.5	23.6316	24.8864	24.3160
4	Title	-6.0	48.9971	45.7975	45.5394
5	Title	-6.5	80.1095	76.7135	77.6939
6	Title	-7.0	91.0655	92.2249	91.6710
7	Title	-7.5	87.3321	91.9077	91.8120
8	Title	-8.5	95.9876	96.7810	94.9074

	Nonlin fit Table of results	A run RN004 4-24-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	0.1788
4	TOP	95.29
5	LOGIC50	-6.003
6	HILLSLOPE	-1.138
7	Std. Error	
8	BOTTOM	1.999
9	TOP	1.236
10	LOGIC50	0.01975
11	HILLSLOPE	0.07997
12	95% Confidence Intervals	
13	BOTTOM	-4.040 to 4.398
14	TOP	92.68 to 97.90
15	LOGIC50	-6.044 to -5.961
16	HILLSLOPE	-1.307 to -0.9694
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9946
20	Absolute Sum of Squares	137.4
.1	Sy.x	2.843
22	Number of points	
23	Analyzed	21

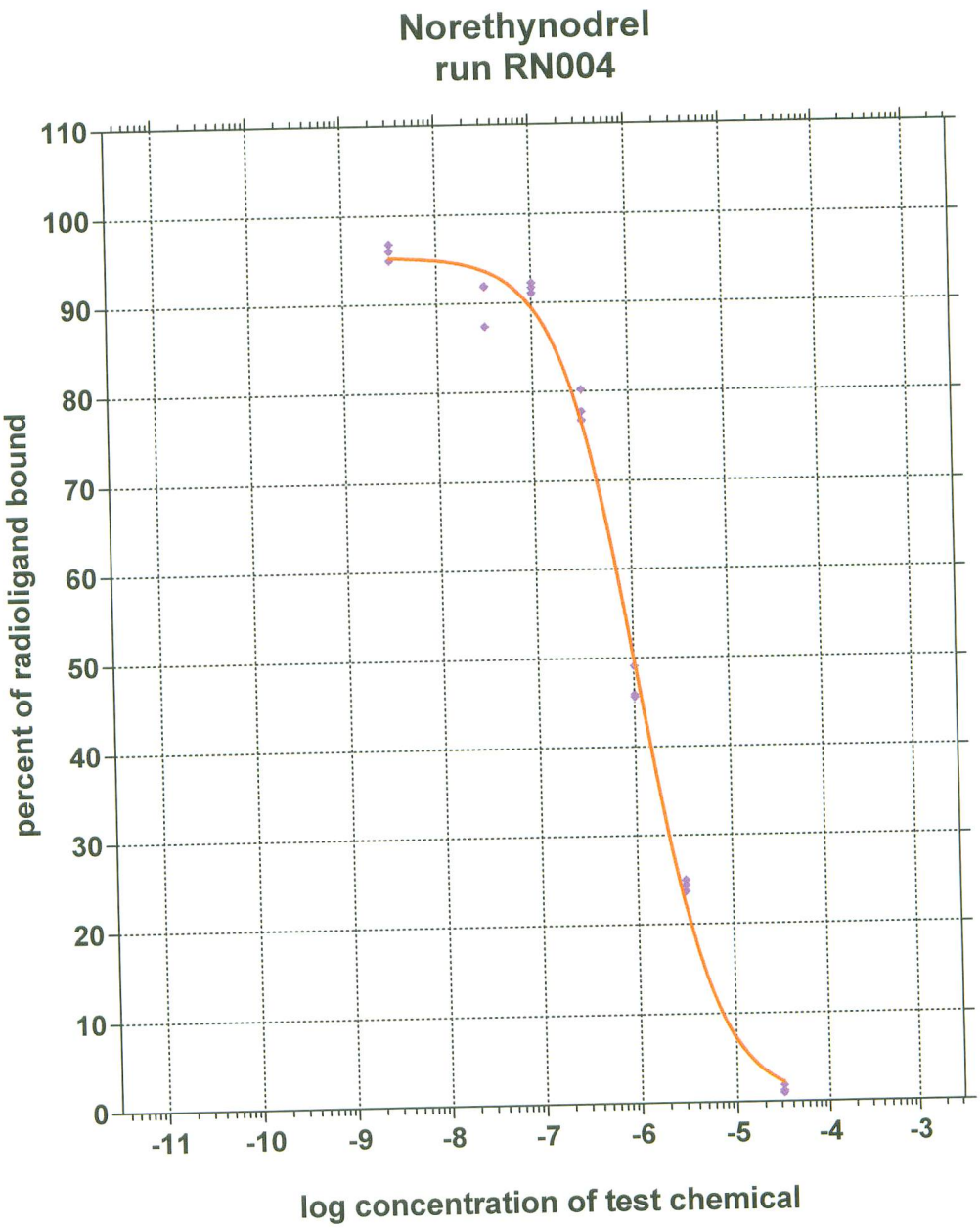

Norethynodrel
run RN004
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 6 run RN004		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			-0.1429
3	Title	-5.0	11.1880	10.6082	10.1642
4	Title	-6.0	55.2440	55.0340	57.0909
5	Title	-7.0	89.3812	89.6125	93.7255
6	Title	-8.0	102.3664	99.7305	97.2523
7	Title	-9.0	93.8801	94.2038	91.9514
8	Title	-10.0	95.8306	96.8416	87.1488

	Nonlin fit Table of results	A st chemical 6 run RNC
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	0.4869
4	TOP	95.65
5	LOGIC50	-5.897
6	HILLSLOPE	-1.104
7	Std. Error	
8	BOTTOM	3.564
9	TOP	1.170
10	LOGIC50	0.03454
11	HILLSLOPE	0.1521
12	95% Confidence Intervals	
13	BOTTOM	-7.107 to 8.081
14	TOP	93.16 to 98.14
15	LOGIC50	-5.971 to -5.823
16	HILLSLOPE	-1.428 to -0.7798
17	Goodness of Fit	
18	Degrees of Freedom	15
19	R^2	0.9920
20	Absolute Sum of Squares	186.4
21	Sy.x	3.525
22	Number of points	
23	Analyzed	19

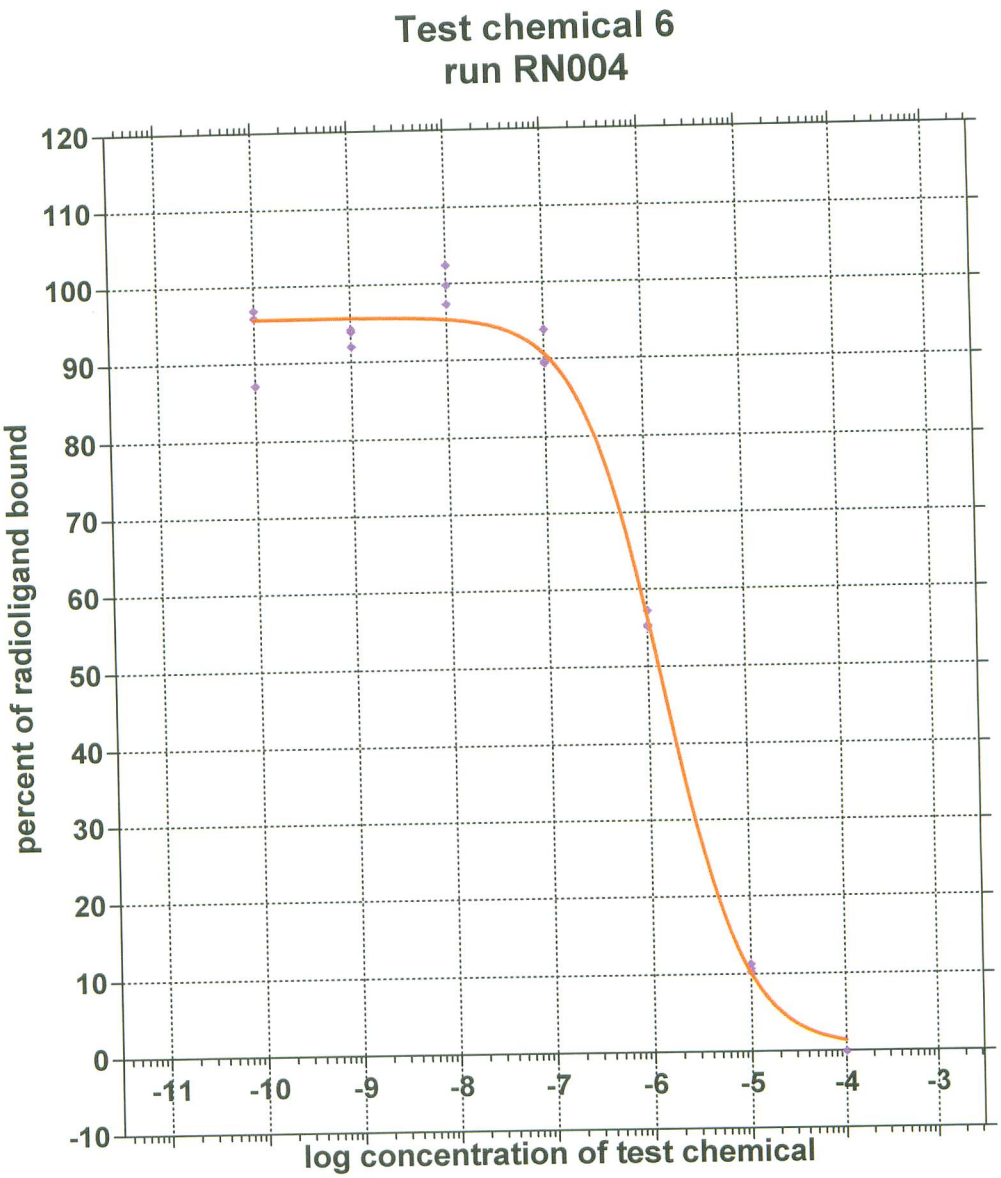

Test chemical 6
run RN004
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 7 run RN004		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	2.4771	2.8306	
2	Title	-4.0	62.4350	64.7992	67.1136
3	Title	-5.0	93.7570	92.7876	95.5832
4	Title	-6.0	95.0976	78.2379	108.2588
5	Title	-7.0	92.7058	102.1191	96.3323
6	Title	-8.0	104.9140	99.0369	101.4347
7	Title	-9.0	70.2814	100.4673	91.3743
8	Title	-10.0	96.9568	98.5478	102.9464

	Nonlin fit Table of results	A Test chemical 7 run RN004
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-7.978
4	TOP	95.97
5	LOGIC50	-3.796
6	HILLSLOPE	-1.311
7	Std. Error	
8	BOTTOM	35.44
9	TOP	2.270
10	LOGIC50	0.1665
11	HILLSLOPE	1.197
12	95% Confidence Intervals	
13	BOTTOM	-82.15 to 66.19
14	TOP	91.22 to 100.7
15	LOGIC50	-4.145 to -3.448
16	HILLSLOPE	-3.817 to 1.194
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9227
20	Absolute Sum of Squares	1406
21	Sy.x	8.601
22	Number of points	
23	Analyzed	23

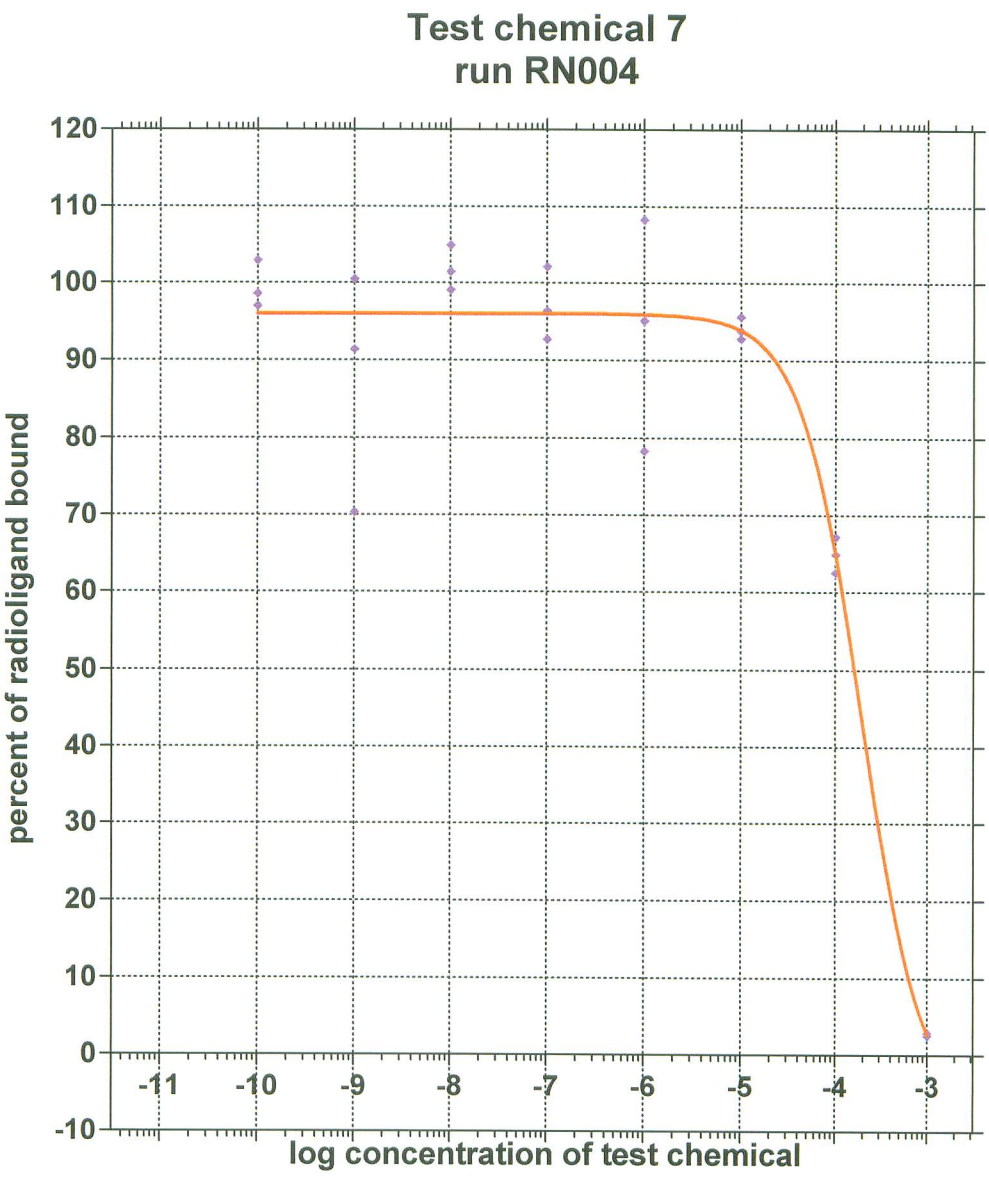
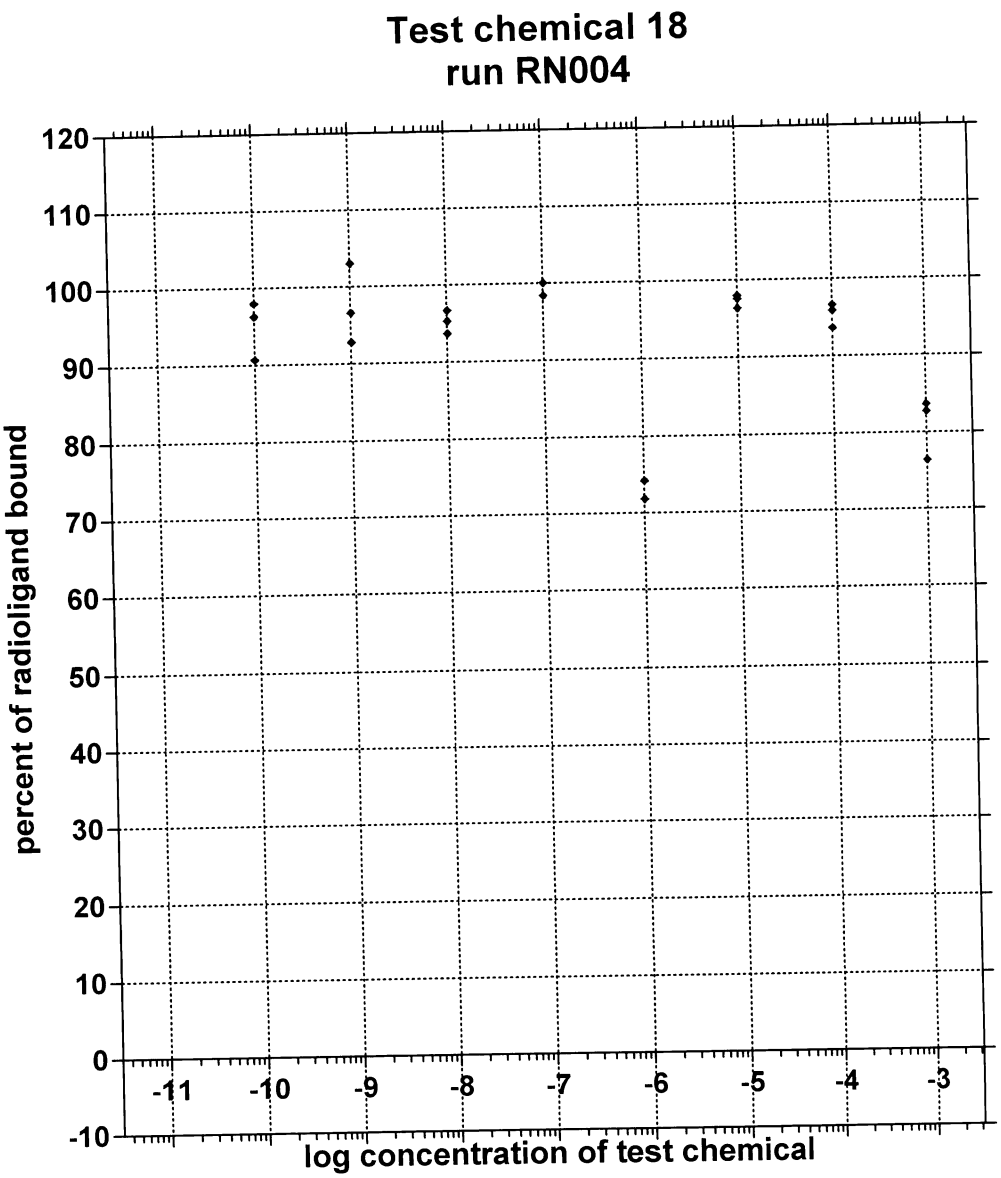

Test chemical 7
run RN004
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 18 run RN004		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	76.2866	83.4996	82.5829
2	Title	-4.0	93.5150	96.5114	95.7681
3	Title	-5.0	97.9038	97.4798	96.2349
4	Title	-6.0	74.1209		71.7555
5	Title	-7.0	99.9952		98.3535
6	Title	-8.0	93.6576	96.6355	95.2503
7	Title	-9.0	96.5403	102.9945	92.7539
8	Title	-10.0	96.2377	97.9317	90.6844

	Nonlin fit Table of results	A Test chemical 18 run RN004
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	72.94
4	TOP	99.17
5	LOGIC50	-5.603
6	HILLSLOPE	-1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	22


Test chemical 18
run RN004
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

APPENDIX S

Excel and Prism Files of the Run Identifier RN005

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	RN005	
Assay start date:	5/1/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.97	Ci/mmole
Cytosol vial or lot identification:	RUC2-21-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.63	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.87	M
RBA, norethynodrel (Weak Positive):	1.734E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-5.65	6.0
RBA, Unknown 1:	1.679E-03	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-3.57	7.0
RBA, Unknown 2:	8.710E-06	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-5.88	13.0
RBA, Unknown 3:	1.778E-03	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	n/a	18.0
RBA, Unknown 4:	n/a	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 3.73%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	6	4001.8	209.18	Total Binding, solvent control, tubes
Hot	6	107401.8	4849.77	Total hot E2 added to each tube
NSB	6	275.5	25.77	Nonspecific Binding
Specific EtOH	6	3726.3	209.18	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.04	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.07	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.5
R1881	#N/A	not applicable
norethynodrel	-4.5	3.9
Unknown 1	-5.0	17.3
Unknown 2	-3.0	3.9
Unknown 3	-4.0	1.7
Unknown 4	-7.0	72.2

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

% binding >105 or < 0.95 highlighted in red font, points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2591.00	3886.50	TRUE			—	96.9	3886.5	3611.0	103790.8	96.9	101.2	96.9
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2678.00	4017.00	TRUE			—	100.4	4017	3741.5	103660.3	100.4		100.4
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2827.00	4240.50	TRUE			—	106.4	4240.5	3965.0	103436.8	106.4		106.4
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	200.00	300.00	TRUE		cold E2	-7.0	0.7	300	24.5	107377.3	0.7	0.5	0.7
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	189.00	283.50	TRUE		cold E2	-7.0	0.2	283.5	8.0	107393.8	0.2		0.2
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	201.00	301.50	TRUE		cold E2	-7.0	0.7	301.5	26.0	107375.8	0.7		0.7
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	706.00	1059.00	TRUE		cold E2	-8.0	21.0	1059	783.5	106618.3	21.0	19.6	21.0
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	660.00	990.00	TRUE		cold E2	-8.0	19.2	990	714.5	106687.3	19.2		19.2
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	644.00	966.00	TRUE		cold E2	-8.0	18.5	966	690.5	106711.3	18.5		18.5
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1210.00	1815.00	TRUE		cold E2	-8.5	41.3	1815	1539.5	105862.3	41.3	41.2	41.3
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1209.00	1813.50	TRUE		cold E2	-8.5	41.3	1813.5	1538.0	105863.8	41.3		41.3
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1205.00	1807.50	TRUE		cold E2	-8.5	41.1	1807.5	1532.0	105869.8	41.1		41.1
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1853.00	2779.50	TRUE		cold E2	-9.0	67.2	2779.5	2504.0	104897.8	67.2	67.7	67.2
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	873.00	1309.50	FALSE	mispipet	cold E2	-9.0							
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	1879.00	2818.50	TRUE		cold E2	-9.0	68.2	2818.5	2543.0	104858.8	68.2		68.2
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2343.00	3514.50	TRUE		cold E2	-9.5	86.9	3514.5	3239.0	104162.8	86.9	89.5	86.9
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2474.00	3711.00	TRUE		cold E2	-9.5	92.2	3711	3435.5	103966.3	92.2		92.2
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2401.00	3601.50	TRUE		cold E2	-9.5	89.3	3601.5	3326.0	104075.8	89.3		89.3
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2341.00	3511.50	TRUE		cold E2	-10.0	86.8	3511.5	3236.0	104165.8	86.8	89.1	86.8
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	1800.00	2700.00	FALSE	mispipet	cold E2	-10.0							
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2453.00	3679.50	TRUE		cold E2	-10.0	91.4	3679.5	3404.0	103997.8	91.4		91.4
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	2343.00	3514.50	TRUE		cold E2	-11.0	86.9	3514.5	3239.0	104162.8	86.9	93.5	86.9
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	2626.00	3939.00	TRUE		cold E2	-11.0	98.3	3939	3663.5	103738.3	98.3		98.3
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	2548.00	3822.00	TRUE		cold E2	-11.0	95.2	3822	3546.5	103855.3	95.2		95.2
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

| | | |

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 3.73%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font, points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2499.00	3748.50	TRUE		Unknown 2	-10.0	93.2	3748.5	3473.0	103928.8	93.2		93.2
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	17089.00	25633.50	TRUE		Unknown 3	-3.0	680.5	25633.5	25358.0	82043.8	680.5	651.8	
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	18601.00	27901.50	TRUE		Unknown 3	-3.0	741.4	27901.5	27626.0	79775.8	741.4		
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	13434.00	20151.00	TRUE		Unknown 3	-3.0	533.4	20151	19875.5	87526.3	533.4		
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	182.00	273.00	TRUE		Unknown 3	-4.0	-0.1	273	-2.5	107404.3	-0.1	1.7	-0.1
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	276.00	414.00	TRUE		Unknown 3	-4.0	3.7	414	138.5	107263.3	3.7		3.7
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	223.00	334.50	TRUE		Unknown 3	-4.0	1.6	334.5	59.0	107342.8	1.6		1.6
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	314.00	471.00	TRUE		Unknown 3	-5.0	5.2	471	195.5	107206.3	5.2	4.8	5.2
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	306.00	459.00	TRUE		Unknown 3	-5.0	4.9	459	183.5	107218.3	4.9		4.9
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	287.00	430.50	TRUE		Unknown 3	-5.0	4.2	430.5	155.0	107246.8	4.2		4.2
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1463.00	2194.50	TRUE		Unknown 3	-6.0	51.5	2194.5	1919.0	105482.8	51.5	61.9	51.5
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1903.00	2854.50	TRUE		Unknown 3	-6.0	69.2	2854.5	2579.0	104822.8	69.2		69.2
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1800.00	2700.00	TRUE		Unknown 3	-6.0	65.1	2700	2424.5	104977.3	65.1		65.1
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2438.00	3657.00	TRUE		Unknown 3	-7.0	90.7	3657	3381.5	104020.3	90.7	93.3	90.7
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2409.00	3613.50	TRUE		Unknown 3	-7.0	89.6	3613.5	3338.0	104063.8	89.6		89.6
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2657.00	3985.50	TRUE		Unknown 3	-7.0	99.6	3985.5	3710.0	103691.8	99.6		99.6
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2240.00	3360.00	TRUE		Unknown 3	-8.0	82.8	3360	3084.5	104317.3	82.8	91.5	82.8
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2738.00	4107.00	TRUE		Unknown 3	-8.0	102.8	4107	3831.5	103570.3	102.8		102.8
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2392.00	3588.00	TRUE		Unknown 3	-8.0	88.9	3588	3312.5	104089.3	88.9		88.9
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2318.00	3477.00	TRUE		Unknown 3	-9.0	85.9	3477	3201.5	104200.3	85.9	91.8	85.9
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2620.00	3930.00	TRUE		Unknown 3	-9.0	98.1	3930	3654.5	103747.3	98.1		98.1
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2454.00	3681.00	TRUE		Unknown 3	-9.0	91.4	3681	3405.5	103996.3	91.4		91.4
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2392.00	3588.00	TRUE		Unknown 3	-10.0	88.9	3588	3312.5	104089.3	88.9	85.1	88.9
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2258.00	3387.00	TRUE		Unknown 3	-10.0	83.5	3387	3111.5	104290.3	83.5		83.5
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2241.00	3361.50	TRUE		Unknown 3	-10.0	82.8	3361.5	3086.0	104315.8	82.8		82.8
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2500.00	3750.00	TRUE		Unknown 4	-3.0	93.2	3750	3474.5	103927.3	93.2	105.8	93.2
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3344.00	5016.00	TRUE		Unknown 4	-3.0	127.2	5016	4740.5	102661.3	127.2		127.2
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2590.00	3885.00	TRUE		Unknown 4	-3.0	96.9	3885	3609.5	103792.3	96.9		96.9
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2307.00	3460.50	TRUE		Unknown 4	-4.0	85.5	3460.5	3185.0	104216.8	85.5	87.9	85.5
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2496.00	3744.00	TRUE		Unknown 4	-4.0	93.1	3744	3468.5	103933.3	93.1		93.1
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2296.00	3444.00	TRUE		Unknown 4	-4.0	85.0	3444	3168.5	104233.3	85.0		85.0
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2525.00	3787.50	TRUE		Unknown 4	-5.0	94.3	3787.5	3512.0	103889.8	94.3	97.6	94.3
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2418.00	3627.00	TRUE		Unknown 4	-5.0	89.9	3627	3351.5	104050.3	89.9		89.9
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2881.00	4321.50	TRUE		Unknown 4	-5.0	108.6	4321.5	4046.0	103355.8	108.6		108.6
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2329.00	3493.50	TRUE		Unknown 4	-6.0	86.4	3493.5	3218.0	104183.8	86.4	90.2	86.4
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2619.00	3928.50	TRUE		Unknown 4	-6.0	98.0	3928.5	3653.0	103748.8	98.0		98.0
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2324.00	3486.00	TRUE		Unknown 4	-6.0	86.2	3486	3210.5	104191.3	86.2		86.2
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	629.00	943.50	FALSE	mispipet	Unknown 4	-7.0						72.2	
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2260.00	3390.00	TRUE		Unknown 4	-7.0	83.6	3390	3114.5	104287.3	83.6		83.6
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1696.00	2544.00	TRUE		Unknown 4	-7.0	60.9	2544	2268.5	105133.3	60.9		101.1
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2309.00	3463.50	TRUE		Unknown 4	-8.0	85.6	3463.5	3188.0	104213.8	85.6	79.6	85.6
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2003.00	3004.50	TRUE		Unknown 4	-8.0	73.2	3004.5	2729.0	104672.8	73.2		73.2
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2174.00	3261.00	TRUE		Unknown 4	-8.0	80.1	3261	2985.5	104416.3	80.1		80.1
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1617.00	2425.50	TRUE		Unknown 4	-9.0	57.7	2425.5	2150.0	105251.8	57.7	76.4	57.7
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2242.00	3363.00	TRUE		Unknown 4	-9.0	82.9	3363	3087.5	104314.3	82.9		82.9
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2388.00	3582.00	TRUE		Unknown 4	-9.0	88.7	3582	3306.5	104095.3	88.7		88.7
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2474.00	3711.00	TRUE		Unknown 4	-10.0	92.2	3711	3435.5	103966.3	92.2	89.5	92.2
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2500.00	3750.00	TRUE		Unknown 4	-10.0	93.2	3750	3474.5	103927.3	93.2		93.2
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2245.00	3367.50	TRUE		Unknown 4	-10.0	83.0	3367.5	3092.0	104309.8	83.0		83.0
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	156.00	234.00	TRUE		cold E2	-7.0	-1.1	234	-41.5	107443.3	-1.1	-0.5	-1.1
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	183.00	274.50	TRUE		cold E2	-7.0	0.0	274.5	-1.0	107402.8	0.0		0.0
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	173.00	259.50	TRUE		cold E2	-7.0	-0.4	259.5	-16.0	107417.8	-0.4		-0.4
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2446.00	3669.00	TRUE			—	91.1	3669	3393.5	104008.3	91.1	98.8	91.1
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2797.00	4195.50	TRUE			—	105.2	4195.5	3920.0	103481.8	105.2		105.2
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2668.00	4002.00	TRUE			—	100.0	4002	3726.5	103675.3	100.0		100.0
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	103150.00	103150.00	TRUE					103150	102874.5				
179	2	none	Hot		—	—	—	10	—	—	—	—	108930.00	108930.00	TRUE					108930	108654.5				
180	3	none	Hot		—	—	—	10	—	—	—	—	105551.00	105551.00	TRUE					105551	105275.5				
181	1	none	Hot		—	—	—	10	—	—	—	—	116533.00	116533.00	TRUE					116533	116257.5				
182	2	none	Hot		—	—	—	10	—	—	—	—	105160.00	105160.00	TRUE					105160	104884.5				
183	3	none	Hot		—	—	—	10	—	—	—	—	105087.00	105087.00	TRUE					105087	104811.5				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				6.0				7.0				13.0				18.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	0.6575	0.2147	0.6978	-4.0				-3.0				-3.0	1.8249	6.4140	3.5961	-3.0				-3.0	93.2439	127.2191	96.8668	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-1.1137	-0.0268	-0.4294	-4.5	3.9181	3.7974	4.1194	-4.0				-4.0	66.5548	76.0148	68.4066	-4.0	-0.0671	3.7169	1.5834	-4.0	85.4747	93.0829	85.0319	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	21.0265	19.1748	18.5307	-5.5	32.5797	29.6813	32.4992	-5.0	14.5455	20.3019	17.0413	-5.0	89.3794	94.7333	74.3241	-5.0	5.2466	4.9245	4.1597	-5.0	94.2503	89.9430	108.5810	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	41.3150	41.2747	41.1137	-6.0	52.2241	52.4254	54.3173	-6.0	61.1204	60.4361	68.1248	-6.0	90.5065	91.0299	103.5089	-6.0	51.4995	69.2117	65.0654	-6.0	86.3603	98.0342	86.1590	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	67.1989		68.2456	-6.5	81.1271	96.7461	84.2268	-7.0	93.5257	95.4982	88.6548	-7.0	95.2566	96.1825		-7.0	90.7481	89.5807	99.5639	-7.0		83.5827	101.1338	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	86.9239	92.1972	89.2586	-7.0	91.6739	98.0342	98.6380	-8.0	101.4559	102.1000	92.7206	-8.0	102.0597	104.7165	96.6253	-8.0	82.7776	102.8246	88.8963	-8.0	85.5552	73.2372	80.1208	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	86.8433		91.3519	-7.5	88.3730	92.0362	95.8202	-9.0	91.9960	85.6357	91.0701	-9.0	93.9282		98.7991	-9.0	85.9175	98.0745	91.3922	-9.0	57.6988	82.8581	88.7353	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	86.9239	98.3160	95.1761	-8.5	89.1379	92.3583	95.5787	-10.0	94.9346	93.0023	85.0721	-10.0	95.9410	97.1084	93.2036	-10.0	88.8963	83.5022	82.8178	-10.0	92.1972	93.2439	82.9789	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve	RN005 5-1-08			Positive Control	RN005 5-1-08			6.0	RN005 5-1-08			7.0	RN005 5-1-08			13.0	RN005 5-1-08			18.0	RN005 5-1-08			Negative Control	Prism run ID & date		
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values	bad initial values			Best-fit values			
13	1	BOTTOM	-1.639			BOTTOM	3.712			BOTTOM	16.27			BOTTOM	-1148000			BOTTOM	2.451			BOTTOM	76.43			BOTTOM			
14	2	TOP	94.45			TOP	95.25			TOP	93.08			TOP	97.29			TOP	90.49			TOP	105.8			TOP			
15	3	LOGIC50	-8.628			LOGIC50	-5.867			LOGIC50	-5.853			LOGIC50	-3.568			LOGIC50	-5.878			LOGIC50	n/a			LOGIC50			
16	1	HILLSLOPE	-1.06			HILLSLOPE	-1.267			HILLSLOPE	-2.063			HILLSLOPE	-0.5173			HILLSLOPE	-2.063			HILLSLOPE	n/a			HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM	1.684			BOTTOM	3.836			BOTTOM	9.101			BOTTOM	1.195E+10			BOTTOM	3.638			BOTTOM				BOTTOM			
19	1	TOP	1.606			TOP	2.18			TOP	1.695			TOP	1.927			TOP	1.855			TOP				TOP			
20	2	LOGIC50	0.02437			LOGIC50	0.03802			LOGIC50	0.2381			LOGIC50	0.1031			LOGIC50	0.08724			LOGIC50				LOGIC50			
21	3	HILLSLOPE	0.08421			HILLSLOPE	0.1818			HILLSLOPE	3.539			HILLSLOPE	0.2461			HILLSLOPE	1.397			HILLSLOPE				HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.8

Provide information in all blue cells.
Laboratory Code: B

	run	xgroup	rep	x	y
Compound ID:	Estradiol	1	1	1	-7.0	0.7
Run ID:	RN005	1	1	2	-7.0	0.2
Assay start date:	5/1/08	1	1	3	-7.0	0.7
Technician ID:	RN	1	2	1	-8.0	21.0
		1	2	2	-8.0	19.2
		1	2	3	-8.0	18.5
		1	3	1	-8.5	41.3
		1	3	2	-8.5	41.3
		1	3	3	-8.5	41.1
		1	4	1	-9.0	67.2
		1	4	2		
		1	4	3	-9.0	68.2
		1	5	1	-9.5	86.9
		1	5	2	-9.5	92.2
		1	5	3	-9.5	89.3
		1	6	1	-10.0	86.8
		1	6	2		
		1	6	3	-10.0	91.4
		1	7	1	-11.0	86.9
		1	7	2	-11.0	98.3
		1	7	3	-11.0	95.2
		1	8	1		
		1	8	2		
		1	8	3		
		1	9	1		
		1	9	2		
		1	9	3		
		1	10	1		
		1	10	2		
		1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.2

Provide information in all blue cells.
Laboratory Code: B
Compound ID: Norethynodrel
Run ID: RN005
Assay start date: 5/1/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-4.0	111.1
1	1	2	-4.0	112.5
1	1	3	-4.0	102.7
1	2	1	-4.5	3.9
1	2	2	-4.5	3.8
1	2	3	-4.5	4.1
1	3	1	-5.5	32.6
1	3	2	-5.5	29.7
1	3	3	-5.5	32.5
1	4	1	-6.0	52.2
1	4	2	-6.0	52.4
1	4	3	-6.0	54.3
1	5	1	-6.5	81.1
1	5	2	-6.5	96.7
1	5	3	-6.5	84.2
1	6	1	-7.0	91.7
1	6	2	-7.0	98.0
1	6	3	-7.0	98.6
1	7	1	-7.5	88.4
1	7	2	-7.5	92.0
1	7	3	-7.5	95.8
1	8	1	-8.5	89.1
1	8	2	-8.5	92.4
1	8	3	-8.5	95.6
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 22.5

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 6
Run ID: RN005
Assay start date: 5/1/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	1462.9
1	1	2	-3.0	1572.1
1	1	3	-3.0	1475.6
1	2	1		
1	2	2	-4.0	105.6
1	2	3	-4.0	127.8
1	3	1	-5.0	14.5
1	3	2	-5.0	20.3
1	3	3	-5.0	17.0
1	4	1	-6.0	61.1
1	4	2	-6.0	60.4
1	4	3	-6.0	68.1
1	5	1	-7.0	93.5
1	5	2	-7.0	95.5
1	5	3	-7.0	88.7
1	6	1	-8.0	101.5
1	6	2	-8.0	102.1
1	6	3	-8.0	92.7
1	7	1	-9.0	92.0
1	7	2	-9.0	85.6
1	7	3	-9.0	91.1
1	8	1	-10.0	94.9
1	8	2	-10.0	93.0
1	8	3	-10.0	85.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.7

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 7
Run ID: RN005
Assay start date: 5/1/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	1.8
1	1	2	-3.0	6.4
1	1	3	-3.0	3.6
1	2	1	-4.0	66.6
1	2	2	-4.0	76.0
1	2	3	-4.0	68.4
1	3	1	-5.0	89.4
1	3	2	-5.0	94.7
1	3	3	-5.0	74.3
1	4	1	-6.0	90.5
1	4	2	-6.0	91.0
1	4	3	-6.0	103.5
1	5	1	-7.0	95.3
1	5	2	-7.0	96.2
1	5	3		
1	6	1	-8.0	102.1
1	6	2	-8.0	104.7
1	6	3	-8.0	96.6
1	7	1	-9.0	93.9
1	7	2		
1	7	3	-9.0	98.8
1	8	1	-10.0	95.9
1	8	2	-10.0	97.1
1	8	3	-10.0	93.2
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.2

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 13
Run ID: RN005
Assay start date: 5/1/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1		
1	1	2		
1	1	3		
1	2	1	-4.0	-0.1
1	2	2	-4.0	3.7
1	2	3	-4.0	1.6
1	3	1	-5.0	5.2
1	3	2	-5.0	4.9
1	3	3	-5.0	4.2
1	4	1	-6.0	51.5
1	4	2	-6.0	69.2
1	4	3	-6.0	65.1
1	5	1	-7.0	90.7
1	5	2	-7.0	89.6
1	5	3	-7.0	99.6
1	6	1	-8.0	82.8
1	6	2	-8.0	102.8
1	6	3	-8.0	88.9
1	7	1	-9.0	85.9
1	7	2	-9.0	98.1
1	7	3	-9.0	91.4
1	8	1	-10.0	88.9
1	8	2	-10.0	83.5
1	8	3	-10.0	82.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 11.4

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 18
Run ID: RN005
Assay start date: 5/1/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	93.2
1	1	2	-3.0	127.2
1	1	3	-3.0	96.9
1	2	1	-4.0	85.5
1	2	2	-4.0	93.1
1	2	3	-4.0	85.0
1	3	1	-5.0	94.3
1	3	2	-5.0	89.9
1	3	3	-5.0	108.6
1	4	1	-6.0	86.4
1	4	2	-6.0	98.0
1	4	3	-6.0	86.2
1	5	1		
1	5	2	-7.0	83.6
1	5	3	-7.0	60.9
1	6	1	-8.0	85.6
1	6	2	-8.0	73.2
1	6	3	-8.0	80.1
1	7	1	-9.0	57.7
1	7	2	-9.0	82.9
1	7	3	-9.0	88.7
1	8	1	-10.0	92.2
1	8	2	-10.0	93.2
1	8	3	-10.0	83.0
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run RN005 5-1-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.6575	0.2147	0.6978
2	Title	-7.0	-1.1137	-0.0268	-0.4294
3	Title	-8.0	21.0265	19.1748	18.5307
4	Title	-8.5	41.3150	41.2747	41.1137
5	Title	-9.0	67.1989		68.2456
6	Title	-9.5	86.9239	92.1972	89.2586
7	Title	-10.0	86.8433		91.3519
8	Title	-11.0	86.9239	98.3160	95.1761

	Nonlin fit Table of results	A run RN005 5-1-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.639
4	TOP	94.45
5	LOGIC50	-8.628
6	HILLSLOPE	-1.060
7	Std. Error	
8	BOTTOM	1.684
9	TOP	1.606
10	LOGIC50	0.02437
11	HILLSLOPE	0.08421
12	95% Confidence Intervals	
13	BOTTOM	-5.178 to 1.900
14	TOP	91.08 to 97.83
15	LOGIC50	-8.679 to -8.576
16	HILLSLOPE	-1.237 to -0.8829
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9942
20	Absolute Sum of Squares	185.7
21	Sy.x	3.212
22	Number of points	
23	Analyzed	22

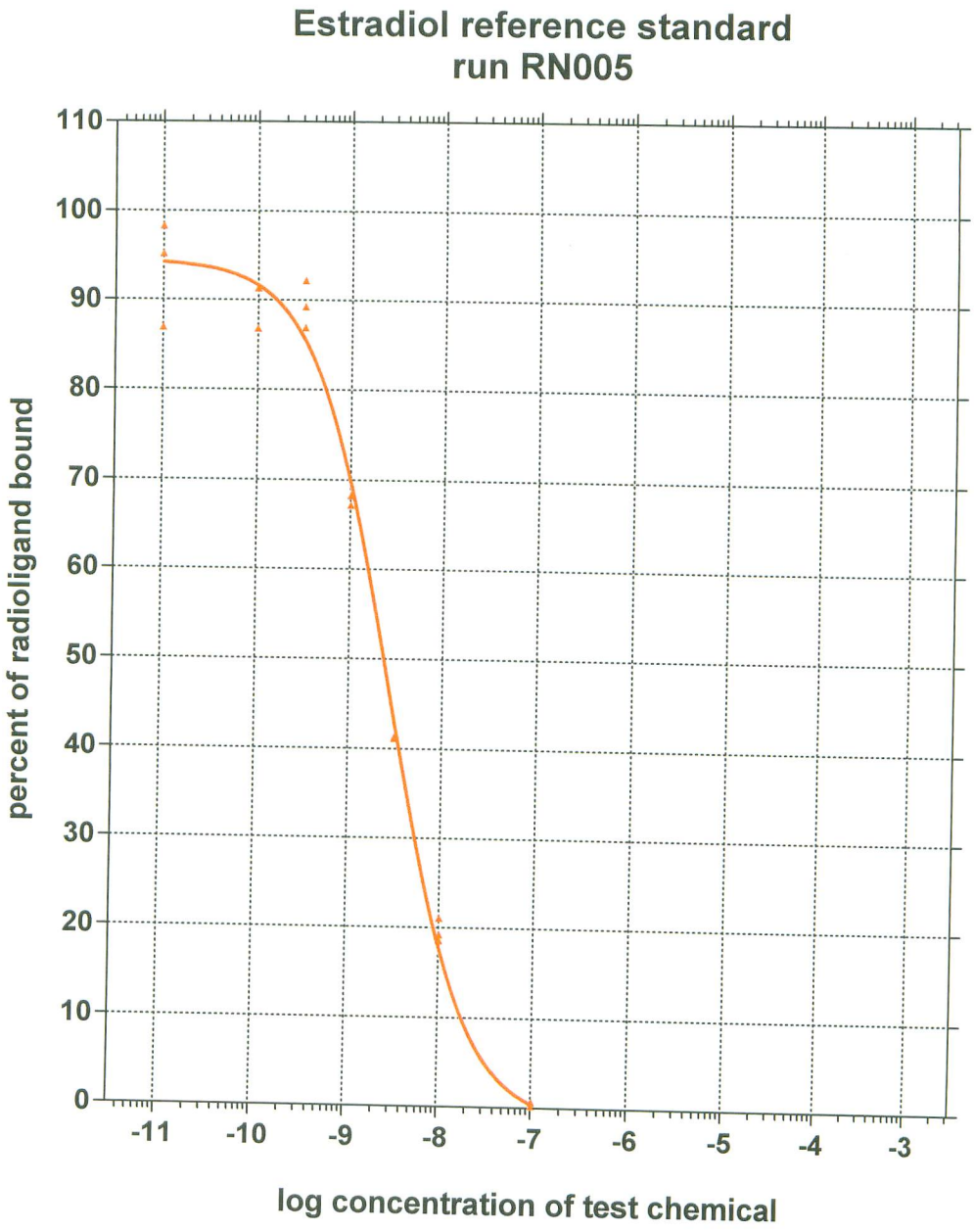

Estradiol reference standard
run RN005
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run RN005 5-1-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	3.9181	3.7974	4.1194
3	Title	-5.5	32.5797	29.6813	32.4992
4	Title	-6.0	52.2241	52.4254	54.3173
5	Title	-6.5	81.1271	96.7461	84.2268
6	Title	-7.0	91.6739	98.0342	98.6380
7	Title	-7.5	88.3730	92.0362	95.8202
8	Title	-8.5	89.1379	92.3583	95.5787

	Nonlin fit Table of results	A run RN005 5-1-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	3.712
4	TOP	95.25
5	LOGIC50	-5.867
6	HILLSLOPE	-1.267
7	Std. Error	
8	BOTTOM	3.836
9	TOP	2.180
10	LOGIC50	0.03802
11	HILLSLOPE	0.1818
12	95% Confidence Intervals	
13	BOTTOM	-4.381 to 11.81
14	TOP	90.65 to 99.85
15	LOGIC50	-5.947 to -5.787
16	HILLSLOPE	-1.651 to -0.8833
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9781
20	Absolute Sum of Squares	527.3
21	Sy.x	5.570
22	Number of points	
23	Analyzed	21

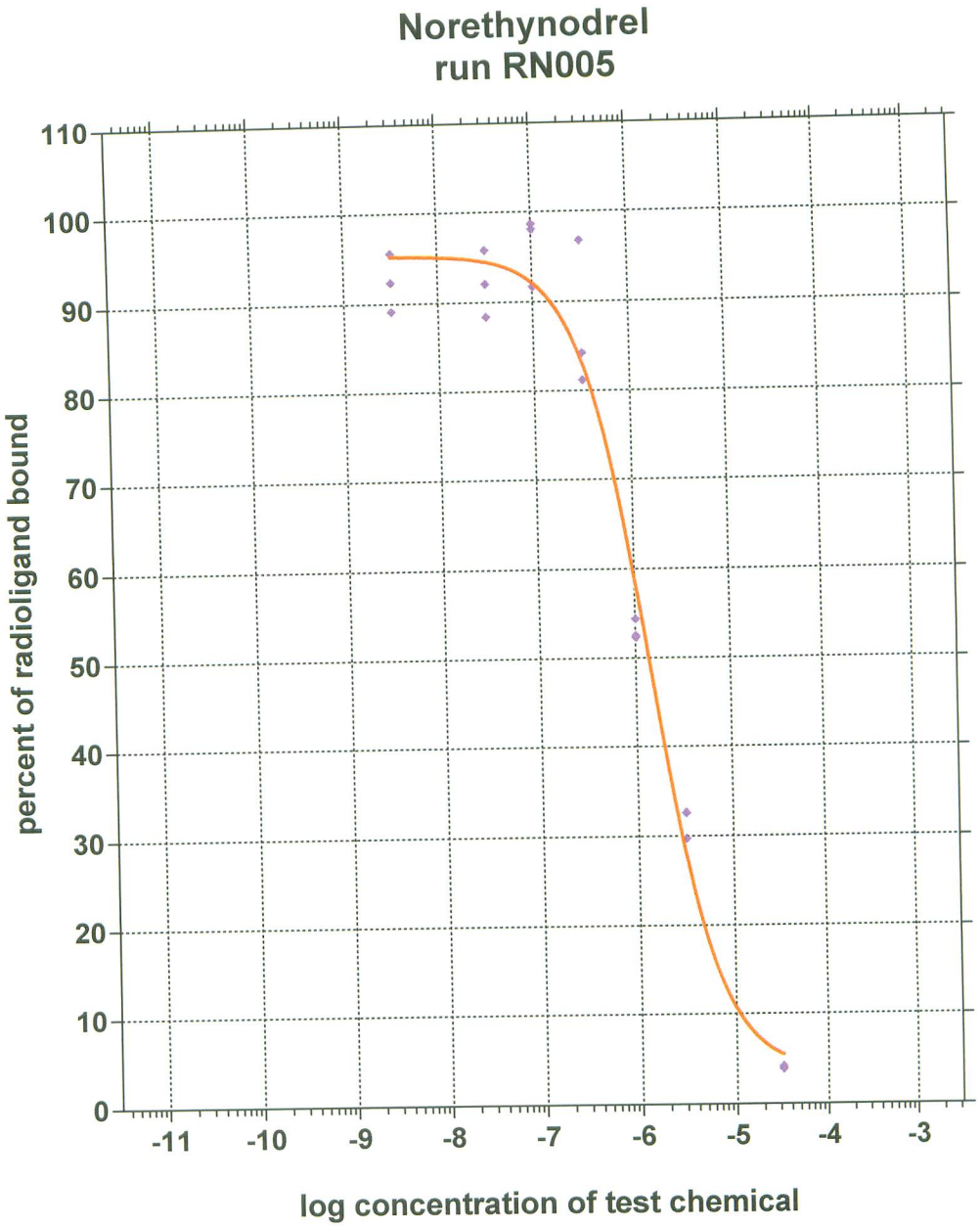

Norethynodrel
run RN005
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 6 run RN005		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	14.5455	20.3019	17.0413
4	Title	-6.0	61.1204	60.4361	68.1248
5	Title	-7.0	93.5257	95.4982	88.6548
6	Title	-8.0	101.4559	102.1000	92.7206
7	Title	-9.0	91.9960	85.6357	91.0701
8	Title	-10.0	94.9346	93.0023	85.0721

	Nonlin fit Table of results	A Test chemical 6 run RN005
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	16.27
4	TOP	93.08
5	LOGIC50	-5.853
6	HILLSLOPE	-2.063
7	Std. Error	
8	BOTTOM	9.101
9	TOP	1.695
10	LOGIC50	0.2381
11	HILLSLOPE	3.539
12	95% Confidence Intervals	
13	BOTTOM	-3.256 to 35.79
14	TOP	89.44 to 96.71
15	LOGIC50	-6.364 to -5.343
16	HILLSLOPE	-9.655 to 5.528
17	Goodness of Fit	
18	Degrees of Freedom	14
19	R^2	0.9756
20	Absolute Sum of Squares	357.7
21	Sy.x	5.055
22	Number of points	
23	Analyzed	18

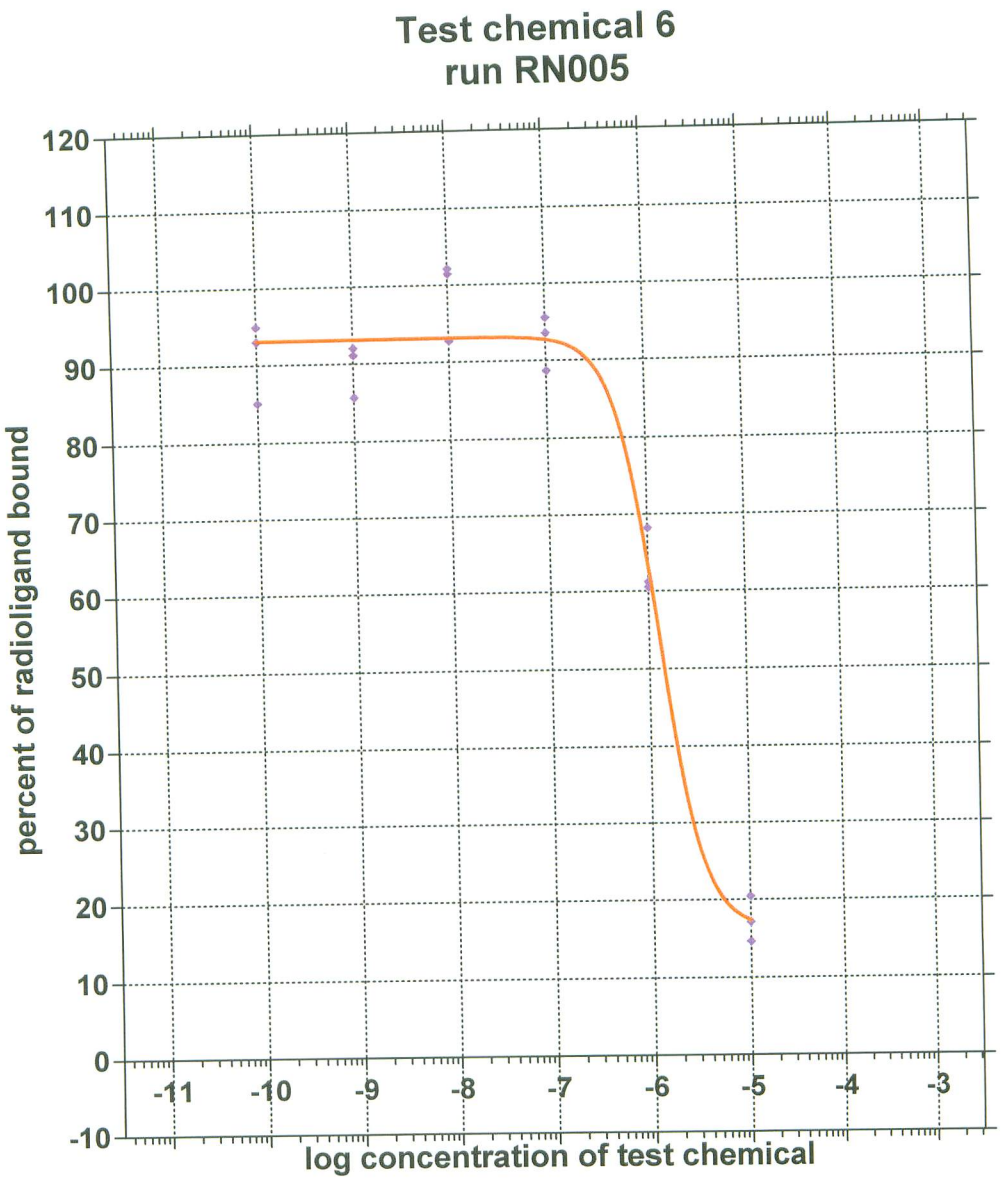

Test chemical 6
run RN005
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 7 run RN005		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	1.8249	6.4140	3.5961
2	Title	-4.0	66.5548	76.0148	68.4066
3	Title	-5.0	89.3794	94.7333	74.3241
4	Title	-6.0	90.5065	91.0299	103.5089
5	Title	-7.0	95.2566	96.1825	
6	Title	-8.0	102.0597	104.7165	96.6253
7	Title	-9.0	93.9282		98.7991
8	Title	-10.0	95.9410	97.1084	93.2036

	Nonlin fit Table of results	A Test chemical 7 run RN005
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.148e+006
4	TOP	97.29
5	LOGIC50	-3.568
6	HILLSLOPE	-0.5173
7	Std. Error	
8	BOTTOM	1.195e+010
9	TOP	1.927
10	LOGIC50	0.1031
11	HILLSLOPE	0.2461
12	95% Confidence Intervals	
13	BOTTOM	-2.512e+010 to 2.512e+010
14	TOP	93.24 to 101.3
15	LOGIC50	-3.785 to -3.352
16	HILLSLOPE	-1.034 to -0.0003633
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9756
20	Absolute Sum of Squares	535.9
21	Sy.x	5.456
22	Number of points	
23	Analyzed	22

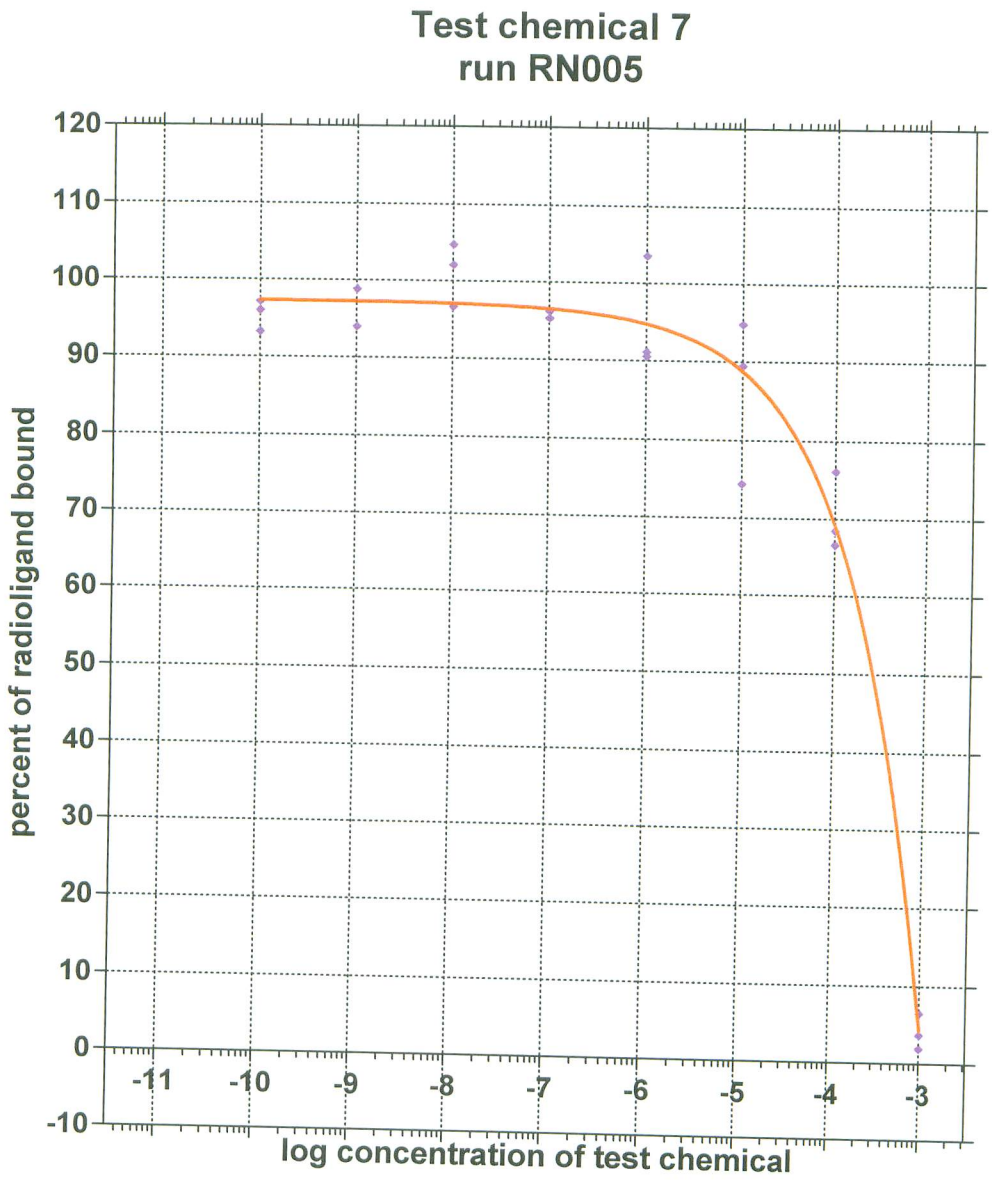

Test chemical 7
run RN005
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 13 run RN005		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-0.0671	3.7169	1.5834
3	Title	-5.0	5.2466	4.9245	4.1597
4	Title	-6.0	51.4995	69.2117	65.0654
5	Title	-7.0	90.7481	89.5807	99.5639
6	Title	-8.0	82.7776	102.8246	88.8963
7	Title	-9.0	85.9175	98.0745	91.3922
8	Title	-10.0	88.8963	83.5022	82.8178

	Nonlin fit Table of results	A Test chemical 13 run RN005
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	2.451
4	TOP	90.49
5	LOGIC50	-5.878
6	HILLSLOPE	-2.063
7	Std. Error	
8	BOTTOM	3.638
9	TOP	1.855
10	LOGIC50	0.08724
11	HILLSLOPE	1.397
12	95% Confidence Intervals	
13	BOTTOM	-5.225 to 10.13
14	TOP	86.57 to 94.40
15	LOGIC50	-6.062 to -5.694
16	HILLSLOPE	-5.012 to 0.8850
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9782
20	Absolute Sum of Squares	676.2
21	Sy.x	6.307
22	Number of points	
23	Analyzed	21

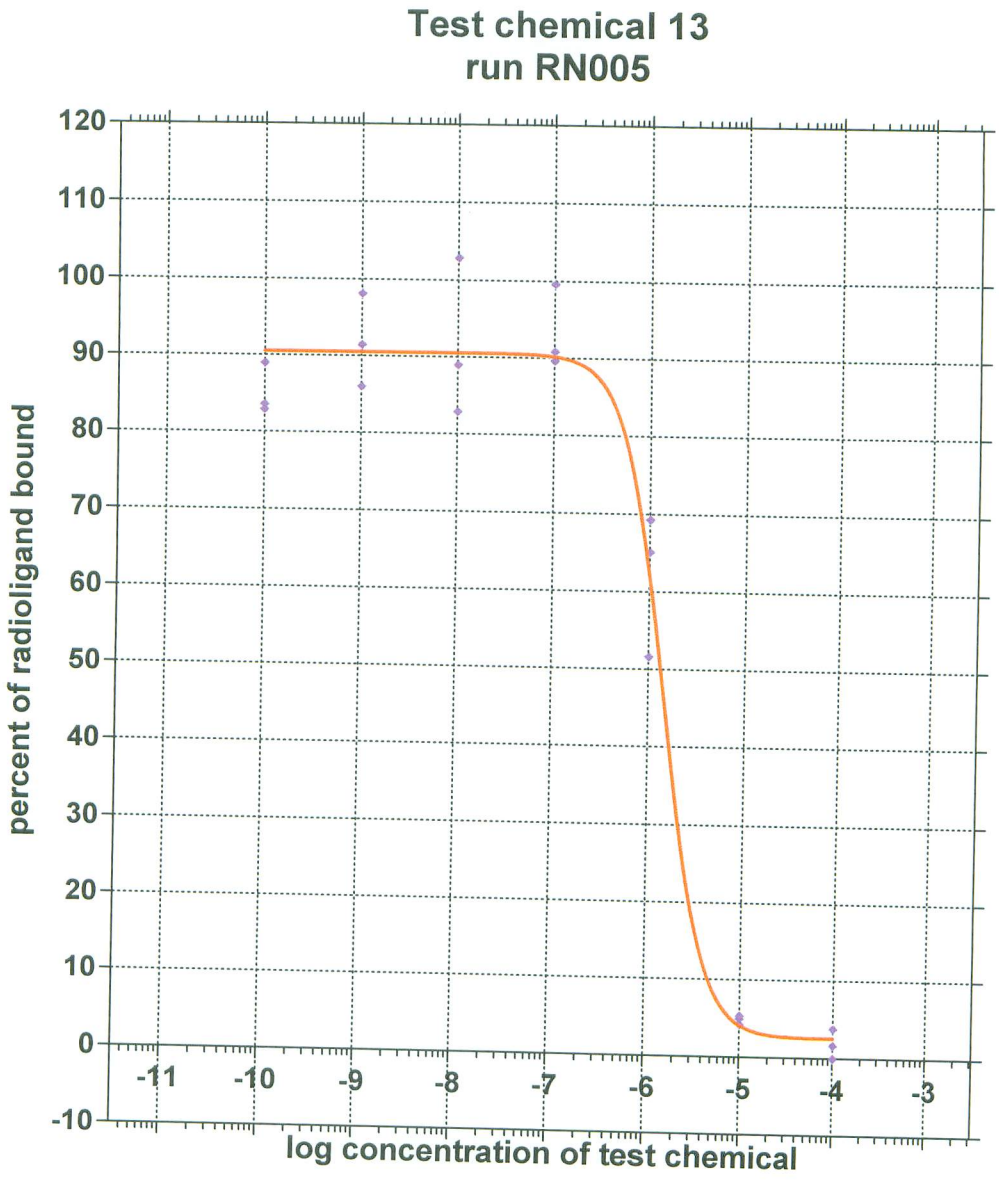
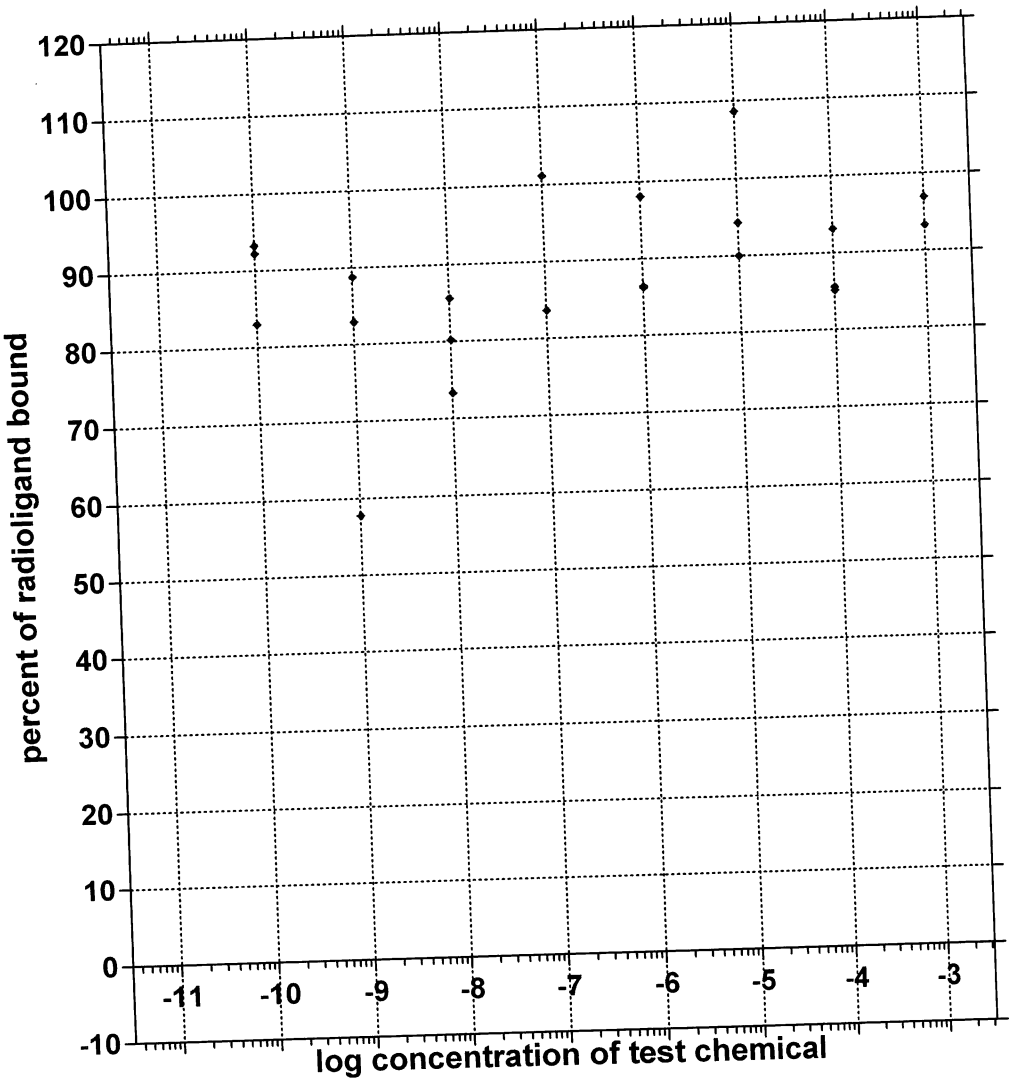

Test chemical 13
run RN005
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 18 run RN005		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	93.2439	127.2191	96.8668
2	Title	-4.0	85.4747	93.0829	85.0319
3	Title	-5.0	94.2503	89.9430	108.5810
4	Title	-6.0	86.3603	98.0342	86.1590
5	Title	-7.0		83.5827	101.1338
6	Title	-8.0	85.5552	73.2372	80.1208
7	Title	-9.0	57.6988	82.8581	88.7353
8	Title	-10.0	92.1972	93.2439	82.9789

	Nonlin fit Table of results	A Test chemical 18 run RN005
		Y
1	logIC50	Bad initial values
2	Best-fit values	
3	BOTTOM	76.43
4	TOP	105.8
5	LOGIC50	-6.423
6	HILLSLOPE	1.000
7	Std. Error	
8	BOTTOM	
9	TOP	
10	LOGIC50	
11	HILLSLOPE	
12	95% Confidence Intervals	
13	BOTTOM	
14	TOP	
15	LOGIC50	
16	HILLSLOPE	
17	Goodness of Fit	
18	Degrees of Freedom	
19	R^2	
20	Absolute Sum of Squares	
21	Sy.x	
22	Number of points	
23	Analyzed	23

Test chemical 18
run RN005



APPENDIX T

Excel and Prism Files of the Run Identifier RN006

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.

If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software.

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	RN006	
Assay start date:	5/1/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.97	Ci/mmole
Cytosol vial or lot identification:	RUC2-21-08	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.67	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.84	M
RBA, norethynodrel (Weak Positive):	1.493E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-5.74	6.0
RBA, Unknown 1:	1.183E-03	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-3.68	7.0
RBA, Unknown 2:	1.021E-05	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-5.66	13.0
RBA, Unknown 3:	9.772E-04	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	-3.04	18.0
RBA, Unknown 4:	2.344E-06	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 5.05%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values

	n	Mean	SD	
EtOH	4	4839.9	129.62	Total Binding, solvent control, tubes
Hot	6	95793.0	8226.13	Total hot E2 added to each tube
NSB	6	399.0	13.84	Nonspecific Binding
Specific EtOH	4	4440.9	129.62	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.05	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.08	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.1
R1881	#N/A	not applicable
norethynodrel	-4.0	-0.4
Unknown 1	-5.0	16.6
Unknown 2	-3.0	10.9
Unknown 3	-4.0	1.3
Unknown 4	-3.0	34.1

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2300.12	3450.18	FALSE	mispipet		—						103.8	
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3338.63	5007.95	TRUE			—	103.8	5007.945	4608.9	91184.0	103.8		103.8
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2144.98	3217.47	FALSE	mispipet		—							
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	271.63	407.45	TRUE		cold E2	-7.0	0.2	407.445	8.4	95784.5	0.2	0.1	0.2
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	267.53	401.30	TRUE		cold E2	-7.0	0.1	401.295	2.3	95790.7	0.1		0.1
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	271.26	406.89	TRUE		cold E2	-7.0	0.2	406.89	7.9	95785.1	0.2		0.2
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	733.06	1099.59	TRUE		cold E2	-8.0	15.8	1099.59	700.6	95092.4	15.8	17.2	15.8
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	787.00	1180.50	TRUE		cold E2	-8.0	17.6	1180.5	781.5	95011.5	17.6		17.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	802.81	1204.22	TRUE		cold E2	-8.0	18.1	1204.215	805.2	94987.8	18.1		18.1
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1354.39	2031.59	TRUE		cold E2	-8.0	36.8	2031.585	1632.6	94160.4	36.8	38.7	36.8
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	2144.17	3216.26	FALSE	mispipet	cold E2	-8.0							
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1467.57	2201.36	TRUE		cold E2	-8.0	40.6	2201.355	1802.3	93990.6	40.6		40.6
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2257.82	3386.73	TRUE		cold E2	-9.0	67.3	3386.73	2987.7	92805.3	67.3	70.1	67.3
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2363.60	3545.40	TRUE		cold E2	-9.0	70.9	3545.4	3146.4	92646.6	70.9		70.9
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2403.40	3605.10	TRUE		cold E2	-9.0	72.2	3605.1	3206.1	92586.9	72.2		72.2
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2825.35	4238.03	TRUE		cold E2	-9.5	86.4	4238.025	3839.0	91954.0	86.4	87.5	86.4
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2843.66	4265.49	TRUE		cold E2	-9.5	87.1	4265.49	3866.5	91926.5	87.1		87.1
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2901.76	4352.64	TRUE		cold E2	-9.5	89.0	4352.64	3953.6	91839.4	89.0		89.0
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3135.20	4702.80	TRUE		cold E2	-10.0	96.9	4702.8	4303.8	91489.2	96.9	97.4	96.9
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3169.87	4754.81	TRUE		cold E2	-10.0	98.1	4754.805	4355.8	91437.2	98.1		98.1
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3144.45	4716.68	TRUE		cold E2	-10.0	97.2	4716.675	4317.7	91475.3	97.2		97.2
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3191.38	4787.07	TRUE		cold E2	-11.0	98.8	4787.07	4388.1	91404.9	98.8	102.6	98.8
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3424.89	5137.34	TRUE		cold E2	-11.0	106.7	5137.335	4738.3	91054.7	106.7		106.7
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3292.11	4938.17	TRUE		cold E2	-11.0	102.2	4938.165	4539.2	91253.8	102.2		102.2
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A

Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 5.05%

If the ratio of EtOH / Hot is ... there are problems with the ...

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a t... curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2893.02	4339.53	FALSE	mispipet	norethynodrel	-4.0						-0.4	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	261.45	392.18	TRUE		norethynodrel	-4.0	-0.2	392.175	-6.8	95799.8	-0.2		-0.2
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	246.12	369.18	TRUE		norethynodrel	-4.0	-0.7	369.18	-29.8	95822.8	-0.7		-0.7
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	301.08	451.62	TRUE		norethynodrel	-4.5	1.2	451.62	52.6	95740.4	1.2	2.8	1.2
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	411.21	616.82	TRUE		norethynodrel	-4.5	4.9	616.815	217.8	95575.2	4.9		4.9
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	334.05	501.08	TRUE		norethynodrel	-4.5	2.3	501.075	102.1	95690.9	2.3		2.3
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1096.40	1644.60	TRUE		norethynodrel	-5.5	28.0	1644.6	1245.6	94547.4	28.0	30.4	28.0
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1247.93	1871.90	TRUE		norethynodrel	-5.5	33.2	1871.895	1472.9	94320.1	33.2		33.2
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1158.13	1737.20	TRUE		norethynodrel	-5.5	30.1	1737.195	1338.2	94454.8	30.1		30.1
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1939.07	2908.61	TRUE		norethynodrel	-6.0	56.5	2908.605	2509.6	93283.4	56.5	57.5	56.5
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2005.77	3008.66	TRUE		norethynodrel	-6.0	58.8	3008.655	2609.6	93183.3	58.8		58.8
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1959.29	2938.94	TRUE		norethynodrel	-6.0	57.2	2938.935	2539.9	93253.1	57.2		57.2
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2823.63	4235.45	TRUE		norethynodrel	-6.5	86.4	4235.445	3836.4	91956.5	86.4	88.3	86.4
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2870.18	4305.27	TRUE		norethynodrel	-6.5	88.0	4305.27	3906.3	91886.7	88.0		88.0
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2942.38	4413.57	TRUE		norethynodrel	-6.5	90.4	4413.57	4014.6	91778.4	90.4		90.4
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2966.36	4449.54	TRUE		norethynodrel	-7.0	91.2	4449.54	4050.5	91742.5	91.2	90.5	91.2
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3008.89	4513.34	TRUE		norethynodrel	-7.0	92.6	4513.335	4114.3	91678.7	92.6		92.6
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2862.26	4293.39	TRUE		norethynodrel	-7.0	87.7	4293.39	3894.4	91898.6	87.7		87.7
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	2554.52	3831.78	FALSE	mispipet	norethynodrel	-7.5						98.9	
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3245.24	4867.86	TRUE		norethynodrel	-7.5	100.6	4867.86	4468.9	91324.1	100.6		100.6
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3144.82	4717.23	TRUE		norethynodrel	-7.5	97.2	4717.23	4318.2	91474.8	97.2		97.2
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3228.34	4842.51	TRUE		norethynodrel	-8.5	100.1	4842.51	4443.5	91349.5	100.1	101.1	100.1
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3292.15	4938.23	TRUE		norethynodrel	-8.5	102.2	4938.225	4539.2	91253.8	102.2		102.2
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3254.64	4881.96	TRUE		norethynodrel	-8.5	100.9	4881.96	4483.0	91310.0	100.9		100.9
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	35930.92	53896.38	TRUE		Unknown 1	-3.0	1204.7	53896.38	53497.4	42295.6	1204.7	1218.3	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	14784.15	22176.23	FALSE	mispipet	Unknown 1	-3.0							
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	36735.55	55103.33	TRUE		Unknown 1	-3.0	1231.8	55103.325	54704.3	41088.7	1231.8		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2977.89	4466.84	TRUE		Unknown 1	-4.0	91.6	4466.835	4067.8	91725.2	91.6	91.0	
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2763.83	4145.75	TRUE		Unknown 1	-4.0	84.4	4145.745	3746.7	92046.2	84.4		
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3140.20	4710.30	TRUE		Unknown 1	-4.0	97.1	4710.3	4311.3	91481.7	97.1		
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	754.91	1132.37	TRUE		Unknown 1	-5.0	16.5	1132.365	733.4	95059.6	16.5	16.6	16.5
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	757.10	1135.65	TRUE		Unknown 1	-5.0	16.6	1135.65	736.6	95056.3	16.6		16.6
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	761.21	1141.82	TRUE		Unknown 1	-5.0	16.7	1141.815	742.8	95050.2	16.7		16.7
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2089.20	3133.80	TRUE		Unknown 1	-6.0	61.6	3133.8	2734.8	93058.2	61.6	64.0	61.6
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2125.46	3188.19	TRUE		Unknown 1	-6.0	62.8	3188.19	2789.2	93003.8	62.8		62.8
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2270.35	3405.53	TRUE		Unknown 1	-6.0	67.7	3405.525	3006.5	92786.5	67.7		67.7
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3041.85	4562.78	TRUE		Unknown 1	-7.0	93.8	4562.775	4163.8	91629.2	93.8	98.1	93.8
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3151.15	4726.73	TRUE		Unknown 1	-7.0	97.5	4726.725	4327.7	91465.3	97.5		97.5
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3321.10	4981.65	TRUE		Unknown 1	-7.0	103.2	4981.65	4582.6	91210.3	103.2		103.2
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3178.77	4768.16	TRUE		Unknown 1	-8.0	98.4	4768.155	4369.1	91423.8	98.4	103.2	98.4
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3463.01	5194.52	TRUE		Unknown 1	-8.0	108.0	5194.515	4795.5	90997.5	108.0		108.0
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3320.01	4980.02	TRUE		Unknown 1	-8.0	103.2	4980.015	4581.0	91212.0	103.2		103.2
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3770.44	5655.66	TRUE		Unknown 1	-9.0	118.4	5655.66	5256.7	90536.3	118.4	111.1	118.4
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3390.20	5085.30	TRUE		Unknown 1	-9.0	105.5	5085.3	4686.3	91106.7	105.5		105.5
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3504.21	5256.32	TRUE		Unknown 1	-9.0	109.4	5256.315	4857.3	90935.7	109.4		109.4
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3182.10	4773.15	TRUE		Unknown 1	-10.0	98.5	4773.15	4374.1	91418.8	98.5	102.5	98.5
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3353.96	5030.94	TRUE		Unknown 1	-10.0	104.3	5030.94	4631.9	91161.1	104.3		104.3
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3362.32	5043.48	TRUE		Unknown 1	-10.0	104.6	5043.48	4644.5	91148.5	104.6		104.6
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	622.99	934.49	TRUE		Unknown 2	-3.0	12.1	934.485	535.5	95257.5	12.1	10.9	12.1
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	555.28	832.92	TRUE		Unknown 2	-3.0	9.8	832.92	433.9	95359.1	9.8		9.8
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	588.50	882.75	TRUE		Unknown 2	-3.0	10.9	882.75	483.7	95309.2	10.9		10.9
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2251.48	3377.22	TRUE		Unknown 2	-4.0	67.1	3377.22	2978.2	92814.8	67.1	69.3	67.1
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2349.11	3523.67	TRUE		Unknown 2	-4.0	70.4	3523.665	3124.7	92668.3	70.4		70.4
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2352.65	3528.98	TRUE		Unknown 2	-4.0	70.5	3528.975	3130.0	92663.0	70.5		70.5
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2914.56	4371.84	TRUE		Unknown 2	-5.0	89.5	4371.84	3972.8	91820.2	89.5	96.7	89.5
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3337.74	5006.61	TRUE		Unknown 2	-5.0	103.8	5006.61	4607.6	91185.4	103.8		103.8
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3134.89	4702.34	TRUE		Unknown 2	-5.0	96.9	4702.335	4303.3	91489.7	96.9		96.9
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3083.57	4625.36	TRUE		Unknown 2	-6.0	95.2	4625.355	4226.3	91566.6	95.2	98.8	95.2
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1197.37	1796.06	FALSE	mispipet	Unknown 2	-6.0							
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3299.10	4948.65	TRUE		Unknown 2	-6.0	102.4	4948.65	4549.6	91243.3	102.4		102.4
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3202.68	4804.02	TRUE		Unknown 2	-7.0	99.2	4804.02	4405.0	91388.0	99.2	101.3	99.2
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2116.69	3175.04	FALSE	mispipet	Unknown 2	-7.0							
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3328.59	4992.89	TRUE		Unknown 2	-7.0	103.4	4992.885	4593.9	91199.1	103.4		103.4
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3199.45	4799.18	TRUE		Unknown 2	-8.0	99.1	4799.175	4400.2	91392.8	99.1	100.9	99.1
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3373.71	5060.57	TRUE		Unknown 2	-8.0	105.0	5060.565	4661.6	91131.4	105.0		105.0
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3183.71	4775.57	TRUE		Unknown 2	-8.0	98.6	4775.565	4376.6	91416.4	98.6		98.6
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3228.42	4842.63	TRUE		Unknown 2	-9.0	100.1	4842.63	4443.6	91349.4	100.1	101.7	100.1
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3346.90	5020.35	TRUE		Unknown 2	-9.0	104.1	5020.35	4621.3	91171.6	104.1		104.1
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3254.79	4882.19	TRUE		Unknown 2	-9.0	101.0	4882.185	4483.2	91309.8	101.0		101.0
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3097.39	4646.09	TRUE		Unknown 2	-10.0	95.6	4646.085	4247.1	91545.9	95.6	99.4	95.6
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3267.21	4900.82	TRUE		Unknown 2	-10.0	101.4	4900.815	4501.8	91291.2	101.4		101.4
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3264.03	4896.05	TRUE		Unknown 2	-10.0	101.3	4896.045	4497.0	91295.9	101.3		101.3
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	12982.35	19473.53	TRUE		Unknown 3	-3.0	429.5	19473.525	19074.5	76718.5	429.5	435.5	
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	12970.69	19456.04	TRUE		Unknown 3	-3.0	429.1	19456.035	19057.0	76736.0	429.1		
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	13527.52	20291.28	TRUE		Unknown 3	-3.0	447.9	20291.28	19892.3	75900.7	447.9		
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	312.60	468.90	TRUE		Unknown 3	-4.0	1.6	468.9	69.9	95723.1	1.6	1.3	1.6
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	753.32	1129.98	FALSE	mispipet	Unknown 3	-4.0							
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	294.24	441.36	TRUE		Unknown 3	-4.0	1.0	441.36	42.4	95750.6	1.0		1.0
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	391.97	587.96	TRUE		Unknown 3	-5.0	4.3	587.955	188.9	95604.0	4.3	5.0	4.3

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 5.05% — If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped curve (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	438.78	658.17	TRUE		Unknown 3	-5.0	5.8	658.17	259.2	95533.8	5.8		5.8
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	413.62	620.43	TRUE		Unknown 3	-5.0	5.0	620.43	221.4	95571.6	5.0		5.0
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2763.74	4145.61	TRUE		Unknown 3	-6.0	84.4	4145.61	3746.6	92046.4	84.4	83.2	84.4
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2640.25	3960.38	TRUE		Unknown 3	-6.0	80.2	3960.375	3561.4	92231.6	80.2		80.2
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2784.58	4176.87	TRUE		Unknown 3	-6.0	85.1	4176.87	3777.9	92015.1	85.1		85.1
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2987.91	4481.87	TRUE		Unknown 3	-7.0	91.9	4481.865	4082.9	91710.1	91.9	96.1	91.9
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3173.08	4759.62	TRUE		Unknown 3	-7.0	98.2	4759.62	4360.6	91432.4	98.2		98.2
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3174.81	4762.22	TRUE		Unknown 3	-7.0	98.3	4762.215	4363.2	91429.8	98.3		98.3
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3000.05	4500.08	TRUE		Unknown 3	-8.0	92.3	4500.075	4101.1	91691.9	92.3	96.9	92.3
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3212.26	4818.39	TRUE		Unknown 3	-8.0	99.5	4818.39	4419.4	91373.6	99.5		99.5
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3191.24	4786.86	TRUE		Unknown 3	-8.0	98.8	4786.86	4387.9	91405.1	98.8		98.8
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3122.17	4683.26	TRUE		Unknown 3	-9.0	96.5	4683.255	4284.2	91508.7	96.5	106.1	96.5
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3764.82	5647.23	TRUE		Unknown 3	-9.0	118.2	5647.23	5248.2	90544.8	118.2		118.2
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3335.65	5003.48	TRUE		Unknown 3	-9.0	103.7	5003.475	4604.5	91188.5	103.7		103.7
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3043.93	4565.90	TRUE		Unknown 3	-10.0	93.8	4565.895	4166.9	91626.1	93.8	99.7	93.8
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3286.57	4929.86	TRUE		Unknown 3	-10.0	102.0	4929.855	4530.8	91262.1	102.0		102.0
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3319.48	4979.22	TRUE		Unknown 3	-10.0	103.1	4979.22	4580.2	91212.8	103.1		103.1
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1350.93	2026.40	TRUE		Unknown 4	-3.0	36.6	2026.395	1627.4	94165.6	36.6	34.1	36.6
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1267.22	1900.83	TRUE		Unknown 4	-3.0	33.8	1900.83	1501.8	94291.2	33.8		33.8
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1205.23	1807.85	TRUE		Unknown 4	-3.0	31.7	1807.845	1408.8	94384.1	31.7		31.7
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3002.57	4503.86	TRUE		Unknown 4	-4.0	92.4	4503.855	4104.8	91688.1	92.4	97.1	92.4
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3178.97	4768.46	TRUE		Unknown 4	-4.0	98.4	4768.455	4369.4	91423.5	98.4		98.4
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3236.51	4854.77	TRUE		Unknown 4	-4.0	100.3	4854.765	4455.8	91337.2	100.3		100.3
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2942.81	4414.22	TRUE		Unknown 4	-5.0	90.4	4414.215	4015.2	91777.8	90.4	95.3	90.4
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3119.01	4678.52	TRUE		Unknown 4	-5.0	96.4	4678.515	4279.5	91513.5	96.4		96.4
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3200.62	4800.93	TRUE		Unknown 4	-5.0	99.1	4800.93	4401.9	91391.1	99.1		99.1
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2996.55	4494.83	TRUE		Unknown 4	-6.0	92.2	4494.825	4095.8	91697.2	92.2	95.5	92.2
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3199.68	4799.52	TRUE		Unknown 4	-6.0	99.1	4799.52	4400.5	91392.5	99.1		99.1
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3088.01	4632.02	TRUE		Unknown 4	-6.0	95.3	4632.015	4233.0	91560.0	95.3		95.3
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2949.30	4423.95	TRUE		Unknown 4	-7.0	90.6	4423.95	4024.9	91768.0	90.6	86.4	90.6
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2697.46	4046.19	TRUE		Unknown 4	-7.0	82.1	4046.19	3647.2	92145.8	82.1		82.1
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3948.01	5922.02	FALSE	mispipet	Unknown 4	-7.0							
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3028.59	4542.89	TRUE		Unknown 4	-8.0	93.3	4542.885	4143.9	91649.1	93.3	96.2	93.3
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3193.46	4790.19	TRUE		Unknown 4	-8.0	98.9	4790.19	4391.2	91401.8	98.9		98.9
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3122.10	4683.15	TRUE		Unknown 4	-8.0	96.5	4683.15	4284.1	91508.8	96.5		96.5
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2994.50	4491.75	TRUE		Unknown 4	-9.0	92.2	4491.75	4092.7	91700.2	92.2	96.9	92.2
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3151.11	4726.67	TRUE		Unknown 4	-9.0	97.5	4726.665	4327.7	91465.3	97.5		97.5
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3258.84	4888.26	TRUE		Unknown 4	-9.0	101.1	4888.26	4489.3	91303.7	101.1		101.1
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3027.99	4541.99	TRUE		Unknown 4	-10.0	93.3	4541.985	4143.0	91650.0	93.3	97.5	93.3
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3210.38	4815.57	TRUE		Unknown 4	-10.0	99.5	4815.57	4416.6	91376.4	99.5		99.5
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3218.47	4827.71	TRUE		Unknown 4	-10.0	99.7	4827.705	4428.7	91364.3	99.7		99.7
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	248.32	372.48	TRUE		cold E2	-7.0	-0.6	372.48	-26.5	95819.5	-0.6	-0.1	-0.6
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	264.33	396.50	TRUE		cold E2	-7.0	-0.1	396.495	-2.5	95795.5	-0.1		-0.1
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	272.97	409.46	TRUE		cold E2	-7.0	0.2	409.455	10.4	95782.5	0.2		0.2
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3138.79	4708.19	TRUE			—	97.0	4708.185	4309.2	91483.8	97.0	98.7	97.0
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3184.08	4776.12	TRUE			—	98.6	4776.12	4377.1	91415.9	98.6		98.6
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3244.80	4867.20	TRUE			—	100.6	4867.2	4468.2	91324.8	100.6		100.6
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	93511.66	93511.66	TRUE					93511.66	93112.7				
179	2	none	Hot		—	—	—	10	—	—	—	—	100633.70	100633.70	TRUE					100633.7	100234.7				
180	3	none	Hot		—	—	—	10	—	—	—	—	99396.61	99396.61	TRUE					99396.61	98997.6				
181	1	none	Hot		—	—	—	10	—	—	—	—	80519.00	80519.00	TRUE					80519	80120.0				
182	2	none	Hot		—	—	—	10	—	—	—	—	103659.30	103659.30	TRUE					103659.3	103260.3				
183	3	none	Hot		—	—	—	10	—	—	—	—	97037.62	97037.62	TRUE					97037.62	96638.6				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				6.0				7.0				13.0				18.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	0.1899	0.0515	0.1774	-4.0		-0.1539	-0.6717	-3.0				-3.0	12.0579	9.7709	10.8930	-3.0				-3.0	36.6458	33.8183	31.7244	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.5974	-0.0566	0.2352	-4.5	1.1847	4.9046	2.2983	-4.0				-4.0	67.0639	70.3616	70.4812	-4.0	1.5738		0.9536	-4.0	92.4000	98.4000	100.3000	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	15.7758	17.5977	18.1318	-5.5	28.0484	33.1667	30.1335	-5.0	16.5138	16.5878	16.7266	-5.0	89.4610	103.7549	96.9031	-5.0	4.2547	5.8358	4.9860	-5.0	90.4152	96.3667	99.1233	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	36.7626		40.5856	-6.0	56.5116	58.7645	57.1945	-6.0	61.5825	62.8073	67.7013	-6.0	95.1697		102.4497	-6.0	84.3667	80.1955	85.0706	-6.0	92.2304	99.0916	95.3196	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	67.2781	70.8510	72.1954	-6.5	86.3896	87.9619	90.4007	-7.0	93.7605	97.4523	103.1928	-7.0	99.1929		103.4458	-7.0	91.9385	98.1931	98.2515	-7.0	90.6344	82.1279		-7.0	#N/A	#N/A	#N/A
6	3	-9.5	86.4477	87.0662	89.0286	-7.0	91.2106	92.6472	87.6944	-8.0	98.3853	107.9861	103.1560	-8.0	99.0838	104.9698	98.5521	-8.0	92.3486	99.5165	98.8065	-8.0	93.3126	98.8815	96.4711	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	96.9136	98.0847	97.2260	-7.5		100.6305	97.2385	-9.0	118.3703	105.5268	109.3778	-9.0	100.0623	104.0643	100.9530	-9.0	96.4735	118.1805	103.6843	-9.0	92.1611	97.4510	101.0898	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	98.8112	106.6985	102.2136	-8.5	100.0596	102.2149	100.9480	-10.0	98.4978	104.3027	104.5851	-10.0	95.6365	101.3725	101.2651	-10.0	93.8307	102.0265	103.1381	-10.0	93.2923	99.4530	99.7262	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		RN006 5-6-08		Positive Control		RN006 5-6-08		6.0		RN006 5-6-08		7.0		RN006 5-6-08		13.0		RN006 5-6-08		18.0		RN006 5-6-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values		ambiguous		Best-fit values			
13	1	BOTTOM		-2.407		BOTTOM		-0.8947		BOTTOM		-1.399		BOTTOM		-11.68		BOTTOM		1.009		BOTTOM		~ -8.477e+011		BOTTOM			
14	2	TOP		102.7		TOP		99.98		TOP		106.1		TOP		100.6		TOP		99.76		TOP		~ 95.42		TOP			
15	3	LOGIC50		-8.668		LOGIC50		-5.842		LOGIC50		-5.741		LOGIC50		-3.677		LOGIC50		-5.658		LOGIC50		-3.038		LOGIC50			
16	1	HILLSLOPE		-0.9726		HILLSLOPE		-1.086		HILLSLOPE		-0.8889		HILLSLOPE		-1.012		HILLSLOPE		-2.048		HILLSLOPE		~ -3.400		HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.067		BOTTOM		1.836		BOTTOM		14.89		BOTTOM		20.51		BOTTOM		4.224		BOTTOM		~		BOTTOM			
19	1	TOP		1.04		TOP		1.449		TOP		1.895		TOP		0.9979		TOP		1.704		TOP		~ 1.667e+006		TOP			
20	2	LOGIC50		0.01472		LOGIC50		0.02209		LOGIC50		0.07092		LOGIC50		0.0677		LOGIC50		0.09449		LOGIC50		~ 2.870e+022		LOGIC50			
21	3	HILLSLOPE		0.04214		HILLSLOPE		0.08228		HILLSLOPE		0.2291		HILLSLOPE		0.274		HILLSLOPE		0.5723		HILLSLOPE		~ 2.565e+024		HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.1

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Estradiol
Run ID: RN006
Assay start date: 5/6/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-7.0	0.2
1	1	2	-7.0	0.1
1	1	3	-7.0	0.2
1	2	1	-8.0	15.8
1	2	2	-8.0	17.6
1	2	3	-8.0	18.1
1	3	1	-8.5	36.8
1	3	2		
1	3	3	-8.5	40.6
1	4	1	-9.0	67.3
1	4	2	-9.0	70.9
1	4	3	-9.0	72.2
1	5	1	-9.5	86.4
1	5	2	-9.5	87.1
1	5	3	-9.5	89.0
1	6	1	-10.0	96.9
1	6	2	-10.0	98.1
1	6	3	-10.0	97.2
1	7	1	-11.0	98.8
1	7	2	-11.0	106.7
1	7	3	-11.0	102.2
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.9

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Norethynodrel
Run ID: RN006
Assay start date: 5/6/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1		
1	1	2	-4.0	-0.2
1	1	3	-4.0	-0.7
1	2	1	-4.5	1.2
1	2	2	-4.5	4.9
1	2	3	-4.5	2.3
1	3	1	-5.5	28.0
1	3	2	-5.5	33.2
1	3	3	-5.5	30.1
1	4	1	-6.0	56.5
1	4	2	-6.0	58.8
1	4	3	-6.0	57.2
1	5	1	-6.5	86.4
1	5	2	-6.5	88.0
1	5	3	-6.5	90.4
1	6	1	-7.0	91.2
1	6	2	-7.0	92.6
1	6	3	-7.0	87.7
1	7	1		
1	7	2	-7.5	100.6
1	7	3	-7.5	97.2
1	8	1	-8.5	100.1
1	8	2	-8.5	102.2
1	8	3	-8.5	100.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.7

Provide information in all blue cells.
Laboratory Code: B

		run	xgroup	rep	x	y
Compound ID:	Chem Code 6	1	1	1	-3.0	1204.7
Run ID:	RN006	1	1	2		
Assay start date:	5/6/08	1	1	3	-3.0	1231.8
Technician ID:	RN	1	2	1	-4.0	91.6
		1	2	2	-4.0	84.4
		1	2	3	-4.0	97.1
		1	3	1	-5.0	16.5
		1	3	2	-5.0	16.6
		1	3	3	-5.0	16.7
		1	4	1	-6.0	61.6
		1	4	2	-6.0	62.8
		1	4	3	-6.0	67.7
		1	5	1	-7.0	93.8
		1	5	2	-7.0	97.5
		1	5	3	-7.0	103.2
		1	6	1	-8.0	98.4
		1	6	2	-8.0	108.0
		1	6	3	-8.0	103.2
		1	7	1	-9.0	118.4
		1	7	2	-9.0	105.5
		1	7	3	-9.0	109.4
		1	8	1	-10.0	98.5
		1	8	2	-10.0	104.3
		1	8	3	-10.0	104.6
		1	9	1		
		1	9	2		
		1	9	3		
		1	10	1		
		1	10	2		
		1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.8

Provide information in all blue cells.
Laboratory Code: B

		run	xgroup	rep	x	y
Compound ID:	Chem Code 7	1	1	1	-3.0	12.1
Run ID:	RN006	1	1	2	-3.0	9.8
Assay start date:	5/6/08	1	1	3	-3.0	10.9
Technician ID:	RN	1	2	1	-4.0	67.1
		1	2	2	-4.0	70.4
		1	2	3	-4.0	70.5
		1	3	1	-5.0	89.5
		1	3	2	-5.0	103.8
		1	3	3	-5.0	96.9
		1	4	1	-6.0	95.2
		1	4	2		
		1	4	3	-6.0	102.4
		1	5	1	-7.0	99.2
		1	5	2		
		1	5	3	-7.0	103.4
		1	6	1	-8.0	99.1
		1	6	2	-8.0	105.0
		1	6	3	-8.0	98.6
		1	7	1	-9.0	100.1
		1	7	2	-9.0	104.1
		1	7	3	-9.0	101.0
		1	8	1	-10.0	95.6
		1	8	2	-10.0	101.4
		1	8	3	-10.0	101.3
		1	9	1		
		1	9	2		
		1	9	3		
		1	10	1		
		1	10	2		
		1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.3

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 13
Run ID: RN006
Assay start date: 5/6/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	429.5
1	1	2	-3.0	429.1
1	1	3	-3.0	447.9
1	2	1	-4.0	1.6
1	2	2		
1	2	3	-4.0	1.0
1	3	1	-5.0	4.3
1	3	2	-5.0	5.8
1	3	3	-5.0	5.0
1	4	1	-6.0	84.4
1	4	2	-6.0	80.2
1	4	3	-6.0	85.1
1	5	1	-7.0	91.9
1	5	2	-7.0	98.2
1	5	3	-7.0	98.3
1	6	1	-8.0	92.3
1	6	2	-8.0	99.5
1	6	3	-8.0	98.8
1	7	1	-9.0	96.5
1	7	2	-9.0	118.2
1	7	3	-9.0	103.7
1	8	1	-10.0	93.8
1	8	2	-10.0	102.0
1	8	3	-10.0	103.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.9

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 18
Run ID: RN006
Assay start date: 5/6/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	36.6
1	1	2	-3.0	33.8
1	1	3	-3.0	31.7
1	2	1	-4.0	92.4
1	2	2	-4.0	98.4
1	2	3	-4.0	100.3
1	3	1	-5.0	90.4
1	3	2	-5.0	96.4
1	3	3	-5.0	99.1
1	4	1	-6.0	92.2
1	4	2	-6.0	99.1
1	4	3	-6.0	95.3
1	5	1	-7.0	90.6
1	5	2	-7.0	82.1
1	5	3		
1	6	1	-8.0	93.3
1	6	2	-8.0	98.9
1	6	3	-8.0	96.5
1	7	1	-9.0	92.2
1	7	2	-9.0	97.5
1	7	3	-9.0	101.1
1	8	1	-10.0	93.3
1	8	2	-10.0	99.5
1	8	3	-10.0	99.7
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run RN006 5-6-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	0.1899	0.0515	0.1774
2	Title	-7.0	-0.5974	-0.0566	0.2352
3	Title	-8.0	15.7758	17.5977	18.1318
4	Title	-8.5	36.7626		40.5856
5	Title	-9.0	67.2781	70.8510	72.1954
6	Title	-9.5	86.4477	87.0662	89.0286
7	Title	-10.0	96.9136	98.0847	97.2260
8	Title	-11.0	98.8112	106.6985	102.2136

	Nonlin fit Table of results	A run RN006 5-6-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.407
4	TOP	102.7
5	LOGIC50	-8.668
6	HILLSLOPE	-0.9726
7	Std. Error	
8	BOTTOM	1.067
9	TOP	1.040
10	LOGIC50	0.01472
11	HILLSLOPE	0.04214
12	95% Confidence Intervals	
13	BOTTOM	-4.640 to -0.1734
14	TOP	100.5 to 104.9
15	LOGIC50	-8.699 to -8.637
16	HILLSLOPE	-1.061 to -0.8845
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9981
20	Absolute Sum of Squares	74.47
21	Sy.x	1.980
22	Number of points	
23	Analyzed	23

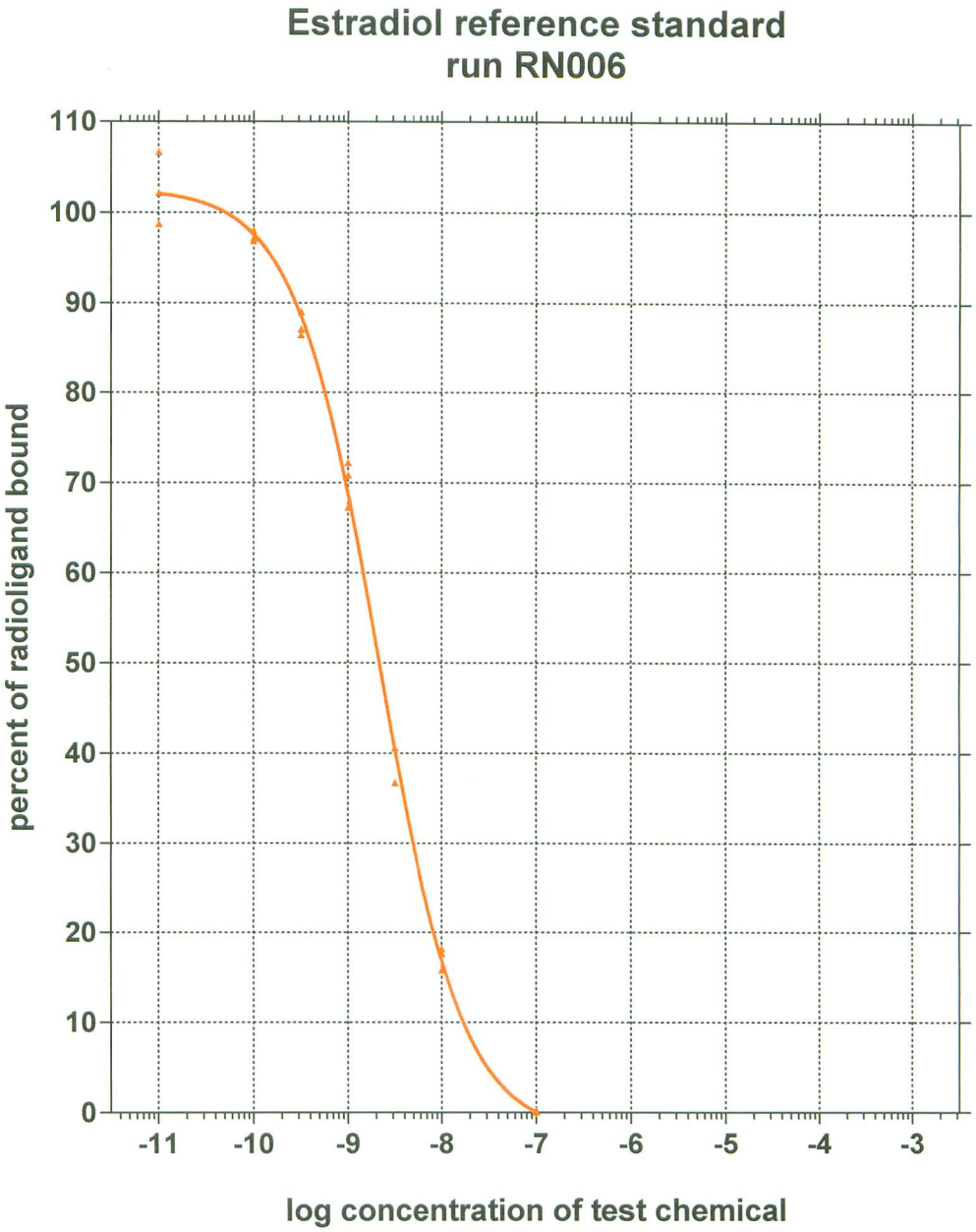

Estradiol reference standard
run RN006
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run RN006 5-6-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0		-0.1539	-0.6717
2	Title	-4.5	1.1847	4.9046	2.2983
3	Title	-5.5	28.0484	33.1667	30.1335
4	Title	-6.0	56.5116	58.7645	57.1945
5	Title	-6.5	86.3896	87.9619	90.4007
6	Title	-7.0	91.2106	92.6472	87.6944
7	Title	-7.5		100.6305	97.2385
8	Title	-8.5	100.0596	102.2149	100.9480

	Nonlin fit Table of results	A run RN006 5-6-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.8947
4	TOP	99.98
5	LOGIC50	-5.842
6	HILLSLOPE	-1.086
7	Std. Error	
8	BOTTOM	1.836
9	TOP	1.449
10	LOGIC50	0.02209
11	HILLSLOPE	0.08228
12	95% Confidence Intervals	
13	BOTTOM	-4.752 to 2.963
14	TOP	96.93 to 103.0
15	LOGIC50	-5.888 to -5.795
16	HILLSLOPE	-1.259 to -0.9129
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9943
20	Absolute Sum of Squares	188.0
21	Sy.x	3.232
22	Number of points	
23	Analyzed	22

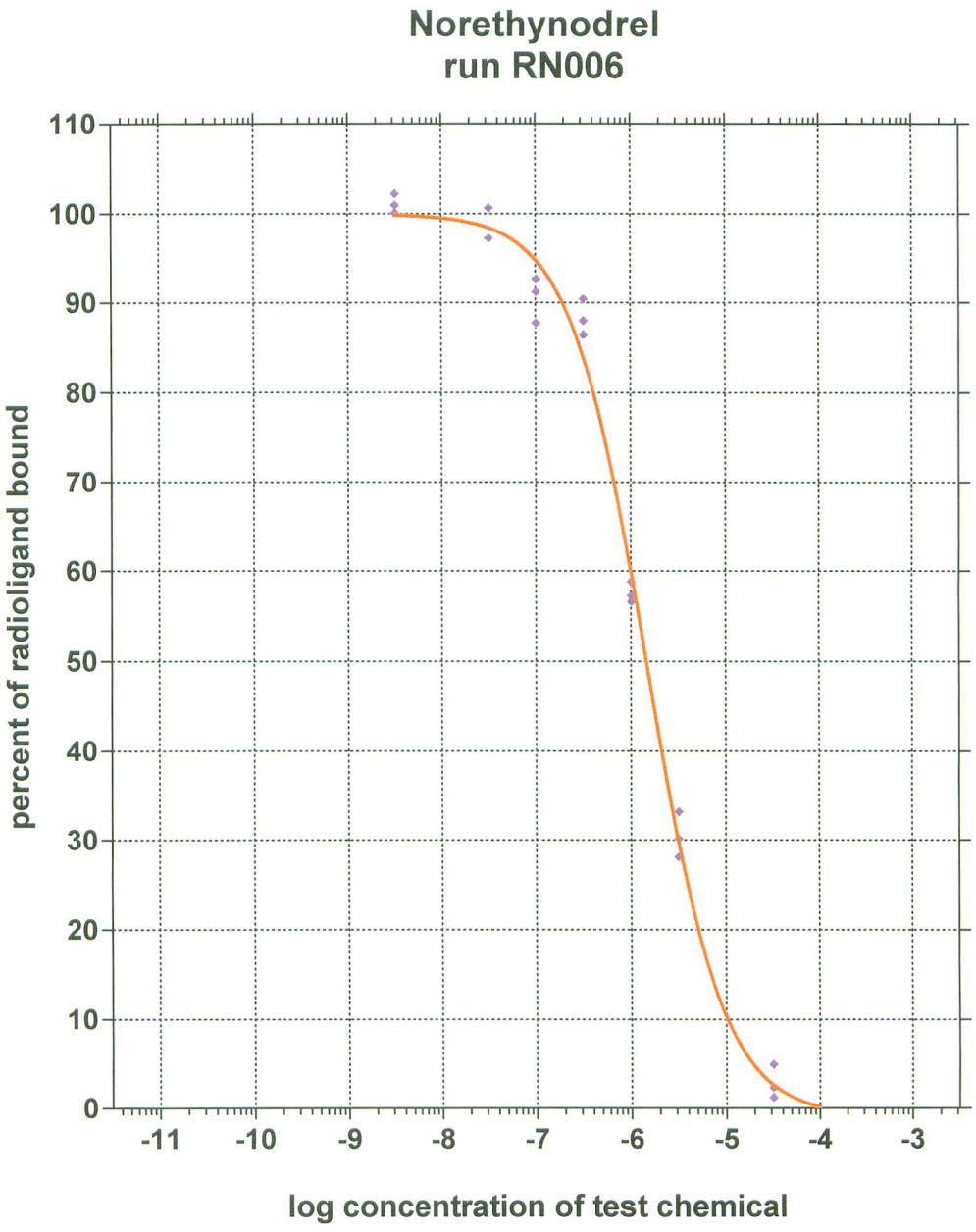

Norethynodrel
run RN006
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 6 run RN006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0			
3	Title	-5.0	16.5138	16.5878	16.7266
4	Title	-6.0	61.5825	62.8073	67.7013
5	Title	-7.0	93.7605	97.4523	103.1928
6	Title	-8.0	98.3853	107.9861	103.1560
7	Title	-9.0	118.3703	105.5268	109.3778
8	Title	-10.0	98.4978	104.3027	104.5851

	Nonlin fit Table of results	A Test chemical 6 run RN006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-1.399
4	TOP	106.1
5	LOGIC50	-5.741
6	HILLSLOPE	-0.8889
7	Std. Error	
8	BOTTOM	14.89
9	TOP	1.895
10	LOGIC50	0.07092
11	HILLSLOPE	0.2291
12	95% Confidence Intervals	
13	BOTTOM	-33.33 to 30.53
14	TOP	102.0 to 110.2
15	LOGIC50	-5.893 to -5.589
16	HILLSLOPE	-1.380 to -0.3975
17	Goodness of Fit	
18	Degrees of Freedom	14
19	R^2	0.9824
20	Absolute Sum of Squares	351.1
21	Sy.x	5.008
22	Number of points	
23	Analyzed	18

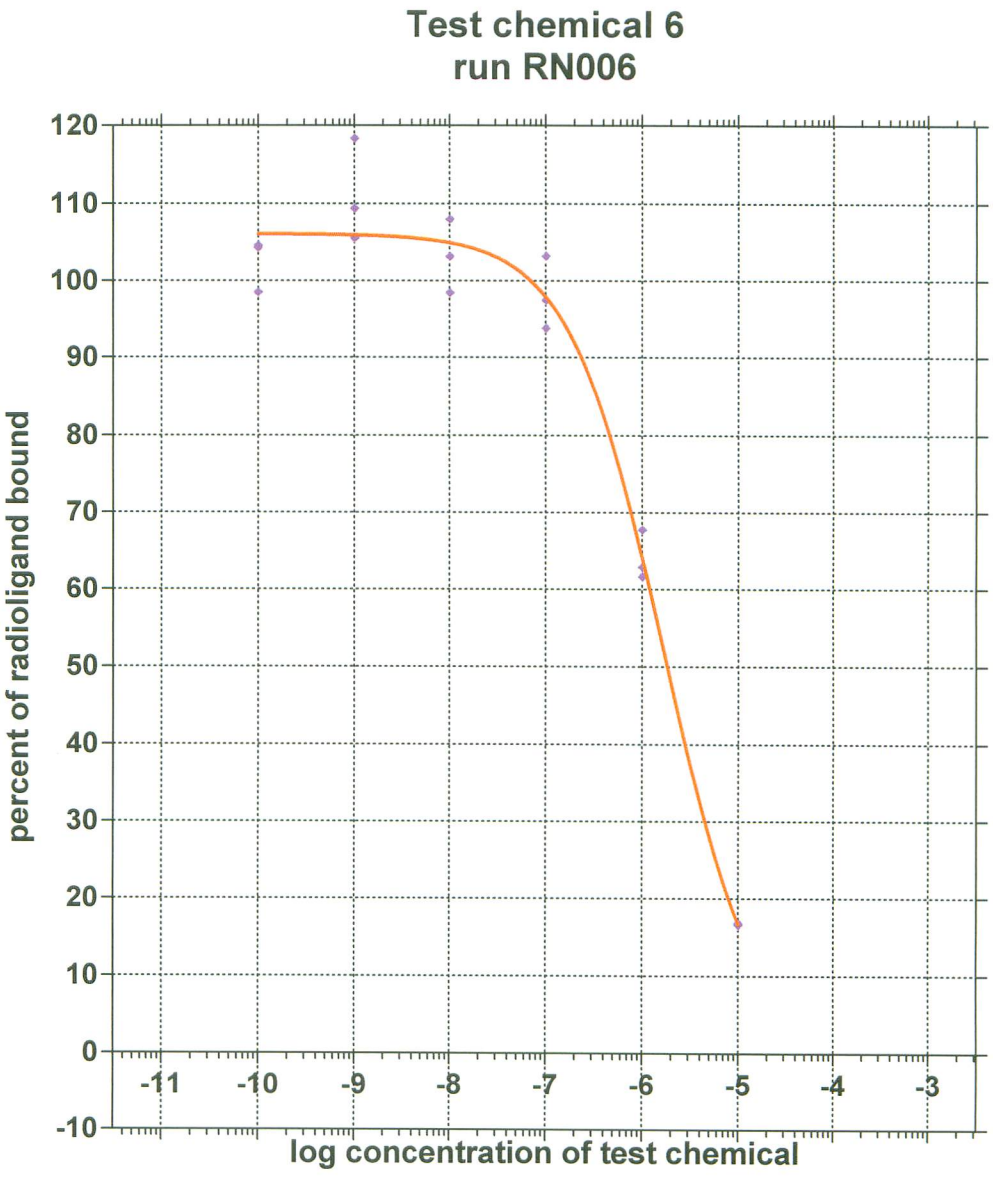

Test chemical 6
run RN006
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 7 run RN006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	12.0579	9.7709	10.8930
2	Title	-4.0	67.0639	70.3616	70.4812
3	Title	-5.0	89.4610	103.7549	96.9031
4	Title	-6.0	95.1697		102.4497
5	Title	-7.0	99.1929		103.4458
6	Title	-8.0	99.0838	104.9698	98.5521
7	Title	-9.0	100.0623	104.0643	100.9530
8	Title	-10.0	95.6365	101.3725	101.2651

	Nonlin fit Table of results	A Test chemical 7 run RN006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-11.68
4	TOP	100.6
5	LOGIC50	-3.677
6	HILLSLOPE	-1.012
7	Std. Error	
8	BOTTOM	20.51
9	TOP	0.9979
10	LOGIC50	0.06770
11	HILLSLOPE	0.2740
12	95% Confidence Intervals	
13	BOTTOM	-54.77 to 31.42
14	TOP	98.49 to 102.7
15	LOGIC50	-3.819 to -3.534
16	HILLSLOPE	-1.588 to -0.4367
17	Goodness of Fit	
18	Degrees of Freedom	18
19	R^2	0.9896
20	Absolute Sum of Squares	216.6
21	Sy.x	3.469
22	Number of points	
23	Analyzed	22

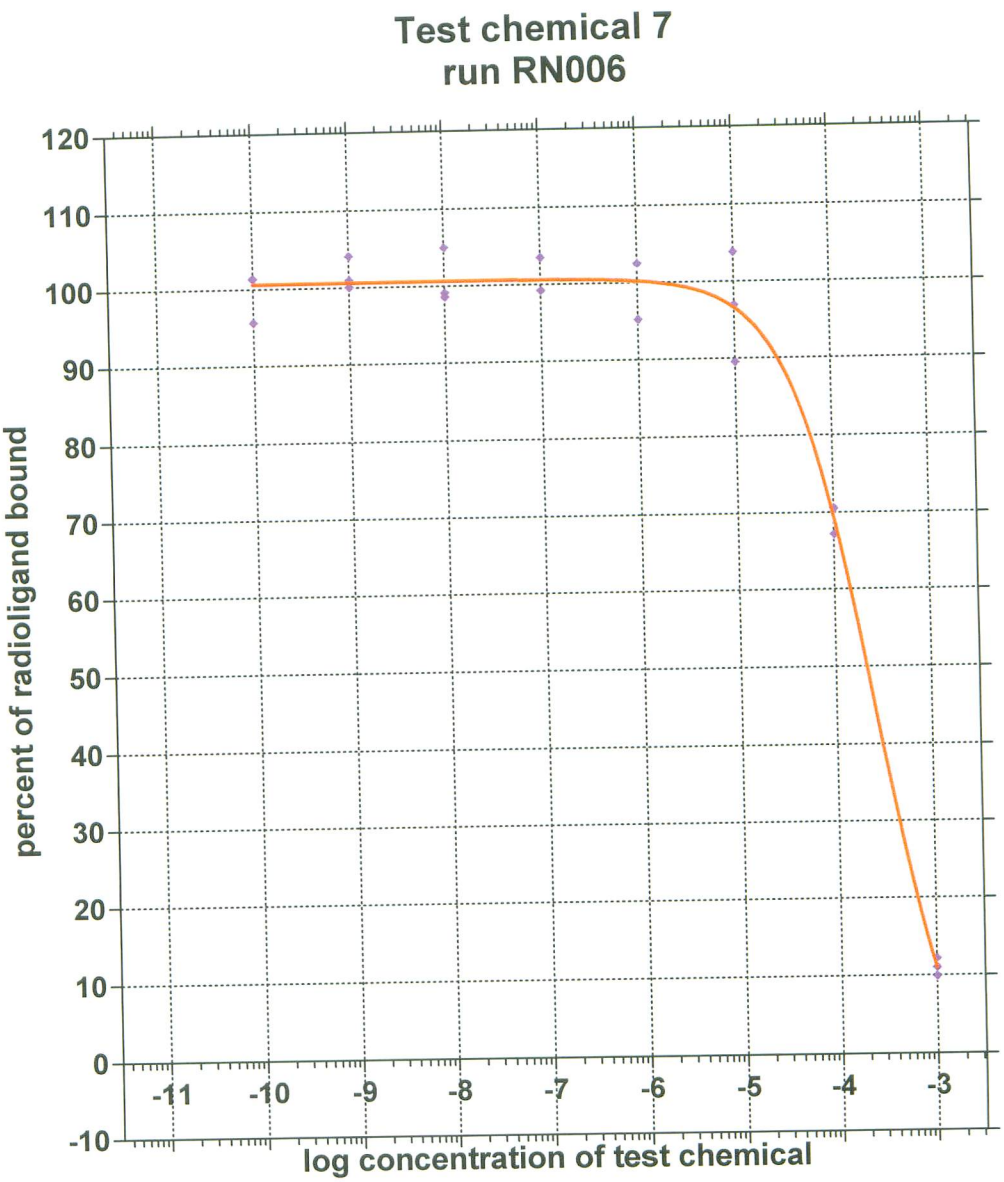

Test chemical 7
run RN006
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
percent of radioligand bound
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 13 run RN006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	1.5738		0.9536
3	Title	-5.0	4.2547	5.8358	4.9860
4	Title	-6.0	84.3667	80.1955	85.0706
5	Title	-7.0	91.9385	98.1931	98.2515
6	Title	-8.0	92.3486	99.5165	98.8065
7	Title	-9.0	96.4735	118.1805	103.6843
8	Title	-10.0	93.8307	102.0265	103.1381

	Nonlin fit Table of results	A Test chemical 13 run RN006
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	1.009
4	TOP	99.76
5	LOGIC50	-5.658
6	HILLSLOPE	-2.048
7	Std. Error	
8	BOTTOM	4.224
9	TOP	1.704
10	LOGIC50	0.09449
11	HILLSLOPE	0.5723
12	95% Confidence Intervals	
13	BOTTOM	-7.945 to 9.963
14	TOP	96.15 to 103.4
15	LOGIC50	-5.859 to -5.458
16	HILLSLOPE	-3.262 to -0.8350
17	Goodness of Fit	
18	Degrees of Freedom	16
19	R^2	0.9836
0	Absolute Sum of Squares	550.7
21	Sy.x	5.867
22	Number of points	
23	Analyzed	20

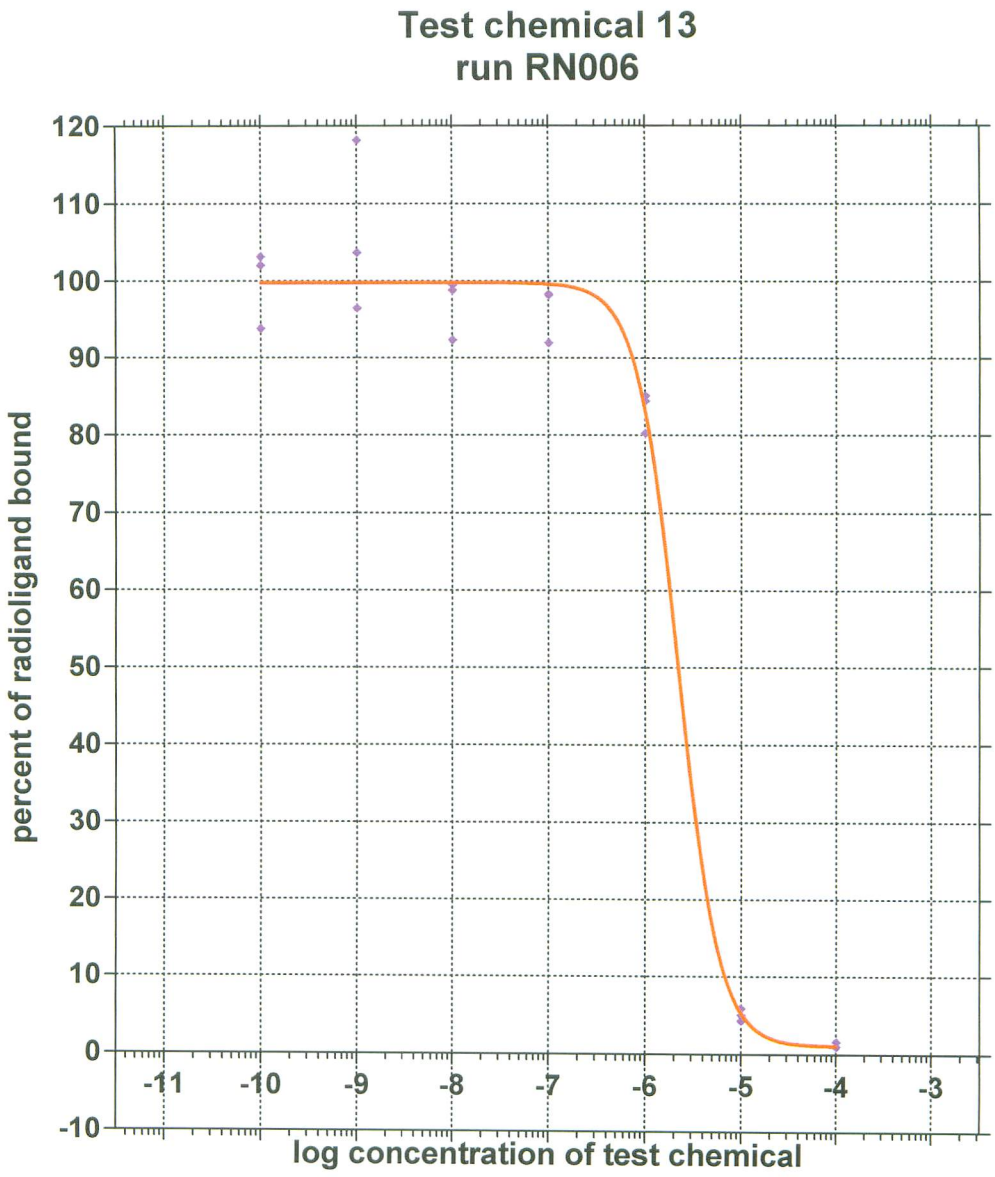
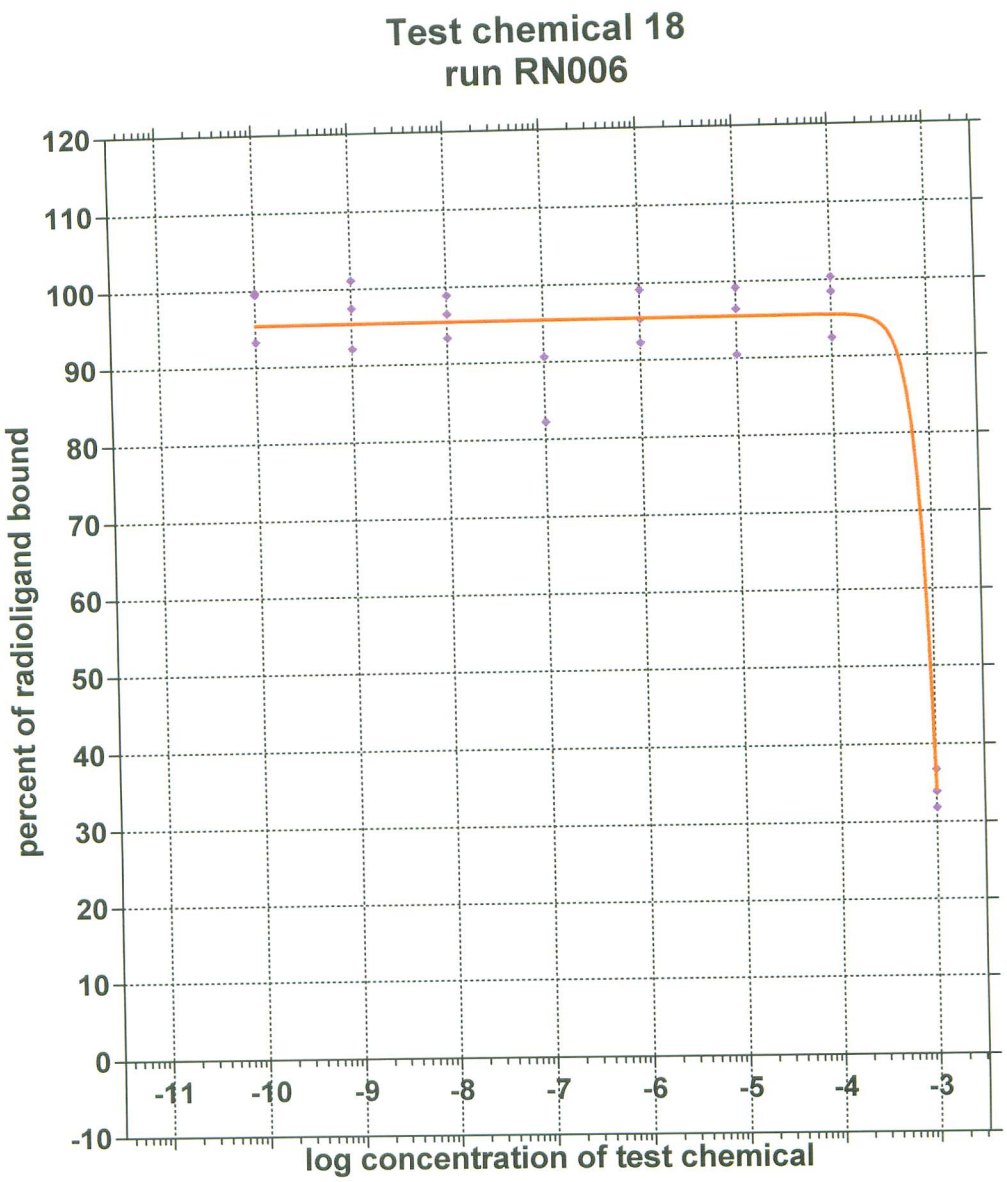
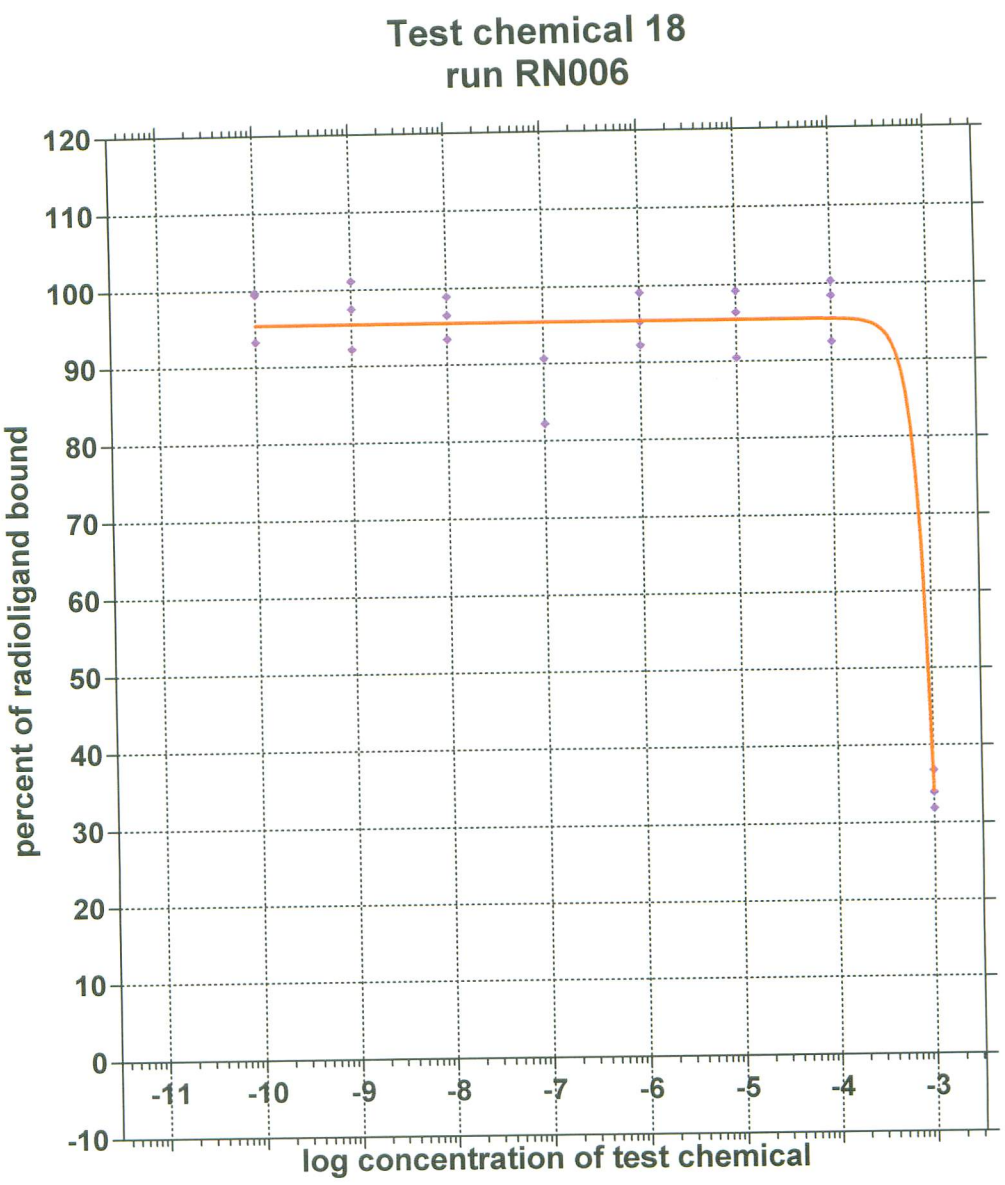

Test chemical 13
run RN006
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 18 run RN006		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	36.6458	33.8183	31.7244
2	Title	-4.0	92.4000	98.4000	100.3000
3	Title	-5.0	90.4152	96.3667	99.1233
4	Title	-6.0	92.2304	99.0916	95.3196
5	Title	-7.0	90.6344	82.1279	
6	Title	-8.0	93.3126	98.8815	96.4711
7	Title	-9.0	92.1611	97.4510	101.0898
8	Title	-10.0	93.2923	99.4530	99.7262

	Nonlin fit Table of results	A Test chemical 18 run RN006
		Y
1	logIC50	Ambiguous
2	Best-fit values	
3	BOTTOM	~ -8.477e+011
4	TOP	~ 95.42
5	LOGIC50	~ -3.038
6	HILLSLOPE	~ -3.400
7	Std. Error	
8	BOTTOM	~
9	TOP	~ 1.667e+006
10	LOGIC50	~ 2.870e+022
11	HILLSLOPE	~ 2.565e+024
12	95% Confidence Intervals	
13	BOTTOM	(Very wide)
14	TOP	(Very wide)
15	LOGIC50	(Very wide)
16	HILLSLOPE	(Very wide)
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9589
20	Absolute Sum of Squares	420.9
21	Sy.x	4.707
22	Number of points	
23	Analyzed	23


Test chemical 18
run RN006
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 18
run RN006
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

APPENDIX U

Excel and Prism Files of the Run Identifier RN008

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.

If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software, and the maximum concentrations for each of the unknowns.

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	RN008	
Assay start date:	5/12/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.79	Ci/mmole
Cytosol vial or lot identification:	RUC3-3-08A	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.67	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.77	M
RBA, norethynodrel (Weak Positive):	1.262E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-8.72	2.0
RBA, Unknown 1:	1.122E+00	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-4.67	8.0
RBA, Unknown 2:	1.012E-04	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-3.04	10.0
RBA, Unknown 3:	2.388E-06	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	-5.22	11.0
RBA, Unknown 4:	3.606E-04	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Check the 10% rule: 6.06%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Summary values	n	Mean	SD	
EtOH	6	4873.2	703.02	Total Binding, solvent control, tubes
Hot	6	80397.5	5656.98	Total hot E2 added to each tube
NSB	5	345.2	49.79	Nonspecific Binding
Specific EtOH	6	4528.0	703.02	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values			
EtOH / Hot	0.06	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.07	around 0.25 ?	

	log conc'n at min hot binding	min hot (%)
cold E2	-7.0	0.4
R1881	#N/A	not applicable
norethynodrel	-4.5	2.7
Unknown 1	-6.0	-2.7
Unknown 2	-4.0	31.0
Unknown 3	-3.0	46.8
Unknown 4	-4.0	6.6

Values for analysis by nonlinear regression

Standard Curve

Negative Control

Weak Positive

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Competitive Assay Tube Layout - Four Test Chemicals

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3360.52	5040.78	TRUE			—	103.7	5040.78	4695.6	75702.0	103.7	106.1	103.7
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3425.01	5137.52	TRUE			—	105.8	5137.515	4792.3	75605.3	105.8		105.8
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3516.72	5275.08	TRUE			—	108.9	5275.08	4929.9	75467.7	108.9		108.9
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	284.07	426.11	TRUE		cold E2	-7.0	1.8	426.105	80.9	80316.7	1.8	0.4	1.8
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	233.97	350.96	FALSE	mispipet	cold E2	-7.0							
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	199.08	298.62	TRUE		cold E2	-7.0	-1.0	298.62	-46.6	80444.2	-1.0		-1.0
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	793.86	1190.79	TRUE		cold E2	-8.0	18.7	1190.79	845.6	79552.0	18.7	16.8	18.7
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	730.51	1095.77	TRUE		cold E2	-8.0	16.6	1095.765	750.5	79647.0	16.6		16.6
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	685.67	1028.51	TRUE		cold E2	-8.0	15.1	1028.505	683.3	79714.3	15.1		15.1
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1356.04	2034.06	TRUE		cold E2	-8.5	37.3	2034.06	1688.8	78708.7	37.3	38.2	37.3
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1414.51	2121.77	TRUE		cold E2	-8.5	39.2	2121.765	1776.5	78621.0	39.2		39.2
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1377.31	2065.97	TRUE		cold E2	-8.5	38.0	2065.965	1720.7	78676.8	38.0		38.0
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2291.22	3436.83	TRUE		cold E2	-9.0	68.3	3436.83	3091.6	77305.9	68.3	70.8	68.3
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2424.90	3637.35	TRUE		cold E2	-9.0	72.7	3637.35	3292.1	77105.4	72.7		72.7
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2381.93	3572.90	TRUE		cold E2	-9.0	71.3	3572.895	3227.7	77169.9	71.3		71.3
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2917.06	4375.59	TRUE		cold E2	-9.5	89.0	4375.59	4030.4	76367.2	89.0	95.8	89.0
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3313.69	4970.54	TRUE		cold E2	-9.5	102.1	4970.535	4625.3	75772.2	102.1		102.1
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	3137.67	4706.51	TRUE		cold E2	-9.5	96.3	4706.505	4361.3	76036.3	96.3		96.3
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2001.13	3001.70	FALSE	mispipet	cold E2	-10.0						102.8	
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2378.29	3567.44	FALSE	mispipet	cold E2	-10.0							
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	3334.17	5001.26	TRUE		cold E2	-10.0	102.8	5001.255	4656.0	75741.5	102.8		102.8
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3549.12	5323.68	TRUE		cold E2	-11.0	109.9	5323.68	4978.5	75419.1	109.9	105.9	109.9
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3244.88	4867.32	TRUE		cold E2	-11.0	99.9	4867.32	4522.1	75875.5	99.9		99.9
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3484.31	5226.47	TRUE		cold E2	-11.0	107.8	5226.465	4881.2	75516.3	107.8		107.8
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 6.06%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE	
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE	
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE	
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE	
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE	
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE	
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE	
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE	
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE	
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE	
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	1899.25	2848.88	TRUE	
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	233.06	349.59	TRUE	
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	2161.22	3241.83	TRUE	
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	320.96	481.44	TRUE	
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	301.12	451.68	TRUE	
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	308.40	462.60	TRUE	
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1204.97	1807.46	TRUE	
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1344.31	2016.47	TRUE	
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1434.00	2151.00	TRUE	
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2050.24	3075.36	TRUE	
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2010.65	3015.98	TRUE	
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2104.10	3156.15	TRUE	
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2821.85	4232.78	TRUE	
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2929.78	4394.67	TRUE	
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	3043.56	4565.34	TRUE	
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3102.81	4654.22	TRUE	
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3386.46	5079.69	TRUE	
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3357.37	5036.06	TRUE	
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3259.34	4889.01	TRUE	
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3263.65	4895.48	TRUE	
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	3092.65	4638.98	TRUE	
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3193.43	4790.15	TRUE	
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3546.08	5319.12	TRUE	
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3534.30	5301.45	TRUE	
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7444.55	11166.83	TRUE	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7411.85	11117.78	TRUE	
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6980.62	10470.93	TRUE	
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	514.93	772.40	TRUE	
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	395.03	592.55	TRUE	
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	384.81	577.22	TRUE	
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	152.31	228.47	TRUE	
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	158.95	238.43	TRUE	
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	147.37	221.06	TRUE	
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	137.14	205.71	TRUE	
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	134.92	202.38	TRUE	
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	171.91	257.87	TRUE	
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	171.21	256.82	TRUE	
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	188.77	283.16	TRUE	
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	182.00	273.00	TRUE	
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	597.60	896.40	TRUE	
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	605.68	908.52	TRUE	
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	600.75	901.13	TRUE	
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2156.18	3234.27	TRUE	
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2403.75	3605.63	TRUE	
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2274.14	3411.21	TRUE	
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2422.44	3633.66	TRUE	
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3360.51	5040.77	TRUE	
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3378.47	5067.71	TRUE	
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6875.24	10312.86	TRUE	
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	10242.18	15363.27	TRUE	
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	10773.38	16160.07	TRUE	
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1042.51	1563.77	TRUE	
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	303.58	455.37	FALSE	mispipet
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1287.45	1931.18	TRUE	
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2000.78	3001.17	TRUE	
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2216.36	3324.54	TRUE	
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2575.19	3862.79	TRUE	
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2955.17	4432.76	TRUE	
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3303.72	4955.58	TRUE	
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3233.73	4850.60	TRUE	
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2981.47	4472.21	TRUE	
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3414.07	5121.11	TRUE	
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3261.17	4891.76	TRUE	
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2610.49	3915.74	TRUE	
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2872.63	4308.95	TRUE	
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3388.32	5082.48	TRUE	
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3203.03	4804.55	TRUE	
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3432.70	5149.05	TRUE	
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3519.80	5279.70	TRUE	
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3368.69	5053.04	TRUE	
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3441.18	5161.77	TRUE	

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
R1881	-7.0							#N/A
R1881	-8.0						no average	#N/A
R1881	-8.0							#N/A
R1881	-8.0							#N/A
R1881	-9.0						no average	#N/A
R1881	-9.0							#N/A
R1881	-9.0							#N/A
R1881	-10.0						no average	#N/A
R1881	-10.0							#N/A
R1881	-10.0							#N/A
norethynodrel	-4.0	55.3	2848.875	2503.7	77893.9	55.3	39.8	
norethynodrel	-4.0	0.1	349.59	4.4	80393.2	0.1		
norethynodrel	-4.0	64.0	3241.83	2896.6	77500.9	64.0		
norethynodrel	-4.5	3.0	481.44	136.2	80261.3	3.0	2.7	3.0
norethynodrel	-4.5	2.4	451.68	106.5	80291.1	2.4		2.4
norethynodrel	-4.5	2.6	462.6	117.4	80280.2	2.6		2.6
norethynodrel	-5.5	32.3	1807.455	1462.2	78935.3	32.3	36.4	32.3
norethynodrel	-5.5	36.9	2016.465	1671.2	78726.3	36.9		36.9
norethynodrel	-5.5	39.9	2151	1805.8	78591.8	39.9		39.9
norethynodrel	-6.0	60.3	3075.36	2730.1	77667.4	60.3	60.5	60.3
norethynodrel	-6.0	59.0	3015.975	2670.8	77726.8	59.0		59.0
norethynodrel	-6.0	62.1	3156.15	2810.9	77586.6	62.1		62.1
norethynodrel	-6.5	85.9	4232.775	3887.6	76510.0	85.9	89.5	85.9
norethynodrel	-6.5	89.4	4394.67	4049.4	76348.1	89.4		89.4
norethynodrel	-6.5	93.2	4565.34	4220.1	76177.4	93.2		93.2
norethynodrel	-7.0	95.2	4654.215	4309.0	76088.6	95.2	101.1	95.2
norethynodrel	-7.0	104.6	5079.69	4734.5	75663.1	104.6		104.6
norethynodrel	-7.0	103.6	5036.055	4690.8	75706.7	103.6		103.6
norethynodrel	-7.5	100.3	4889.01	4543.8	75853.8	100.3	98.6	100.3
norethynodrel	-7.5	100.5	4895.475	4550.3	75847.3	100.5		100.5
norethynodrel	-7.5	94.8	4638.975	4293.8	76103.8	94.8		94.8
norethynodrel	-8.5	98.2	4790.145	4444.9	75952.6	98.2	105.8	98.2
norethynodrel	-8.5	109.8	5319.12	4973.9	75423.7	109.8		109.8
norethynodrel	-8.5	109.5	5301.45	4956.2	75441.3	109.5		109.5
Unknown 1	-3.0	239.0	11166.825	10821.6	69575.9	239.0	233.5	
Unknown 1	-3.0	237.9	11117.775	10772.6	69625.0	237.9		
Unknown 1	-3.0	223.6	10470.93	10125.7	70271.8	223.5		
Unknown 1	-4.0	9.4	772.395	427.2	79970.4	9.4	6.7	9.4
Unknown 1	-4.0	5.5	592.545	247.3	80150.2	5.5		5.5
Unknown 1	-4.0	5.1	577.215	232.0	80165.6	5.1		5.1
Unknown 1	-5.0	-2.6	228.465	-116.8	80514.3	-2.6	-2.6	-2.6
Unknown 1	-5.0	-2.4	238.425	-106.8	80504.3	-2.4		-2.4
Unknown 1	-5.0	-2.7	221.055	-124.2	80521.7	-2.7		-2.7
Unknown 1	-6.0	-3.1	205.71	-139.5	80537.1	-3.1	-2.7	-3.1
Unknown 1	-6.0	-3.2	202.38	-142.8	80540.4	-3.2		-3.2
Unknown 1	-6.0	-1.9	257.865	-87.4	80484.9	-1.9		-1.9
Unknown 1	-7.0	-2.0	256.815	-88.4	80486.0	-2.0	-1.6	-2.0
Unknown 1	-7.0	-1.4	283.155	-62.1	80459.6	-1.4		-1.4
Unknown 1	-7.0	-1.6	273	-72.2	80469.8	-1.6		-1.6
Unknown 1	-8.0	12.2	896.4	551.2	79846.4	12.2	12.3	12.2
Unknown 1	-8.0	12.4	908.52	563.3	79834.3	12.4		12.4
Unknown 1	-8.0	12.3	901.125	555.9	79841.6	12.3		12.3
Unknown 1	-9.0	63.8	3234.27	2889.0	77508.5	63.8	67.8	63.8
Unknown 1	-9.0	72.0	3605.625	3260.4	77137.1	72.0		72.0
Unknown 1	-9.0	67.7	3411.21	3066.0	77331.6	67.7		67.7
Unknown 1	-10.0	72.6	3633.66	3288.4	77109.1	72.6	93.5	72.6
Unknown 1	-10.0	103.7	5040.765	4695.5	75702.0	103.7		103.7
Unknown 1	-10.0	104.3	5067.705	4722.5	75675.1	104.3		104.3
Unknown 2	-3.0	220.1	10312.86	9967.6	70429.9	220.1	300.4	
Unknown 2	-3.0	331.7	15363.27	15018.0	65379.5	331.7		
Unknown 2	-3.0	349.3	16160.07	15814.8	64582.7	349.3		
Unknown 2	-4.0	26.9	1563.765	1218.5	79179.0	26.9	31.0	26.9
Unknown 2	-4.0							
Unknown 2	-4.0	35.0	1931.175	1586.0	78811.6	35.0		35.0
Unknown 2	-5.0	58.7	3001.17	2655.9	77741.6	58.7	67.4	58.7
Unknown 2	-5.0	65.8	3324.54	2979.3	77418.2	65.8		65.8
Unknown 2	-5.0	77.7	3862.785	3517.6	76880.0	77.7		77.7
Unknown 2	-6.0	90.3	4432.755	4087.5	76310.0	90.3	97.2	90.3
Unknown 2	-6.0	101.8	4955.58	4610.4	75787.2	101.8		101.8
Unknown 2	-6.0	99.5	4850.595	4505.4	75892.2	99.5		99.5
Unknown 2	-7.0	91.1	4472.205	4127.0	76270.6	91.1	99.0	91.1
Unknown 2	-7.0	105.5	5121.105	4775.9	75621.7	105.5		105.5
Unknown 2	-7.0	100.4	4891.755	4546.5	75851.0	100.4		100.4
Unknown 2	-8.0	78.9	3915.735	3570.5	76827.0	78.9	90.3	78.9
Unknown 2	-8.0	87.5	4308.945	3963.7	76433.8	87.5		87.5
Unknown 2	-8.0	104.6	5082.48	4737.3	75660.3	104.6		104.6
Unknown 2	-9.0	98.5	4804.545	4459.3	75938.2	98.5	104.5	98.5
Unknown 2	-9.0	106.1	5149.05	4803.8	75593.7	106.1		106.1
Unknown 2	-9.0	109.0	5279.7	4934.5	75463.1	109.0		109.0
Unknown 2	-10.0	104.0	5053.035	4707.8	75689.7	104.0	105.3	104.0
Unknown 2	-10.0	106.4	5161.77	4816.5	75581.0	106.4		106.4

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 6.06%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3412.42	5118.63	TRUE	
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1412.87	2119.31	TRUE	
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1734.96	2602.44	TRUE	
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	1783.17	2674.76	TRUE	
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2927.77	4391.66	TRUE	
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2712.55	4068.83	TRUE	
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	3201.49	4802.24	TRUE	
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3369.08	5053.62	TRUE	
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	3462.37	5193.56	TRUE	
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2974.57	4461.86	TRUE	
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3168.90	4753.35	TRUE	
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3404.97	5107.46	TRUE	
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	3321.56	4982.34	TRUE	
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3069.86	4604.79	TRUE	
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3555.91	5333.87	TRUE	
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3341.25	5011.88	TRUE	
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3186.14	4779.21	TRUE	
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3422.98	5134.47	TRUE	
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3424.30	5136.45	TRUE	
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3213.21	4819.82	TRUE	
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3423.26	5134.89	TRUE	
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3410.76	5116.14	TRUE	
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3273.74	4910.61	TRUE	
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3186.38	4779.57	TRUE	
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3549.08	5323.62	TRUE	
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	3931.64	5897.46	TRUE	
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	4792.43	7188.65	TRUE	
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	2573.84	3860.76	TRUE	
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	421.75	632.63	TRUE	
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	437.38	656.07	TRUE	
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	432.77	649.16	TRUE	
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1327.75	1991.63	TRUE	
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1240.06	1860.09	TRUE	
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1487.73	2231.60	TRUE	
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2453.37	3680.06	TRUE	
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2801.66	4202.49	TRUE	
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2807.25	4210.88	TRUE	
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2953.23	4429.85	TRUE	
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3158.51	4737.77	TRUE	
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	3064.70	4597.05	TRUE	
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3183.59	4775.39	TRUE	
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	3148.00	4722.00	TRUE	
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2877.30	4315.95	TRUE	
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3087.12	4630.68	TRUE	
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2994.17	4491.26	TRUE	
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	3317.50	4976.25	TRUE	
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2612.41	3918.62	TRUE	
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	3201.30	4801.95	TRUE	
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2422.28	3633.42	TRUE	
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	216.61	324.92	TRUE	
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	237.88	356.82	TRUE	
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	213.10	319.65	TRUE	
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2298.79	3448.19	TRUE	
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3483.01	5224.52	TRUE	
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	3408.87	5113.31	TRUE	
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE	
178	1	none	Hot		—	—	—	10	—	—	—	—	75345.75	75345.75	TRUE	
179	2	none	Hot		—	—	—	10	—	—	—	—	74155.27	74155.27	TRUE	
180	3	none	Hot		—	—	—	10	—	—	—	—	77465.35	77465.35	TRUE	
181	1	none	Hot		—	—	—	10	—	—	—	—	81540.49	81540.49	TRUE	
182	2	none	Hot		—	—	—	10	—	—	—	—	86817.80	86817.80	TRUE	
183	3	none	Hot		—	—	—	10	—	—	—	—	87060.63	87060.63	TRUE	

Unknown

	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
Unknown 2	-10.0	105.4	5118.63	4773.4	75624.1	105.4		105.4
Unknown 3	-3.0	39.2	2119.305	1774.1	78623.5	39.2	46.8	39.2
Unknown 3	-3.0	49.9	2602.44	2257.2	78140.3	49.9		49.9
Unknown 3	-3.0	51.4	2674.755	2329.5	78068.0	51.4		51.4
Unknown 3	-4.0	89.4	4391.655	4046.4	76351.1	89.4	90.0	89.4
Unknown 3	-4.0	82.2	4068.825	3723.6	76673.9	82.2		82.2
Unknown 3	-4.0	98.4	4802.235	4457.0	75940.5	98.4		98.4
Unknown 3	-5.0	104.0	5053.62	4708.4	75689.2	104.0	100.7	104.0
Unknown 3	-5.0	107.1	5193.555	4848.3	75549.2	107.1		107.1
Unknown 3	-5.0	90.9	4461.855	4116.6	76280.9	90.9		90.9
Unknown 3	-6.0	97.4	4753.35	4408.1	75989.4	97.4	101.6	97.4
Unknown 3	-6.0	105.2	5107.455	4762.2	75635.3	105.2		105.2
Unknown 3	-6.0	102.4	4982.34	4637.1	75760.4	102.4		102.4
Unknown 3	-7.0	94.1	4604.79	4259.6	76138.0	94.1	102.4	94.1
Unknown 3	-7.0	110.2	5333.865	4988.6	75408.9	110.2		110.2
Unknown 3	-7.0	103.1	5011.875	4666.7	75730.9	103.1		103.1
Unknown 3	-8.0	97.9	4779.21	4434.0	75963.6	97.9	103.2	97.9
Unknown 3	-8.0	105.8	5134.47	4789.2	75608.3	105.8		105.8
Unknown 3	-8.0	105.8	5136.45	4791.2	75606.3	105.8		105.8
Unknown 3	-9.0	98.8	4819.815	4474.6	75923.0	98.8	103.3	98.8
Unknown 3	-9.0	105.8	5134.89	4789.7	75607.9	105.8		105.8
Unknown 3	-9.0	105.4	5116.14	4770.9	75626.6	105.4		105.4
Unknown 3	-10.0	100.8	4910.61	4565.4	75832.2	100.8	102.9	100.8
Unknown 3	-10.0	97.9	4779.57	4434.3	75963.2	97.9		97.9
Unknown 3	-10.0	109.9	5323.62	4978.4	75419.2	109.9		109.9
Unknown 4	-3.0	122.6	5897.46	5552.2	74845.3	122.6	117.1	
Unknown 4	-3.0	151.1	7188.645	6843.4	73554.1	151.1		
Unknown 4	-3.0	77.6	3860.76	3515.5	76882.0	77.6		
Unknown 4	-4.0	6.3	632.625	287.4	80110.1	6.3	6.6	
Unknown 4	-4.0	6.9	656.07	310.8	80086.7	6.9		3.4
Unknown 4	-4.0	6.7	649.155	303.9	80093.6	6.7		4.2
Unknown 4	-5.0	36.4	1991.625	1646.4	78751.1	36.4	37.2	36.4
Unknown 4	-5.0	33.5	1860.09	1514.9	78882.7	33.5		33.5
Unknown 4	-5.0	41.7	2231.595	1886.4	78511.2	41.7		41.7
Unknown 4	-6.0	73.6	3680.055	3334.8	77062.7	73.6	81.4	73.6
Unknown 4	-6.0	85.2	4202.49	3857.3	76540.3	85.2		85.2
Unknown 4	-6.0	85.4	4210.875	3865.7	76531.9	85.4		85.4
Unknown 4	-7.0	90.2	4429.845	4084.6	76312.9	90.2	93.7	90.2
Unknown 4	-7.0	97.0	4737.765	4392.5	76005.0	97.0		97.0
Unknown 4	-7.0	93.9	4597.05	4251.8	76145.7	93.9		93.9
Unknown 4	-8.0	97.8	4775.385	4430.2	75967.4	97.8	94.1	97.8
Unknown 4	-8.0	96.7	4722	4376.8	76020.8	96.7		96.7
Unknown 4	-8.0	87.7	4315.95	3970.7	76426.8	87.7		87.7
Unknown 4	-9.0	94.6	4630.68	4285.5	76112.1	94.6	96.2	94.6
Unknown 4	-9.0	91.6	4491.255	4146.0	76251.5	91.6		91.6
Unknown 4	-9.0	102.3	4976.25	4631.0	75766.5	102.3		102.3
Unknown 4	-10.0	78.9	3918.615	3573.4	76824.2	78.9	83.3	78.9
Unknown 4	-10.0	98.4	4801.95	4456.7	75940.8	98.4		98.4
Unknown 4	-10.0	72.6	3633.42	3288.2	77109.4	72.6		72.6
cold E2	-7.0	-0.4	324.915	-20.3	80417.9	-0.4	-0.3	-0.4
cold E2	-7.0	0.3	356.82	11.6	80386.0	0.3		0.3
cold E2	-7.0	-0.6	319.65	-25.6	80423.1	-0.6		-0.6
	—	68.5	3448.185	3103.0	77294.6	68.5	93.9	68.5
	—	107.8	5224.515	4879.3	75518.3	107.8		107.8
	—	105.3	5113.305	4768.1	75629.5	105.3		105.3
	-6.0						no average	
	-6.0							
	-6.0							
			75345.75	75000.5				
			74155.27	73810.0				
			77465.35	77120.1				
			81540.49	81195.3				
			86817.8	86472.6				
			87060.63	86715.4				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				2.0				8.0				10.0				11.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	1.7863		-1.0292	-4.0				-3.0				-3.0				-3.0	39.1802	49.8501	51.4472	-3.0				-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-0.4485	0.2561	-0.5648	-4.5	3.0083	2.3511	2.5923	-4.0	9.4340	5.4621	5.1235	-4.0	26.9112		35.0254	-4.0	89.3645	82.2349	98.4321	-4.0	0.0000	3.4000	4.2000	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	18.6742	16.5756	15.0901	-5.5	32.2931	36.9090	39.8802	-5.0	-2.5786	-2.3586	-2.7422	-5.0	58.6560	65.7975	77.6846	-5.0	103.9839	107.0743	90.9149	-5.0	36.3604	33.4555	41.6601	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	37.2976	39.2345	38.0022	-6.0	60.2945	58.9830	62.0787	-6.0	-3.0811	-3.1546	-1.9293	-6.0	90.2722	101.8187	99.5001	-6.0	97.3525	105.1728	102.4097	-6.0	73.6490	85.1869	85.3720	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	68.2774	72.7059	71.2824	-6.5	85.8557	89.4311	93.2003	-7.0	-1.9524	-1.3707	-1.5950	-7.0	91.1435	105.4743	100.4091	-7.0	94.0716	110.1730	103.0619	-7.0	90.2079	97.0083	93.9006	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	89.0097	102.1490	96.3179	-7.0	95.1631	104.5596	103.5960	-8.0	12.1726	12.4403	12.2770	-8.0	78.8539	87.5379	104.6212	-8.0	97.9236	105.7694	105.8132	-8.0	97.8391	96.6601	87.6926	-8.0	#N/A	#N/A	#N/A
7	1	-10.0			102.8274	-7.5	100.3485	100.4913	94.8265	-9.0	63.8040	72.0052	67.7116	-9.0	98.4831	106.0914	108.9768	-9.0	98.8203	105.7787	105.3646	-9.0	94.6433	91.5642	102.2752	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	109.9481	99.8695	107.8011	-8.5	98.1651	109.8474	109.4571	-10.0	72.6244	103.7000	104.2949	-10.0	103.9710	106.3723	105.4196	-10.0	100.8255	97.9315	109.9468	-10.0	78.9176	98.4258	72.6191	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		RN008 5-12-08		Positive Control		RN008 5-12-08		2.0		RN008 5-12-08		8.0		RN008 5-12-08		10.0		RN008 5-12-08		11.0		RN008 5-12-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values			
13	1	BOTTOM		-0.9105		BOTTOM		-2.295		BOTTOM		-0.1944		BOTTOM		26.62		BOTTOM		-187.1		BOTTOM		-2.902		BOTTOM			
14	2	TOP		107.2		TOP		104.9		TOP		95.73		TOP		99.85		TOP		102.9		TOP		91.93		TOP			
15	3	LOGIC50		-8.666		LOGIC50		-5.767		LOGIC50		-8.716		LOGIC50		-4.671		LOGIC50		-3.044		LOGIC50		-5.223		LOGIC50			
16	1	HILLSLOPE		-1.08		HILLSLOPE		-0.9875		HILLSLOPE		-1.23		HILLSLOPE		-1.299		HILLSLOPE		-0.7143		HILLSLOPE		-1.074		HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.776		BOTTOM		4.25		BOTTOM		2.179		BOTTOM		13.44		BOTTOM		2756		BOTTOM		7.358		BOTTOM			
19	1	TOP		1.859		TOP		2.114		TOP		5.204		TOP		2.604		TOP		1.696		TOP		2.291		TOP			
20	2	LOGIC50		0.02158		LOGIC50		0.03368		LOGIC50		0.06968		LOGIC50		0.2088		LOGIC50		0.06432		LOGIC50		0.07521		LOGIC50			
21	3	HILLSLOPE		0.07488		HILLSLOPE		0.1165		HILLSLOPE		0.2302		HILLSLOPE		0.9634		HILLSLOPE		0.7836		HILLSLOPE		0.2739		HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.9

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Estradiol
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y	
1	1	1	-7.0		1.8
1	1	2			
1	1	3	-7.0		-1.0
1	2	1	-8.0		18.7
1	2	2	-8.0		16.6
1	2	3	-8.0		15.1
1	3	1	-8.5		37.3
1	3	2	-8.5		39.2
1	3	3	-8.5		38.0
1	4	1	-9.0		68.3
1	4	2	-9.0		72.7
1	4	3	-9.0		71.3
1	5	1	-9.5		89.0
1	5	2	-9.5		102.1
1	5	3	-9.5		96.3
1	6	1			
1	6	2			
1	6	3	-10.0		102.8
1	7	1	-11.0		109.9
1	7	2	-11.0		99.9
1	7	3	-11.0		107.8
1	8	1			
1	8	2			
1	8	3			
1	9	1			
1	9	2			
1	9	3			
1	10	1			
1	10	2			
1	10	3			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.2

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Norethynodrel
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-4.0	55.3
1	1	2		
1	1	3	-4.0	64.0
1	2	1	-4.5	3.0
1	2	2	-4.5	2.3
1	2	3	-4.5	2.6
1	3	1	-5.5	32.3
1	3	2	-5.5	36.9
1	3	3	-5.5	39.9
1	4	1	-6.0	60.3
1	4	2	-6.0	59.0
1	4	3	-6.0	62.1
1	5	1	-6.5	85.9
1	5	2	-6.5	89.4
1	5	3	-6.5	93.2
1	6	1	-7.0	95.2
1	6	2	-7.0	104.6
1	6	3	-7.0	103.6
1	7	1	-7.5	100.3
1	7	2	-7.5	100.5
1	7	3	-7.5	94.8
1	8	1	-8.5	98.2
1	8	2	-8.5	109.8
1	8	3	-8.5	109.5
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.3

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 2
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	239.0
1	1	2	-3.0	237.9
1	1	3	-3.0	223.6
1	2	1	-4.0	9.4
1	2	2	-4.0	5.5
1	2	3	-4.0	5.1
1	3	1	-5.0	-2.6
1	3	2	-5.0	-2.4
1	3	3	-5.0	-2.7
1	4	1	-6.0	-3.1
1	4	2	-6.0	-3.2
1	4	3	-6.0	-1.9
1	5	1	-7.0	-2.0
1	5	2	-7.0	-1.4
1	5	3	-7.0	-1.6
1	6	1	-8.0	12.2
1	6	2	-8.0	12.4
1	6	3	-8.0	12.3
1	7	1	-9.0	63.8
1	7	2	-9.0	72.0
1	7	3	-9.0	67.7
1	8	1	-10.0	72.6
1	8	2	-10.0	103.7
1	8	3	-10.0	104.3
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
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1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 26.6

Provide information in all blue cells.
Laboratory Code: B
Compound ID: Chem Code 8
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	220.1
1	1	2	-3.0	331.7
1	1	3	-3.0	349.3
1	2	1	-4.0	26.9
1	2	2		
1	2	3	-4.0	35.0
1	3	1	-5.0	58.7
1	3	2	-5.0	65.8
1	3	3	-5.0	77.7
1	4	1	-6.0	90.3
1	4	2	-6.0	101.8
1	4	3	-6.0	99.5
1	5	1	-7.0	91.1
1	5	2	-7.0	105.5
1	5	3	-7.0	100.4
1	6	1	-8.0	78.9
1	6	2	-8.0	87.5
1	6	3	-8.0	104.6
1	7	1	-9.0	98.5
1	7	2	-9.0	106.1
1	7	3	-9.0	109.0
1	8	1	-10.0	104.0
1	8	2	-10.0	106.4
1	8	3	-10.0	105.4
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.5

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 10
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	39.2
1	1	2	-3.0	49.9
1	1	3	-3.0	51.4
1	2	1	-4.0	89.4
1	2	2	-4.0	82.2
1	2	3	-4.0	98.4
1	3	1	-5.0	104.0
1	3	2	-5.0	107.1
1	3	3	-5.0	90.9
1	4	1	-6.0	97.4
1	4	2	-6.0	105.2
1	4	3	-6.0	102.4
1	5	1	-7.0	94.1
1	5	2	-7.0	110.2
1	5	3	-7.0	103.1
1	6	1	-8.0	97.9
1	6	2	-8.0	105.8
1	6	3	-8.0	105.8
1	7	1	-9.0	98.8
1	7	2	-9.0	105.8
1	7	3	-9.0	105.4
1	8	1	-10.0	100.8
1	8	2	-10.0	97.9
1	8	3	-10.0	109.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 14.5

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 11
Run ID: RN008
Assay start date: 5/12/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	122.6
1	1	2	-3.0	151.1
1	1	3	-3.0	77.6
1	2	1	-4.0	6.3
1	2	2	-4.0	6.9
1	2	3	-4.0	6.7
1	3	1	-5.0	36.4
1	3	2	-5.0	33.5
1	3	3	-5.0	41.7
1	4	1	-6.0	73.6
1	4	2	-6.0	85.2
1	4	3	-6.0	85.4
1	5	1	-7.0	90.2
1	5	2	-7.0	97.0
1	5	3	-7.0	93.9
1	6	1	-8.0	97.8
1	6	2	-8.0	96.7
1	6	3	-8.0	87.7
1	7	1	-9.0	94.6
1	7	2	-9.0	91.6
1	7	3	-9.0	102.3
1	8	1	-10.0	78.9
1	8	2	-10.0	98.4
1	8	3	-10.0	72.6
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run RN008 5-12-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	1.7863		-1.0292
2	Title	-7.0	-0.4485	0.2561	-0.5648
3	Title	-8.0	18.6742	16.5756	15.0901
4	Title	-8.5	37.2976	39.2345	38.0022
5	Title	-9.0	68.2774	72.7059	71.2824
6	Title	-9.5	89.0097	102.1490	96.3179
7	Title	-10.0			102.8274
8	Title	-11.0	109.9481	99.8695	107.8011

	Nonlin fit Table of results	A run RN008 5-12-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.9105
4	TOP	107.2
5	LOGIC50	-8.666
6	HILLSLOPE	-1.080
7	Std. Error	
8	BOTTOM	1.776
9	TOP	1.859
10	LOGIC50	0.02158
11	HILLSLOPE	0.07488
12	95% Confidence Intervals	
13	BOTTOM	-4.658 to 2.837
14	TOP	103.3 to 111.1
15	LOGIC50	-8.711 to -8.620
16	HILLSLOPE	-1.238 to -0.9217
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9944
20	Absolute Sum of Squares	200.4
21	Sy.x	3.434
22	Number of points	
23	Analyzed	21

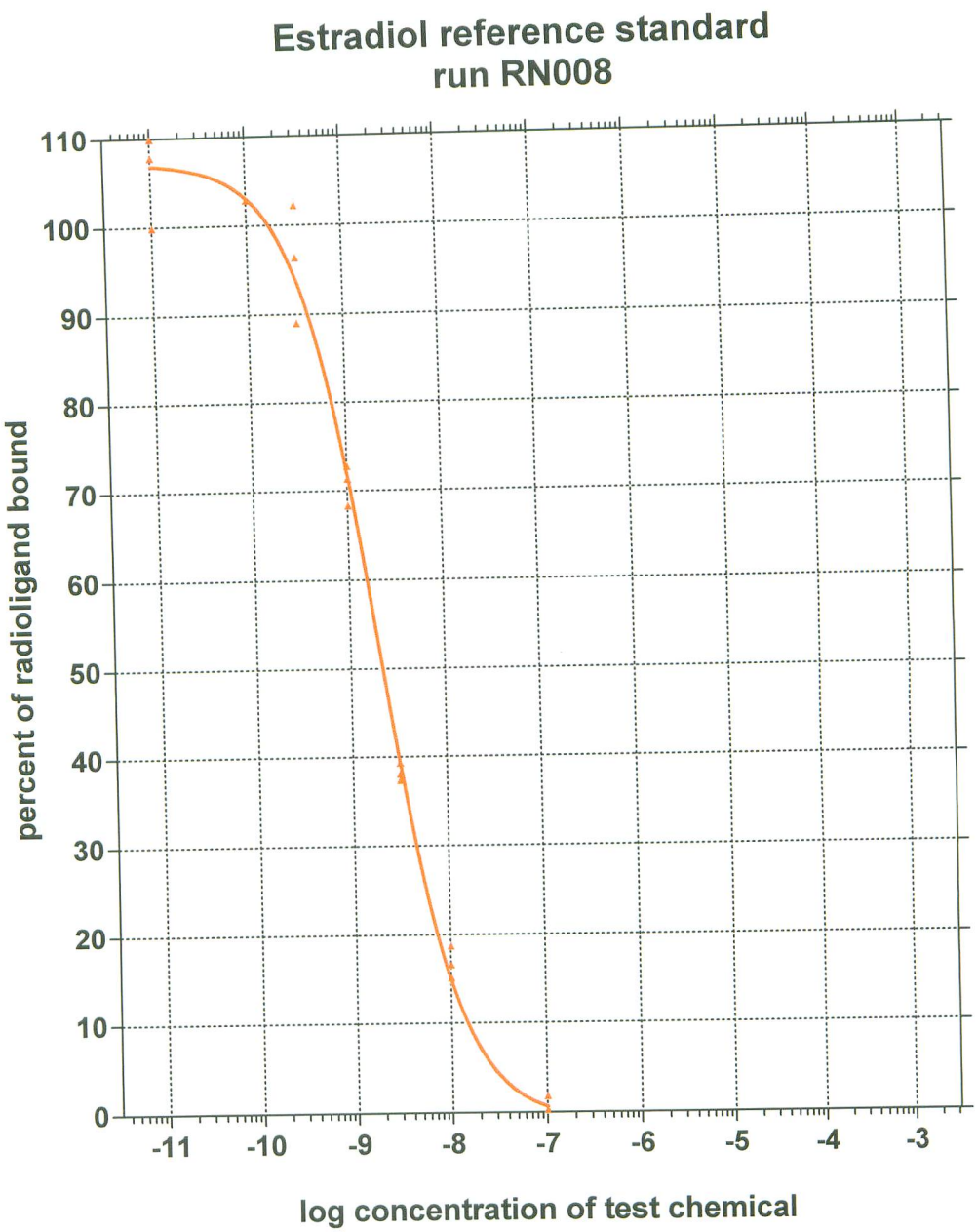

Estradiol reference standard
run RN008
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run RN008 5-12-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0			
2	Title	-4.5	3.0083	2.3511	2.5923
3	Title	-5.5	32.2931	36.9090	39.8802
4	Title	-6.0	60.2945	58.9830	62.0787
5	Title	-6.5	85.8557	89.4311	93.2003
6	Title	-7.0	95.1631	104.5596	103.5960
7	Title	-7.5	100.3485	100.4913	94.8265
8	Title	-8.5	98.1651	109.8474	109.4571

	Nonlin fit Table of results	A run RN008 5-12-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.295
4	TOP	104.9
5	LOGIC50	-5.767
6	HILLSLOPE	-0.9875
7	Std. Error	
8	BOTTOM	4.250
9	TOP	2.114
10	LOGIC50	0.03368
11	HILLSLOPE	0.1165
12	95% Confidence Intervals	
13	BOTTOM	-11.26 to 6.671
14	TOP	100.4 to 109.3
15	LOGIC50	-5.838 to -5.696
16	HILLSLOPE	-1.233 to -0.7418
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9868
20	Absolute Sum of Squares	367.2
21	Sy.x	4.648
22	Number of points	
23	Analyzed	21

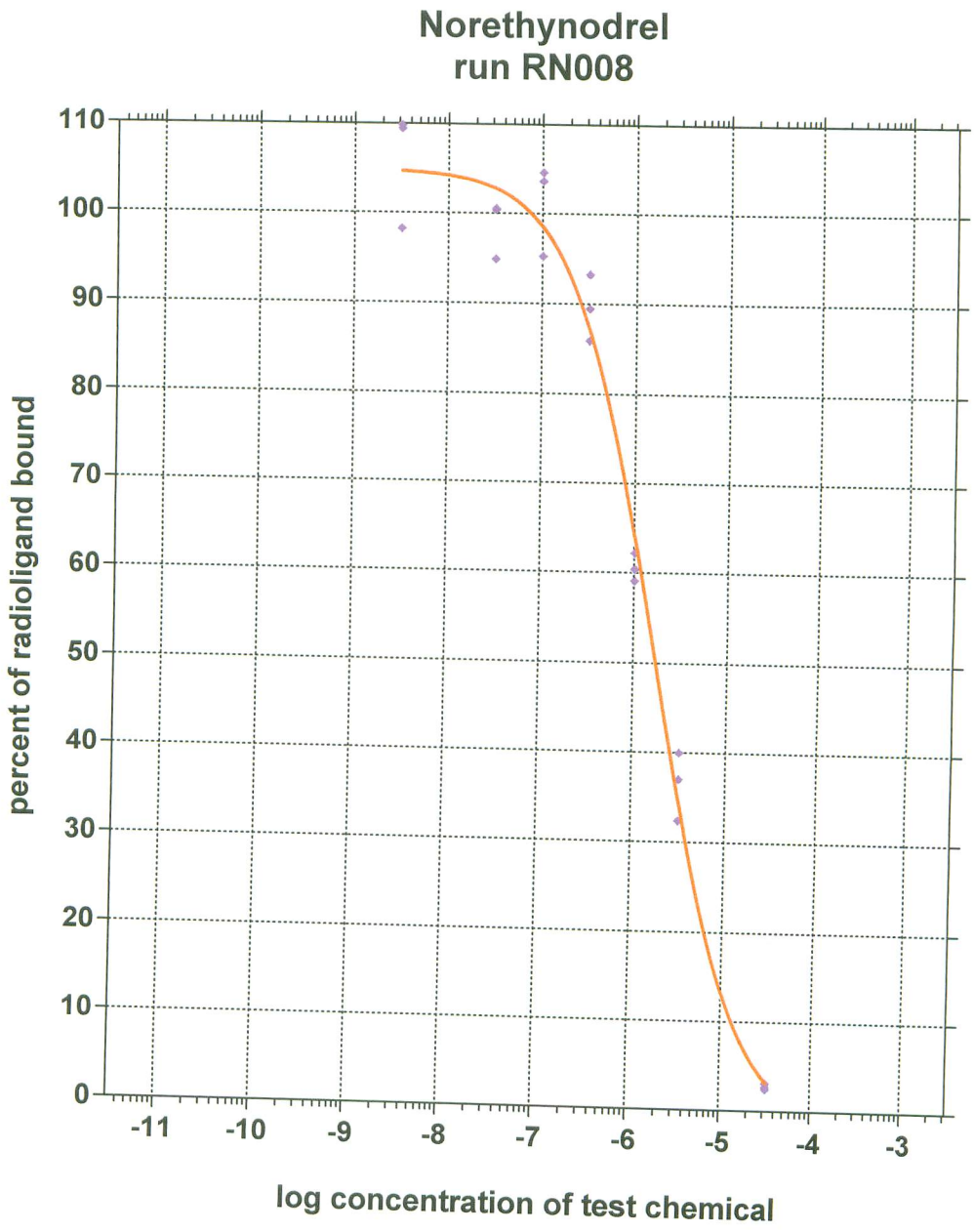

Norethynodrel
run RN008
percent of radioligand bound
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 2 run RN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	9.4340	5.4621	5.1235
3	Title	-5.0	-2.5786	-2.3586	-2.7422
4	Title	-6.0	-3.0811	-3.1546	-1.9293
5	Title	-7.0	-1.9524	-1.3707	-1.5950
6	Title	-8.0	12.1726	12.4403	12.2770
7	Title	-9.0	63.8040	72.0052	67.7116
8	Title	-10.0	72.6244	103.7000	104.2949

	Nonlin fit Table of results	A Test chemical 2 run RN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.1944
4	TOP	95.73
5	LOGIC50	-8.716
6	HILLSLOPE	-1.230
7	Std. Error	
8	BOTTOM	2.179
9	TOP	5.204
10	LOGIC50	0.06968
11	HILLSLOPE	0.2302
12	95% Confidence Intervals	
13	BOTTOM	-4.791 to 4.402
14	TOP	84.75 to 106.7
15	LOGIC50	-8.863 to -8.569
16	HILLSLOPE	-1.716 to -0.7446
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9685
20	Absolute Sum of Squares	896.7
21	Sy.x	7.263
22	Number of points	
23	Analyzed	21

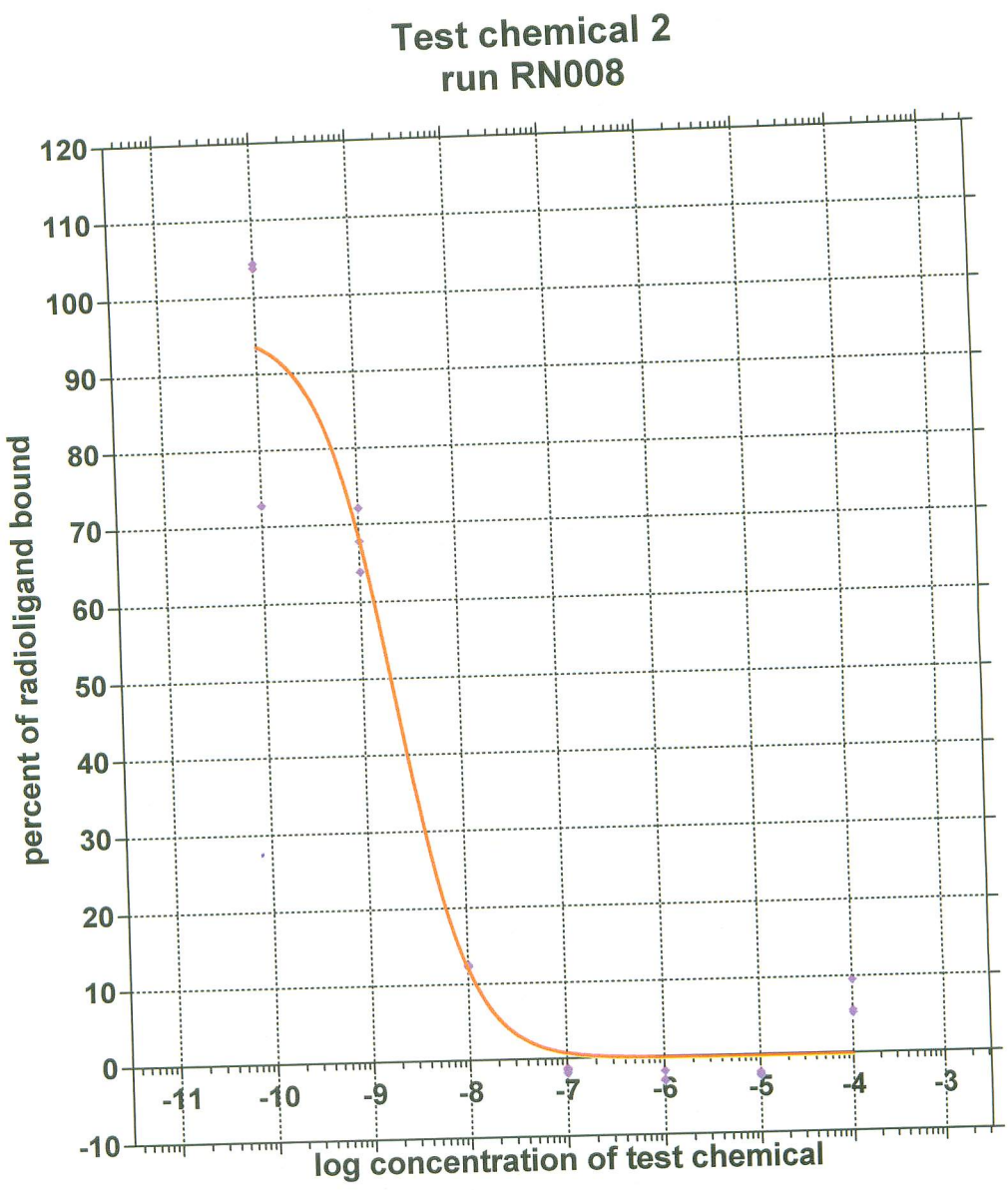

Test chemical 2
run RN008
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 8 run RN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	26.9112		35.0254
3	Title	-5.0	58.6560	65.7975	77.6846
4	Title	-6.0	90.2722	101.8187	99.5001
5	Title	-7.0	91.1435	105.4743	100.4091
6	Title	-8.0	78.8539	87.5379	104.6212
7	Title	-9.0	98.4831	106.0914	108.9768
8	Title	-10.0	103.9710	106.3723	105.4196

	Nonlin fit Table of results	A Test chemical 8 run RN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	26.62
4	TOP	99.85
5	LOGIC50	-4.671
6	HILLSLOPE	-1.299
7	Std. Error	
8	BOTTOM	13.44
9	TOP	2.604
10	LOGIC50	0.2088
11	HILLSLOPE	0.9634
12	95% Confidence Intervals	
13	BOTTOM	-1.876 to 55.11
14	TOP	94.33 to 105.4
15	LOGIC50	-5.113 to -4.228
16	HILLSLOPE	-3.341 to 0.7432
17	Goodness of Fit	
18	Degrees of Freedom	16
19	R^2	0.8875
20	Absolute Sum of Squares	1229
21	Sy.x	8.764
22	Number of points	
23	Analyzed	20

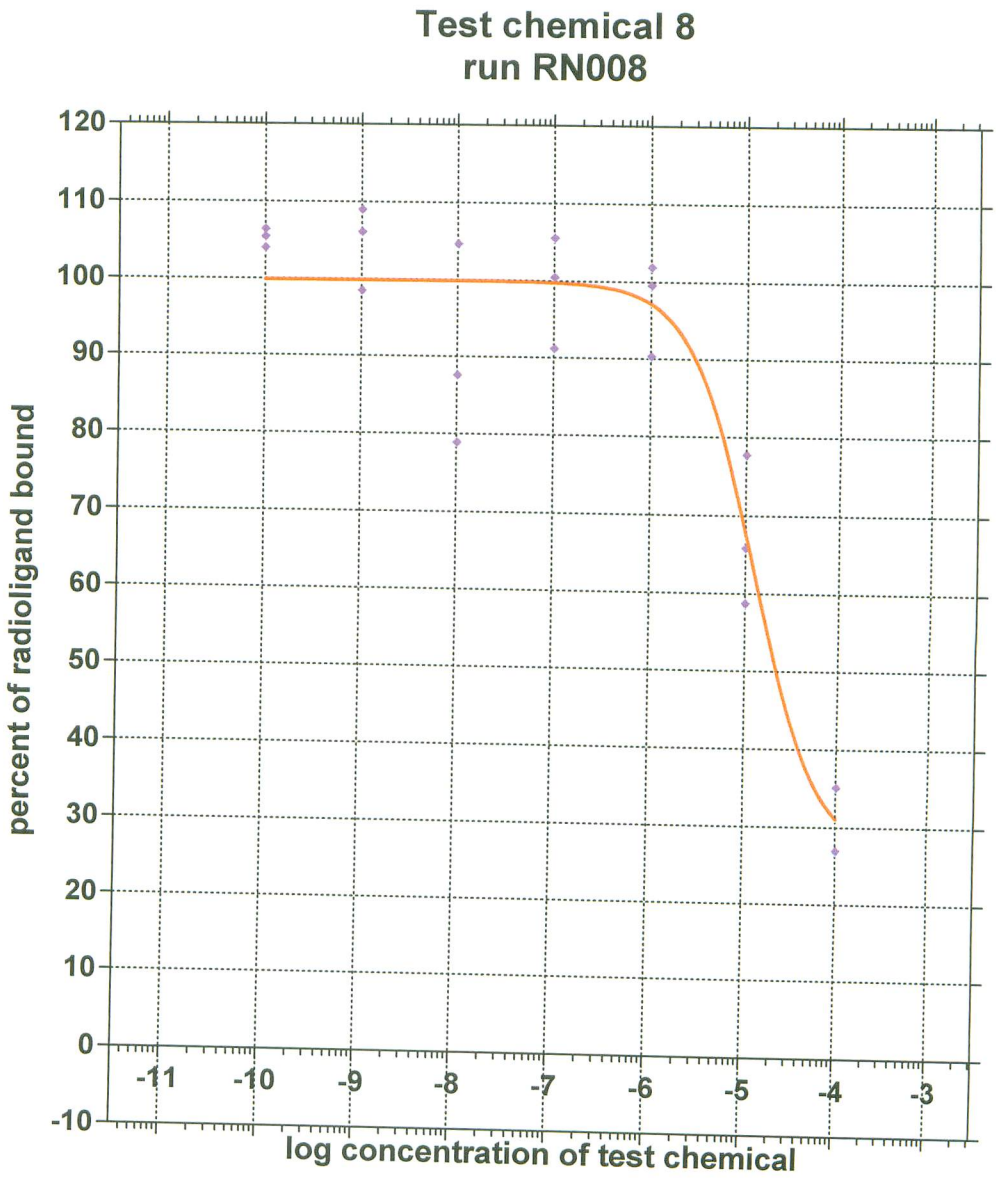

Test chemical 8
run RN008
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 10 run RN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	39.1802	49.8501	51.4472
2	Title	-4.0	89.3645	82.2349	98.4321
3	Title	-5.0	103.9839	107.0743	90.9149
4	Title	-6.0	97.3525	105.1728	102.4097
5	Title	-7.0	94.0716	110.1730	103.0619
6	Title	-8.0	97.9236	105.7694	105.8132
7	Title	-9.0	98.8203	105.7787	105.3646
8	Title	-10.0	100.8255	97.9315	109.9468

	Nonlin fit Table of results	A Test chemical 10 run RN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-187.1
4	TOP	102.9
5	LOGIC50	-3.044
6	HILLSLOPE	-0.7143
7	Std. Error	
8	BOTTOM	2756
9	TOP	1.696
10	LOGIC50	0.06432
11	HILLSLOPE	0.7836
12	95% Confidence Intervals	
13	BOTTOM	-5936 to 5561
14	TOP	99.34 to 106.4
15	LOGIC50	-3.178 to -2.909
16	HILLSLOPE	-2.349 to 0.9203
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9213
20	Absolute Sum of Squares	683.2
21	Sy.x	5.845
22	Number of points	
23	Analyzed	24

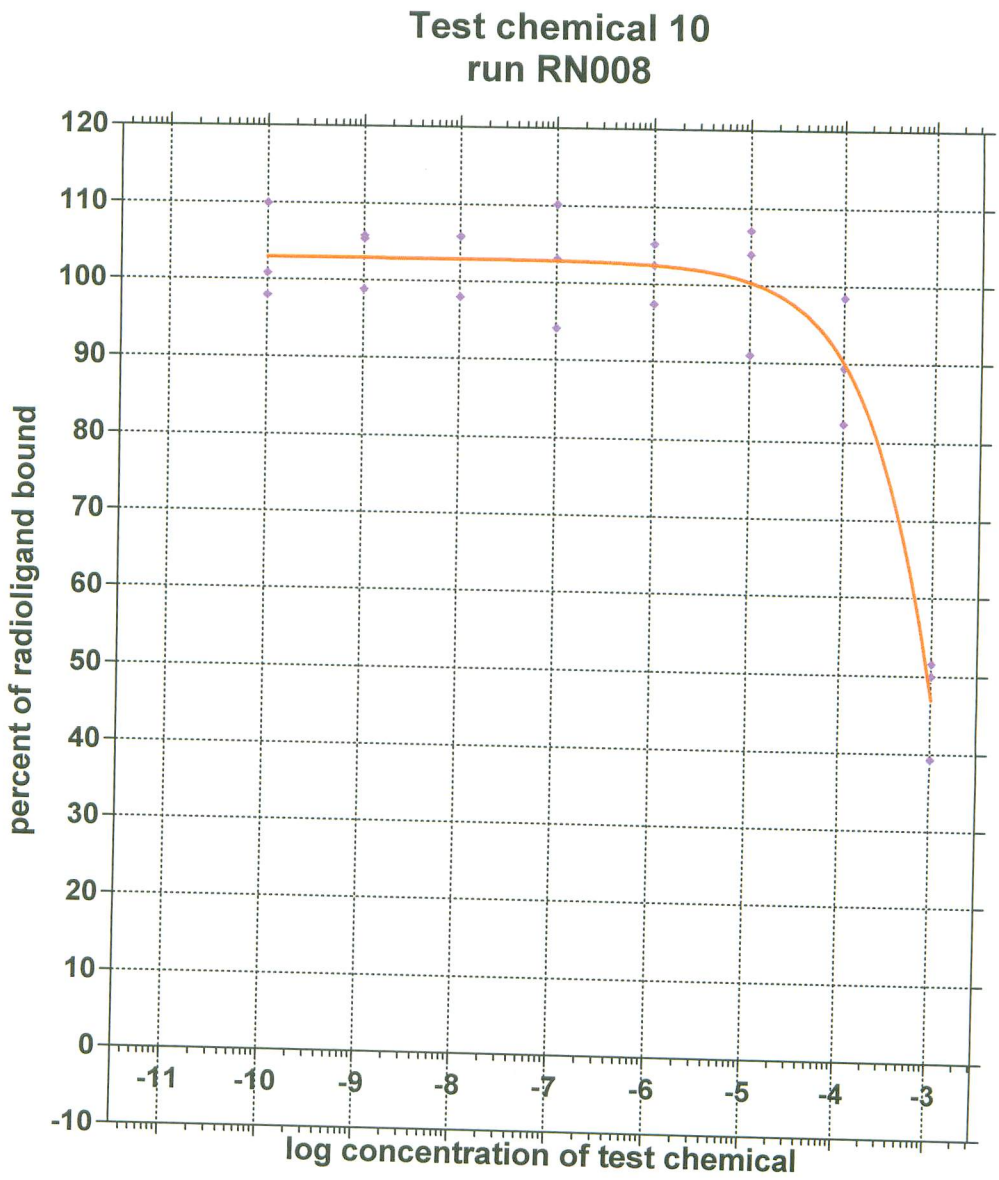
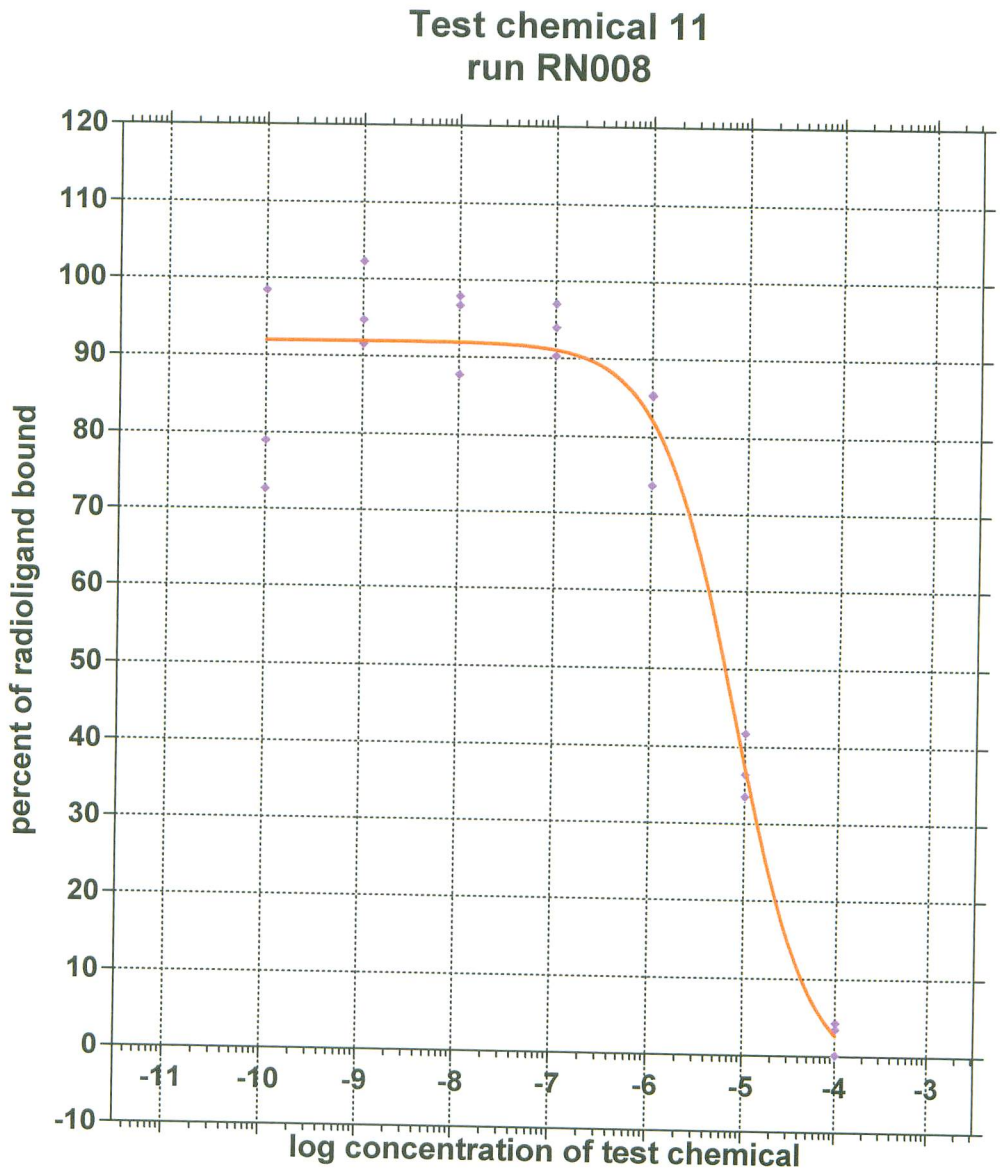

Test chemical 10
run RN008
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 11 run RN008		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	0.0000	3.4000	4.2000
3	Title	-5.0	36.3604	33.4555	41.6601
4	Title	-6.0	73.6490	85.1869	85.3720
5	Title	-7.0	90.2079	97.0083	93.9006
6	Title	-8.0	97.8391	96.6601	87.6926
7	Title	-9.0	94.6433	91.5642	102.2752
8	Title	-10.0	78.9176	98.4258	72.6191

	Nonlin fit Table of results	A Test chemical 11 run RN008
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.902
4	TOP	91.93
5	LOGIC50	-5.223
6	HILLSLOPE	-1.074
7	Std. Error	
8	BOTTOM	7.358
9	TOP	2.291
10	LOGIC50	0.07521
11	HILLSLOPE	0.2739
12	95% Confidence Intervals	
13	BOTTOM	-18.43 to 12.62
14	TOP	87.09 to 96.76
15	LOGIC50	-5.382 to -5.064
16	HILLSLOPE	-1.651 to -0.4956
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9600
20	Absolute Sum of Squares	956.5
21	Sy.x	7.501
22	Number of points	
23	Analyzed	21


Test chemical 11
run RN008
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

APPENDIX V

Excel and Prism Files of the Run Identifier RN009

ER Competitive Assay - Four Unknowns

183 assay tubes

Provide information in all blue cells in this column

Laboratory Code:	TRL B	
Run identification:	RN009	
Assay start date:	5/13/2008	
Tracer lot number:	3589791/1	
Specific activity on day of assay:	108.77	Ci/mmole
Cytosol vial or lot identification:	RUC3-3-08A	
Protein (cytosol):	50	microgram per tube
log(IC50), Cold E2 (Standard Curve):	-8.45	M
Weak Positive, Max Concentration:	5.00E-03	M
log(IC50), norethynodrel (Weak Positive):	-5.51	M
RBA, norethynodrel (Weak Positive):	1.132E-03	relative to E2=1.0
Max Concentration, Unknown 1:	1.00E-03	M
log(IC50), Unknown 1:	-8.50	2.0
RBA, Unknown 1:	1.102E+00	relative to E2=1.0
Max Concentration, Unknown 2:	1.00E-03	M
log(IC50), Unknown 2:	-8.91	3.0
RBA, Unknown 2:	2.831E+00	relative to E2=1.0
Max Concentration, Unknown 3:	1.00E-03	M
log(IC50), Unknown 3:	-3.78	10.0
RBA, Unknown 3:	2.113E-05	relative to E2=1.0
Max Concentration, Unknown 4:	1.00E-03	M
log(IC50), Unknown 4:	-4.89	23.0
RBA, Unknown 4:	2.723E-04	relative to E2=1.0
volume of ethanol counted:	1	mL
multiply DPM in sample by :	1.5	

Provide information in all blue cells in column O, and cells AE43 : AY43 and AE48 : AY48.
If the DPM value for a tube was judged unreliable, include the DPM value in column O and provide a reason in column R; the "TRUE" in column Q will change to "FALSE".

Columns AC through AZ contain values to be analyzed by nonlinear regression software

Column O, Rows 10 through 24 will contain output parameters from the nonlinear regression software and the maximum concentrations for each of the unknowns.

Summary values

	n	Mean	SD	
EtOH	6	3614.8	430.13	Total Binding, solvent control, tubes
Hot	6	86335.4	1982.57	Total hot E2 added to each tube
NSB	6	369.2	28.72	Nonspecific Binding
Specific EtOH	6	3245.6	430.13	Specific Total Binding
R1881	0	#DIV/0!	#DIV/0!	Negative control (at 1.0 E-6 M only)

Assay Characterization Values

EtOH / Hot	0.04	less than 0.1?	
R1881 / EtOH	#DIV/0!	close to 1.0 ?	at 1.0 E-6 M only
NSB / EtOH	0.10	around 0.25 ?	

log conc'n at min hot binding		min hot (%)
cold E2	-7.0	-0.2
R1881	#N/A	not applicable
norethynodrel	-4.0	-1.3
Unknown 1	-5.0	-4.5
Unknown 2	-5.0	-3.9
Unknown 3	-3.0	9.6
Unknown 4	-3.0	6.9

Values for analysis by nonlinear regression

Standard Curve
Negative Control
Weak Positive
Unknown

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 4.19%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
1	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2600.09	3900.14	TRUE			—	108.8	3900.135	3530.9	82804.5	108.8	107.1	108.8
2	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2765.56	4148.34	TRUE			—	116.4	4148.34	3779.1	82556.3	116.4		116.4
3	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2322.23	3483.35	TRUE			—	95.9	3483.345	3114.1	83221.3	95.9		95.9
4	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	231.63	347.45	TRUE		cold E2	-7.0	-0.7	347.445	-21.8	86357.2	-0.7	-0.2	-0.7
5	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	244.87	367.31	TRUE		cold E2	-7.0	-0.1	367.305	-1.9	86337.3	-0.1		-0.1
6	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	246.10	369.15	TRUE		cold E2	-7.0	0.0	369.15	-0.1	86335.5	0.0		0.0
7	1	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	747.96	1121.94	TRUE		cold E2	-8.0	23.2	1121.94	752.7	85582.7	23.2	24.4	23.2
8	2	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	742.42	1113.63	TRUE		cold E2	-8.0	22.9	1113.63	744.4	85591.0	22.9		22.9
9	3	cold E2	S	2	5.00E-07	100	380	10	10	250	500	1.0E-08	829.77	1244.66	TRUE		cold E2	-8.0	27.0	1244.655	875.4	85460.0	27.0		27.0
10	1	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1303.89	1955.84	TRUE		cold E2	-8.5	48.9	1955.835	1586.6	84748.8	48.9	52.6	48.9
11	2	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1369.33	2054.00	TRUE		cold E2	-8.5	51.9	2053.995	1684.8	84650.6	51.9		51.9
12	3	cold E2	S	3	1.58E-07	100	380	10	10	250	500	3.2E-09	1479.83	2219.75	TRUE		cold E2	-8.5	57.0	2219.745	1850.5	84484.9	57.0		57.0
13	1	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2121.83	3182.75	TRUE		cold E2	-9.0	86.7	3182.745	2813.5	83521.9	86.7	86.8	86.7
14	2	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2147.13	3220.70	TRUE		cold E2	-9.0	87.9	3220.695	2851.5	83483.9	87.9		87.9
15	3	cold E2	S	4	5.00E-08	100	380	10	10	250	500	1.0E-09	2102.68	3154.02	TRUE		cold E2	-9.0	85.8	3154.02	2784.8	83550.6	85.8		85.8
16	1	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2601.70	3902.55	TRUE		cold E2	-9.5	108.9	3902.55	3533.3	82802.1	108.9	111.1	108.9
17	2	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2617.81	3926.72	TRUE		cold E2	-9.5	109.6	3926.715	3557.5	82777.9	109.6		109.6
18	3	cold E2	S	5	1.58E-08	100	380	10	10	250	500	3.2E-10	2729.29	4093.94	TRUE		cold E2	-9.5	114.8	4093.935	3724.7	82610.7	114.8		114.8
19	1	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2856.16	4284.24	TRUE		cold E2	-10.0	120.6	4284.24	3915.0	82420.4	120.6	122.9	120.6
20	2	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2967.26	4450.89	TRUE		cold E2	-10.0	125.8	4450.89	4081.7	82253.7	125.8		125.8
21	3	cold E2	S	6	5.00E-09	100	380	10	10	250	500	1.0E-10	2891.97	4337.96	TRUE		cold E2	-10.0	122.3	4337.955	3968.7	82366.7	122.3		122.3
22	1	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3135.06	4702.59	TRUE		cold E2	-11.0	133.5	4702.59	4333.4	82002.0	133.5	130.2	133.4
23	2	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3048.79	4573.19	TRUE		cold E2	-11.0	129.5	4573.185	4203.9	82131.5	129.5		129.5
24	3	cold E2	S	7	5.00E-10	100	380	10	10	250	500	1.0E-11	3004.61	4506.92	TRUE		cold E2	-11.0	127.5	4506.915	4137.7	82197.7	127.5		127.5
25	1	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0						no average	#N/A
26	2	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
27	3	R1881	N	1	5.00E-02	100	380	10	10	250	500	1.0E-03		0.00	TRUE		R1881	-3.0							#N/A
28	1	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0						no average	#N/A
29	2	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
30	3	R1881	N	2	5.00E-03	100	380	10	10	250	500	1.0E-04		0.00	TRUE		R1881	-4.0							#N/A
31	1	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0						no average	#N/A
32	2	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
33	3	R1881	N	3	5.00E-04	100	380	10	10	250	500	1.0E-05		0.00	TRUE		R1881	-5.0							#N/A
34	1	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0						no average	#N/A
35	2	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
36	3	R1881	N	4	5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE		R1881	-6.0							#N/A
37	1	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0						no average	#N/A
38	2	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 4.19%

If the ratio of EtOH / ... 0% then there are problems with ... say

Unknown

% binding >105 or < 0.95 highlighted in red font; points in the ri... a U-shaped (binding > 10% higher than at avg minimum) are flagged by yello...

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"		concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
39	3	R1881	N	5	5.00E-06	100	380	10	10	250	500	1.0E-07		0.00	TRUE		R1881	-7.0							#N/A
40	1	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0						no average	#N/A
41	2	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
42	3	R1881	N	6	5.00E-07	100	380	10	10	250	500	1.0E-08		0.00	TRUE		R1881	-8.0							#N/A
43	1	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0						no average	#N/A
44	2	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
45	3	R1881	N	7	5.00E-08	100	380	10	10	250	500	1.0E-09		0.00	TRUE		R1881	-9.0							#N/A
46	1	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0						no average	#N/A
47	2	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
48	3	R1881	N	8	5.00E-09	100	380	10	10	250	500	1.0E-10		0.00	TRUE		R1881	-10.0							#N/A
49	1	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	197.50	296.25	TRUE		norethynodrel	-4.0	-2.2	296.25	-73.0	86408.4	-2.2	-1.3	-2.2
50	2	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	219.64	329.46	TRUE		norethynodrel	-4.0	-1.2	329.46	-39.8	86375.2	-1.2		-1.2
51	3	norethynodrel	P	1	5.00E-03	100	380	10	10	250	500	1.0E-04	237.75	356.63	TRUE		norethynodrel	-4.0	-0.4	356.625	-12.6	86348.0	-0.4		-0.4
52	1	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	289.04	433.56	TRUE		norethynodrel	-4.5	2.0	433.56	64.3	86271.1	2.0	3.4	2.0
53	2	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	330.81	496.22	TRUE		norethynodrel	-4.5	3.9	496.215	127.0	86208.4	3.9		3.9
54	3	norethynodrel	P	2	1.58E-03	100	380	10	10	250	500	3.2E-05	338.84	508.26	TRUE		norethynodrel	-4.5	4.3	508.26	139.0	86196.4	4.3		4.3
55	1	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1345.67	2018.51	TRUE		norethynodrel	-5.5	50.8	2018.505	1649.3	84686.1	50.8	51.9	50.8
56	2	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1365.12	2047.68	TRUE		norethynodrel	-5.5	51.7	2047.68	1678.4	84657.0	51.7		51.7
57	3	norethynodrel	P	3	1.58E-04	100	380	10	10	250	500	3.2E-06	1395.56	2093.34	TRUE		norethynodrel	-5.5	53.1	2093.34	1724.1	84611.3	53.1		53.1
58	1	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2097.10	3145.65	TRUE		norethynodrel	-6.0	85.5	3145.65	2776.4	83559.0	85.5	80.5	85.5
59	2	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1789.96	2684.94	TRUE		norethynodrel	-6.0	71.3	2684.94	2315.7	84019.7	71.3		71.3
60	3	norethynodrel	P	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2074.94	3112.41	TRUE		norethynodrel	-6.0	84.5	3112.41	2743.2	83592.2	84.5		84.5
61	1	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2486.19	3729.29	TRUE		norethynodrel	-6.5	103.5	3729.285	3360.0	82975.4	103.5	111.6	103.5
62	2	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2781.31	4171.97	TRUE		norethynodrel	-6.5	117.2	4171.965	3802.7	82532.7	117.2		117.2
63	3	norethynodrel	P	5	1.58E-05	100	380	10	10	250	500	3.2E-07	2715.42	4073.13	TRUE		norethynodrel	-6.5	114.1	4073.13	3703.9	82631.5	114.1		114.1
64	1	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2842.24	4263.36	TRUE		norethynodrel	-7.0	120.0	4263.36	3894.1	82441.3	120.0	122.8	120.0
65	2	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	2847.43	4271.15	TRUE		norethynodrel	-7.0	120.2	4271.145	3901.9	82433.5	120.2		120.2
66	3	norethynodrel	P	6	5.00E-06	100	380	10	10	250	500	1.0E-07	3017.80	4526.70	TRUE		norethynodrel	-7.0	128.1	4526.7	4157.5	82177.9	128.1		128.1
67	1	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	2672.73	4009.10	TRUE		norethynodrel	-7.5	112.1	4009.095	3639.9	82695.5	112.1	110.9	112.1
68	2	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	2302.19	3453.29	TRUE		norethynodrel	-7.5	95.0	3453.285	3084.0	83251.4	95.0		95.0
69	3	norethynodrel	P	7	1.58E-06	100	380	10	10	250	500	3.2E-08	2959.29	4438.94	TRUE		norethynodrel	-7.5	125.4	4438.935	4069.7	82265.7	125.4		125.4
70	1	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	1595.36	2393.04	TRUE		norethynodrel	-8.5	62.4	2393.04	2023.8	84311.6	62.4	75.0	62.4
71	2	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	2140.74	3211.11	TRUE		norethynodrel	-8.5	87.6	3211.11	2841.9	83493.5	87.6		87.6
72	3	norethynodrel	P	8	1.58E-07	100	380	10	10	250	500	3.2E-09	3031.88	4547.82	FALSE	mispipet	norethynodrel	-8.5							
73	1	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6369.74	9554.61	TRUE		Unknown 1	-3.0	283.0	9554.61	9185.4	77150.0	283.0	312.8	
74	2	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	6824.09	10236.14	TRUE		Unknown 1	-3.0	304.0	10236.135	9866.9	76468.5	304.0		
75	3	Unknown 1	U1	1	5.00E-02	100	380	10	10	250	500	1.0E-03	7846.14	11769.21	TRUE		Unknown 1	-3.0	351.2	11769.21	11400.0	74935.4	351.2		
76	1	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	240.30	360.45	TRUE		Unknown 1	-4.0	-0.3	360.45	-8.8	86344.2	-0.3	0.7	-0.3
77	2	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	302.36	453.54	TRUE		Unknown 1	-4.0	2.6	453.54	84.3	86251.1	2.6		2.6
78	3	Unknown 1	U1	2	5.00E-03	100	380	10	10	250	500	1.0E-04	243.10	364.65	TRUE		Unknown 1	-4.0	-0.1	364.65	-4.6	86340.0	-0.1		-0.1
79	1	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	150.84	226.26	TRUE		Unknown 1	-5.0	-4.4	226.26	-143.0	86478.4	-4.4	-4.5	-4.4
80	2	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	143.73	215.60	TRUE		Unknown 1	-5.0	-4.7	215.595	-153.6	86489.0	-4.7		-4.7
81	3	Unknown 1	U1	3	5.00E-04	100	380	10	10	250	500	1.0E-05	149.58	224.37	TRUE		Unknown 1	-5.0	-4.5	224.37	-144.9	86480.3	-4.5		-4.5
82	1	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	174.65	261.98	TRUE		Unknown 1	-6.0	-3.3	261.975	-107.3	86442.7	-3.3	-3.6	-3.3
83	2	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	158.46	237.69	TRUE		Unknown 1	-6.0	-4.1	237.69	-131.6	86466.9	-4.1		-4.1
84	3	Unknown 1	U1	4	5.00E-05	100	380	10	10	250	500	1.0E-06	169.15	253.73	TRUE		Unknown 1	-6.0	-3.6	253.725	-115.5	86450.9	-3.6		-3.6
85	1	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	262.49	393.74	TRUE		Unknown 1	-7.0	0.8	393.735	24.5	86310.9	0.8	0.1	0.8
86	2	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	256.33	384.50	TRUE		Unknown 1	-7.0	0.5	384.495	15.3	86320.1	0.5		0.5
87	3	Unknown 1	U1	5	5.00E-06	100	380	10	10	250	500	1.0E-07	228.98	343.47	TRUE		Unknown 1	-7.0	-0.8	343.47	-25.8	86361.2	-0.8		-0.8
88	1	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	690.41	1035.62	TRUE		Unknown 1	-8.0	20.5	1035.615	666.4	85669.0	20.5	20.5	20.5
89	2	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	666.02	999.03	TRUE		Unknown 1	-8.0	19.4	999.03	629.8	85705.6	19.4		19.4
90	3	Unknown 1	U1	6	5.00E-07	100	380	10	10	250	500	1.0E-08	713.16	1069.74	TRUE		Unknown 1	-8.0	21.6	1069.74	700.5	85634.9	21.6		21.6
91	1	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2003.39	3005.09	TRUE		Unknown 1	-9.0	81.2	3005.085	2635.8	83699.6	81.2	81.0	81.2
92	2	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2093.48	3140.22	TRUE		Unknown 1	-9.0	85.4	3140.22	2771.0	83564.4	85.4		85.4
93	3	Unknown 1	U1	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1901.35	2852.03	TRUE		Unknown 1	-9.0	76.5	2852.025	2482.8	83852.6	76.5		76.5
94	1	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2596.10	3894.15	TRUE		Unknown 1	-10.0	108.6	3894.15	3524.9	82810.5	108.6	103.6	108.6
95	2	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2335.58	3503.37	TRUE		Unknown 1	-10.0	96.6	3503.37	3134.1	83201.3	96.6		96.6
96	3	Unknown 1	U1	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2533.59	3800.39	TRUE		Unknown 1	-10.0	105.7	3800.385	3431.1	82904.3	105.7		105.7
97	1	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	336.38	504.57	TRUE		Unknown 2	-3.0	4.2	504.57	135.3	86200.1	4.2	6.1	
98	2	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	397.58	596.37	TRUE		Unknown 2	-3.0	7.0	596.37	227.1	86108.3	7.0		
99	3	Unknown 2	U2	1	5.00E-02	100	380	10	10	250	500	1.0E-03	399.93	599.90	TRUE		Unknown 2	-3.0	7.1	599.895	230.7	86104.7	7.1		
100	1	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	167.90	251.85	TRUE		Unknown 2	-4.0	-3.6	251.85	-117.4	86452.8	-3.6	-3.6	-3.6
101	2	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	147.75	221.63	TRUE		Unknown 2	-4.0	-4.5	221.625	-147.6	86483.0	-4.5		-4.5
102	3	Unknown 2	U2	2	5.00E-03	100	380	10	10	250	500	1.0E-04	187.98	281.97	TRUE		Unknown 2	-4.0	-2.7	281.97	-87.3	86422.7	-2.7		-2.7
103	1	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	139.16	208.74	TRUE		Unknown 2	-5.0	-4.9	208.74	-160.5	86495.9	-4.9	-3.9	-4.9
104	2	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	182.81	274.22	TRUE		Unknown 2	-5.0	-2.9	274.215	-95.0	86430.4	-2.9		-2.9
105	3	Unknown 2	U2	3	5.00E-04	100	380	10	10	250	500	1.0E-05	161.75	242.63	TRUE		Unknown 2	-5.0	-3.9	242.625	-126.6	86462.0	-3.9		-3.9
106	1	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	179.08	268.62	TRUE		Unknown 2	-6.0	-3.1	268.62	-100.6	86436.0	-3.1	-3.5	-3.1
107	2	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	168.32	252.48	TRUE		Unknown 2	-6.0	-3.6	252.48	-116.8	86452.2	-3.6		-3.6
108	3	Unknown 2	U2	4	5.00E-05	100	380	10	10	250	500	1.0E-06	164.25	246.38	TRUE		Unknown 2	-6.0	-3.8	246.375	-122.9	86458.3	-3.8		-3.8
109	1	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	193.36	290.04	TRUE		Unknown 2	-7.0	-2.4	290.04	-79.2	86414.6	-2.4	-1.9	-2.4
110	2	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	234.17	351.26	TRUE		Unknown 2	-7.0	-0.6	351.255	-18.0	86353.4	-0.6		-0.6
111	3	Unknown 2	U2	5	5.00E-06	100	380	10	10	250	500	1.0E-07	187.06	280.59	TRUE		Unknown 2	-7.0	-2.7	280.59	-88.7	86424.0	-2.7		-2.7
112	1	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	436.76	655.14	TRUE		Unknown 2	-8.0	8.8	655.14	285.9	86049.5	8.8	7.5	8.8
113	2	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	364.12	546.18	TRUE		Unknown 2	-8.0	5.5	546.18	176.9	86158.5	5.5		5.5
114	3	Unknown 2	U2	6	5.00E-07	100	380	10	10	250	500	1.0E-08	422.64	633.96	TRUE		Unknown 2	-8.0	8.2	633.96	264.7	86070.7	8.2		8.2
115	1	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1507.78	2261.67	TRUE		Unknown 2	-9.0	58.3	2261.67	1892.4	84443.0	58.3	56.2	58.3
116	2	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1517.74	2276.61	TRUE		Unknown 2	-9.0	58.8	2276.61	1907.4	84428.0	58.8		58.8
117	3	Unknown 2	U2	7	5.00E-08	100	380	10	10	250	500	1.0E-09	1363.02	2044.53	TRUE		Unknown 2	-9.0	51.6	2044.53	1675.3	84660.1	51.6		51.6
118	1	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2335.31	3502.97	TRUE		Unknown 2	-10.0	96.6	3502.965	3133.7	83201.7	96.6	101.8	96.6
119	2	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2586.56	3879.84	TRUE		Unknown 2	-10.0	108.2	3879.84	3510.6	82824.8	108.2		108.2

Competitive Assay Tube Layout - Four Test Chemicals

Check the 10% rule: 4.19%

If the ratio of EtOH / Hot is > 10% then there are problems with the assay

% binding >105 or < 0.95 highlighted in red font; points in the right leg of a U-shaped (binding > 10% higher than at avg minimum) are flagged by yellow cell.

Position	Replicate	Competitor	Competitor Code	Concentration Code	Competitor Initial Concentration (M)	Uterine Cytosol (uL)	Buffer Volume (uL)	Tracer (Hot E2) Volume (uL)	Competitor Volume (uL)	HAP Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	DPM as sampled	DPM for 1.5 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Unknown	concentration (logM)	percent of hot E2 bound (including data points on right leg of U-shaped curves)	Usable DPM values	Specific Binding (Total - NSB)	Free DPM (mean total add - total bound)	Percent Binding	Replicate average	percent of hot E2 bound (excluding data points on right leg of U-shaped curves)
120	3	Unknown 2	U2	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2427.55	3641.33	TRUE		Unknown 2	-10.0	100.8	3641.325	3272.1	83063.3	100.8		100.8
121	1	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	280.06	420.09	TRUE		Unknown 3	-3.0	1.6	420.09	50.9	86284.5	1.6	9.6	1.6
122	2	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	464.07	696.11	TRUE		Unknown 3	-3.0	10.1	696.105	326.9	86008.5	10.1		10.1
123	3	Unknown 3	U3	1	5.00E-02	100	380	10	10	250	500	1.0E-03	618.10	927.15	TRUE		Unknown 3	-3.0	17.2	927.15	557.9	85777.5	17.2		17.2
124	1	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1747.84	2621.76	TRUE		Unknown 3	-4.0	69.4	2621.76	2252.5	84082.9	69.4	76.7	69.4
125	2	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	2008.67	3013.01	TRUE		Unknown 3	-4.0	81.5	3013.005	2643.8	83691.6	81.5		81.5
126	3	Unknown 3	U3	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1959.04	2938.56	TRUE		Unknown 3	-4.0	79.2	2938.56	2569.3	83766.1	79.2		79.2
127	1	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2640.24	3960.36	TRUE		Unknown 3	-5.0	110.6	3960.36	3591.1	82744.3	110.6	115.6	110.6
128	2	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2775.72	4163.58	TRUE		Unknown 3	-5.0	116.9	4163.58	3794.3	82541.1	116.9		116.9
129	3	Unknown 3	U3	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2825.01	4237.52	TRUE		Unknown 3	-5.0	119.2	4237.515	3868.3	82467.1	119.2		119.2
130	1	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2756.02	4134.03	TRUE		Unknown 3	-6.0	116.0	4134.03	3764.8	82570.6	116.0	118.1	116.0
131	2	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2818.81	4228.22	TRUE		Unknown 3	-6.0	118.9	4228.215	3859.0	82476.4	118.9		118.9
132	3	Unknown 3	U3	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2831.95	4247.93	TRUE		Unknown 3	-6.0	119.5	4247.925	3878.7	82456.7	119.5		119.5
133	1	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2760.16	4140.24	TRUE		Unknown 3	-7.0	116.2	4140.24	3771.0	82564.4	116.2	109.5	116.2
134	2	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2791.37	4187.06	TRUE		Unknown 3	-7.0	117.6	4187.055	3817.8	82517.6	117.6		117.6
135	3	Unknown 3	U3	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2297.88	3446.82	TRUE		Unknown 3	-7.0	94.8	3446.82	3077.6	83257.8	94.8		94.8
136	1	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2631.15	3946.73	TRUE		Unknown 3	-8.0	110.2	3946.725	3577.5	82757.9	110.2	119.2	110.2
137	2	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2951.29	4426.94	TRUE		Unknown 3	-8.0	125.0	4426.935	4057.7	82277.7	125.0		125.0
138	3	Unknown 3	U3	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2893.30	4339.95	TRUE		Unknown 3	-8.0	122.3	4339.95	3970.7	82364.7	122.3		122.3
139	1	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2632.47	3948.71	TRUE		Unknown 3	-9.0	110.3	3948.705	3579.5	82755.9	110.3	118.4	110.3
140	2	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2922.79	4384.19	TRUE		Unknown 3	-9.0	123.7	4384.185	4014.9	82320.5	123.7		123.7
141	3	Unknown 3	U3	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2868.95	4303.43	TRUE		Unknown 3	-9.0	121.2	4303.425	3934.2	82401.2	121.2		121.2
142	1	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2629.72	3944.58	TRUE		Unknown 3	-10.0	110.2	3944.58	3575.3	82760.1	110.2	116.1	110.2
143	2	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2785.99	4178.99	TRUE		Unknown 3	-10.0	117.4	4178.985	3809.7	82525.7	117.4		117.4
144	3	Unknown 3	U3	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2862.04	4293.06	TRUE		Unknown 3	-10.0	120.9	4293.06	3923.8	82411.6	120.9		120.9
145	1	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	390.70	586.05	TRUE		Unknown 4	-3.0	6.7	586.05	216.8	86118.6	6.7	6.9	6.7
146	2	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	380.82	571.23	TRUE		Unknown 4	-3.0	6.2	571.23	202.0	86133.4	6.2		6.2
147	3	Unknown 4	U4	1	5.00E-02	100	380	10	10	250	500	1.0E-03	417.30	625.95	TRUE		Unknown 4	-3.0	7.9	625.95	256.7	86078.7	7.9		7.9
148	1	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	951.01	1426.52	TRUE		Unknown 4	-4.0	32.6	1426.515	1057.3	85278.1	32.6	34.1	
149	2	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	999.49	1499.24	TRUE		Unknown 4	-4.0	34.8	1499.235	1130.0	85205.4	34.8		3.4
150	3	Unknown 4	U4	2	5.00E-03	100	380	10	10	250	500	1.0E-04	1004.67	1507.01	TRUE		Unknown 4	-4.0	35.1	1507.005	1137.8	85197.6	35.1		4.2
151	1	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	2148.94	3223.41	TRUE		Unknown 4	-5.0	87.9	3223.41	2854.2	83481.2	87.9	74.7	87.9
152	2	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1912.11	2868.17	TRUE		Unknown 4	-5.0	77.0	2868.165	2498.9	83836.5	77.0		77.0
153	3	Unknown 4	U4	3	5.00E-04	100	380	10	10	250	500	1.0E-05	1525.77	2288.66	TRUE		Unknown 4	-5.0	59.1	2288.655	1919.4	84416.0	59.1		59.1
154	1	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2256.93	3385.40	TRUE		Unknown 4	-6.0	92.9	3385.395	3016.2	83319.2	92.9	93.2	92.9
155	2	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	2633.83	3950.75	TRUE		Unknown 4	-6.0	110.4	3950.745	3581.5	82753.9	110.4		110.4
156	3	Unknown 4	U4	4	5.00E-05	100	380	10	10	250	500	1.0E-06	1896.53	2844.80	TRUE		Unknown 4	-6.0	76.3	2844.795	2475.6	83859.8	76.3		76.3
157	1	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1982.11	2973.17	TRUE		Unknown 4	-7.0	80.2	2973.165	2603.9	83731.5	80.2	71.6	80.2
158	2	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	2269.54	3404.31	TRUE		Unknown 4	-7.0	93.5	3404.31	3035.1	83300.3	93.5		93.5
159	3	Unknown 4	U4	5	5.00E-06	100	380	10	10	250	500	1.0E-07	1135.26	1702.89	TRUE		Unknown 4	-7.0	41.1	1702.89	1333.7	85001.7	41.1		41.1
160	1	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2642.43	3963.65	TRUE		Unknown 4	-8.0	110.7	3963.645	3594.4	82741.0	110.7	113.9	110.7
161	2	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2903.60	4355.40	TRUE		Unknown 4	-8.0	122.8	4355.4	3986.2	82349.2	122.8		122.8
162	3	Unknown 4	U4	6	5.00E-07	100	380	10	10	250	500	1.0E-08	2586.94	3880.41	TRUE		Unknown 4	-8.0	108.2	3880.41	3511.2	82824.2	108.2		108.2
163	1	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2612.62	3918.93	TRUE		Unknown 4	-9.0	109.4	3918.93	3549.7	82785.7	109.4	107.8	109.4
164	2	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2605.83	3908.75	TRUE		Unknown 4	-9.0	109.1	3908.745	3539.5	82795.9	109.1		109.1
165	3	Unknown 4	U4	7	5.00E-08	100	380	10	10	250	500	1.0E-09	2519.32	3778.98	TRUE		Unknown 4	-9.0	105.1	3778.98	3409.7	82925.7	105.1		105.1
166	1	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2590.09	3885.14	TRUE		Unknown 4	-10.0	108.3	3885.135	3515.9	82819.5	108.3	107.9	108.3
167	2	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2721.78	4082.67	TRUE		Unknown 4	-10.0	114.4	4082.67	3713.4	82622.0	114.4		114.4
168	3	Unknown 4	U4	8	5.00E-09	100	380	10	10	250	500	1.0E-10	2433.01	3649.52	TRUE		Unknown 4	-10.0	101.1	3649.515	3280.3	83055.1	101.1		101.1
169	1	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	221.00	331.50	TRUE		cold E2	-7.0	-1.2	331.5	-37.7	86373.1	-1.2	0.2	-1.2
170	2	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	258.17	387.26	TRUE		cold E2	-7.0	0.6	387.255	18.0	86317.4	0.6		0.6
171	3	cold E2	NSB	1	5.00E-06	100	380	10	10	250	500	1.0E-07	275.19	412.79	TRUE		cold E2	-7.0	1.3	412.785	43.5	86291.9	1.3		1.3
172	1	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2103.21	3154.82	TRUE			—	85.8	3154.815	2785.6	83549.8	85.8	92.9	85.8
173	2	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2074.04	3111.06	TRUE			—	84.5	3111.06	2741.8	83593.6	84.5		84.5
174	3	ethanol	EtOH	0	—	100	380	10	10	250	500	—	2594.09	3891.14	TRUE			—	108.5	3891.135	3521.9	82813.5	108.5		108.5
175	1	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0						no average	
176	2	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
177	3	R1881	N		5.00E-05	100	380	10	10	250	500	1.0E-06		0.00	TRUE			-6.0							
178	1	none	Hot		—	—	—	10	—	—	—	—	82816.96	82816.96	TRUE					82816.96	82447.7				
179	2	none	Hot		—	—	—	10	—	—	—	—	87962.99	87962.99	TRUE					87962.99	87593.8				
180	3	none	Hot		—	—	—	10	—	—	—	—	85199.62	85199.62	TRUE					85199.62	84830.4				
181	1	none	Hot		—	—	—	10	—	—	—	—	87611.45	87611.45	TRUE					87611.45	87242.2				
182	2	none	Hot		—	—	—	10	—	—	—	—	86915.78	86915.78	TRUE					86915.78	86546.5				
183	3	none	Hot		—	—	—	10	—	—	—	—	87505.58	87505.58	TRUE					87505.58	87136.3				

Prism data, excluding data points in the right leg of U-shaped curves

		standard curve				weak positive				2.0				3.0				10.0				23.0				negative control			
Position	Replicate	concentration (logM)	Y1-SC	Y2-SC	Y3-SC	concentration (logM)	y1-PC	y2-PC	y3-PC	concentration (logM)	y1-U1	y2-U1	y3-U1	concentration (logM)	y1-U2	y2-U2	y3-U2	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	y1-U3	y2-U3	y3-U3	concentration (logM)	Y1-NC	Y2-NC	Y3-NC
1	1	-7.0	-0.6715	-0.0596	-0.0028	-4.0	-2.2489	-1.2257	-0.3887	-3.0				-3.0				-3.0	1.5668	10.0711	17.1899	-3.0	6.6802	6.2236	7.9096	-3.0	#N/A	#N/A	#N/A
2	2	-7.0	-1.1628	0.5551	1.3417	-4.5	1.9818	3.9123	4.2834	-4.0	-0.2708	2.5974	-0.1414	-4.0	-3.6169	-4.5482	-2.6889	-4.0	69.4030	81.4578	79.1640	-4.0	0.0000	3.4000	4.2000	-4.0	#N/A	#N/A	#N/A
3	3	-8.0	23.1916	22.9356	26.9727	-5.5	50.8160	51.7149	53.1217	-5.0	-4.4054	-4.7340	-4.4636	-5.0	-4.9452	-2.9278	-3.9012	-5.0	110.6470	116.9085	119.1865	-5.0	87.9406	76.9951	59.1396	-5.0	#N/A	#N/A	#N/A
4	1	-8.5	48.8850	51.9095	57.0164	-6.0	85.5447	71.3497	84.5206	-6.0	-3.3050	-4.0532	-3.5592	-6.0	-3.1002	-3.5975	-3.7856	-6.0	115.9980	118.8999	119.5072	-6.0	92.9316	110.3507	76.2750	-6.0	#N/A	#N/A	#N/A
5	2	-9.0	86.6877	87.8570	85.8026	-6.5	103.5273	117.1668	114.1216	-7.0	0.7547	0.4700	-0.7940	-7.0	-2.4403	-0.5541	-2.7314	-7.0	116.1893	117.6318	94.8242	-7.0	80.2303	93.5144	41.0915	-7.0	#N/A	#N/A	#N/A
6	3	-9.5	108.8658	109.6103	114.7626	-7.0	119.9828	120.2227	128.0966	-8.0	20.5319	19.4046	21.5833	-8.0	8.8089	5.4517	8.1564	-8.0	110.2269	125.0228	122.3426	-8.0	110.7482	122.8187	108.1836	-8.0	#N/A	#N/A	#N/A
7	1	-10.0	120.6261	125.7608	122.2812	-7.5	112.1486	95.0234	125.3925	-9.0	81.2137	85.3774	76.4978	-9.0	58.3082	58.7685	51.6178	-9.0	110.2879	123.7056	121.2173	-9.0	109.3705	109.0567	105.0584	-9.0	#N/A	#N/A	#N/A
8	2	-11.0	133.5160	129.5289	127.4870	-8.5	62.3559	87.5616		-10.0	108.6070	96.5665	105.7180	-10.0	96.5541	108.1661	100.8171	-10.0	110.1608	117.3831	120.8979	-10.0	108.3292	114.4155	101.0695	-10.0	#N/A	#N/A	#N/A
9	3																												
10	1	Standard Curve		RN009 5-13-08		Positive Control		RN009 5-13-08		2.0		RN009 5-13-08		3.0		RN009 5-13-08		10.0		RN009 5-13-08		23.0		RN009 5-13-08		Negative Control		Prism run ID & date	
11	2	logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model				logIC50 model			
12	3	Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values				Best-fit values			
13	1	BOTTOM		-4.459		BOTTOM		-0.6397		BOTTOM		-2.46		BOTTOM		-3.601		BOTTOM		7.623		BOTTOM		4.732		BOTTOM			
14	2	TOP		131.2		TOP		107.7		TOP		106.2		TOP		111.6		TOP		116.3		TOP		98.9		TOP			
15	3	LOGIC50		-8.454		LOGIC50		-5.508		LOGIC50		-8.496		LOGIC50		-8.906		LOGIC50		-3.779		LOGIC50		-4.889		LOGIC50			
16	1	HILLSLOPE		-0.8863		HILLSLOPE		-1.442		HILLSLOPE		-1.092		HILLSLOPE		-1.002		HILLSLOPE		-1.971		HILLSLOPE		~ -4.466		HILLSLOPE			
17	2	Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error				Std. Error			
18	3	BOTTOM		1.369		BOTTOM		7.449		BOTTOM		0.9939		BOTTOM		0.8047		BOTTOM		15.06		BOTTOM		10.15		BOTTOM			
19	1	TOP		1.291		TOP		5.461		TOP		2.408		TOP		3.345		TOP		1.878		TOP		4.575		TOP			
20	2	LOGIC50		0.01426		LOGIC50		0.09461		LOGIC50		0.02975		LOGIC50		0.02185		LOGIC50		0.3154		LOGIC50		~ 16.49		LOGIC50			
21	3	HILLSLOPE		0.03571		HILLSLOPE		0.5671		HILLSLOPE		0.07593		HILLSLOPE		0.08376		HILLSLOPE		3.059		HILLSLOPE		~ 665.6		HILLSLOPE			
22	1	95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals				95% Confidence Intervals			
23	2	BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM				BOTTOM			
24	3	TOP				TOP				TOP				TOP				TOP				TOP				TOP			
25	1	LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50				LOGIC50			
26	2	HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE				HILLSLOPE			
27	3	Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit				Goodness of Fit			
28	1	Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom				Degrees of Freedom			
29	2	R^2				R^2				R^2				R^2				R^2				R^2				R^2			
30	3	Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares				Absolute Sum of Squares			
31	1	Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x				Sy.x			
32	2	Data				Data				Data				Data				Data				Data				Data			
33	3	Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values				Number of X values			
34	1	Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates				Number of Y replicates			
35	2	Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values				Total number of values			
36	3	Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values				Number of missing values			
37	1																												

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.7

Provide information in all blue cells.
Laboratory Code: B
Compound ID: Estradiol
Run ID: RN009
Assay start date: 5/13/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-7.0	-0.7
1	1	2	-7.0	-0.1
1	1	3	-7.0	0.0
1	2	1	-8.0	23.2
1	2	2	-8.0	22.9
1	2	3	-8.0	27.0
1	3	1	-8.5	48.9
1	3	2	-8.5	51.9
1	3	3	-8.5	57.0
1	4	1	-9.0	86.7
1	4	2	-9.0	87.9
1	4	3	-9.0	85.8
1	5	1	-9.5	108.9
1	5	2	-9.5	109.6
1	5	3	-9.5	114.8
1	6	1	-10.0	120.6
1	6	2	-10.0	125.8
1	6	3	-10.0	122.3
1	7	1	-11.0	133.5
1	7	2	-11.0	129.5
1	7	3	-11.0	127.5
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 8.4

Provide information in all blue cells.

Laboratory Code:	B
Compound ID:	Norethynodrel
Run ID:	RN009
Assay start date:	5/13/08
Technician ID:	RN

run	xgroup	rep	x	y
1	1	1	-4.0	-2.2
1	1	2	-4.0	-1.2
1	1	3	-4.0	-0.4
1	2	1	-4.5	2.0
1	2	2	-4.5	3.9
1	2	3	-4.5	4.3
1	3	1	-5.5	50.8
1	3	2	-5.5	51.7
1	3	3	-5.5	53.1
1	4	1	-6.0	85.5
1	4	2	-6.0	71.3
1	4	3	-6.0	84.5
1	5	1	-6.5	103.5
1	5	2	-6.5	117.2
1	5	3	-6.5	114.1
1	6	1	-7.0	120.0
1	6	2	-7.0	120.2
1	6	3	-7.0	128.1
1	7	1	-7.5	112.1
1	7	2	-7.5	95.0
1	7	3	-7.5	125.4
1	8	1	-8.5	62.4
1	8	2	-8.5	87.6
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 12.7

Provide information in all blue cells.
Laboratory Code: B
Compound ID: Chem Code 2
Run ID: RN009
Assay start date: 5/13/08
Technician ID: RN

run	xgroup	rep	x	y	
1	1	1	-3.0	283.0	
1	1	2	-3.0	304.0	
1	1	3	-3.0	351.2	
1	2	1	-4.0	-0.3	
1	2	2	-4.0	2.6	
1	2	3	-4.0	-0.1	
1	3	1	-5.0	-4.4	
1	3	2	-5.0	-4.7	
1	3	3	-5.0	-4.5	
1	4	1	-6.0	-3.3	
1	4	2	-6.0	-4.1	
1	4	3	-6.0	-3.6	
1	5	1	-7.0	0.8	
1	5	2	-7.0	0.5	
1	5	3	-7.0	-0.8	
1	6	1	-8.0	20.5	
1	6	2	-8.0	19.4	
1	6	3	-8.0	21.6	
1	7	1	-9.0	81.2	
1	7	2	-9.0	85.4	
1	7	3	-9.0	76.5	
1	8	1	-10.0	108.6	
1	8	2	-10.0	96.6	
1	8	3	-10.0	105.7	
1	9	1			
1	9	2			
1	9	3			
1	10	1			
1	10	2			
1	10	3			

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.7

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 3
Run ID: RN009
Assay start date: 5/13/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	4.2
1	1	2	-3.0	7.0
1	1	3	-3.0	7.1
1	2	1	-4.0	-3.6
1	2	2	-4.0	-4.5
1	2	3	-4.0	-2.7
1	3	1	-5.0	-4.9
1	3	2	-5.0	-2.9
1	3	3	-5.0	-3.9
1	4	1	-6.0	-3.1
1	4	2	-6.0	-3.6
1	4	3	-6.0	-3.8
1	5	1	-7.0	-2.4
1	5	2	-7.0	-0.6
1	5	3	-7.0	-2.7
1	6	1	-8.0	8.8
1	6	2	-8.0	5.5
1	6	3	-8.0	8.2
1	7	1	-9.0	58.3
1	7	2	-9.0	58.8
1	7	3	-9.0	51.6
1	8	1	-10.0	96.6
1	8	2	-10.0	108.2
1	8	3	-10.0	100.8
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
This spreadsheet is used to estimate within-run standard deviation for data from a single run.
Within-run standard deviation (SDwithin-run) is a measure of variation of y at each concentration in a single run.
It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:
1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
   Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
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   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.3

Provide information in all blue cells.
Laboratory Code: B

Compound ID:	Chem Code 10
Run ID:	RN009
Assay start date:	5/13/08
Technician ID:	RN

run	xgroup	rep	x	y
1	1	1	-3.0	1.6
1	1	2	-3.0	10.1
1	1	3	-3.0	17.2
1	2	1	-4.0	69.4
1	2	2	-4.0	81.5
1	2	3	-4.0	79.2
1	3	1	-5.0	110.6
1	3	2	-5.0	116.9
1	3	3	-5.0	119.2
1	4	1	-6.0	116.0
1	4	2	-6.0	118.9
1	4	3	-6.0	119.5
1	5	1	-7.0	116.2
1	5	2	-7.0	117.6
1	5	3	-7.0	94.8
1	6	1	-8.0	110.2
1	6	2	-8.0	125.0
1	6	3	-8.0	122.3
1	7	1	-9.0	110.3
1	7	2	-9.0	123.7
1	7	3	-9.0	121.2
1	8	1	-10.0	110.2
1	8	2	-10.0	117.4
1	8	3	-10.0	120.9
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

SDwithin-run calculation for the ER Competitive Binding Assay
Data & results worksheet

Instruction:
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   A template for data entry titled "ER-RUC data entry templates.xls" is provided.
   x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
   y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:
1. SDwithin-run is estimated separately for each run.
   SDwithin-run (estradiol) should be < 5.0%.
   SDwithin-run (norethynodrel) should be < 5.7%.
   SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy all of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 13.0

Provide information in all blue cells.
Laboratory Code: B

Compound ID: Chem Code 23
Run ID: RN009
Assay start date: 5/13/08
Technician ID: RN

run	xgroup	rep	x	y
1	1	1	-3.0	6.7
1	1	2	-3.0	6.2
1	1	3	-3.0	7.9
1	2	1	-4.0	32.6
1	2	2	-4.0	34.8
1	2	3	-4.0	35.1
1	3	1	-5.0	87.9
1	3	2	-5.0	77.0
1	3	3	-5.0	59.1
1	4	1	-6.0	92.9
1	4	2	-6.0	110.4
1	4	3	-6.0	76.3
1	5	1	-7.0	80.2
1	5	2	-7.0	93.5
1	5	3	-7.0	41.1
1	6	1	-8.0	110.7
1	6	2	-8.0	122.8
1	6	3	-8.0	108.2
1	7	1	-9.0	109.4
1	7	2	-9.0	109.1
1	7	3	-9.0	105.1
1	8	1	-10.0	108.3
1	8	2	-10.0	114.4
1	8	3	-10.0	101.1
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Table format: XY		X	A		
		log concentration	run RN009 5-13-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-7.0	-0.6715	-0.0596	-0.0028
2	Title	-7.0	-1.1628	0.5551	1.3417
3	Title	-8.0	23.1916	22.9356	26.9727
4	Title	-8.5	48.8850	51.9095	57.0164
5	Title	-9.0	86.6877	87.8570	85.8026
6	Title	-9.5	108.8658	109.6103	114.7626
7	Title	-10.0	120.6261	125.7608	122.2812
8	Title	-11.0	133.5160	129.5289	127.4870

	Nonlin fit Table of results	A run RN009 5-13-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-4.459
4	TOP	131.2
5	LOGIC50	-8.454
6	HILLSLOPE	-0.8863
7	Std. Error	
8	BOTTOM	1.369
9	TOP	1.291
10	LOGIC50	0.01426
11	HILLSLOPE	0.03571
12	95% Confidence Intervals	
13	BOTTOM	-7.314 to -1.604
14	TOP	128.6 to 133.9
15	LOGIC50	-8.484 to -8.425
16	HILLSLOPE	-0.9608 to -0.8118
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9983
20	Absolute Sum of Squares	104.7
21	Sy.x	2.288
22	Number of points	
23	Analyzed	24

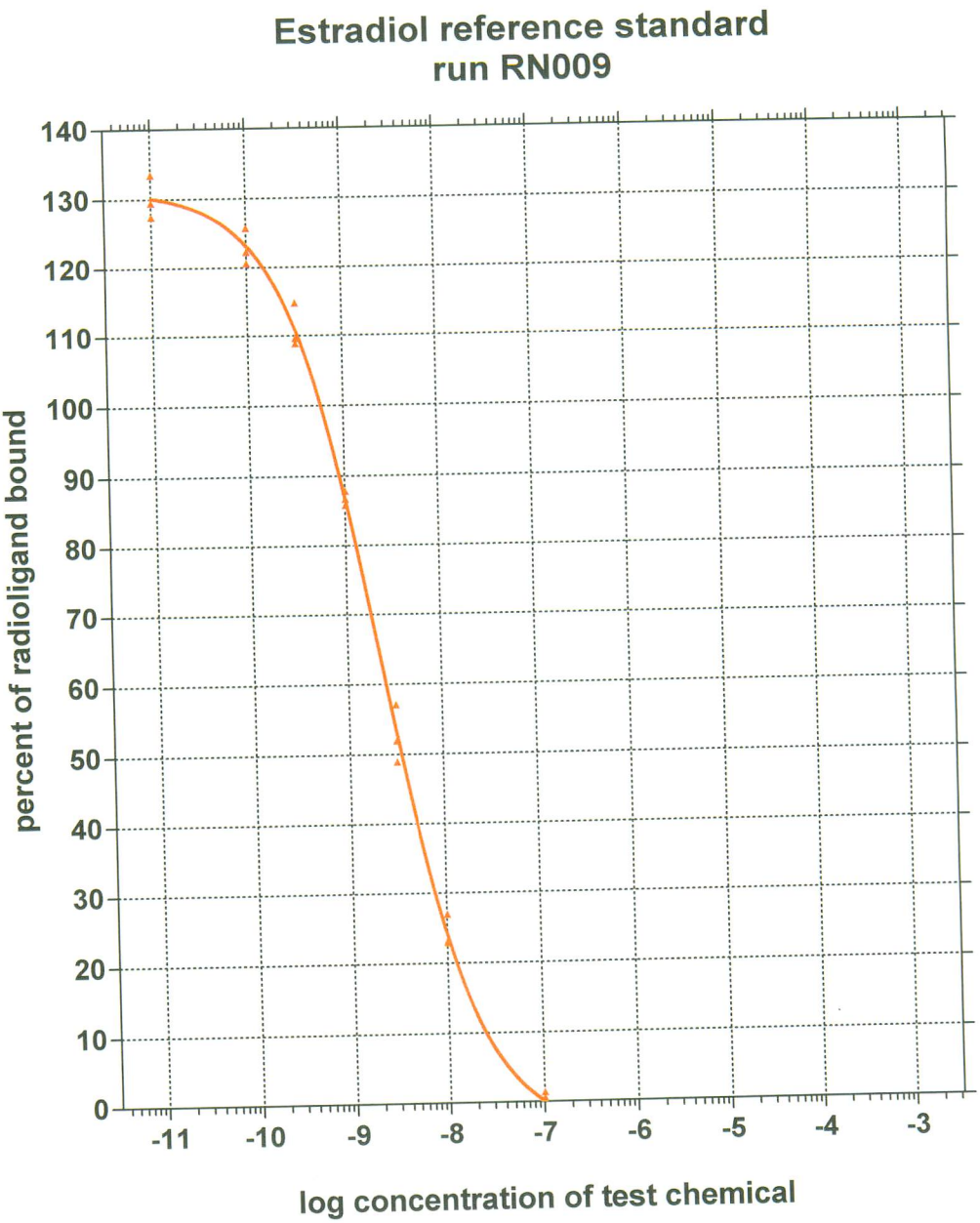

Estradiol reference standard
run RN009
percent of radioligand bound
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	run RN009 5-13-08		
		X	A:Y1	A:Y2	A:Y3
1	Title	-4.0	-2.2489	-1.2257	-0.3887
2	Title	-4.5	1.9818	3.9123	4.2834
3	Title	-5.5	50.8160	51.7149	53.1217
4	Title	-6.0	85.5447	71.3497	84.5206
5	Title	-6.5	103.5273	117.1668	114.1216
6	Title	-7.0	119.9828	120.2227	128.0966
7	Title	-7.5	112.1486	95.0234	125.3925
8	Title	-8.5	62.3559	87.5616	

	Nonlin fit Table of results	A run RN009 5-13-08
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-0.6397
4	TOP	107.7
5	LOGIC50	-5.508
6	HILLSLOPE	-1.442
7	Std. Error	
8	BOTTOM	7.449
9	TOP	5.461
10	LOGIC50	0.09461
11	HILLSLOPE	0.5671
12	95% Confidence Intervals	
13	BOTTOM	-16.23 to 14.95
14	TOP	96.32 to 119.2
15	LOGIC50	-5.706 to -5.310
16	HILLSLOPE	-2.629 to -0.2546
17	Goodness of Fit	
18	Degrees of Freedom	19
19	R^2	0.9111
0	Absolute Sum of Squares	4400
21	Sy.x	15.22
22	Number of points	
23	Analyzed	23

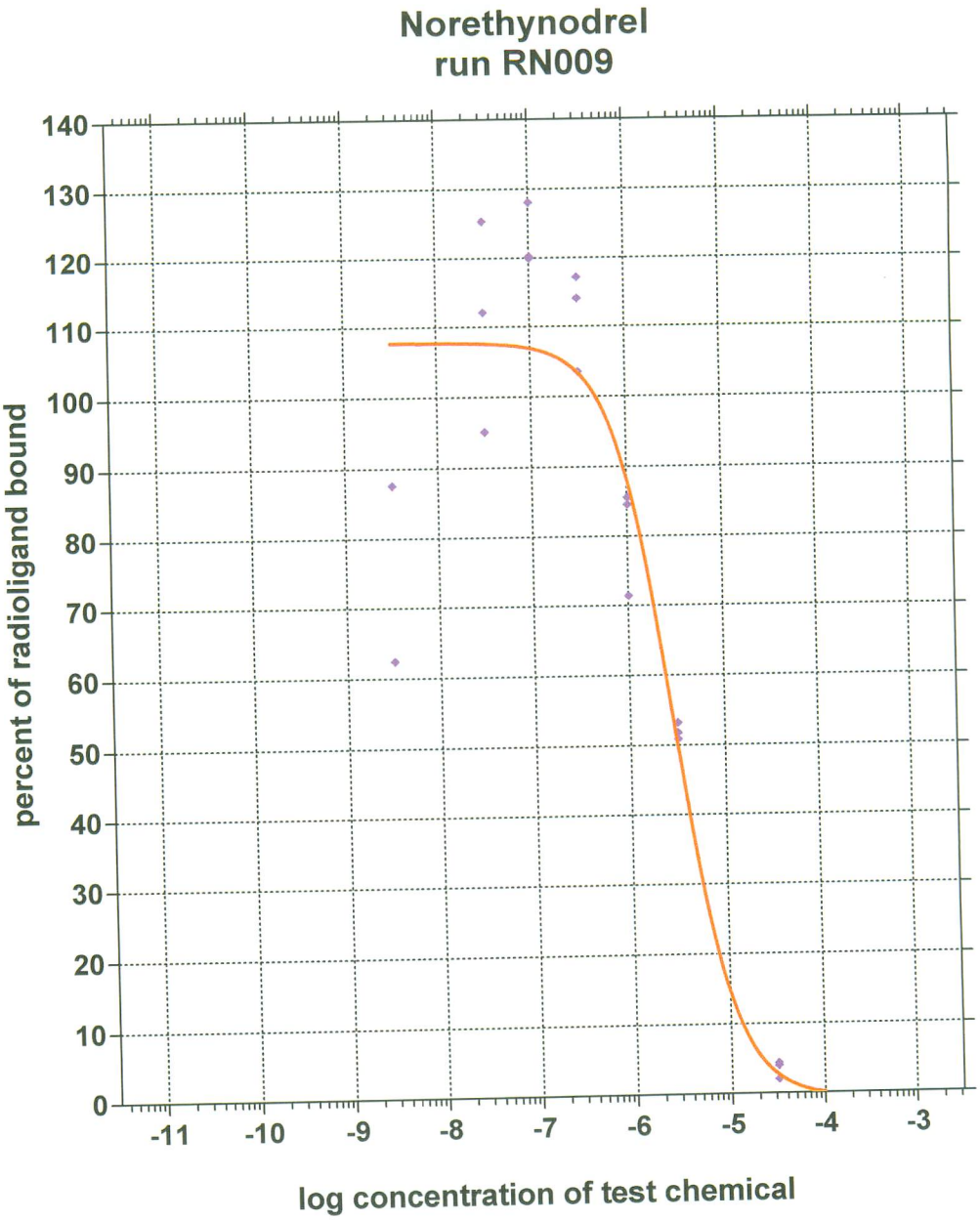

Norethynodrel
run RN009
percent of radioligand bound
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 2 run RN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-0.2708	2.5974	-0.1414
3	Title	-5.0	-4.4054	-4.7340	-4.4636
4	Title	-6.0	-3.3050	-4.0532	-3.5592
5	Title	-7.0	0.7547	0.4700	-0.7940
6	Title	-8.0	20.5319	19.4046	21.5833
7	Title	-9.0	81.2137	85.3774	76.4978
8	Title	-10.0	108.6070	96.5665	105.7180

	Nonlin fit Table of results	A Test chemical 2 run RN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-2.460
4	TOP	106.2
5	LOGIC50	-8.496
6	HILLSLOPE	-1.092
7	Std. Error	
8	BOTTOM	0.9939
9	TOP	2.408
10	LOGIC50	0.02975
11	HILLSLOPE	0.07593
12	95% Confidence Intervals	
13	BOTTOM	-4.558 to -0.3633
14	TOP	101.1 to 111.3
15	LOGIC50	-8.559 to -8.433
16	HILLSLOPE	-1.252 to -0.9314
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9952
20	Absolute Sum of Squares	177.3
21	Sy.x	3.229
22	Number of points	
23	Analyzed	21

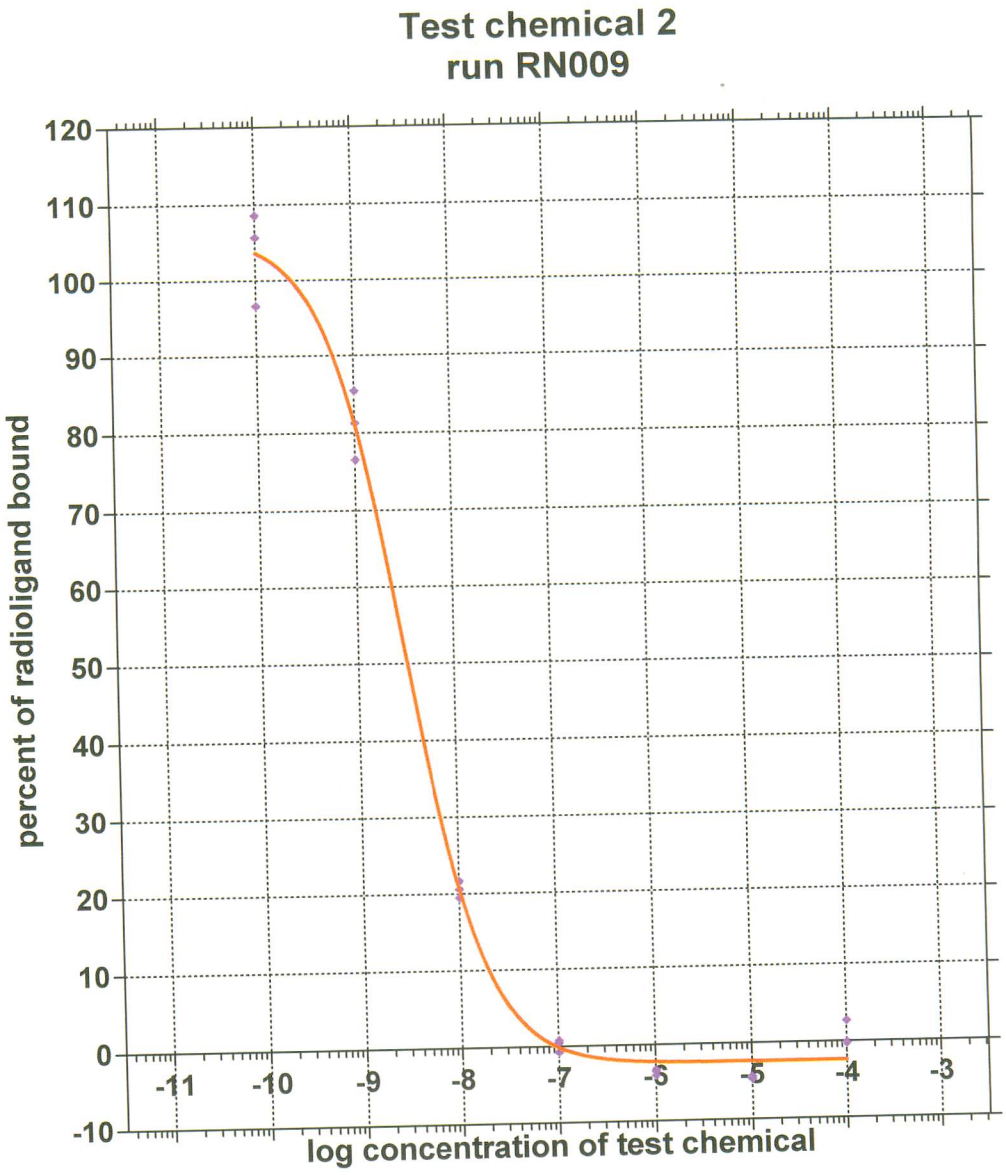

Test chemical 2
run RN009
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 3 run RN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0			
2	Title	-4.0	-3.6169	-4.5482	-2.6889
3	Title	-5.0	-4.9452	-2.9278	-3.9012
4	Title	-6.0	-3.1002	-3.5975	-3.7856
5	Title	-7.0	-2.4403	-0.5541	-2.7314
6	Title	-8.0	8.8089	5.4517	8.1564
7	Title	-9.0	58.3082	58.7685	51.6178
8	Title	-10.0	96.5541	108.1661	100.8171

	Nonlin fit Table of results	A Test chemical 3 run RN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	-3.601
4	TOP	111.6
5	LOGIC50	-8.906
6	HILLSLOPE	-1.002
7	Std. Error	
8	BOTTOM	0.8047
9	TOP	3.345
10	LOGIC50	0.02185
11	HILLSLOPE	0.08376
12	95% Confidence Intervals	
13	BOTTOM	-5.299 to -1.903
14	TOP	104.5 to 118.6
15	LOGIC50	-8.953 to -8.860
16	HILLSLOPE	-1.178 to -0.8250
17	Goodness of Fit	
18	Degrees of Freedom	17
19	R^2	0.9963
0	Absolute Sum of Squares	115.2
21	Sy.x	2.604
22	Number of points	
23	Analyzed	21

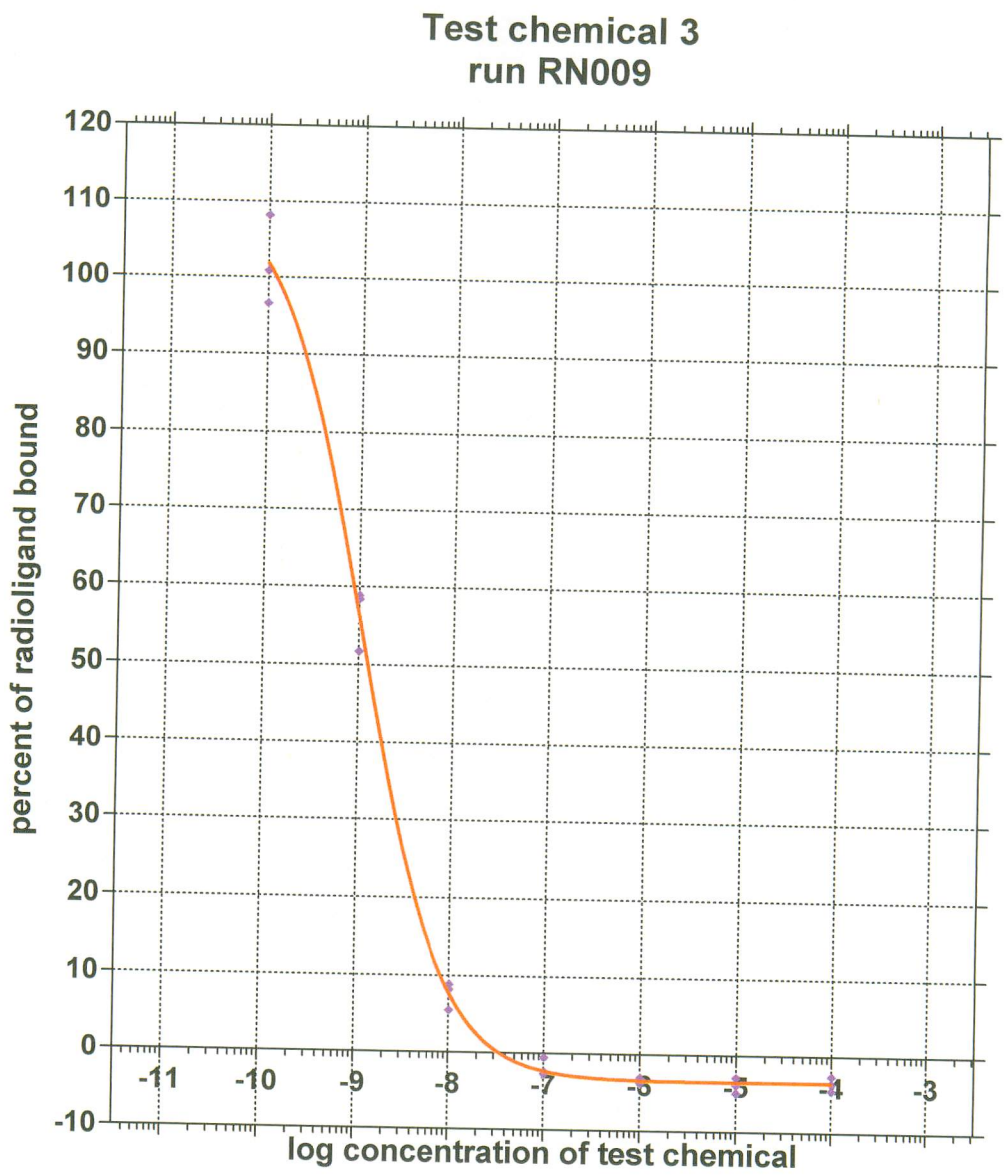

Test chemical 3
run RN009
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 10 run RN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	1.5668	10.0711	17.1899
2	Title	-4.0	69.4030	81.4578	79.1640
3	Title	-5.0	110.6470	116.9085	119.1865
4	Title	-6.0	115.9980	118.8999	119.5072
5	Title	-7.0	116.1893	117.6318	94.8242
6	Title	-8.0	110.2269	125.0228	122.3426
7	Title	-9.0	110.2879	123.7056	121.2173
8	Title	-10.0	110.1608	117.3831	120.8979

	Nonlin fit Table of results	A Test chemical 10 run RN009
		Y
1	logIC50	
2	Best-fit values	
3	BOTTOM	7.623
4	TOP	116.3
5	LOGIC50	-3.779
6	HILLSLOPE	-1.971
7	Std. Error	
8	BOTTOM	15.06
9	TOP	1.878
10	LOGIC50	0.3154
11	HILLSLOPE	3.059
12	95% Confidence Intervals	
13	BOTTOM	-23.79 to 39.03
14	TOP	112.4 to 120.2
15	LOGIC50	-4.437 to -3.121
16	HILLSLOPE	-8.353 to 4.410
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.9670
20	Absolute Sum of Squares	1048
21	Sy.x	7.240
22	Number of points	
23	Analyzed	24

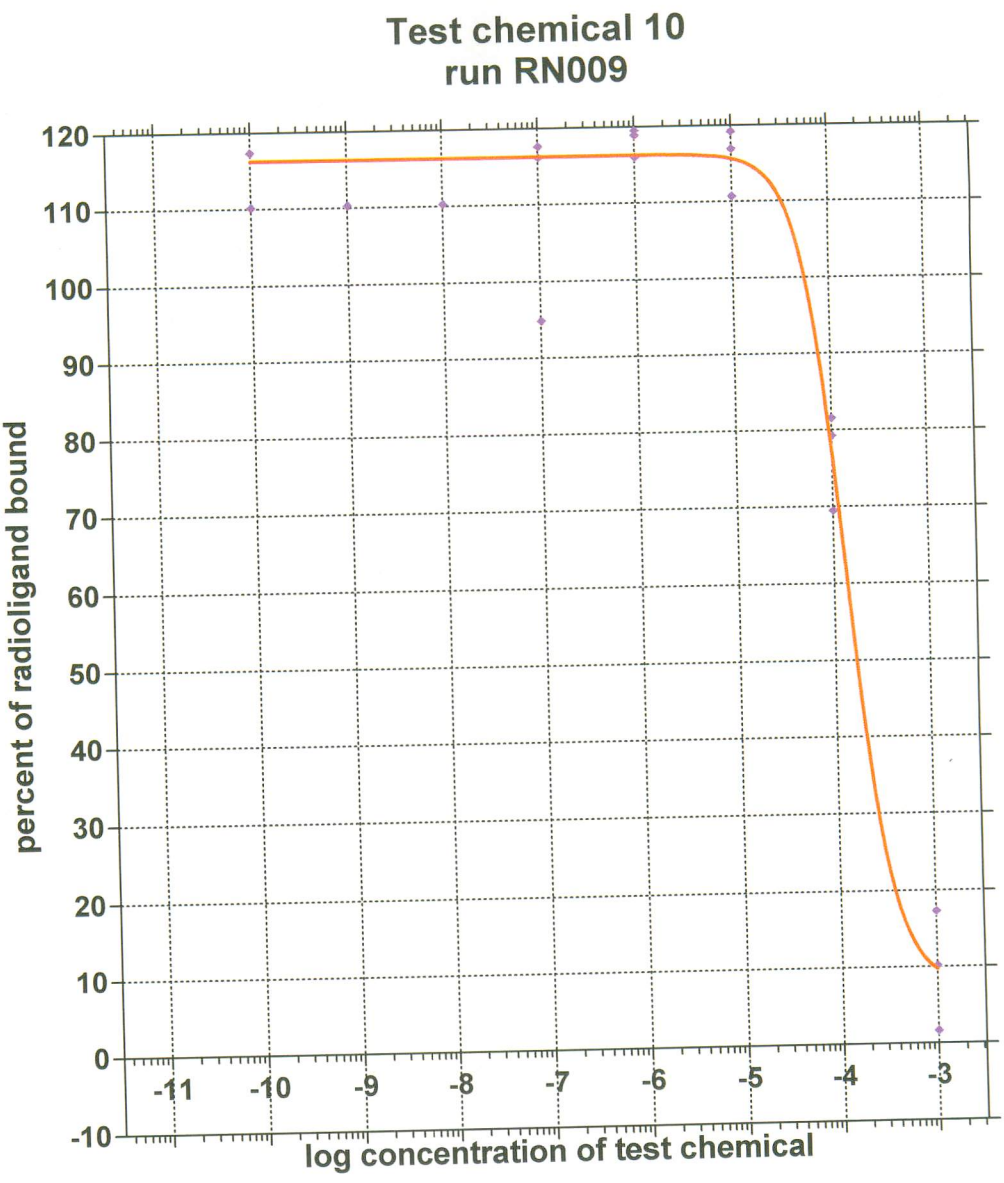
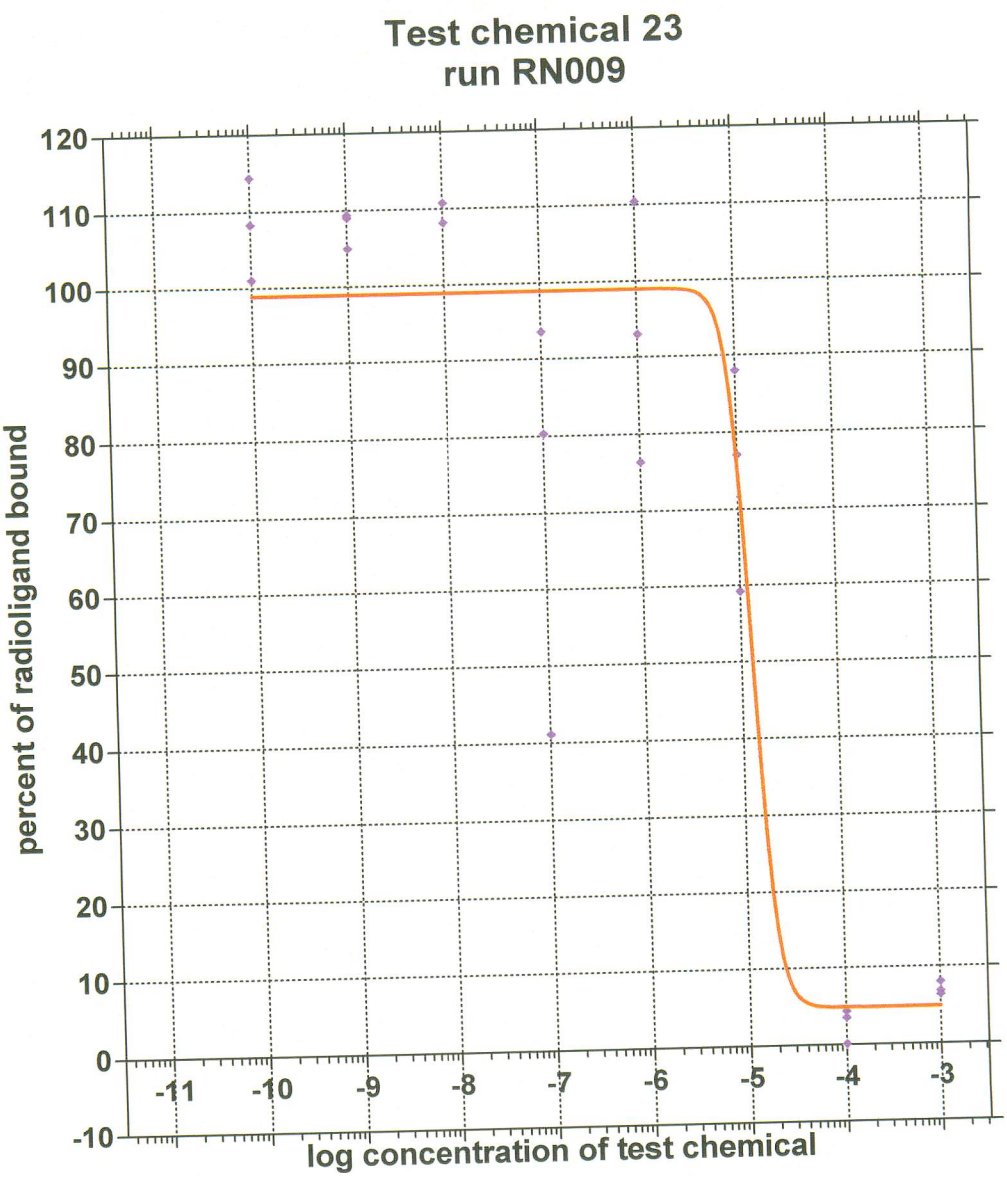

Test chemical 10
run RN009
percent of radioligand bound
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-11
-10
-9
-8
-7
-6
-5
-4
-3
log concentration of test chemical

Table format: XY		X	A		
		log concentration	Test chemical 23 run RN009		
		X	A:Y1	A:Y2	A:Y3
1	Title	-3.0	6.6802	6.2236	7.9096
2	Title	-4.0	0.0000	3.4000	4.2000
3	Title	-5.0	87.9406	76.9951	59.1396
4	Title	-6.0	92.9316	110.3507	76.2750
5	Title	-7.0	80.2303	93.5144	41.0915
6	Title	-8.0	110.7482	122.8187	108.1836
7	Title	-9.0	109.3705	109.0567	105.0584
8	Title	-10.0	108.3292	114.4155	101.0695

	Nonlin fit Table of results	A Test chemical 23 run RN009
		Y
1	logIC50	Ambiguous
2	Best-fit values	
3	BOTTOM	4.732
4	TOP	98.90
5	LOGIC50	~ -4.889
6	HILLSLOPE	~ -4.466
7	Std. Error	
8	BOTTOM	10.15
9	TOP	4.575
10	LOGIC50	~ 16.49
11	HILLSLOPE	~ 665.6
12	95% Confidence Intervals	
13	BOTTOM	-16.43 to 25.90
14	TOP	89.35 to 108.4
15	LOGIC50	(Very wide)
16	HILLSLOPE	(Very wide)
17	Goodness of Fit	
18	Degrees of Freedom	20
19	R^2	0.8589
20	Absolute Sum of Squares	6245
21	Sy.x	17.67
22	Number of points	
23	Analyzed	24


Test chemical 23
run RN009
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

APPENDIX W

Protocol and Amendment

Contract No.: 6-340-0210114
Task Order: 7
UIC/TRL Study No.: 525A

CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

1.0 PURPOSE:

The purpose of this study is to perform the chemical testing of unknown test chemicals on the basis of application of the estrogen receptor binding assay in rat uterine cytosol validated under UIC/TRL Study No. 525.

2.0 SPONSOR:

2.1	Sponsor Name:	Laboratory of Reproductive and Endocrine Toxicology Health Sciences Science and Engineering Group RTI International
2.2	Address:	3040 Cornwallis Rd. PO Box 12194 Research Triangle Park, NC 27709
2.3	Representative:	Carol D. Sloan, M.S. Phone: 919-541-6337 Fax: 919-541-6499 e-mail: css@rti.org

3.0 TESTING FACILITY:

3.1	Name:	Toxicology Research Laboratory (TRL)
3.2	Address:	University of Illinois at Chicago (UIC) Department of Pharmacology (M/C 868) 808 S. Wood Street, Room 1306 Chicago, IL 60612
3.3	Study Director:	Daniel M. Tessier, Ph.D., D.A.B.T.. Tel: 312-996-3781 Fax: 312-996-7755 E-mail: dmt@uic.edu

4.0 PROPOSED STUDY DATES:

4.1	Proposed Initiation Date:	3/11/08

REVISED PAGE
STUDY NO: 525A INITIAL: DMT
DATE: 7-8-08

4.2 Proposed Completion Date: 5/22/08

4.3 Proposed Draft Report Date: 5/29/08

5.0 PERSONNEL:

Study Director:	Daniel M Tessier, Ph.D., D.A.B.T.
Principal Investigator	Alexander V. Lyubimov, M.D., Ph.D., D.A.B.T.
Receptor Binding Analyst	Thuly Nguyen, B.S.

6.0 REFERENCE CHEMICAL, WEAK POSITIVE AND NEGATIVE CONTROL ARTICLES, AND TESTING CHEMICALS:

6.1 Reference Chemical:

6.1.1 Name: 17β-estradiol

6.1.2 Lot No.: 026K1611

6.1.3 Physical Description: White to off-white powder

6.1.4 Storage Conditions to Maintain Stability:

6.1.4.1 Storage Temperature: Ambient

6.1.4.2 Humidity: Ambient

6.1.4.3 Light: Ambient

6.1.4.4 Special Requirements: Keep tightly closed

6.2 Weak Positive Control:

6.2.1 Name: Norethynodrel

6.2.2 Lot No.: G

6.2.3 Physical Description: White or nearly white crystalline powder

6.2.4 Storage Conditions to Maintain Stability:

6.2.4.1 Storage Temperature: Ambient

6.2.4.2 Humidity: Ambient

REVISED PAGE
STUDY NO: 525A INITIAL: DMT
DATE: 7-8-08

6.2.4.2 Humidity: Ambient

6.2.4.3 Light: Ambient

6.2.4.4 Special Requirements: Store in tight container

6.3 Weak Negative Control:

6.3.1 Name: R1881

6.3.2 Lot No.: TBD

6.2.3 Physical Description: TBD

6.3.4 Storage Conditions to Maintain Stability:

6.3.4.1 Storage Temperature: TBD

6.3.4.2 Humidity: TBD

6.3.4.3 Light: TBD

6.3.4.4 Special Requirements: TBD

6.4 Test Chemicals:

Twenty three chemicals will be tested in this study. The test chemicals will be tested as unknowns; that is, the persons conducting the experiments will not know the identity of the chemicals being tested. The reference chemical (17ß-estradiol) and control chemicals (norethynodrel and R1881) shall not be coded when used as reference chemicals; they shall be coded when tested as test chemicals. The test chemicals will be supplied as coded chemicals by the Sponsor. All chemicals will be tested using ethanol as solvent, with the exception of benz(a)anthracene, which will be tested using dimethyl sulfoxide (DMSO) as solvent. Storage conditions to maintain stability will be provided by the Sponsor and will be documented in the raw data.

6.5 Identification, Purity, and Stability: Identification, purity, stability and any other characteristics that define the reference chemical and weak positive and negative controls, and test chemicals are the sole responsibility of the Sponsor and will not be included in the TRL study report.

6.6 Special Handling Procedures: Standard safety precautions including gloves, labcoat, mask and eye protection.

6.5 Logs of Reference Chemical, Weak Positive and Negative Controls and Test Chemicals: The amount, date, identity of person(s) removing aliquots and the purpose for which

each aliquot of the reference chemical, weak positive and negative controls, and test chemicals was removed from the batch will be documented.

6.6 Reference Chemical, Weak Positive and Negative Controls, and Test Chemicals Retention Samples: Since the in-life portion of this study is less than four weeks in duration, TRL will not retain a reserved sample of the reference chemical, weak positive and negative controls, and test chemicals.

7.0 STUDY MATERIALS REQUESTED FROM SPONSOR:

7.1 The Sponsor is requested to submit the test chemicals, reference chemical, and weak positive and negative controls and other useful data for the reference chemical and weak positive and negative controls used in the study.

8.0 TEST SYSTEM:

8.1 Rat uterine cytosol prepared from uteri of ovariectomized Sprague-Dawley female rats (85 -100 day old).

8.2 Justification for Selection of Test System: The rat uterine cytosol is a standard and accepted test system for estrogen receptor binding assay, and it is specified by the Sponsor.

9.0 EXPERIMENTAL DESIGN:

9.1 The chemicals testing will be performed per estrogen receptor binding assay validated under UIC/TRL Study No. 525.

9.2 To obtain the estrogen receptor binding data, Liquid Scintillation Analyzer (Model TRI-CARB 3100TR, Packard BioScience Company, Serial No. 424664) with QuantaSmart™ software (version 1.31) will be used.

9.3. Runs that do not meet the within-run performance criteria for estradiol, norethynodrel, and R1881 will be repeated, including the test chemicals. No more than five runs will be performed to obtain three acceptable runs on a given chemical.

10.0 EVALUATION OF TEST RESULTS:

10.1 Analytical and Statistical Determination: Data analysis will be performed using QuantaSmart (TM) software (version 1.31), Microsoft Excel and GraphPad Prism (version 5).

11.0 PROTOCOL AMENDMENTS:

Changes to the approved protocol will be in the form of amendments approved by the Study Director and the Sponsor Representative. Amendments will clearly describe the protocol changes and will include the effective date of the change and the justification for the change. The Study Director and Sponsor Representative may authorize protocol changes by telephone, facsimile machine or other electronic means if he/she is not physically present at the time urgent or critical changes are required. Any authorization for such changes made as above must be appropriately documented and followed by a properly written protocol amendment. The amendment must be signed and dated by the Study Director and Sponsor Representative as soon as possible.

12.0 REPORT:

As soon as feasible after final data collection and/or receipt, a draft report will be submitted to the Sponsor. The report will include all items in the protocol as well as a comprehensive presentation of all data collected in the study. The report will include, but not be limited to, the following information: materials and methods, results, reference chemical and weak positive and negative control certificates of analysis, protocol and protocol amendments (if applicable), deviations (if applicable), and representative graphs of the estrogen receptor binding data. The final report will be provided to the Sponsor both in hard copy and electronically.

13.0 RECORDS TO BE MAINTAINED/ARCHIVAL OF DATA:

All data generated during the conduct of the study, except those that are generated as direct computer input, shall be recorded directly, promptly, and accurately in ink in bound books with prenumbered pages or on worksheets that shall be bound during or at the conclusion of the nonclinical laboratory study. All appropriate computer and machine output shall be bound during or at the conclusion of the study. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data.

Any changes in entries for whatever reason (e.g., to correct an error or transposition) shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed by the responsible individual. In automated data collection systems, the individual responsible for direct data input shall be identified at the time of data input. Any changes in automated data entries for whatever reason (e.g., to correct an error or transposition) shall be made in such manner so as not to obscure the original entry, and if possible, shall indicate the reason for such change, and shall be dated and the responsible individual shall be identified.

Upon completion of the study and submission of the final report, all raw data, documentation, and other materials necessary to reconstruct the study will be stored in the UIC/TRL archives maintained by Quality Assurance. These materials will be shipped to EPA upon request.

14.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE:

This study will be performed within the spirit of the UIC/TRL Quality Assurance Program designed to conform with FDA Good Laboratory Practice Regulations.
Will this study be submitted to a regulatory agency? Yes
If so, to which agency(ies)? EPA
Does the Sponsor request that samples of the test article/carrier mixture(s) be sent to the Sponsor? No
Does the Sponsor request that test article samples be returned? No

Does the Sponsor request that samples of test article/carrier mixture(s) be sent to the Sponsor? No.

15.0 PROTOCOL APPROVAL:

STUDY DIRECTOR: _____ 3/11/08
Kasim K. Kabirov, M.D., Ph.D. — Date

PRINCIPAL INVESTIGATOR: _____ 3/11/08
Alexander V. Lyubimov, M.D., Ph.D., D.A.B.T. — Date

TOXICOLOGIST: Daniel M. Tessier 3-11-08
Daniel M. Tessier, Ph.D., D.A.B.T. — Date

SPONSOR REPRESENTATIVE: _____ _____
Carol D. Sloan, M.S. — Date

PROTOCOL AMENDMENT – 1

Study No.: 525A

Title: CHEMICALS TESTING ON BASIS OF THE ESTROGEN RECEPTOR BINDING ASSAY IN RAT UTERINE CYTOSOL

Item(s):

1. Page 1, Section 3.3, Study Director:

   Change: Study Director, Kasim K Kabirov, M.D., Ph.D., to Daniel M Tessier, Ph.D., D.A.B.T. Dr. Tessier has replaced Dr. Kabirov as Study Director.

2. Page 2, Section 5.0, Personnel:

   Change: Study Director, Kasim K Kabirov, M.D., Ph.D., to Daniel M Tessier, Ph.D., D.A.B.T. Delete "Toxicologist: Daniel M Tessier, Ph.D., D.A.B.T."

APPROVALS:

STUDY DIRECTOR: Daniel M Tessier, Ph.D., D.A.B.T. — Date: 7-8-08

FORMER STUDY DIRECTOR: Kasim K Kabirov, M.D., Ph.D. — Date: 07/08/08

PRINCIPLE INVESTIGATOR: Alexander V. Lyubimov, M.D., Ph.D., D.A.B.T. — Date: 7/8/08

SPONSOR: Carol D. Sloan, M.S. — Date: 7-10-08