

DRAFT REPORT

**ANDROGEN RECEPTOR COMPETITIVE BINDING ASSAY USING
RAT VENTRAL PROSTATE CYTOSOL**

BATTELLE STUDY NO. WA 4-11

ABC LABORATORIES STUDY NO. 49063

Analytical Site:	ABC Laboratories, Inc. 7200 E. ABC Lane Columbia, Missouri 65202 (573) 474-8579
Study Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, Ohio 43201-2693
ABC Study Initiation Date:	November 17, 2004
ABC Study Completion Date:	<i>To be determined</i>

1.0 STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study, identified as "Androgen Receptor Competitive Binding Assay Using Rat Ventral Prostate Cytosol, Battelle Study No. WA 4-11," ABC Laboratories' Study Number 49063, on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Study Sponsor: Battelle Memorial Institute

Company Agent: _____
David P. Houchens, Ph.D. Date

2.0 GLP COMPLIANCE STATEMENT

The ABC Laboratories, Inc. study number 49063 (portions conducted at ABC Laboratories, Inc.) was conducted in compliance with U.S. EPA FIFRA (40 CFR Part 160) Good Laboratory Practice Standards, which are compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM (98),17, OECD, Paris 1998, with the following exceptions:

The dose solutions used in the study were not analyzed; however, the stability of the methyltrienolone and dexamethasone in ethanol were evaluated and are reported separately by Battelle.

The graphing and data analysis to determine Bmax, KD, IC50, and RBA were conducted by Battelle and reported to ABC Laboratories but a signed report was not provided. However, this information was audited by Battelle EDSP QAU and a QA statement was provided by Battelle.

The LSC record was prepared on February 8, 2005, but the sample identifications were not recorded until September 14, 2005. Although the data was not recorded promptly, it is accurately represented.

The paperwork could not be located for the TRIS buffer prepared on February 3, 2005 and March 8, 2005, and therefore, not added to the raw data. According to paperwork present for the other TRIS buffer preparations prepared by the same analyst, the TRIS buffer was made appropriately, as is verified by the accurate completion of the experiment.

Chanda Baker, B.S.
Study Director
Staff Scientist

Date

3.0 SIGNATURE PAGE

**ABC Laboratories, Inc.
DMPK and Bioanalytical Services
7200 E. ABC Lane
Columbia, MO 65202
(573) 474-8579**

Prepared By:

Chanda Baker, B.S.
Study Director
Staff Scientist

Date

Reviewed By:

Michelle Haines, B.A.
Quality Assurance Associate II

Date

Approved By:

Amy Mize, Ph.D.
Director, DMPK and Bioanalytical Services

Date

**Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693**

David P. Houchens, Ph.D.

Date

4.0 QUALITY ASSURANCE STATEMENT

Quality Assurance statement for ABC Laboratories, Inc. study number 49063, "Androgen Receptor Competitive Binding Assay Using a Standard Rat Ventral Prostate Cytosol."

The report was reviewed by ABC's Quality Assurance Unit. The following inspections were conducted on study number 49063.

Date of Inspection	Phase Inspected	Date Reported to Study Director	Date Reported to Management
23 Nov 2004	Protocol	29 Nov 2004	03 Jan 2005
15 Mar 2005	Procedure Transferring	15 Mar 2005	20 Jun 2005
27 Jan 2006	Raw Data and Draft Report	27 Jan 2006	
	Final Report		

The undersigned indicates that the report submitted is an accurate reflection of the raw data.

Michelle Haines, B.A.
Quality Assurance Associate II

Date

5.0 STUDY PERSONNEL

The following ABC Laboratories' personnel assisted with various portions of this study:

Name	Title
Robert Howell, B.S.	Senior Scientist
Camelia Gliser, B.S.	Associate Scientist
Wes Kabler	Senior Technician
Chanda Baker, B.S.	Staff Scientist

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7.0 LIST OF ABBREVIATIONS

AR	Androgen Receptor
Bmax	Density of the Receptor
°C	Degrees Celsius
cpm	Counts per Minute
dpm	Disintegrations per minute
EPA	Environmental Protection Agency
EDTA	Ethylenediaminetetraacetic Acid
HAP	Hydroxylapatite Slurry
hr	Hour
IC50	Half Life
Kd	Dissociation constant
kg	Kilogram
LSC	Liquid Scintillation Counter
mg	Milligram
mL	Milliliter
μCi	Micro Curie
μL	Microliter
μg	Microgram
min	Minute
n	Number
NC	No Comment
ng	Nanogram
RBA	Relative Binding Affinity

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10.0 INTRODUCTION

Test Substance:	Dexamethasone
Reference:	R1881 (Methyltrienolone)
Marker:	Radiolabeled R1881 (³ H-Methyltrienolone)
Test System:	Rat prostate cytosol

The saturation assay was used to calculate the K_d (dissociation constant) and the number of receptors for saturation binding. The competitive assay was used to determine the IC₅₀ for R1881 and dexamethasone as well as the relative binding affinity (RBA) for dexamethasone as compared to R1881.

11.0 SAMPLE RECEIPT

A total of 42 study rat prostate cytosol samples were received in acceptable condition; frozen, on dry ice (solid CO₂) on January 6, 2005. The samples consisted of prostate gland tissue harvested from male Sprague-Dawley rats (90-100 days of age). Rats were castrated, killed, and the ventral prostate excised. Tissue was minced and homogenated. A detailed description of the extraction is included in Section 6.0 of the protocol. Samples were collected at Battelle Pacific Northwest Laboratory, then shipped by Federal Express to ABC Laboratories and stored at < -70°C.

12.0 BRIEF DESCRIPTION OF THE PROTOCOL

This Battelle study is being conducted to determine the ability of Dexamethasone to compete with [³H] ligand for binding in rat ventral prostate tissue homogenate. Saturation and competitive binding assay experiments using R1881 were performed according to the protocol included in Appendix II.

13.0 METHODOLOGY

13.1 Saturation Assay Procedure

Day 1: The assay consisted of 48 tubes at 8 concentrations in triplicate each with and without 100X inert R1881. Approximately 7.5-45 µL of Hot R1881 and 50 µL of triamcinolone acetonide were added to each glass tube. An aliquot of each concentration of [³H]R1881 was added to tubes #49-72. Tubes 1-48 were dried and 300 µL of diluted cytosol (final concentration – 0.6 mg/mL) was added to each tube before vortexing. Samples were kept on ice during procedure. Samples were kept in the refrigerator overnight in a rotor.

Day 2: HAP slurry tubes were prepared and 100 µL of each assay tube was pipetted into individual HAP slurry tubes. After vortexing, centrifuging, aspirating the supernatant, and repeating a TRIS washing procedure multiple times, the supernatants were decanted and placed in scintillation vials for analysis. Samples were on ice the entire time.

13.2 Competitive Assay Procedure

Day 1: The assay consisted of 57 tubes. Approximately 30 μL of Hot R1881 and 50 μL of triamcinolone acetonide was added to each glass tube and 10 μL of compound stocks. Three tubes at the beginning and end of the assay also had 30 μL of 100x inert R1881 added for determining nonspecific binding. Tubes were dried, and 300 μL of diluted cytosol (final concentration – 1.0 mg/mL) was added to each tube before vortexing and storing in the refrigerator in a rotor overnight.

Day 2: HAP slurry tubes were prepared and 100 μL of each assay tube was pipetted into individual HAP slurry tubes. After vortexing, centrifuging, aspirating the supernatant, and repeating a TRIS washing procedure multiple times, the supernatants were decanted and placed in scintillation vials for analysis.

13.3 List of Test Substances

Dexamethasone (CAS 50-02-2)			
Formula	Molecular Weight	Lot Number	Chemical Purity
$\text{C}_{22}\text{H}_{29}\text{FO}_5$	392.5	P4311	99%
Storage: Room Temperature		Supplied By: Battelle	
Physical Nature: White Solid			
Reference Substance			
R-1881 (Methyltrienolone; CAS 965-93-5)			
Formula	Molecular Weight	Lot Number	Chemical Purity
$\text{C}_{19}\text{H}_{24}\text{O}_2$	284.38	3411228	NG
Storage: -20 °C away from light		Supplied By: Battelle	
Physical Nature: Solid			
Marker			
Radiolabeled R-1881 (^3H-Methyltrienolone; CAS 68-23-5)			
Formula	Molecular Weight	Lot Number	Chemical Purity
$\text{C}_{19}\text{H}_{24}\text{O}_2$	284.4	3538 497	NG
Storage: -20 °C away from light		Supplied By: Battelle	
Physical Nature: in Ethanol			

13.4 List of Solutions Received

Substance/Chemical	Concentration	Date Made
Cytosol	5.206 mg/mL	10/27/04
Cold R-1881 Saturation	1.00E-05 M	1/10/05
Cold R-1881 Saturation	1.00E-06 M	1/10/05
NSB	1.00E-05 M	1/10/05
Standard 1	3.00E-06 M	1/10/05
Standard 2	3.00E-07 M	1/10/05
Standard 3	3.00E-08 M	1/10/05
Standard 4	3.00E-09 M	1/10/05
Standard 5	3.00E-10 M	1/10/05
Weak Positive	3.00E-02 M	1/11/05
Weak Positive	3.00E-03 M	1/11/05
Weak Positive	3.00E-04 M	1/11/05
Weak Positive	3.00E-05 M	1/11/05
Weak Positive	3.00E-06 M	1/11/05
Weak Positive	3.00E-07 M	1/11/05
Weak Positive	3.00E-08 M	1/11/05
Weak Positive	3.00E-09 M	1/11/05
Labeled R-1881 Tracer	1.00E-07 M	1/11/05
Labeled R-1881 Tracer	1.00E-08 M	1/11/05

13.5 Equipment and Calibration

A Beckman Model LS 6500 liquid scintillation counter (LSC; Beckman Instruments, Inc; Schaumburg, IL) was used to record the radioactivity levels of each sample after assay. The LSC is equipped with the H number method for cpm (counts per minute) to dpm (disintegrations per minute) conversion. Prior to analysis, the LSC calibration was checked daily to ensure proper functioning of the instrument.

13.6 Analytical Run Sequence

Each analytical batch was analyzed using three blank samples at the beginning of the run, followed by analysis of the assayed samples. The LSC data was corrected for background by subtracting the dpm value measured from the analysis of blank samples. All samples were analyzed in triplicate. Samples were counted for 5 minutes.

14.0 STUDY SAMPLE RESULTS

Graph pad prism was used by Nancy Holter of Battelle/PNL to calculate the dissociation constant (Kd), the density of the receptor (Bmax), the half life (IC₅₀) for R1881 and dexamethasone, and the Relative Binding Affinity (RBA) for dexamethasone as compared to R1881 from the competitive binding study portion.

Density of Receptor (Bmax) and Dissociation Constant (Kd) of Saturation Experiments

Experiment No.	Date	Bmax (fmole / 100 µg)	Kd (nM)
Saturation #3	2-Feb-05	2.23e-010	8.32e-010
Saturation #4	7-Feb-05	1.72e-010	6.85e-010
Saturation #5	22-Feb-05	1.84e-010	7.24e-010

Note: Experiments 1 and 2 were not reportable.
Bmax and Kd values are taken from Appendix I.

IC₅₀ for R1881 and Dexamethasone

Experiment No.	Date	IC ₅₀ for R1881	IC ₅₀ for Dexamethasone
Competitive #1	7-Mar-05	1.74e-09	2.94e-05
Competitive #2	9-Mar-05	1.46e-09	2.84e-05
Competitive #3	14-Mar-05	1.99e-09	3.37e-05

Relative Binding Affinity (RBA)

Experiment No.	Date	RBA
Competitive #1	7-Mar-05	5.93e-05
Competitive #2	9-Mar-05	5.15e-05
Competitive #3	14-Mar-05	5.91e-05

15.0 CONCLUSIONS

Saturation experiments were conducted on February 2, 7, and 22, 2005. Competitive experiments were conducted on March 7, 9, and 14, 2005. Results of the experiments indicate that saturation experiments 3 through 5 had a Bmax of 1.72e-10 to 2.23e-010. The Kd ranged from 6.85e-010 to 8.32e-010. Results of the competitive experiments indicate an IC₅₀ ranging from 1.46e-09 to 1.99e-09 for R1881 and 2.84e-05 to 3.37e-05 for dexamethasone. The RBA for the competitive experiments ranged from 5.15e-05 to 5.93e-05.

16.0 SAMPLES, STUDY DATA AND REPORT ARCHIVAL

At the sponsor's discretion, study samples will be destroyed upon finalization of the report.

The protocol, any amendments, the final report and all raw data collected as a result of this study will be archived by Battelle-Richland. The associated facility records will also be archived as required by Battelle-Richland SOPs.

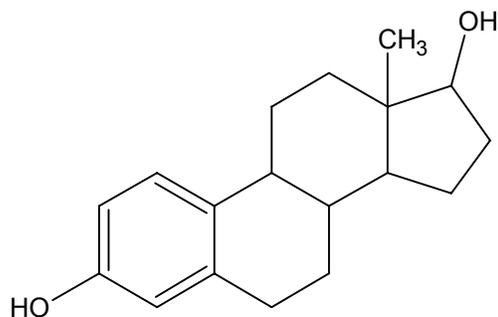
17.0 REFERENCES

U.S. EPA FIFRA (40 CFR Part 160) Good Laboratory Practice Standards

FIGURES

Figure 1. Structure of Dexamethasone, R1881, and Radiolabeled R1881

Test Compound:	Dexamethasone
Supplier:	Battelle
CAS No. :	CAS 50-02-2
Formula:	$C_{22}H_{29}FO_5$
Molecular Weight:	392.5
Storage Conditions:	Room Temperature
Structure:	



Reference Compound:	R1881 (Methyltrienolone)
Supplier:	Battelle
CAS No.:	CAS 965-93-5
Chemical Name:	Methyltrienolone
Formula:	$C_{19}H_{24}O_2$
Molecular Weight:	284.4
Storage conditions:	-20°C away from light
Structure:	

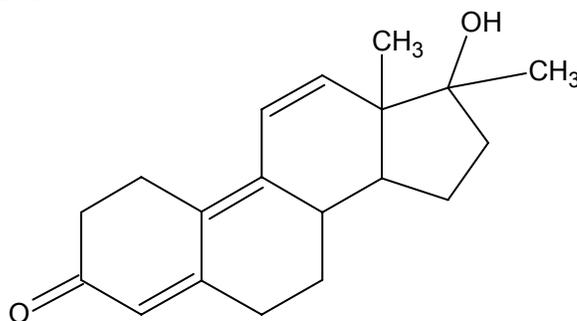
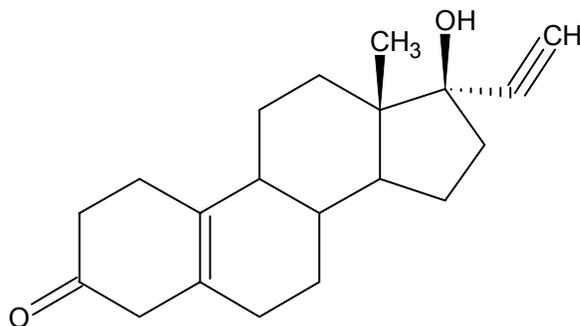


Figure 1. Structure of Dexamethasone, R1881, and Radiolabeled R1881 (continued)

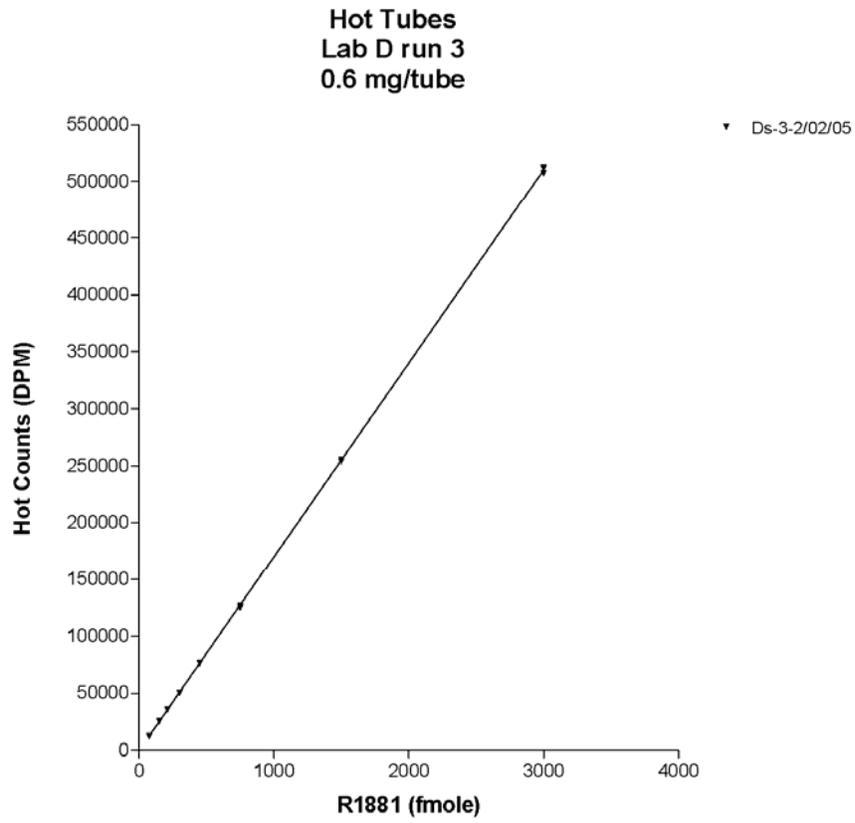
Marker Compound:	Radiolabeled R1881 (³H-Methyltrienolone)
Supplier:	Battelle
CAS No. :	CAS 68-23-5
Chemical Name:	Norethynodrel
Formula:	C ₁₉ H ₂₄ O ₂
Molecular Weight:	284.4
Storage Conditions:	-20°C away from light
Structure:	



APPENDIX I
RAW DATA

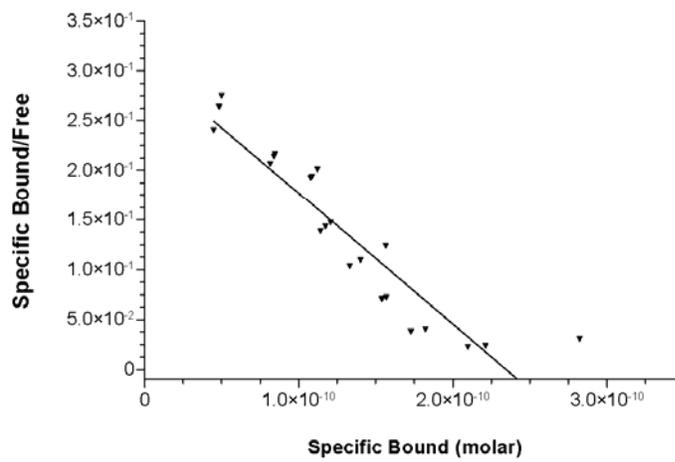
SATURATION ASSAYS

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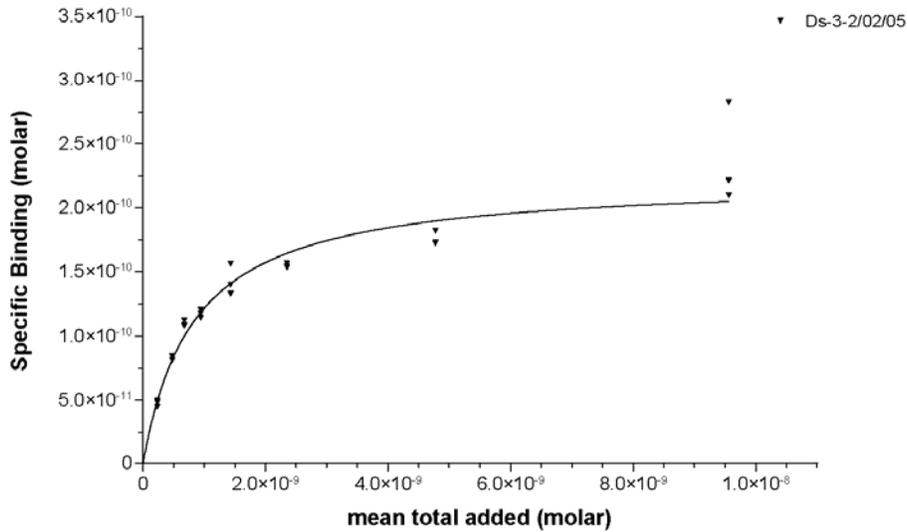


Scatchard Display
Lab D run 3
0.6 mg/tube

▼ Ds-3-2/02/05

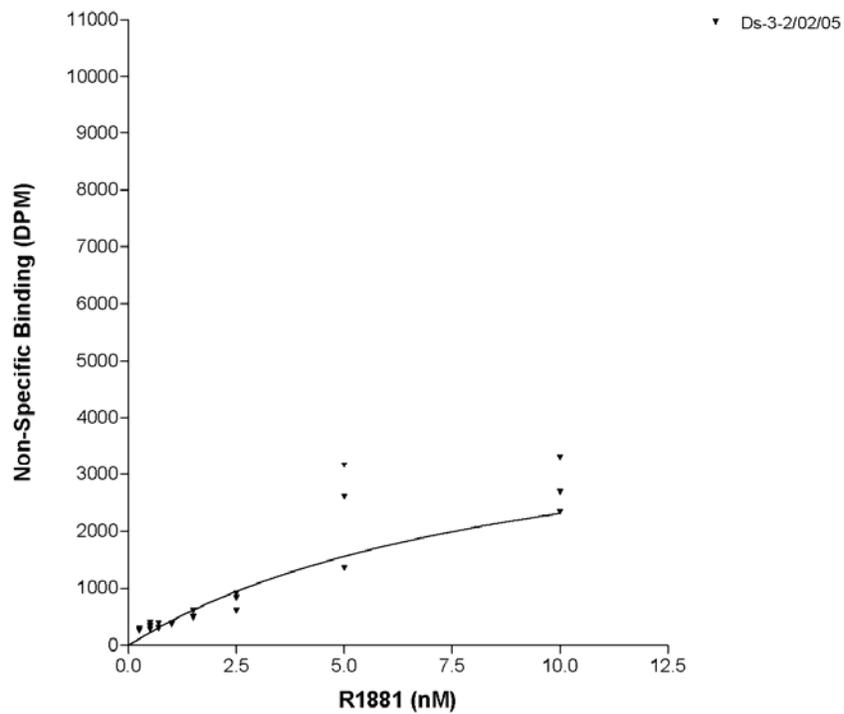


Lab D run 3
0.6 mg/tube



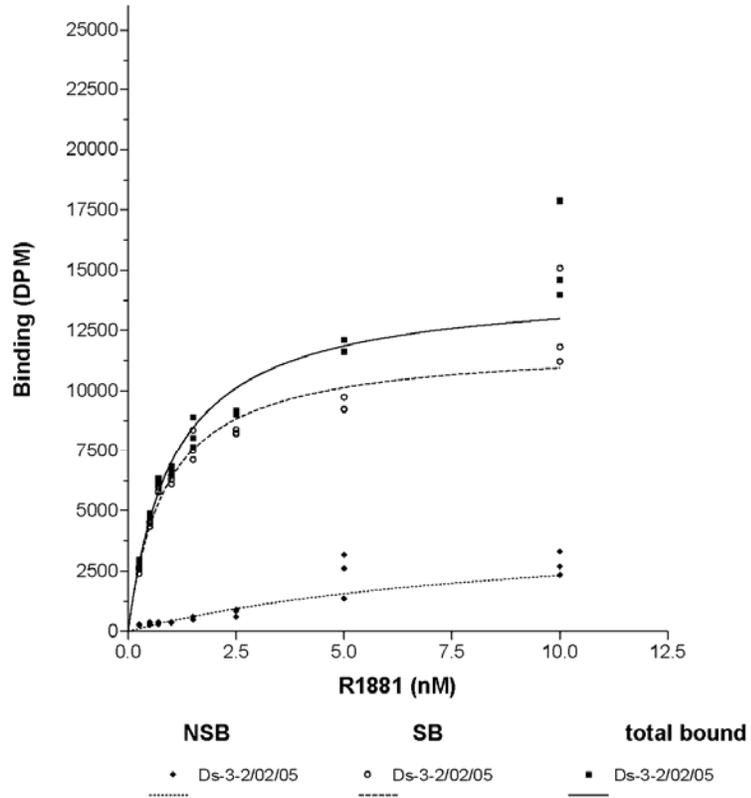
specific bound	Ds-3-2/02/05
BMAX	2.227e-010
KD	8.318e-010
Std. Error	
BMAX	8.231e-012
KD	6.538e-011
95% Confidence Intervals	
BMAX	2.057e-010 to 2.398e-010
KD	6.962e-010 to 9.674e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.8958
Weighted Sum of Squares (1/Y ²)	0.1654
Absolute Sum of Squares	7.838e-021
Sy.x	1.888e-011
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

NSB Tubes
Lab D run 3
0.6 mg/tube

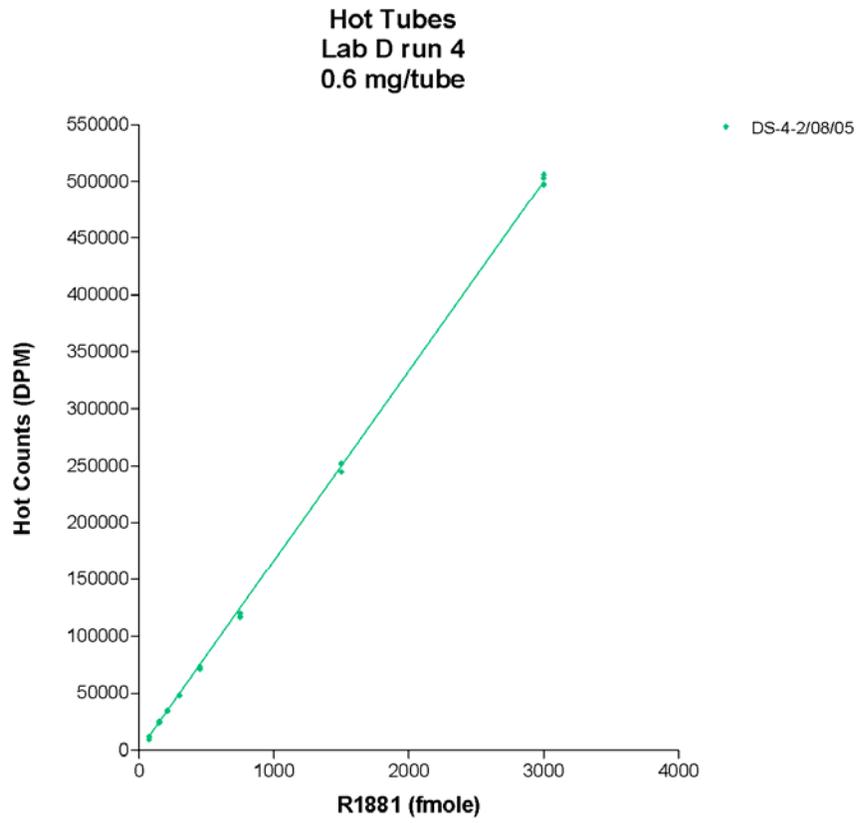


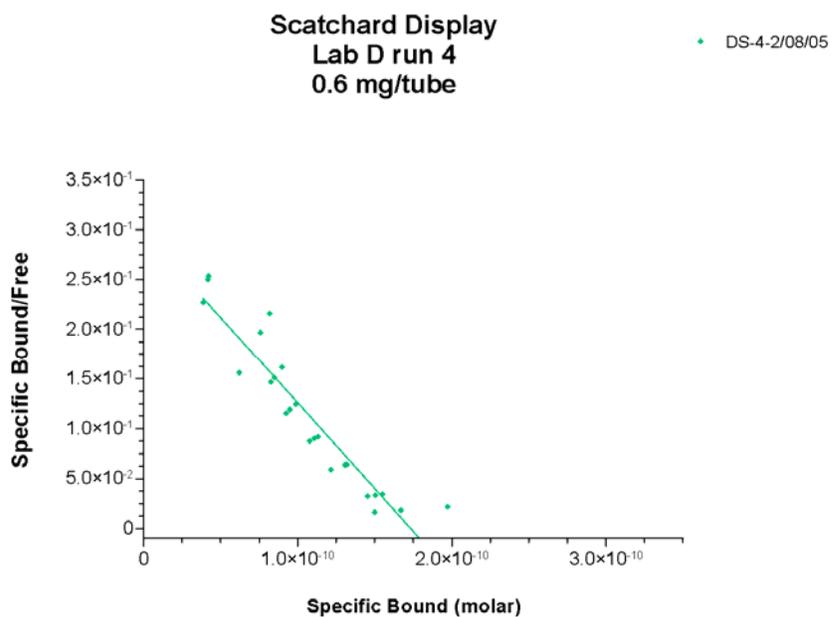
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bound counts
Lab D run 3
0.6 mg/tube

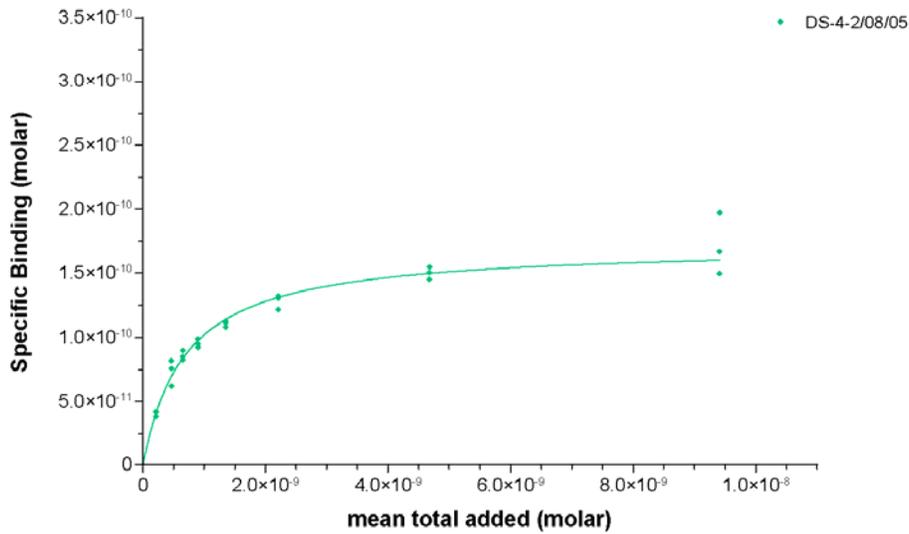


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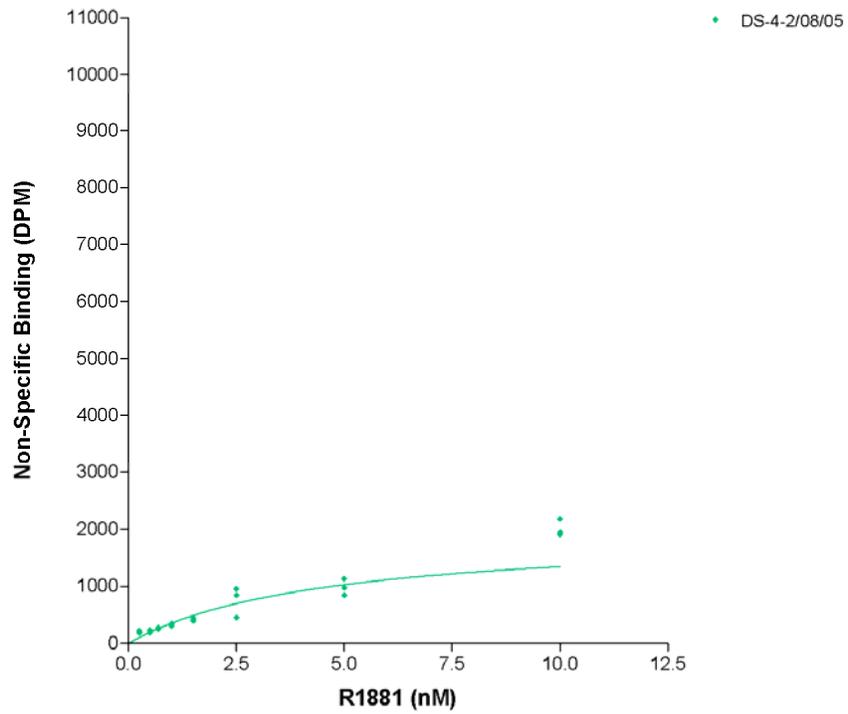
Lab D run 4
0.6 mg/tube



specific bound	DS-4-2/08/05
BMAX	1.719e-010
KD	6.854e-010
Std. Error	
BMAX	5.049e-012
KD	4.546e-011
95% Confidence Intervals	
BMAX	1.614e-010 to 1.823e-010
KD	5.911e-010 to 7.797e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.9478
Weighted Sum of Squares (1/Y ²)	0.1120
Absolute Sum of Squares	2.050e-021
Sy.x	9.654e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

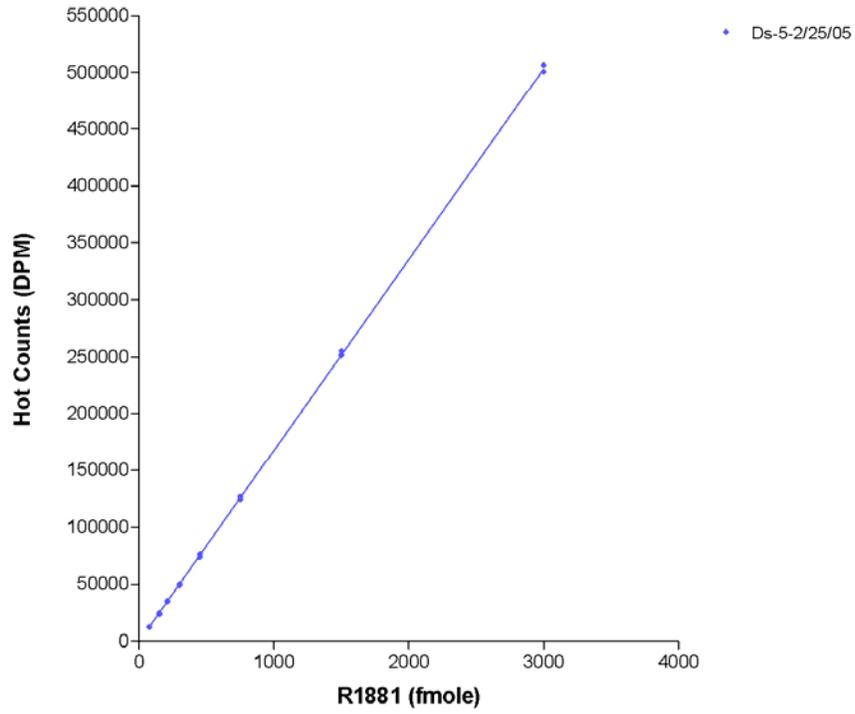
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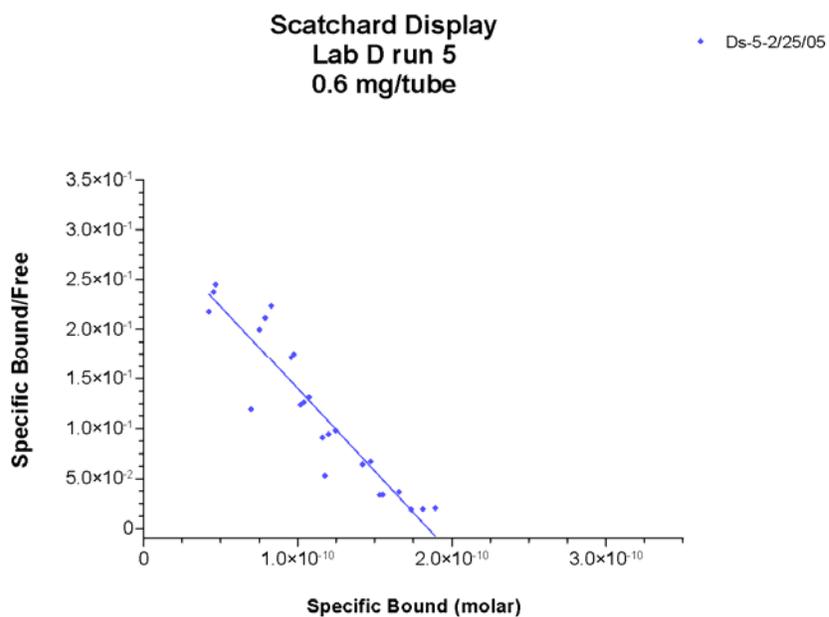
NSB Tubes
Lab D run 4
0.6 mg/tube



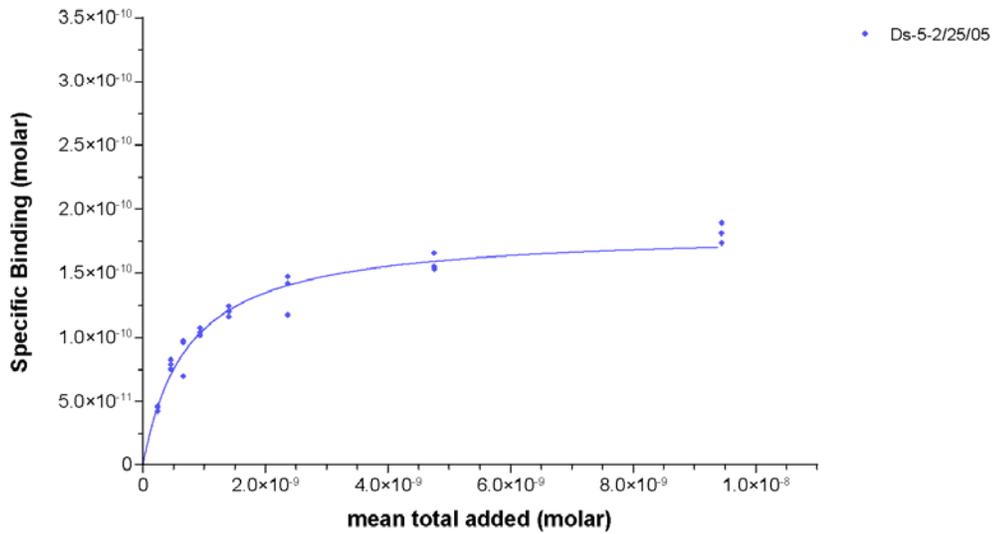
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**Hot Tubes
Lab D run 5
0.6 mg/tube**





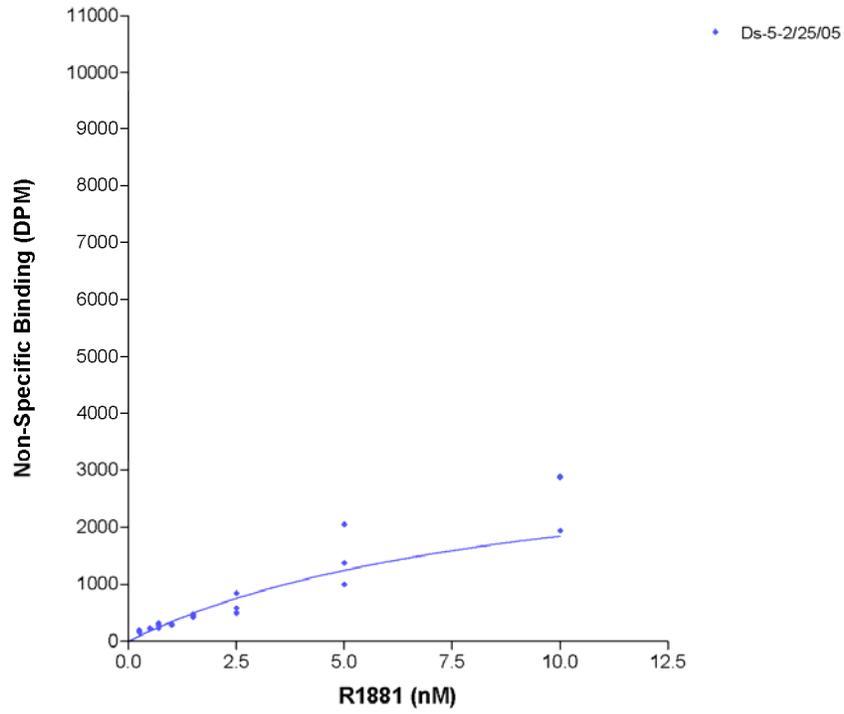
Lab D run 5
0.6 mg/tube



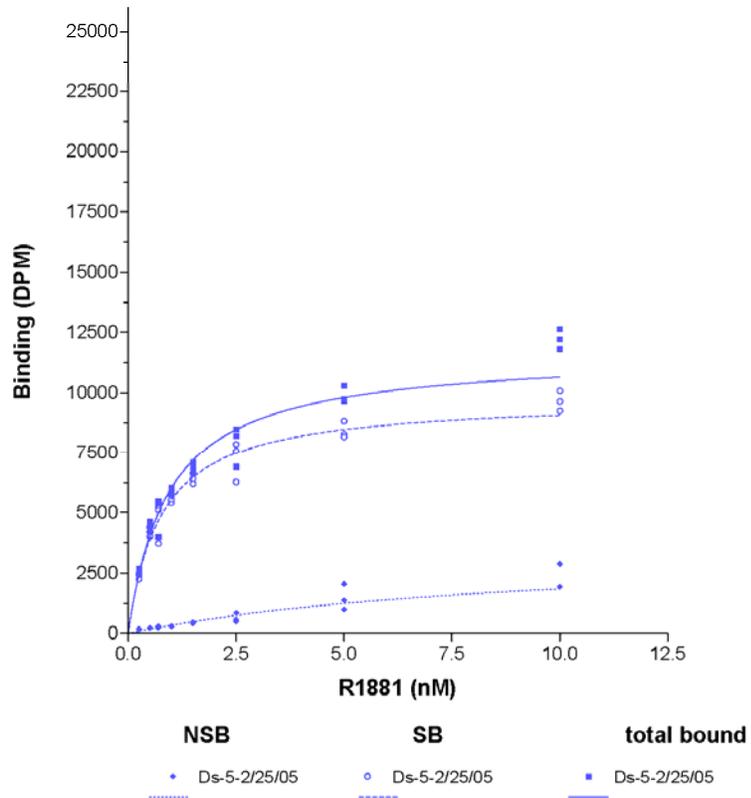
specific bound	Ds-5-2/25/05
BMAX	1.836e-010
KD	7.239e-010
Std. Error	
BMAX	6.973e-012
KD	6.170e-011
95% Confidence Intervals	
BMAX	1.691e-010 to 1.980e-010
KD	5.959e-010 to 8.519e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.9552
Weighted Sum of Squares (1/Y ²)	0.1845
Absolute Sum of Squares	1.910e-021
Sy.x	9.317e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

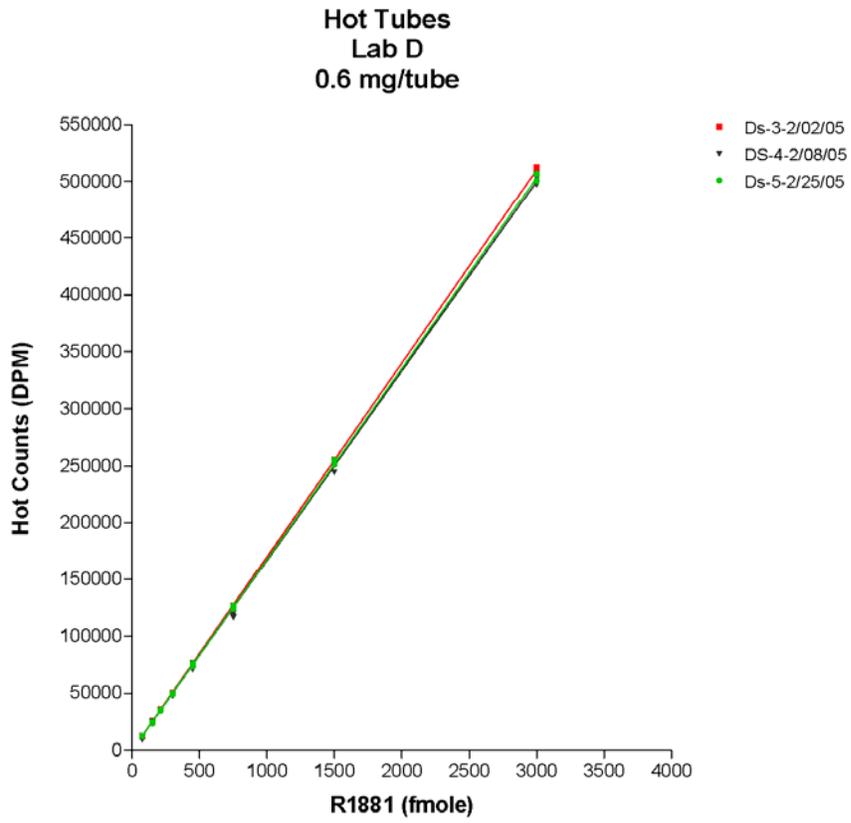
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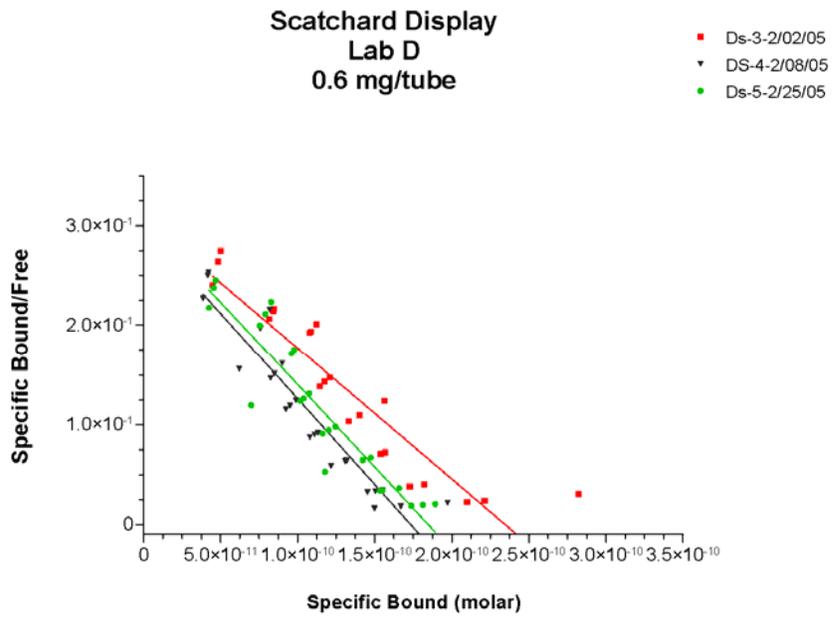
NSB Tubes
Lab D run 5
0.6 mg/tube



bound counts
Lab D run 5
0.6 mg/tube

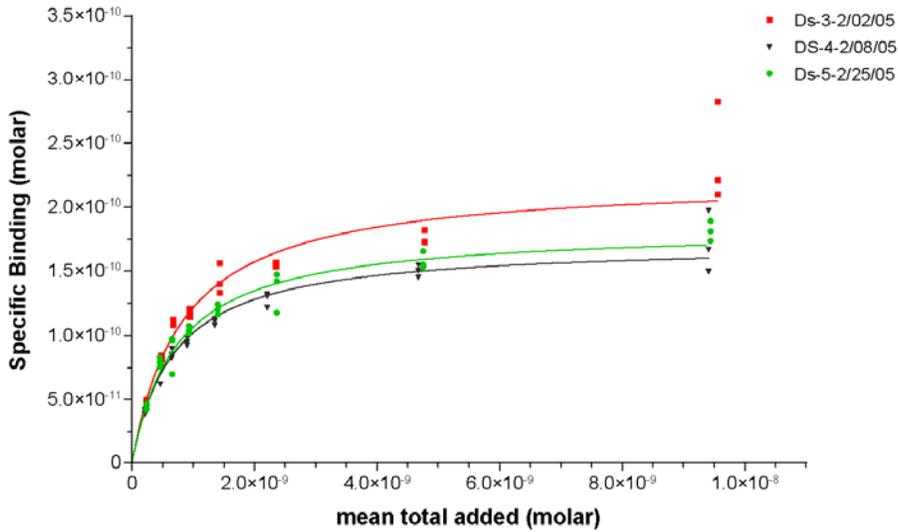






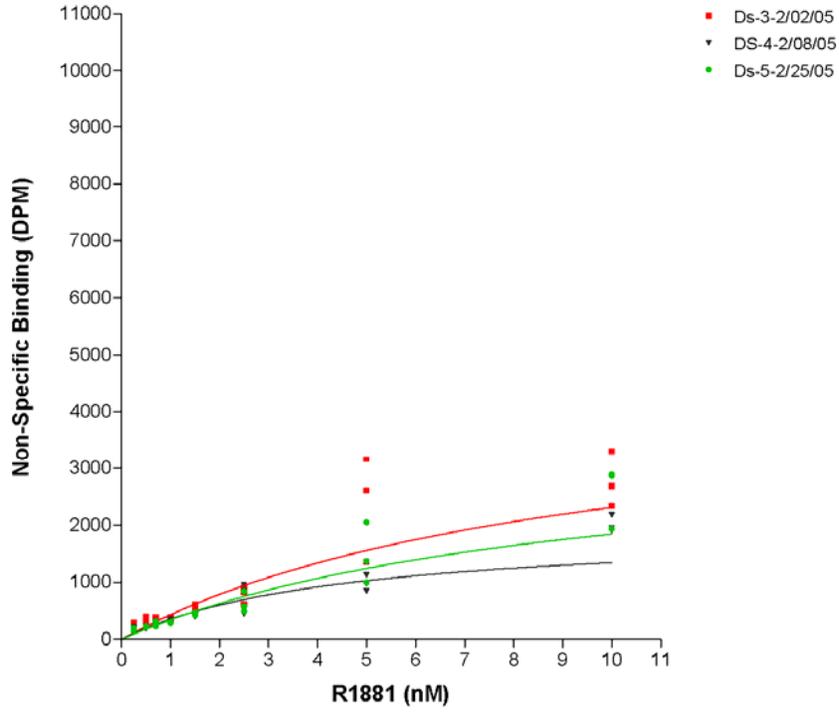
4-11-2-Saturation_D.pzf:D Curve (molar) - Tue Jan 31 08:05:19 2006

Lab D
0.6 mg/tube



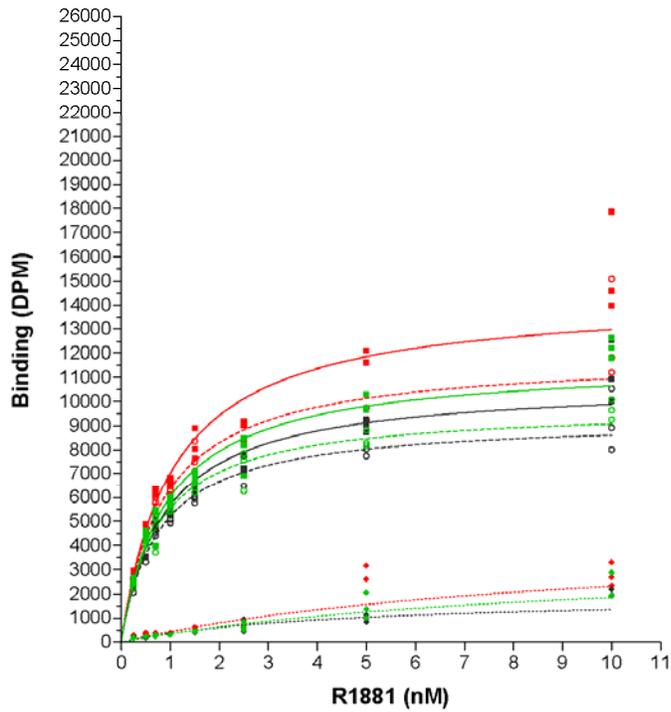
specific bound	Ds-3-2/02/05	DS-4-2/08/05	Ds-5-2/25/05
BMAX	2.227e-010	1.719e-010	1.836e-010
KD	8.318e-010	6.854e-010	7.239e-010
Std. Error			
BMAX	8.231e-012	5.049e-012	6.973e-012
KD	6.538e-011	4.546e-011	6.170e-011
95% Confidence Intervals			
BMAX	2.057e-010 to 2.398e-010	1.614e-010 to 1.823e-010	1.691e-010 to 1.980e-010
KD	6.962e-010 to 9.674e-010	5.911e-010 to 7.797e-010	5.959e-010 to 8.519e-010
Goodness of Fit			
Degrees of Freedom	22	22	22
R ² (unweighted)	0.8958	0.9478	0.9552
Weighted Sum of Squares (1/Y ²)	0.1654	0.1120	0.1845
Absolute Sum of Squares	7.838e-021	2.050e-021	1.910e-021
Sy.x	1.888e-011	9.654e-012	9.317e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	24
Number of missing values	0	0	0

NSB Tubes
Lab D
0.6 mg/tube



4-11-2-Saturation_D.pzf.D data (DPM) - Tue Jan 31 08:05:19 2006

**bound counts
Lab D
0.6 mg/tube**



NSB	SB	total bound
• Ds-3-2/02/05	◦ Ds-3-2/02/05	■ Ds-3-2/02/05
• DS-4-2/08/05	◦ DS-4-2/08/05	■ DS-4-2/08/05
• Ds-5-2/25/05	◦ Ds-5-2/25/05	■ Ds-5-2/25/05

Saturation Data for Experiment #1

Laboratory D
 AR Saturation Assay (cold R1881 dilutions supplied by Battelle)
 72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

The value in column Q will change to FALSE

For your convenience, data reduction is performed in columns

U through BZ, and the values needed for analysis are presented in columns CF through CN

Cells in column S 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: D
 Run identification: 212005
 Assay start date: 2/2/2005

Tracer lot number: 3538-497
 Specific activity on day of assay: 80.27 Ci/mmole

Cytosol lot or vial number: 102704
 protein (cytosol) per tube: 600 ug
 protein (cytosol) per tube: 0.6 mg
 KD: 8.32E-01 nM
 Bmax: 11.14 fmole/100 ug
 total volume in tubes: 300 uL
 volume of ethanol counted: 2 mL
 multiply DPM in sample by: 3

Receptor_Notes
 diluted to 2 mg/ml for use (0.6 mg/300 ul)

protocol calls for counting decanted EtOH supernate
 reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcienone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-E supplied by Battelle to laboratory "E"
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—

dpm as counted	corrected DPM for 2mL	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
893.6	2680.8	TRUE		21.2%	0.25	2680.8	284.5
955.22	2865.66	TRUE		22.7%	0.25	2865.7	284.5
983.8	2951.4	TRUE		23.3%	0.25	2951.4	284.5
1622.81	4868.43	TRUE		18.9%	0.5	4868.4	340.6
1564.02	4692.06	TRUE		18.2%	0.5	4692.1	340.6
1608.43	4825.29	TRUE		18.7%	0.5	4825.3	340.6
2108.42	6325.26	TRUE		17.5%	0.7	6325.3	334.1
2045.36	6136.08	TRUE		17.0%	0.7	6136.1	334.1
2033.94	6101.82	TRUE		16.9%	0.7	6101.8	334.1
2275.51	6826.53	TRUE		13.5%	1	6826.5	377.6
2220.24	6660.72	TRUE		13.2%	1	6660.7	377.6
2159.88	6479.64	TRUE		12.8%	1	6479.6	377.6
2548.96	7646.88	TRUE		10.0%	1.5	7646.9	533.2
2963.48	8890.44	TRUE		11.6%	1.5	8890.4	533.2
2674.37	8023.11	TRUE		10.5%	1.5	8023.1	533.2

Saturation Data for Experiment #1 (continued)

Saturation Assay Tube Layout												
Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot RT881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold RT881 Volume (uL)	Cold Final Concentration (nM)	Triselenone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial supplied by Bottle	Full on Vials in set 1-1-E supplied by Bottle to laboratory "E"
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—

dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
3001.3	9003.9	TRUE	
3010.89	9032.67	TRUE	
3057.91	9173.73	TRUE	
4040.11	12120.33	TRUE	
3867.47	11602.41	TRUE	
3875.12	11625.36	TRUE	
4664.92	13994.76	TRUE	
5957.34	17872.02	TRUE	
4866.43	14599.29	TRUE	
87.52	262.56	TRUE	
98.05	294.15	TRUE	
98.9	296.7	TRUE	
113.56	340.68	TRUE	
131.9	395.7	TRUE	
95.09	285.27	TRUE	
129.53	388.59	TRUE	
102.42	307.26	TRUE	
102.14	306.42	TRUE	
123.23	369.69	TRUE	
130.38	391.14	TRUE	
123.98	371.94	TRUE	
203.23	609.69	TRUE	
161.81	485.43	TRUE	
168.13	504.39	TRUE	
204.33	612.99	TRUE	
277.94	833.82	TRUE	
303.33	909.99	TRUE	
869.68	2609.04	TRUE	
453.25	1359.75	TRUE	
1055.28	3165.84	TRUE	
896.05	2688.15	TRUE	
780.45	2341.35	TRUE	
1103.17	3309.51	TRUE	
12584.12	12584.12	TRUE	
12771.56	12771.56	TRUE	
12593.87	12593.87	TRUE	
25538.73	25538.73	TRUE	
26157.87	26157.87	TRUE	
25591.1	25591.1	TRUE	
36014.45	36014.45	TRUE	
36194.12	36194.12	TRUE	
36085.32	36085.32	TRUE	
50315.2	50315.2	TRUE	
50450.59	50450.59	TRUE	
50917.08	50917.08	TRUE	
76231.29	76231.29	TRUE	
76517.73	76517.73	TRUE	
76886.88	76886.88	TRUE	
126890.8	126890.8	TRUE	
125332.3	125332.3	TRUE	
124842.7	124842.7	TRUE	
255392.3	255392.3	TRUE	
255715.3	255715.3	TRUE	
253941.1	253941.1	TRUE	
507814.8	507814.8	TRUE	
511772.5	511772.5	TRUE	
513128.2	513128.2	TRUE	

Saturation Data for Experiment #1 (continued)

Ten Percent Rule	Saturation X values, Bound y values, NSB y values			Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)													
				Tube Identification		Assay tube contents											Total Volume (ul)
				Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial (nM)	Hot R1881 Volume (ul)	Cold R1881 Conc. Initial (mM)	Cold R1881 volume (ul)	Triamcetenone Acetate (ul)	Cytosol (ul)	Hot Conc. Final (nM)	Cold Conc. Final (nM)	
21.2%	0.25	2680.8	284.5	2E+05	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22.7%	0.25	2865.7	284.5	2E+05	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23.3%	0.25	2951.4	284.5	2E+05	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
18.9%	0.5	4868.4	340.6	2E+05	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
18.2%	0.5	4692.1	340.6	2E+05	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
18.7%	0.5	4825.3	340.6	2E+05	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
17.5%	0.7	6325.3	334.1	2E+05	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
17.0%	0.7	6136.1	334.1	2E+05	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
16.9%	0.7	6101.8	334.1	2E+05	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
13.5%	1	6826.5	377.6	2E+05	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
13.2%	1	6660.7	377.6	2E+05	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
12.8%	1	6479.6	377.6	2E+05	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
10.0%	1.5	7646.9	533.2	2E+05	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
11.6%	1.5	8890.4	533.2	2E+05	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
10.5%	1.5	8023.1	533.2	2E+05	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
7.2%	2.5	9003.9	785.6	2E+05	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
7.2%	2.5	9032.7	785.6	2E+05	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
7.3%	2.5	9173.7	785.6	2E+05	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
4.8%	5	12120.3	2378.2	2E+05	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
4.5%	5	11602.4	2378.2	2E+05	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
4.6%	5	11625.4	2378.2	2E+05	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
2.7%	10	13994.8	2779.7	2E+05	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
3.5%	10	17872.0	2779.7	2E+05	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
2.9%	10	14599.3	2779.7	2E+05	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

Saturation Data for Experiment #1 (continued)

Run	Position								Number of molecules					Ratio
		Total Counts (dpm)	Non Specific Binding (Mean of reps in pos. 25-48) (dpm)	Specific Binding (Total - Non Specific) (dpm)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72) (dpm)	Free (total added - bound) (dpm)	Total Binding molecules (fmole)	Non Specific Binding molecules (fmole)	Specific Binding molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (fmole)	Free (total added - bound) (fmole)	Specific Bound /Free
2E+05	1	2680.8	284.5	2396.3	10.6%	21.2%	12649.9	9969.1	16	2	14	74	59	0.24
2E+05	2	2865.7	284.5	2581.2	9.9%	22.7%	12649.9	9784.2	17	2	15	74	58	0.26
2E+05	3	2951.4	284.5	2666.9	9.6%	23.3%	12649.9	9698.5	17	2	16	74	57	0.27
2E+05	4	4868.4	340.6	4527.9	7.0%	18.9%	25762.6	20894.1	29	2	27	151	123	0.22
2E+05	5	4692.1	340.6	4351.5	7.3%	18.2%	25762.6	21070.5	28	2	26	151	124	0.21
2E+05	6	4825.3	340.6	4484.7	7.1%	18.7%	25762.6	20937.3	28	2	26	151	123	0.21
2E+05	7	6325.3	334.1	5991.2	5.3%	17.5%	36098.0	29772.7	37	2	35	212	175	0.20
2E+05	8	6136.1	334.1	5802.0	5.4%	17.0%	36098.0	29961.9	36	2	34	212	176	0.19
2E+05	9	6101.8	334.1	5767.7	5.5%	16.9%	36098.0	29961.9	36	2	34	212	176	0.19
2E+05	10	6826.5	377.6	6448.9	5.5%	13.5%	50561.0	43734.4	40	2	38	297	257	0.15
2E+05	11	6660.7	377.6	6283.1	5.7%	13.2%	50561.0	43900.2	39	2	37	297	258	0.14
2E+05	12	6479.6	377.6	6102.1	5.8%	12.8%	50561.0	44081.3	38	2	36	297	259	0.14
2E+05	13	7646.9	533.2	7113.7	7.0%	10.0%	76545.3	68898.4	45	3	42	450	405	0.10
2E+05	14	8890.4	533.2	8357.3	6.0%	11.6%	76545.3	67654.9	52	3	49	450	398	0.12
2E+05	15	8023.1	533.2	7489.9	6.6%	10.5%	76545.3	68522.2	47	3	44	450	403	0.11
2E+05	16	9003.9	785.6	8218.3	8.7%	7.2%	125688.6	116684.7	53	5	48	739	686	0.07
2E+05	17	9032.7	785.6	8247.1	8.7%	7.2%	125688.6	116655.9	53	5	48	739	686	0.07
2E+05	18	9173.7	785.6	8388.1	8.6%	7.3%	125688.6	116514.9	54	5	49	739	685	0.07
2E+05	19	12120.3	2378.2	9742.1	19.6%	4.8%	255016.2	242895.9	71	14	57	1499	1428	0.04
2E+05	20	11602.4	2378.2	9224.2	20.5%	4.5%	255016.2	243413.8	68	14	54	1499	1431	0.04
2E+05	21	11625.4	2378.2	9247.2	20.5%	4.6%	255016.2	243390.9	68	14	54	1499	1431	0.04
2E+05	22	13994.8	2779.7	11215.1	19.9%	2.7%	510905.2	496910.4	82	16	66	3003	2921	0.02
2E+05	23	17872.0	2779.7	15092.4	15.6%	3.5%	510905.2	493033.1	105	16	89	3003	2898	0.03
2E+05	24	14599.3	2779.7	11819.6	19.0%	2.9%	510905.2	496305.9	86	16	69	3003	2917	0.02

Saturation Data for Experiment #1 (continued)

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol													
Run	Position	Tube Identification			Assay tube contents								Scintillation Results		
		Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial		Cold R1881 Conc. Initial		Triamcelenone Acetate	Cytosol	Hot Conc. Final		Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)			(nM)	(nM)			
2E+05	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	262.6	284.5	
2E+05	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	294.2	284.5	
2E+05	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	296.7	284.5	
2E+05	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	340.7	340.6	
2E+05	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	395.7	340.6	
2E+05	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	285.3	340.6	
2E+05	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	388.6	334.1	
2E+05	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	307.3	334.1	
2E+05	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	306.4	334.1	
2E+05	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	369.7	377.6	
2E+05	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	391.1	377.6	
2E+05	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	371.9	377.6	
2E+05	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	609.7	533.2	
2E+05	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	485.4	533.2	
2E+05	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	504.4	533.2	
2E+05	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	613.0	785.6	
2E+05	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	833.8	785.6	
2E+05	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	910.0	785.6	
2E+05	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2609.0	2378.2	
2E+05	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	1359.8	2378.2	
2E+05	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	3165.8	2378.2	
2E+05	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	2688.2	2779.7	
2E+05	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	2341.4	2779.7	
2E+05	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	3309.5	2779.7	

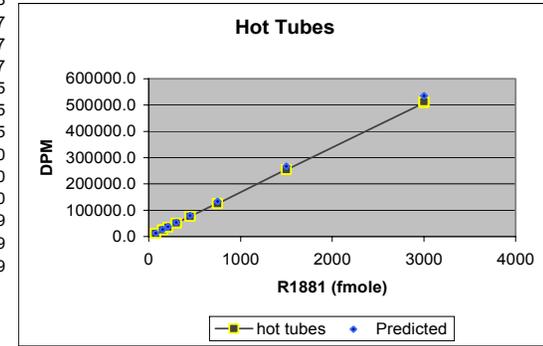
Saturation Data for Experiment #1 (continued)

		Free -- Positions 49-72, radiolabeled R1881 without cytosol								
Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Scintillation Vial (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (dpm)
2E+05	49	1	Hot	c1	10	7.5	75	12584.1	74	12649.9
2E+05	50	2	Hot	c1	10	7.5	75	12771.6	75	12649.9
2E+05	51	3	Hot	c1	10	7.5	75	12593.9	74	12649.9
2E+05	52	1	Hot	c2	10	15	150	25538.7	150	25762.6
2E+05	53	2	Hot	c2	10	15	150	26157.9	154	25762.6
2E+05	54	3	Hot	c2	10	15	150	25591.1	150	25762.6
2E+05	55	1	Hot	c3	10	21	210	36014.5	212	36098.0
2E+05	56	2	Hot	c3	10	21	210	36194.1	213	36098.0
2E+05	57	3	Hot	c3	10	21	210	36085.3	212	36098.0
2E+05	58	1	Hot	c4	10	30	300	50315.2	296	50561.0
2E+05	59	2	Hot	c4	10	30	300	50450.6	297	50561.0
2E+05	60	3	Hot	c4	10	30	300	50917.1	299	50561.0
2E+05	61	1	Hot	c5	10	45	450	76231.3	448	76545.3
2E+05	62	2	Hot	c5	10	45	450	76517.7	450	76545.3
2E+05	63	3	Hot	c5	10	45	450	76886.9	452	76545.3
2E+05	64	1	Hot	c6	100	7.5	750	126890.8	746	125688.6
2E+05	65	2	Hot	c6	100	7.5	750	125332.3	737	125688.6
2E+05	66	3	Hot	c6	100	7.5	750	124842.7	734	125688.6
2E+05	67	1	Hot	c7	100	15	1500	255392.3	1501	255016.2
2E+05	68	2	Hot	c7	100	15	1500	255715.3	1503	255016.2
2E+05	69	3	Hot	c7	100	15	1500	253941.1	1493	255016.2
2E+05	70	1	Hot	c8	100	30	3000	507814.8	2985	510905.2
2E+05	71	2	Hot	c8	100	30	3000	511772.5	3008	510905.2
2E+05	72	3	Hot	c8	100	30	3000	513128.2	3016	510905.2

predicted dpm

13364
13364
13364
26729
26729
26729
37421
37421
37421
53458
53458
53458
80187
80187
80187
133645
133645
133645
267290
267290
267290
534579
534579
534579

Computation Check	
2/2/05	specific activity date
80.27	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7819E+14	DPM/mmole
1.7819E+11	DPM/nmole
178.2	DPM/fmole
0.005612	fmole/DPM



Linear regression results (LINEST function)	
(Regression line forced through 0,0)	
Slope	170.1137712 dpm/fmole
1/slope	0.005878419 fmole/dpm
x	y
origin	0 0
end point	3016.4 513128.2
SLOPE function, used if missing HOT tubes	
Slope	170.3 dpm/fmole
1/slope	0.005873 fmole/dpm
x	y
origin	0 0
end point	3013.4 513128.2

Saturation Data for Experiment #1 (continued)

Prism input for bound/free		Prism input for specific bound							Hot Final
specific bound/molar	bound/free	average total added molar	specific bound/molar	specific bound/dpm	total added dpm	total bound dpm	NSB dpm	Concentration (nM)	
4.48265E-11	0.24038	2.36632E-10	4.48265E-11	2396.3	12584.1	2680.8	262.6	0.25	
4.82845E-11	0.26381	2.36632E-10	4.82845E-11	2581.2	12771.6	2865.7	294.2	0.25	
4.98884E-11	0.27499	2.36632E-10	4.98884E-11	2666.9	12593.9	2951.4	296.7	0.25	
8.46999E-11	0.21671	4.81923E-10	8.46999E-11	4527.9	25538.7	4868.4	340.7	0.50	
8.14007E-11	0.20652	4.81923E-10	8.14007E-11	4351.5	26157.9	4692.1	395.7	0.50	
8.38929E-11	0.21420	4.81923E-10	8.38929E-11	4484.7	25591.1	4825.3	285.3	0.50	
1.12073E-10	0.20123	6.7526E-10	1.12073E-10	5991.2	36014.5	6325.3	388.6	0.70	
1.08534E-10	0.19365	6.7526E-10	1.08534E-10	5802.0	36194.1	6136.1	307.3	0.70	
1.07893E-10	0.19228	6.7526E-10	1.07893E-10	5767.7	36085.3	6101.8	306.4	0.70	
1.20636E-10	0.14746	9.45809E-10	1.20636E-10	6448.9	50315.2	6826.5	369.7	1.00	
1.17534E-10	0.14312	9.45809E-10	1.17534E-10	6283.1	50450.6	6660.7	391.1	1.00	
1.14147E-10	0.13843	9.45809E-10	1.14147E-10	6102.1	50917.1	6479.6	371.9	1.00	
1.33071E-10	0.10325	1.43188E-09	1.33071E-10	7113.7	76231.3	7646.9	609.7	1.50	
1.56334E-10	0.12353	1.43188E-09	1.56334E-10	8357.3	76517.7	8890.4	485.4	1.50	
1.40109E-10	0.10931	1.43188E-09	1.40109E-10	7489.9	76886.9	8023.1	504.4	1.50	
1.53734E-10	0.07043	2.35117E-09	1.53734E-10	8218.3	126890.8	9003.9	613.0	2.50	
1.54272E-10	0.07070	2.35117E-09	1.54272E-10	8247.1	125332.3	9032.7	833.8	2.50	
1.56911E-10	0.07199	2.35117E-09	1.56911E-10	8388.1	124842.7	9173.7	910.0	2.50	
1.82239E-10	0.04011	4.77041E-09	1.82239E-10	9742.1	255392.3	12120.3	2609.0	5.00	
1.72551E-10	0.03790	4.77041E-09	1.72551E-10	9224.2	255715.3	11602.4	1359.8	5.00	
1.7298E-10	0.03799	4.77041E-09	1.7298E-10	9247.2	253941.1	11625.4	3165.8	5.00	
2.09793E-10	0.02257	9.55715E-09	2.09793E-10	11215.1	507814.8	13994.8	2688.2	10.00	
2.82322E-10	0.03061	9.55715E-09	2.82322E-10	15092.4	511772.5	17872.0	2341.4	10.00	
2.21101E-10	0.02382	9.55715E-09	2.21101E-10	11819.6	513128.2	14599.3	3309.5	10.00	

(total volume in tube uL/1000)	mole to molar conversion value	Free (total added - bound) dpm	Free (total added - bound) molar	specific bound/dpm
0.3	0.0003	9969.1	1.86484E-10	2396.3
		9784.2	1.83026E-10	2581.2
		9698.5	1.81422E-10	2666.9
		20894.1	3.90852E-10	4527.9
		21070.5	3.94151E-10	4351.5
		20937.3	3.91659E-10	4484.7
		29772.7	5.56937E-10	5991.2
		29961.9	5.60476E-10	5802.0
		29996.1	5.61117E-10	5767.7
		43734.4	8.1811E-10	6448.9
		43900.2	8.21211E-10	6283.1
		44081.3	8.24599E-10	6102.1
		68898.4	1.28884E-09	7113.7
		67654.9	1.26557E-09	8357.3
		68522.2	1.2818E-09	7489.9
		116684.7	2.18274E-09	8218.3
		116655.9	2.1822E-09	8247.1
		116514.9	2.17956E-09	8388.1
		242895.9	4.54369E-09	9742.1
		243413.8	4.55337E-09	9224.2
		243390.9	4.55294E-09	9247.2
		496910.4	9.29536E-09	11215.1
		493033.1	9.22283E-09	15092.4
		496305.9	9.28405E-09	11819.6

Saturation Data for Experiment #1 (continued)

mean specific bound/dpm	mean specific bound/molar	sp/free %	Prism input for bound/free				total added dpm	total added molar	mean total added dpm	Prism input for specific bound				NSB dpm	NSB molar
			specific bound/molar	bound/free %	total added dpm	total added molar				average total added molar	specific bound/molar	total bound dpm	total bound/molar		
2548.2	4.76665E-11	0.240376967	4.48265E-11	0.24	12584.1	2.35402E-10	12649.85	2.36632E-10	4.48265E-11	2680.8	5.01479E-11	262.6	4.91153E-12		
	0	0.263812334	4.82845E-11	0.26	12771.6	2.38909E-10	12771.6	2.36632E-10	4.82845E-11	2865.7	5.36059E-11	294.2	5.50246E-12		
	0	0.274985178	4.98884E-11	0.27	12593.9	2.35585E-10	12593.9	2.36632E-10	4.98884E-11	2951.4	5.52098E-11	296.7	5.55016E-12		
4454.7	8.33312E-11	0.216705771	8.46999E-11	0.22	25538.7	4.77735E-10	25762.57	4.81923E-10	8.46999E-11	4868.4	9.10704E-11	340.7	6.37287E-12		
	0	0.206521375	8.14007E-11	0.21	26157.9	4.89317E-10	26157.9	4.81923E-10	8.14007E-11	4692.1	8.77711E-11	395.7	7.40209E-12		
	0	0.214198822	8.38929E-11	0.21	25591.1	4.78715E-10	25591.1	4.81923E-10	8.38929E-11	4825.3	9.02634E-11	285.3	5.33635E-12		
5853.6	1.095E-10	0.201230299	1.12073E-10	0.20	36014.5	6.73697E-10	36097.96	6.7526E-10	1.12073E-10	6325.3	1.18322E-10	388.6	7.26908E-12		
	0	0.193645704	1.08534E-10	0.19	36194.1	6.77058E-10	36194.1	6.7526E-10	1.08534E-10	6136.1	1.14783E-10	307.3	5.7477E-12		
	0	0.192282386	1.07893E-10	0.19	36085.3	6.75023E-10	36085.3	6.7526E-10	1.07893E-10	6101.8	1.14143E-10	306.4	5.73199E-12		
6278.0	1.17439E-10	0.147456832	1.20636E-10	0.15	50315.2	9.41212E-10	50560.96	9.45809E-10	1.20636E-10	6826.5	1.27699E-10	369.7	6.91554E-12		
	0	0.143122919	1.17534E-10	0.14	50450.6	9.43744E-10	50450.6	9.45809E-10	1.17534E-10	6660.7	1.24597E-10	391.1	7.31679E-12		
	0	0.138427127	1.14147E-10	0.14	50917.1	9.52471E-10	50917.1	9.45809E-10	1.14147E-10	6479.6	1.2121E-10	371.9	6.95762E-12		
7653.6	1.43171E-10	0.103249247	1.33071E-10	0.10	76231.3	1.42601E-09	76545.30	1.43188E-09	1.33071E-10	7646.9	1.43045E-10	609.7	1.1405E-11		
	0	0.123528007	1.56334E-10	0.12	76517.7	1.43136E-09	76517.7	1.43188E-09	1.56334E-10	8890.4	1.66307E-10	485.4	9.0806E-12		
	0	0.109306781	1.40109E-10	0.11	76886.9	1.43827E-09	76886.9	1.43188E-09	1.40109E-10	8023.1	1.50083E-10	504.4	9.43528E-12		
8284.5	1.54972E-10	0.070431685	1.53734E-10	0.07	126890.8	2.37366E-09	125688.60	2.35117E-09	1.53734E-10	9003.9	1.6843E-10	613.0	1.14668E-11		
	0	0.070695677	1.54272E-10	0.07	125332.3	2.3444E-09	125332.3	2.35117E-09	1.54272E-10	9032.7	1.68968E-10	833.8	1.55977E-11		
	0	0.071991927	1.56911E-10	0.07	124842.7	2.33535E-09	124842.7	2.35117E-09	1.56911E-10	9173.7	1.71607E-10	910.0	1.70226E-11		
9404.5	1.75923E-10	0.04010821	1.82239E-10	0.04	255392.3	4.77745E-09	255016.23	4.77041E-09	1.82239E-10	12120.3	2.26727E-10	2609.0	4.88055E-11		
	0	0.037895136	1.72551E-10	0.04	255715.3	4.78349E-09	255715.3	4.77041E-09	1.72551E-10	11602.4	2.17038E-10	1359.8	2.54359E-11		
	0	0.037993002	1.7298E-10	0.04	253941.1	4.7503E-09	253941.1	4.77041E-09	1.7298E-10	11625.4	2.17468E-10	3165.8	5.92212E-11		
12709.0	2.37739E-10	0.022569642	2.09793E-10	0.02	507814.8	9.49934E-09	510905.17	9.55715E-09	2.09793E-10	13994.8	2.6179E-10	2688.2	5.02854E-11		
	0	0.030611228	2.82322E-10	0.03	511772.5	9.57337E-09	511772.5	9.55715E-09	2.82322E-10	17872.0	3.3432E-10	2341.4	4.3798E-11		
	0	0.023815193	2.21101E-10	0.02	513128.2	9.59873E-09	513128.2	9.55715E-09	2.21101E-10	14599.3	2.73099E-10	3309.5	6.19087E-11		

Saturation Data for Experiment #1 (continued)

Hot Final Concentration (nM)	Ds-3-2/02/05					
0.25	One site binding (hyperbola)		Bmax molar	2.23E-10	KD molar	8.32E-10
0.25	Best-fit values		mole to molar conversion value	0.0003	molar to nM conversion	1.00E+09
0.25	BMAX	2.23E-10	DPM/mole = (DPM/mmol)*1000	1.78E+17	kd nM =	8.32E-01
0.50	KD	8.32E-10	Bmax molar to Bmax moles	6.6822E-14		
0.50	Std. Error		= DPM/((DPM/mmol)*1000)	6.6822E-14		
0.50	BMAX	8.23E-12	=Bmax DPM	11907.21285		
0.70	KD	6.54E-11				
0.70	95% Confidence Intervals					
0.70	BMAX	2.0567e-010 to 2.3	assay date	2/2/2005		
1.00	KD	6.9616e-010 to 9.6	Bmax(dpm)	11907.21285		
1.00	Goodness of Fit		DPM/Ci (definition)	2.22E+12		
1.00	Degrees of Freedom	22	Ci/mmol	80.27		
1.50	R ² (unweighted)	0.8958	DPM/mmol	1.78E+14		
1.50	Weighted Sum of Square:	0.1654	DPM/pmol	1.78E+05		
1.50	Absolute Sum of Squares	7.84E-21	1/(DPM/mmol)	5.61E-15		
2.50	Sy.x	1.89E-11	1/(DPM/pmol)	5.61E-06		
2.50	Data		SA(dpm/pmol)	1.78E+05		
2.50	Number of X values	24	protein/tube (ug)	600		
5.00	Number of Y replicates	1	protein./tube(mg)	0.6		
5.00	Total number of values	24	bmax pmole	0.066822		
5.00	Number of missing values	0	bmax pmole/mg	0.11137		
10.00			Bmax fmole/mg	111.37		
10.00			Bmax (fmole/100 ug)	11.137		
10.00			Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Saturation Experiment #2

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

The value in column Q will change to FALSE

For your convenience, data reduction is performed in columns

U through BZ, and the values needed for analysis are presented

in columns CF through CN

Cells in column S 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: D
 Run identification: 282005
 Assay start date: 2/8/2005

Tracer lot number: 3538-497
 Specific activity on day of assay: 80.19 Ci/mmmole

Cytosol lot or vial number: 102704
 protein (cytosol) per tube: 600 ug
 protein (cytosol) per tube: 0.6 mg
 KD: 6.85E-01 nM
 Bmax: 8.59 fmole/100 ug
 total volume in tubes: 300 uL
 volume of ethanol counted: 2 mL
 multiply DPM in sample by: 3

Receptor_Notes
 diluted to 2 mg/ml for use (0.6 mg/300 ul)

protocol calls for counting decanted EtOH supe
 reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcetonone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-E supplied by Battelle to laboratory "E"
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—

dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
808.71	2426.13	TRUE	
816.26	2448.78	TRUE	
753.28	2259.84	TRUE	
1177.1	3531.3	TRUE	
1526.82	4580.46	TRUE	
1420.42	4261.26	TRUE	
1557.35	4672.05	TRUE	
1598.47	4795.41	TRUE	
1688.39	5065.17	TRUE	
1755.11	5265.33	TRUE	
1868.66	5605.98	TRUE	
1802.36	5407.08	TRUE	
2113.75	6341.25	TRUE	
2150.53	6451.59	TRUE	
2057.48	6172.44	TRUE	

Saturation Experiment #2 (continued)

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot RT881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold RT881 Volume (uL)	Cold Final Concentration (nM)	Triselenone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial supplied by Battelle	Full on Vials in set 1-1-E supplied by Battelle to laboratory "E"
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—

dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
2413.1	7239.3	TRUE	
2599.59	7798.77	TRUE	
2576.98	7730.94	TRUE	
2911.57	8734.71	TRUE	
3087.49	9262.47	TRUE	
3005.3	9015.9	TRUE	
3338.4	10015.2	TRUE	
3643.13	10929.39	TRUE	
4183.08	12549.24	TRUE	
65.83	197.49	TRUE	
60.67	182.01	TRUE	
74.5	223.5	TRUE	
62.82	188.46	TRUE	
75.77	227.31	TRUE	
79.81	239.43	TRUE	
84.53	253.59	TRUE	
88.8	266.4	TRUE	
92.41	277.23	TRUE	
115.32	345.96	TRUE	
116.05	348.15	TRUE	
101.62	304.86	TRUE	
134.78	404.34	TRUE	
131.5	394.5	TRUE	
148.23	444.69	TRUE	
281.93	845.79	TRUE	
318.2	954.6	TRUE	
149.91	449.73	TRUE	
282.04	846.12	TRUE	
377.74	1133.22	TRUE	
324.28	972.84	TRUE	
636.22	1908.66	TRUE	
648.51	1945.53	TRUE	
726.88	2180.64	TRUE	
9522.67	9522.67	TRUE	
12059.98	12059.98	TRUE	
12374.23	12374.23	TRUE	
25365.87	25365.87	TRUE	
24737.49	24737.49	TRUE	
24276.35	24276.35	TRUE	
34587.47	34587.47	TRUE	
34846.35	34846.35	TRUE	
34838.19	34838.19	TRUE	
47802.89	47802.89	TRUE	
48103.77	48103.77	TRUE	
48324.66	48324.66	TRUE	
70972.52	70972.52	TRUE	
71929.77	71929.77	TRUE	
73535.54	73535.54	TRUE	
116303.8	116303.8	TRUE	
117439.1	117439.1	TRUE	
120166.9	120166.9	TRUE	
252036.5	252036.5	TRUE	
252321.6	252321.6	TRUE	
244642	244642	TRUE	
497527	497527	TRUE	
506483.8	506483.8	TRUE	
503318	503318	TRUE	

Saturation Experiment #2 (continued)

				Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)													
				Tube Identification		Assay tube contents								Total Volume			
Ten Percent Rule	Saturation X values	Bound y values	NSB y values	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcetenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
									(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21.4%	0.25	2426.1	201.0	3E+05	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
21.6%	0.25	2448.8	201.0	3E+05	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
20.0%	0.25	2259.8	201.0	3E+05	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
14.2%	0.5	3531.3	218.4	3E+05	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
18.5%	0.5	4580.5	218.4	3E+05	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
17.2%	0.5	4261.3	218.4	3E+05	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
13.4%	0.7	4672.1	265.7	3E+05	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
13.8%	0.7	4795.4	265.7	3E+05	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
14.6%	0.7	5065.2	265.7	3E+05	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
11.0%	1	5265.3	333.0	3E+05	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
11.7%	1	5606.0	333.0	3E+05	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
11.2%	1	5407.1	333.0	3E+05	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
8.8%	1.5	6341.3	414.5	3E+05	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
8.9%	1.5	6451.6	414.5	3E+05	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
8.6%	1.5	6172.4	414.5	3E+05	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
6.1%	2.5	7239.3	750.0	3E+05	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
6.6%	2.5	7798.8	750.0	3E+05	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
6.6%	2.5	7730.9	750.0	3E+05	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
3.5%	5	8734.7	984.1	3E+05	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
3.7%	5	9262.5	984.1	3E+05	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
3.6%	5	9015.9	984.1	3E+05	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
2.0%	10	10015.2	2011.6	3E+05	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
2.2%	10	10929.4	2011.6	3E+05	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
2.5%	10	12549.2	2011.6	3E+05	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

Saturation Experiment #2 (continued)

Run	Position								Number of molecules					Ratio
		Total Counts (dpm)	Non Specific Binding (Mean of reps in pos. 25-48) (dpm)	Specific Binding (Total- Non Specific) (dpm)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72) (dpm)	Free (total added - bound) (dpm)	Total Binding molecules (fmole)	Non Specific Binding molecules (fmole)	Specific Binding molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (fmole)	Free (total added - bound) (fmole)	Specific Bound /Free
3E+05	1	2426.1	201.0	2225.1	8.3%	21.4%	11319.0	8892.8	15	1	13	68	53	0.25
3E+05	2	2448.8	201.0	2247.8	8.2%	21.6%	11319.0	8870.2	15	1	13	68	53	0.25
3E+05	3	2259.8	201.0	2058.8	8.9%	20.0%	11319.0	9059.1	14	1	12	68	54	0.23
3E+05	4	3531.3	218.4	3312.9	6.2%	14.2%	24793.2	21261.9	21	1	20	149	128	0.16
3E+05	5	4580.5	218.4	4362.1	4.8%	18.5%	24793.2	20212.8	27	1	26	149	121	0.22
3E+05	6	4261.3	218.4	4042.9	5.1%	17.2%	24793.2	20532.0	26	1	24	149	123	0.20
3E+05	7	4672.1	265.7	4406.3	5.7%	13.4%	34757.3	30085.3	28	2	26	209	181	0.15
3E+05	8	4795.4	265.7	4529.7	5.5%	13.8%	34757.3	29961.9	29	2	27	209	180	0.15
3E+05	9	5065.2	265.7	4799.4	5.2%	14.6%	34757.3	29692.2	30	2	29	209	178	0.16
3E+05	10	5265.3	333.0	4932.3	6.3%	11.0%	48077.1	42811.8	32	2	30	289	257	0.12
3E+05	11	5606.0	333.0	5273.0	5.9%	11.7%	48077.1	42471.1	34	2	32	289	255	0.12
3E+05	12	5407.1	333.0	5074.1	6.2%	11.2%	48077.1	42670.0	32	2	30	289	256	0.12
3E+05	13	6341.3	414.5	5926.7	6.5%	8.8%	72145.9	65804.7	38	2	36	433	395	0.09
3E+05	14	6451.6	414.5	6037.1	6.4%	8.9%	72145.9	65694.4	39	2	36	433	394	0.09
3E+05	15	6172.4	414.5	5757.9	6.7%	8.6%	72145.9	65973.5	37	2	35	433	396	0.09
3E+05	16	7239.3	750.0	6489.3	10.4%	6.1%	117969.9	110730.6	43	5	39	708	665	0.06
3E+05	17	7798.8	750.0	7048.7	9.6%	6.6%	117969.9	110171.2	47	5	42	708	661	0.06
3E+05	18	7730.9	750.0	6980.9	9.7%	6.6%	117969.9	110239.0	46	5	42	708	662	0.06
3E+05	19	8734.7	984.1	7750.7	11.3%	3.5%	249666.7	240932.0	52	6	47	1498	1446	0.03
3E+05	20	9262.5	984.1	8278.4	10.6%	3.7%	249666.7	240404.2	56	6	50	1498	1443	0.03
3E+05	21	9015.9	984.1	8031.8	10.9%	3.6%	249666.7	240650.8	54	6	48	1498	1444	0.03
3E+05	22	10015.2	2011.6	8003.6	20.1%	2.0%	502442.9	492427.7	60	12	48	3015	2955	0.02
3E+05	23	10929.4	2011.6	8917.8	18.4%	2.2%	502442.9	491513.5	66	12	54	3015	2950	0.02
3E+05	24	12549.2	2011.6	10537.6	16.0%	2.5%	502442.9	489893.7	75	12	63	3015	2940	0.02

Saturation Experiment #2 (continued)

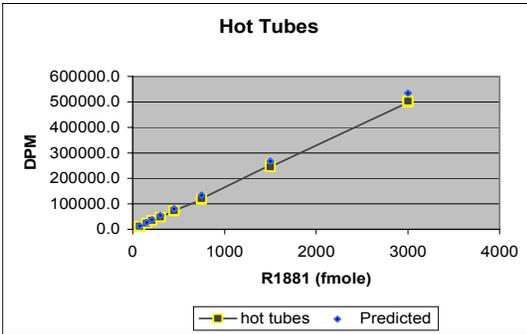
		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
Run	Position	Tube Identification			Assay tube contents								Scintillation Results	
		Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)		
3E+05	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	197.5	201.0
3E+05	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	182.0	201.0
3E+05	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	223.5	201.0
3E+05	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	188.5	218.4
3E+05	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	227.3	218.4
3E+05	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	239.4	218.4
3E+05	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	253.6	265.7
3E+05	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	266.4	265.7
3E+05	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	277.2	265.7
3E+05	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	346.0	333.0
3E+05	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	348.2	333.0
3E+05	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	304.9	333.0
3E+05	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	404.3	414.5
3E+05	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	394.5	414.5
3E+05	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	444.7	414.5
3E+05	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	845.8	750.0
3E+05	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	954.6	750.0
3E+05	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	449.7	750.0
3E+05	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	846.1	984.1
3E+05	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	1133.2	984.1
3E+05	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	972.8	984.1
3E+05	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	1908.7	2011.6
3E+05	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	1945.5	2011.6
3E+05	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2180.6	2011.6

Saturation Experiment #2 (continued)

Free -- Positions 49-72, radiolabeled R1881 without cytosol										
Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Scintillation Vial (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (dpm)
3E+05	49	1	Hot	c1	10	7.5	75	9522.7	57	11319.0
3E+05	50	2	Hot	c1	10	7.5	75	12060.0	72	11319.0
3E+05	51	3	Hot	c1	10	7.5	75	12374.2	74	11319.0
3E+05	52	1	Hot	c2	10	15	150	25365.9	152	24793.2
3E+05	53	2	Hot	c2	10	15	150	24737.5	148	24793.2
3E+05	54	3	Hot	c2	10	15	150	24276.4	146	24793.2
3E+05	55	1	Hot	c3	10	21	210	34587.5	208	34757.3
3E+05	56	2	Hot	c3	10	21	210	34846.4	209	34757.3
3E+05	57	3	Hot	c3	10	21	210	34838.2	209	34757.3
3E+05	58	1	Hot	c4	10	30	300	47802.9	287	48077.1
3E+05	59	2	Hot	c4	10	30	300	48103.8	289	48077.1
3E+05	60	3	Hot	c4	10	30	300	48324.7	290	48077.1
3E+05	61	1	Hot	c5	10	45	450	70972.5	426	72145.9
3E+05	62	2	Hot	c5	10	45	450	71929.8	432	72145.9
3E+05	63	3	Hot	c5	10	45	450	73535.5	441	72145.9
3E+05	64	1	Hot	c6	100	7.5	750	116303.8	698	117969.9
3E+05	65	2	Hot	c6	100	7.5	750	117439.1	705	117969.9
3E+05	66	3	Hot	c6	100	7.5	750	120166.9	721	117969.9
3E+05	67	1	Hot	c7	100	15	1500	252036.5	1513	249666.7
3E+05	68	2	Hot	c7	100	15	1500	252321.6	1514	249666.7
3E+05	69	3	Hot	c7	100	15	1500	244642.0	1468	249666.7
3E+05	70	1	Hot	c8	100	30	3000	497527.0	2986	502442.9
3E+05	71	2	Hot	c8	100	30	3000	506483.8	3040	502442.9
3E+05	72	3	Hot	c8	100	30	3000	503318.0	3021	502442.9

predicted dpm

13352	Computation Check	
13352		
13352	2/8/05	specific activity date
26704	80.19 Ci/mMole 3H R1881	
26704	2.22E+12 DPM/Ci (definition)	
26704		
37386	1.7803E+14 DPM/mmole	
37386	1.7803E+11 DPM/nmole	
37386	178.0 DPM/fmole	
53409	0.005617 fmole/DPM	
53409		



Linear regression results (LINEST function)	
<i>(Regression line forced through 0,0)</i>	
Slope	166.6276229 dpm/fmole
1/slope	0.006001406 fmole/dpm
x	y
origin	0 0
end point	3039.6 506483.8
SLOPE function, used if missing HOT tubes	
Slope	167.9 dpm/fmole
1/slope	0.005956 fmole/dpm
x	y
origin	0 0
end point	3016.6 506483.8

Saturation Experiment #2 (continued)

Prism input for bound/free		Prism input for specific bound							(total volume in tube uL/1000)	mole to molar conversion value	Free (total added - bound)/ dpm	Free (total added - bound)/ molar
specific bound/molar	bound/free	average total added molar	specific bound/molar	specific bound/dpm	total added dpm	total bound dpm	NSB dpm	Hot Final Concentration (nM)				
4.16624E-11	0.25022	2.11931E-10	4.16624E-11	2225.1	9522.7	2426.1	197.5	0.25	0.3	0.0003	8892.8	1.66505E-10
4.20865E-11	0.25341	2.11931E-10	4.20865E-11	2247.8	12060.0	2448.8	182.0	0.25			8870.2	1.66081E-10
3.85488E-11	0.22727	2.11931E-10	3.85488E-11	2058.8	12374.2	2259.8	223.5	0.25			9059.1	1.69619E-10
6.20293E-11	0.15581	4.64218E-10	6.20293E-11	3312.9	25365.9	3531.3	188.5	0.50			21261.9	3.98099E-10
8.16733E-11	0.21581	4.64218E-10	8.16733E-11	4362.1	24737.5	4580.5	227.3	0.50			20212.8	3.78455E-10
7.56967E-11	0.19691	4.64218E-10	7.56967E-11	4042.9	24276.4	4261.3	239.4	0.50			20532.0	3.84432E-10
8.25018E-11	0.14646	6.50781E-10	8.25018E-11	4406.3	34587.5	4672.1	253.6	0.70			30085.3	5.63304E-10
8.48116E-11	0.15118	6.50781E-10	8.48116E-11	4529.7	34846.4	4795.4	266.4	0.70			29961.9	5.60994E-10
8.98624E-11	0.16164	6.50781E-10	8.98624E-11	4799.4	34838.2	5065.2	277.2	0.70			29692.2	5.55943E-10
9.2351E-11	0.11521	9.00175E-10	9.2351E-11	4932.3	47802.9	5265.3	346.0	1.00			42811.8	8.01589E-10
9.87292E-11	0.12415	9.00175E-10	9.87292E-11	5273.0	48103.8	5606.0	348.2	1.00			42471.1	7.95211E-10
9.5005E-11	0.11891	9.00175E-10	9.5005E-11	5074.1	48324.7	5407.1	304.9	1.00			42670.0	7.98935E-10
1.1097E-10	0.09007	1.35083E-09	1.1097E-10	5926.7	70972.5	6341.3	404.3	1.50			65804.7	1.2321E-09
1.13036E-10	0.09190	1.35083E-09	1.13036E-10	6037.1	71929.8	6451.6	394.5	1.50			65694.4	1.23003E-09
1.07809E-10	0.08728	1.35083E-09	1.07809E-10	5757.9	73535.5	6172.4	444.7	1.50			65973.5	1.23526E-09
1.21502E-10	0.05860	2.20882E-09	1.21502E-10	6489.3	116303.8	7239.3	845.8	2.50			110730.6	2.07327E-09
1.31977E-10	0.06398	2.20882E-09	1.31977E-10	7048.7	117439.1	7798.8	954.6	2.50			110171.2	2.0628E-09
1.30707E-10	0.06333	2.20882E-09	1.30707E-10	6980.9	120166.9	7730.9	449.7	2.50			110239.0	2.06407E-09
1.4512E-10	0.03217	4.67465E-09	1.4512E-10	7750.7	252036.5	8734.7	846.1	5.00			240932.0	4.51111E-09
1.55001E-10	0.03444	4.67465E-09	1.55001E-10	8278.4	252321.6	9262.5	1133.2	5.00			240404.2	4.50122E-09
1.50385E-10	0.03338	4.67465E-09	1.50385E-10	8031.8	244642.0	9015.9	972.8	5.00			240650.8	4.50584E-09
1.49856E-10	0.01625	9.40752E-09	1.49856E-10	8003.6	497527.0	10015.2	1908.7	10.00			492427.7	9.22E-09
1.66973E-10	0.01814	9.40752E-09	1.66973E-10	8917.8	506483.8	10929.4	1945.5	10.00			491513.5	9.20289E-09
1.97302E-10	0.02151	9.40752E-09	1.97302E-10	10537.6	503318.0	12549.2	2180.6	10.00			489893.7	9.17256E-09

Saturation Experiment #2 (continued)

specific bound/dpm	mean specific bound/dpm	mean specific bound/molar	sp/free %	Prism input for bound/free		total added dpm	total added molar	mean total added dpm	Prism input for specific bound		total bound dpm	total bound/molar	NSB dpm
				specific bound/molar	bound/free %				average total added molar	specific bound/molar			
2225.1	2177.3	4.07659E-11	0.250216185	4.16624E-11	0.25	9522.7	1.78298E-10	11318.96	2.11931E-10	4.16624E-11	2426.1	4.54258E-11	197.5
2247.8		0	0.253408612	4.20865E-11	0.25	12060.0	2.25806E-10		2.11931E-10	4.20865E-11	2448.8	4.58499E-11	182.0
2058.8		0	0.227267108	3.85488E-11	0.23	12374.2	2.3169E-10		2.11931E-10	3.85488E-11	2259.8	4.23123E-11	223.5
3312.9	3905.9	7.31331E-11	0.155813652	6.20293E-11	0.16	25365.9	4.74939E-10	24793.24	4.64218E-10	6.20293E-11	3531.3	6.61185E-11	188.5
4362.1		0	0.215807065	8.16733E-11	0.22	24737.5	4.63174E-10		4.64218E-10	8.16733E-11	4580.5	8.57625E-11	227.3
4042.9		0	0.196905542	7.56967E-11	0.20	24276.4	4.5454E-10		4.64218E-10	7.56967E-11	4261.3	7.9786E-11	239.4
4406.3	4578.5	8.57253E-11	0.146460629	8.25018E-11	0.15	34587.5	6.47601E-10	34757.34	6.50781E-10	8.25018E-11	4672.1	8.74774E-11	253.6
4529.7		0	0.151180865	8.48116E-11	0.15	34846.4	6.52448E-10		6.50781E-10	8.48116E-11	4795.4	8.97872E-11	266.4
4799.4		0	0.161639602	8.98624E-11	0.16	34838.2	6.52295E-10		6.50781E-10	8.98624E-11	5065.2	9.4838E-11	277.2
4932.3	5093.1	9.53617E-11	0.115209888	9.2351E-11	0.12	47802.9	8.9504E-10	48077.11	9.00175E-10	9.2351E-11	5265.3	9.85857E-11	346.0
5273.0		0	0.1241547	9.87292E-11	0.12	48103.8	9.00674E-10		9.00175E-10	9.87292E-11	5606.0	1.04964E-10	348.2
5074.1		0	0.11891462	9.5005E-11	0.12	48324.7	9.0481E-10		9.00175E-10	9.5005E-11	5407.1	1.0124E-10	304.9
5926.7	5907.3	1.10605E-10	0.090065612	1.1097E-10	0.09	70972.5	1.32886E-09	72145.94	1.35083E-09	1.1097E-10	6341.3	1.18731E-10	404.3
6037.1		0	0.091896483	1.13036E-10	0.09	71929.8	1.34678E-09		1.35083E-09	1.13036E-10	6451.6	1.20797E-10	394.5
5757.9		0	0.087276402	1.07809E-10	0.09	73535.5	1.37685E-09		1.35083E-09	1.07809E-10	6172.4	1.1557E-10	444.7
6489.3	6839.6	1.28062E-10	0.058604018	1.21502E-10	0.06	116303.8	2.17762E-09	117969.93	2.20882E-09	1.21502E-10	7239.3	1.35545E-10	845.8
7048.7		0	0.063979809	1.31977E-10	0.06	117439.1	2.19888E-09		2.20882E-09	1.31977E-10	7798.8	1.46021E-10	954.6
6980.9		0	0.063325143	1.30707E-10	0.06	120166.9	2.24995E-09		2.20882E-09	1.30707E-10	7730.9	1.44751E-10	449.7
7750.7	8020.3	1.50169E-10	0.032169452	1.4512E-10	0.03	252036.5	4.71902E-09	249666.70	4.67465E-09	1.4512E-10	8734.7	1.63545E-10	846.1
8278.4		0	0.034435376	1.55001E-10	0.03	252321.6	4.72436E-09		4.67465E-09	1.55001E-10	9262.5	1.73426E-10	1133.2
8031.8		0	0.033375497	1.50385E-10	0.03	244642.0	4.58057E-09		4.67465E-09	1.50385E-10	9015.9	1.6881E-10	972.8
8003.6	9153.0	1.71377E-10	0.016253329	1.49856E-10	0.02	497527.0	9.31548E-09	502442.93	9.40752E-09	1.49856E-10	10015.2	1.8752E-10	1908.7
8917.8		0	0.018143508	1.66973E-10	0.02	506483.8	9.48318E-09		9.40752E-09	1.66973E-10	10929.4	2.04637E-10	1945.5
10537.6		0	0.021510034	1.97302E-10	0.02	503318.0	9.42391E-09		9.40752E-09	1.97302E-10	12549.2	2.34966E-10	2180.6

Saturation Experiment #2 (continued)

NSB molar	Hot Final Concentration (nM)		DS-4-2/08/05				
3.69772E-12	0.25	One site binding (hyperbola)		Bmax molar	1.72E-10	KD molar	6.85E-10
3.40788E-12	0.25	Best-fit values		mole to molar conversion value	0.0003	molar to nM conversion	1.00E+09
4.18472E-12	0.25	BMAX	1.72E-10	DPM/mole = (DPM/mmmole)*1000	1.78E+17	kd nM =	6.85E-01
3.52864E-12	0.50	KD	6.85E-10	Bmax molar to Bmax moles	5.1555E-14		
4.25605E-12	0.50	Std. Error		= DPM/((DPM/mmmole)*1000)	5.1555E-14		
4.48298E-12	0.50	BMAX	5.05E-12	=Bmax DPM	9178.274191		
4.74811E-12	0.70	KD	4.55E-11				
4.98796E-12	0.70	95% Confidence Intervals		assay date	2/8/2005		
5.19073E-12	0.70	BMAX	1.6138e-010 to 1.8	Bmax(dpm)	9178.274191		
6.4776E-12	1.00	KD	5.9110e-010 to 7.7	DPM/Ci (definition)	2.22E+12		
6.51861E-12	1.00	Goodness of Fit		Ci/mmmole	80.19		
5.70807E-12	1.00	Degrees of Freedom	22	DPM/mmmole	1.78E+14		
7.57069E-12	1.50	R ² (unweighted)	0.9478	DPM/pmmole	1.78E+05		
7.38645E-12	1.50	Weighted Sum of Squares	0.112	1/(DPM/mmmole)	5.62E-15		
8.32618E-12	1.50	Absolute Sum of Squares	2.05E-21	1/(DPM/pmmole)	5.62E-06		
1.58362E-11	2.50	Sy.x	9.65E-12	SA(dpm/pmmole)	1.78E+05		
1.78735E-11	2.50	Data		protein/tube (ug)	600		
8.42055E-12	2.50	Number of X values	24	protein./tube(mg)	0.6		
1.58424E-11	5.00	Number of Y replicates	1	bmax pmole	0.051555		
2.12179E-11	5.00	Total number of values	24	bmax pmole/mg	0.085925		
1.8215E-11	5.00	Number of missing values	0	Bmax fmole/mg	85.925		
3.57369E-11	10.00			Bmax (fmole/100 ug)	8.5925		
3.64273E-11	10.00			Bmax(fmole/100 ug)/Bmax molar	5.00E+10		
4.08294E-11	10.00						

Saturation Experiment #3

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

The value in column Q will change to FALSE

For your convenience, data reduction is performed in columns

U through BZ, and the values needed for analysis are presented in columns CF through CN

Cells in column S 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: E
 Run identification: 2252005
 Assay start date: 2/25/2005

Tracer lot number: 3538-497
 Specific activity on day of assay: 79.98 Ci/mmole

Cytosol lot or vial number: 102704
 protein (cytosol) per tube: 600 ug
 protein (cytosol) per tube: 0.6 mg
 KD: 7.24E-01 nM
 Bmax: 9.18 fmole/100 ug
 total volume in tubes: 300 uL
 volume of ethanol counted: 2 mL
 multiply DPM in sample by: 3

Receptor_Notes
 diluted to 2 mg/ml for use (0.6 mg/300 ul)

protocol calls for counting decanted ETOH supere
 reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcetenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-E supplied by Battelle to laboratory "E"
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—

dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
888.15	2664.45	TRUE	
866.65	2599.95	TRUE	
811.77	2435.31	TRUE	
1414.35	4243.05	TRUE	
1479.43	4438.29	TRUE	
1545.73	4637.19	TRUE	
1822.73	5468.19	TRUE	
1331.01	3993.03	TRUE	
1793.6	5380.8	TRUE	
2004.32	6012.96	TRUE	
1905.38	5716.14	TRUE	
1942.16	5826.48	TRUE	
2283.45	6850.35	TRUE	
2358.77	7076.31	TRUE	
2213.54	6640.62	TRUE	

Saturation Experiment #3 (continued)

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triscentenone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-E supplied by Battelle to laboratory "E"
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	E-1-C8
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	E-1-C7
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	E-1-C6
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	E-1-C5
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	E-1-C4
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	E-1-C3
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	E-1-C2
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	E-1-C1
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—

dpm as counted	corrected DPM for 2nL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
2830.6	8491.8	TRUE	
2302.15	6906.45	TRUE	
2736.86	8210.58	TRUE	
3432.88	10298.64	TRUE	
3245.64	9736.92	TRUE	
3214.11	9642.33	TRUE	
3938.48	11815.44	TRUE	
4070.98	12212.94	TRUE	
4215.13	12645.39	TRUE	
67.11	201.33	TRUE	
55.68	167.04	TRUE	
53.47	160.41	TRUE	
76.47	229.41	TRUE	
75.55	226.65	TRUE	
78.6	235.8	TRUE	
105.05	315.15	TRUE	
78.1	234.3	TRUE	
93.33	279.99	TRUE	
96.76	290.28	TRUE	
98.1	294.3	TRUE	
100.21	300.63	TRUE	
156.72	470.16	TRUE	
150.67	452.01	TRUE	
142.22	426.66	TRUE	
281.47	844.41	TRUE	
193.33	579.99	TRUE	
165.57	496.71	TRUE	
682.83	2048.49	TRUE	
332.67	998.01	TRUE	
457.87	1373.61	TRUE	
644.42	1933.26	TRUE	
956.96	2870.88	TRUE	
962.85	2888.55	TRUE	
12874.8	12874.8	TRUE	
12755.58	12755.58	TRUE	
12784.98	12784.98	TRUE	
24197.63	24197.63	TRUE	
23900.57	23900.57	TRUE	
24893.17	24893.17	TRUE	
34821.17	34821.17	TRUE	
35393.34	35393.34	TRUE	
35189.98	35189.98	TRUE	
49679.88	49679.88	TRUE	
48867.85	48867.85	TRUE	
50324.99	50324.99	TRUE	
74107.2	74107.2	TRUE	
73701.99	73701.99	TRUE	
76381.42	76381.42	TRUE	
126609.8	126609.8	TRUE	
124122	124122	TRUE	
126845	126845	TRUE	
251550.6	251550.6	TRUE	
252042.3	252042.3	TRUE	
255327.6	255327.6	TRUE	
500818.6	500818.6	TRUE	
506795.5	506795.5	TRUE	
500665.8	500665.8	TRUE	

Saturation Experiment #3 (continued)

				Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)													
Ten Percent Rule	Saturation X values	Bound y values	NSB y values	Run	Position	Tube Identification			Assay tube contents								
						Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcetonone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20.8%	0.25	2664.5	176.3	#####	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
20.3%	0.25	2600.0	176.3	#####	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
19.0%	0.25	2435.3	176.3	#####	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
17.4%	0.5	4243.1	230.6	#####	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
18.2%	0.5	4438.3	230.6	#####	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
19.1%	0.5	4637.2	230.6	#####	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
15.6%	0.7	5468.2	276.5	#####	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
11.4%	0.7	3993.0	276.5	#####	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
15.3%	0.7	5380.8	276.5	#####	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
12.1%	1	6013.0	295.1	#####	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
11.5%	1	5716.1	295.1	#####	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
11.7%	1	5826.5	295.1	#####	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
9.2%	1.5	6850.4	449.6	#####	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
9.5%	1.5	7076.3	449.6	#####	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
8.9%	1.5	6640.6	449.6	#####	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
6.7%	2.5	8491.8	640.4	#####	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
5.5%	2.5	6906.5	640.4	#####	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
6.5%	2.5	8210.6	640.4	#####	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
4.1%	5	10298.6	1473.4	#####	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
3.8%	5	9736.9	1473.4	#####	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
3.8%	5	9642.3	1473.4	#####	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
2.4%	10	11815.4	2564.2	#####	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
2.4%	10	12212.9	2564.2	#####	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
2.5%	10	12645.4	2564.2	#####	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

Saturation Experiment #3 (continued)

Run	Position								Number of molecules					Ratio
		Total Counts (dpm)	Non Specific Binding (Mean of reps in pos. 25-48) (dpm)	Specific Binding (Total - Non Specific) (dpm)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72) (dpm)	Free (total added - bound) (dpm)	Total Binding molecules (fmole)	Non Specific Binding molecules (fmole)	Specific Binding molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (fmole)	Free (total added - bound) (fmole)	Specific Bound /Free
2E+06	1	2664.5	176.3	2488.2	6.6%	20.8%	12805.1	10140.7	16	1	15	76	60	0.25
2E+06	2	2600.0	176.3	2423.7	6.8%	20.3%	12805.1	10205.2	15	1	14	76	61	0.24
2E+06	3	2435.3	176.3	2259.1	7.2%	19.0%	12805.1	10369.8	15	1	13	76	62	0.22
2E+06	4	4243.1	230.6	4012.4	5.4%	17.4%	24330.5	20087.4	25	1	24	145	120	0.20
2E+06	5	4438.3	230.6	4207.7	5.2%	18.2%	24330.5	19892.2	26	1	25	145	119	0.21
2E+06	6	4637.2	230.6	4406.6	5.0%	19.1%	24330.5	19693.3	28	1	26	145	117	0.22
2E+06	7	5468.2	276.5	5191.7	5.1%	15.6%	35134.8	29666.6	33	2	31	209	177	0.18
2E+06	8	3993.0	276.5	3716.6	6.9%	11.4%	35134.8	31141.8	24	2	22	209	186	0.12
2E+06	9	5380.8	276.5	5104.3	5.1%	15.3%	35134.8	29754.0	32	2	30	209	177	0.17
2E+06	10	6013.0	295.1	5717.9	4.9%	12.1%	49624.2	43611.3	36	2	34	296	260	0.13
2E+06	11	5716.1	295.1	5421.1	5.2%	11.5%	49624.2	43908.1	34	2	32	296	262	0.12
2E+06	12	5826.5	295.1	5531.4	5.1%	11.7%	49624.2	43797.8	35	2	33	296	261	0.13
2E+06	13	6850.4	449.6	6400.7	6.6%	9.2%	74730.2	67879.9	41	3	38	446	405	0.09
2E+06	14	7076.3	449.6	6626.7	6.4%	9.5%	74730.2	67653.9	42	3	40	446	403	0.10
2E+06	15	6640.6	449.6	6191.0	6.8%	8.9%	74730.2	68089.6	40	3	37	446	406	0.09
2E+06	16	8491.8	640.4	7851.4	7.5%	6.7%	125858.9	117367.1	51	4	47	750	700	0.07
2E+06	17	6906.5	640.4	6266.1	9.3%	5.5%	125858.9	118952.5	41	4	37	750	709	0.05
2E+06	18	8210.6	640.4	7570.2	7.8%	6.5%	125858.9	117648.4	49	4	45	750	701	0.06
2E+06	19	10298.6	1473.4	8825.3	14.3%	4.1%	252973.5	242674.9	61	9	53	1508	1447	0.04
2E+06	20	9736.9	1473.4	8263.6	15.1%	3.8%	252973.5	243236.6	58	9	49	1508	1450	0.03
2E+06	21	9642.3	1473.4	8169.0	15.3%	3.8%	252973.5	243331.2	57	9	49	1508	1451	0.03
2E+06	22	11815.4	2564.2	9251.2	21.7%	2.4%	502760.0	490944.5	70	15	55	2997	2927	0.02
2E+06	23	12212.9	2564.2	9648.7	21.0%	2.4%	502760.0	490547.0	73	15	58	2997	2924	0.02
2E+06	24	12645.4	2564.2	10081.2	20.3%	2.5%	502760.0	490114.6	75	15	60	2997	2922	0.02

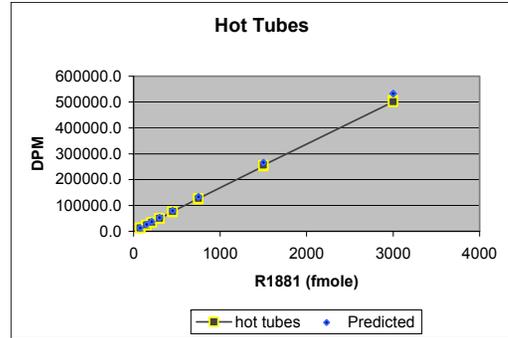
Saturation Experiment #3 (continued)

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol													
Run	Position	Tube Identification			Assay tube contents								Scintillation Results		
		Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial		Cold R1881 Conc. Initial		Triamcelenone Acetate	Cytosol	Hot Conc. Final		Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)			(nM)	(nM)			
2E+06	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	201.3	176.3	
2E+06	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	167.0	176.3	
2E+06	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	160.4	176.3	
2E+06	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	229.4	230.6	
2E+06	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	226.7	230.6	
2E+06	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	235.8	230.6	
2E+06	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	315.2	276.5	
2E+06	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	234.3	276.5	
2E+06	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	280.0	276.5	
2E+06	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	290.3	295.1	
2E+06	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	294.3	295.1	
2E+06	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	300.6	295.1	
2E+06	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	470.2	449.6	
2E+06	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	452.0	449.6	
2E+06	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	426.7	449.6	
2E+06	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	844.4	640.4	
2E+06	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	580.0	640.4	
2E+06	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	496.7	640.4	
2E+06	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2048.5	1473.4	
2E+06	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	998.0	1473.4	
2E+06	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	1373.6	1473.4	
2E+06	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	1933.3	2564.2	
2E+06	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	2870.9	2564.2	
2E+06	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2888.6	2564.2	

Saturation Experiment #3 (continued)

Free -- Positions 49-72, radiolabeled R1881 without cytosol											
Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm
					(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)	
2E+06	49	1	Hot	c1	10	7.5	75	12874.8	77	12805.1	13317
2E+06	50	2	Hot	c1	10	7.5	75	12755.6	76	12805.1	13317
2E+06	51	3	Hot	c1	10	7.5	75	12785.0	76	12805.1	13317
2E+06	52	1	Hot	c2	10	15	150	24197.6	144	24330.5	26635
2E+06	53	2	Hot	c2	10	15	150	23900.6	142	24330.5	26635
2E+06	54	3	Hot	c2	10	15	150	24893.2	148	24330.5	26635
2E+06	55	1	Hot	c3	10	21	210	34821.2	208	35134.8	37289
2E+06	56	2	Hot	c3	10	21	210	35393.3	211	35134.8	37289
2E+06	57	3	Hot	c3	10	21	210	35190.0	210	35134.8	37289
2E+06	58	1	Hot	c4	10	30	300	49679.9	296	49624.2	53269
2E+06	59	2	Hot	c4	10	30	300	48867.9	291	49624.2	53269
2E+06	60	3	Hot	c4	10	30	300	50325.0	300	49624.2	53269
2E+06	61	1	Hot	c5	10	45	450	74107.2	442	74730.2	79904
2E+06	62	2	Hot	c5	10	45	450	73702.0	439	74730.2	79904
2E+06	63	3	Hot	c5	10	45	450	76381.4	455	74730.2	79904
2E+06	64	1	Hot	c6	100	7.5	750	126609.8	755	125858.9	133173
2E+06	65	2	Hot	c6	100	7.5	750	124122.0	740	125858.9	133173
2E+06	66	3	Hot	c6	100	7.5	750	126845.0	756	125858.9	133173
2E+06	67	1	Hot	c7	100	15	1500	251550.6	1500	252973.5	266347
2E+06	68	2	Hot	c7	100	15	1500	252042.3	1503	252973.5	266347
2E+06	69	3	Hot	c7	100	15	1500	255327.6	1522	252973.5	266347
2E+06	70	1	Hot	c8	100	30	3000	500818.6	2986	502760.0	532693
2E+06	71	2	Hot	c8	100	30	3000	506795.5	3021	502760.0	532693
2E+06	72	3	Hot	c8	100	30	3000	500665.8	2985	502760.0	532693

Computation Check	
2/25/05	specific activity date
79.98	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7756E+14	DPM/mmole
1.7756E+11	DPM/nmole
177.6	DPM/fmole
0.005632	fmole/DPM



Linear regression results (LINEST function)	
<i>(Regression line forced through 0,0)</i>	
Slope	167.7425259 dpm/fmole
1/slope	0.005961517 fmole/dpm
x	y
origin	0
end point	3021.3 506795.5
SLOPE function, used if missing HOT tubes	
Slope	167.9 dpm/fmole
1/slope	0.005957 fmole/dpm
x	y
origin	0
end point	3018.8 506795.5

Saturation Experiment #3 (continued)

Prism input for bound/free		Prism input for specific bound							(total volume in tube uL/1000)	mole to molar conversion value	Free (total added - bound)/ dpm	Free (total added - bound)/ molar
specific bound/molar	bound/free	average total added molar	specific bound/molar	specific bound/dpm	total added dpm	total bound dpm	NSB dpm	Hot Final Concentration (nM)				
4.67096E-11	0.24537	2.40385E-10	4.67096E-11	2488.2	12874.8	2664.5	201.3	0.25	0.3	0.0003	10140.7	1.90366E-10
4.54988E-11	0.23750	2.40385E-10	4.54988E-11	2423.7	12755.6	2600.0	167.0	0.25			10205.2	1.91577E-10
4.24081E-11	0.21785	2.40385E-10	4.24081E-11	2259.1	12785.0	2435.3	160.4	0.25			10369.8	1.94688E-10
7.53235E-11	0.19975	4.56744E-10	7.53235E-11	4012.4	24197.6	4243.1	229.4	0.50			20087.4	3.77092E-10
7.89886E-11	0.21152	4.56744E-10	7.89886E-11	4207.7	23900.6	4438.3	226.7	0.50			19892.2	3.73426E-10
8.27225E-11	0.22376	4.56744E-10	8.27225E-11	4406.6	24893.2	4637.2	235.8	0.50			19693.3	3.69693E-10
9.74616E-11	0.17500	6.5957E-10	9.74616E-11	5191.7	34821.2	5468.2	315.2	0.70			29666.6	5.56918E-10
6.97691E-11	0.11934	6.5957E-10	6.97691E-11	3716.6	35393.3	3993.0	234.3	0.70			31141.8	5.84611E-10
9.5821E-11	0.17155	6.5957E-10	9.5821E-11	5104.3	35190.0	5380.8	280.0	0.70			29754.0	5.58559E-10
1.07339E-10	0.13111	9.31573E-10	1.07339E-10	5717.9	49679.9	6013.0	290.3	1.00			43611.3	8.18694E-10
1.01767E-10	0.12346	9.31573E-10	1.01767E-10	5421.1	48867.9	5716.1	294.3	1.00			43908.1	8.24267E-10
1.03839E-10	0.12629	9.31573E-10	1.03839E-10	5531.4	50325.0	5826.5	300.6	1.00			43797.8	8.22195E-10
1.20158E-10	0.09430	1.40288E-09	1.20158E-10	6400.7	74107.2	6850.4	470.2	1.50			67879.9	1.27428E-09
1.244E-10	0.09795	1.40288E-09	1.244E-10	6626.7	73702.0	7076.3	452.0	1.50			67653.9	1.27004E-09
1.16221E-10	0.09092	1.40288E-09	1.16221E-10	6191.0	76381.4	6640.6	426.7	1.50			68089.6	1.27821E-09
1.47391E-10	0.06690	2.36269E-09	1.47391E-10	7851.4	126609.8	8491.8	844.4	2.50			117367.1	2.20328E-09
1.1763E-10	0.05268	2.36269E-09	1.1763E-10	6266.1	124122.0	6906.5	580.0	2.50			118952.5	2.23304E-09
1.42112E-10	0.06435	2.36269E-09	1.42112E-10	7570.2	126845.0	8210.6	496.7	2.50			117648.4	2.20856E-09
1.65673E-10	0.03637	4.74895E-09	1.65673E-10	8825.3	251550.6	10298.6	2048.5	5.00			242674.9	4.55562E-09
1.55128E-10	0.03397	4.74895E-09	1.55128E-10	8263.6	252042.3	9736.9	998.0	5.00			243236.6	4.56617E-09
1.53352E-10	0.03357	4.74895E-09	1.53352E-10	8169.0	255327.6	9642.3	1373.6	5.00			243331.2	4.56794E-09
1.73669E-10	0.01884	9.43808E-09	1.73669E-10	9251.2	500818.6	11815.4	1933.3	10.00			490944.5	9.21628E-09
1.81131E-10	0.01967	9.43808E-09	1.81131E-10	9648.7	506795.5	12212.9	2870.9	10.00			490547.0	9.20881E-09
1.89249E-10	0.02057	9.43808E-09	1.89249E-10	10081.2	500665.8	12645.4	2888.6	10.00			490114.6	9.20069E-09

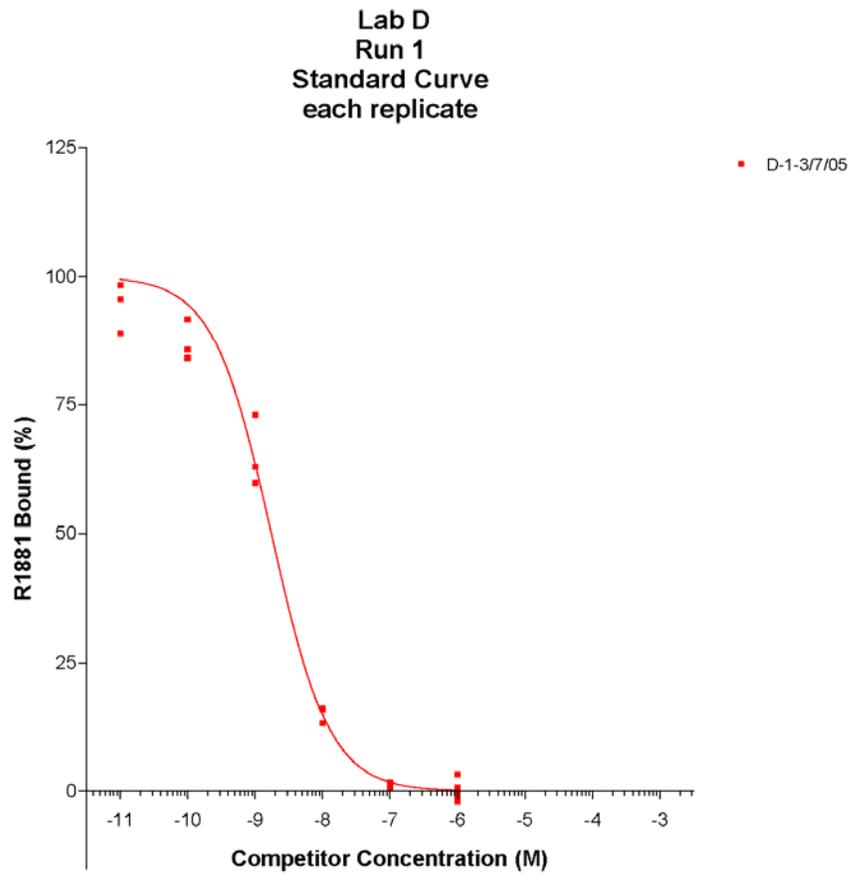
Saturation Experiment #3 (continued)

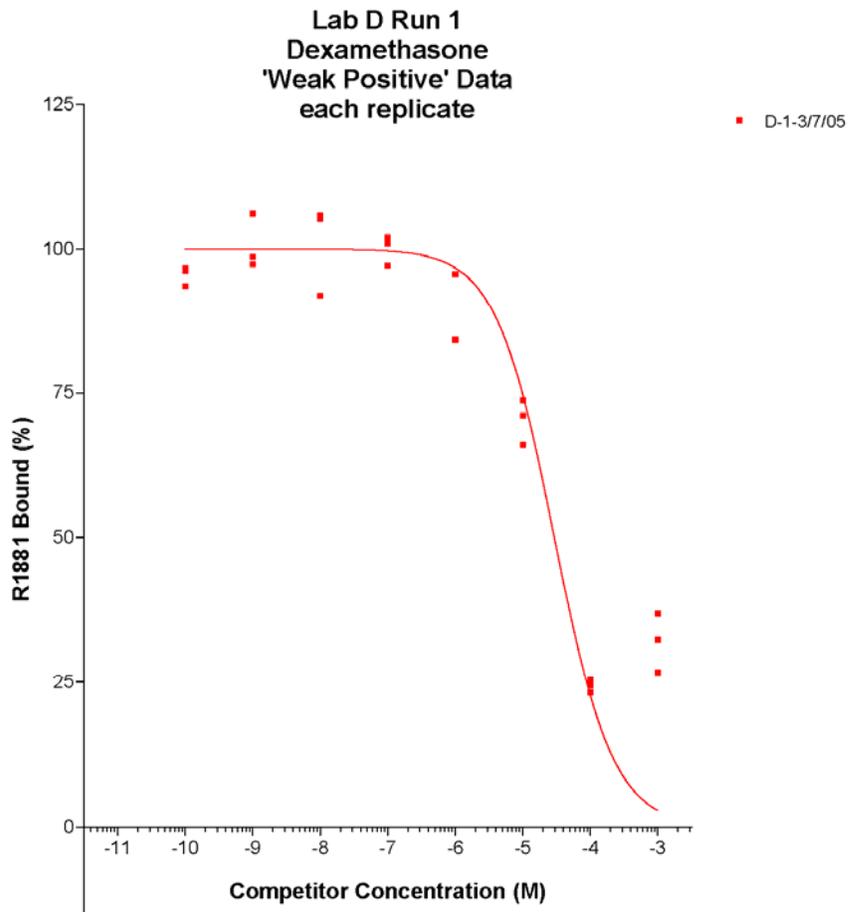
specific bound/dpm	mean specific bound/dpm	mean specific bound/molar	sp/free %	Prism input for bound/free		total added dpm	total added molar	mean total added dpm	Prism input for specific bound		total bound dpm	total bound/molar
				specific bound/molar	bound/free %				average total added molar	specific bound/molar		
2488.2	2390.3	4.48722E-11	0.245367417	4.67096E-11	0.25	12874.8	2.41693E-10	12805.12	2.40385E-10	4.67096E-11	2664.5	5.00185E-11
2423.7				4.54988E-11	0.24	12755.6	2.39455E-10	12785.0	2.40385E-10	4.54988E-11	2600.0	4.88077E-11
2259.1				4.24081E-11	0.22	12785.0	2.40007E-10	24330.46	4.56744E-10	7.53235E-11	4243.1	7.96528E-11
4012.4	4208.9	7.90115E-11	0.199748532	7.53235E-11	0.20	24197.6	4.54251E-10	24330.46	4.56744E-10	7.53235E-11	4438.3	8.3318E-11
4207.7				7.89886E-11	0.21	23900.6	4.48674E-10	24893.2	4.67308E-10	8.27225E-11	4637.2	8.70518E-11
4406.6				8.27225E-11	0.22	34821.2	6.53682E-10	35134.83	6.5957E-10	9.74616E-11	5468.2	1.02652E-10
5191.7	4670.9	8.76839E-11	0.175001618	9.74616E-11	0.18	34821.2	6.53682E-10	35134.83	6.5957E-10	9.74616E-11	3993.0	7.49593E-11
3716.6				6.97691E-11	0.12	35393.3	6.64423E-10	35190.0	6.60605E-10	9.5821E-11	5380.8	1.01011E-10
5104.3				9.5821E-11	0.17	49679.9	9.32617E-10	49624.24	9.31573E-10	1.07339E-10	6013.0	1.12879E-10
5717.9	5556.8	1.04315E-10	0.131110346	1.07339E-10	0.13	49679.9	9.32617E-10	49624.24	9.31573E-10	1.07339E-10	5716.1	1.07306E-10
5421.1				1.01767E-10	0.12	48867.9	9.17374E-10	50325.0	9.44728E-10	1.03839E-10	5826.5	1.09378E-10
5531.4				1.03839E-10	0.13	74107.2	1.39118E-09	74730.20	1.40288E-09	1.20158E-10	6850.4	1.28598E-10
6400.7	6406.2	1.2026E-10	0.094295136	1.20158E-10	0.09	74107.2	1.39118E-09	74730.20	1.40288E-09	1.20158E-10	7076.3	1.3284E-10
6626.7				1.244E-10	0.10	73702.0	1.38357E-09	76381.4	1.43387E-09	1.62221E-10	6640.6	1.24661E-10
6191.0				1.62221E-10	0.09	126609.8	2.37679E-09	125858.93	2.36269E-09	1.47391E-10	8491.8	1.59413E-10
7851.4	7229.2	1.35711E-10	0.066896326	1.47391E-10	0.07	126609.8	2.37679E-09	125858.93	2.36269E-09	1.47391E-10	6906.5	1.29652E-10
6266.1				1.1763E-10	0.05	124122.0	2.33009E-09	124122.0	2.33009E-09	1.1763E-10	6906.5	1.29652E-10
7570.2				1.42112E-10	0.06	126845.0	2.3812E-09	252973.50	4.74895E-09	1.65673E-10	10298.6	1.93332E-10
8825.3	8419.3	1.58051E-10	0.036366643	1.65673E-10	0.04	251550.6	4.72224E-09	252973.50	4.74895E-09	1.65673E-10	9736.9	1.82787E-10
8263.6				1.55128E-10	0.03	252042.3	4.73147E-09	255327.6	4.79315E-09	1.53352E-10	9642.3	1.81011E-10
8169.0				1.53352E-10	0.03	500818.6	9.40164E-09	502759.97	9.43808E-09	1.73669E-10	11815.4	2.21806E-10
9251.2	9660.4	1.81349E-10	0.018843697	1.73669E-10	0.02	500818.6	9.40164E-09	502759.97	9.43808E-09	1.73669E-10	12212.9	2.29268E-10
9648.7				1.81131E-10	0.02	506795.5	9.51384E-09	506795.5	9.51384E-09	1.81131E-10	12212.9	2.29268E-10
10081.2				1.89249E-10	0.02	500665.8	9.39877E-09	500665.8	9.39877E-09	1.89249E-10	12645.4	2.37386E-10

Saturation Experiment #3 (continued)

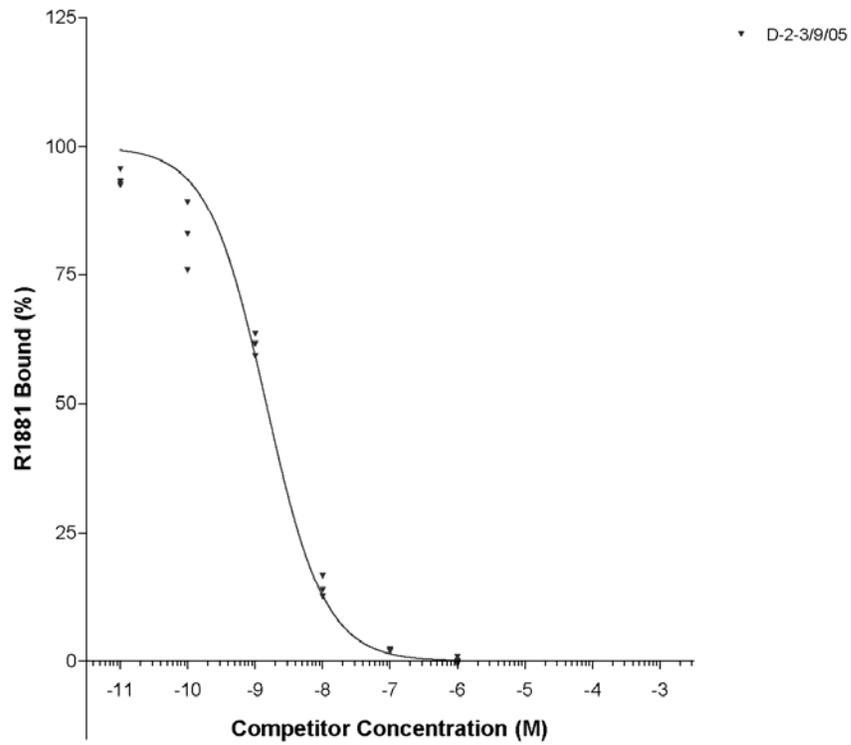
NSB dpm	NSB molar	Hot Final Concentration (nM)		Ds-5-2/25/05				
201.3	3.77948E-12	0.25	One site binding (hyperbola)		Bmax molar	1.84E-10	KD molar	7.24E-10
167.0	3.13576E-12	0.25	Best-fit values		mole to molar conversion value	0.0003	molar to nM conversion	1.00E+09
160.4	3.0113E-12	0.25	BMAX	1.84E-10	DPM/mole = (DPM/mmol)*1000	1.78E+17	kd nM =	7.24E-01
229.4	4.30661E-12	0.50	KD	7.24E-10	Bmax molar to Bmax moles	5.5074E-14		
226.7	4.2548E-12	0.50	Std. Error		= DPM/((DPM/mmol)*1000)	5.5074E-14		
235.8	4.42656E-12	0.50	BMAX	6.97E-12	=Bmax DPM	9779.178195		
315.2	5.91617E-12	0.70	KD	6.17E-11				
234.3	4.39841E-12	0.70	95% Confidence Intervals					
280.0	5.25612E-12	0.70	BMAX	1.6912e-010 to 1.9	assay date	2/25/2005		
290.3	5.44929E-12	1.00	KD	5.9591e-010 to 8.5	Bmax(dpm)	9779.178195		
294.3	5.52476E-12	1.00	Goodness of Fit		DPM/Ci (definition)	2.22E+12		
300.6	5.64359E-12	1.00	Degrees of Freedom	22	Ci/mmol	79.98		
470.2	8.8261E-12	1.50	R ² (unweighted)	0.9552	DPM/mmol	1.78E+14		
452.0	8.48538E-12	1.50	Weighted Sum of Squares	0.1845	DPM/pmol	1.78E+05		
426.7	8.00949E-12	1.50	Absolute Sum of Squares	1.91E-21	1/(DPM/mmol)	5.63E-15		
844.4	1.58517E-11	2.50	Sy.x	9.32E-12	1/(DPM/pmol)	5.63E-06		
580.0	1.08879E-11	2.50	Data		SA(dpm/pmol)	1.78E+05		
496.7	9.32451E-12	2.50	Number of X values	24	protein/tube (ug)	600		
2048.5	3.84554E-11	5.00	Number of Y replicates	1	protein./tube(mg)	0.6		
998.0	1.87352E-11	5.00	Total number of values	24	bmax pmole	0.055074		
1373.6	2.57861E-11	5.00	Number of missing values	0	bmax pmole/mg	0.09179		
1933.3	3.62922E-11	10.00			Bmax fmole/mg	91.79		
2870.9	5.38937E-11	10.00			Bmax (fmole/100 ug)	9.179		
2888.6	5.42254E-11	10.00			Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

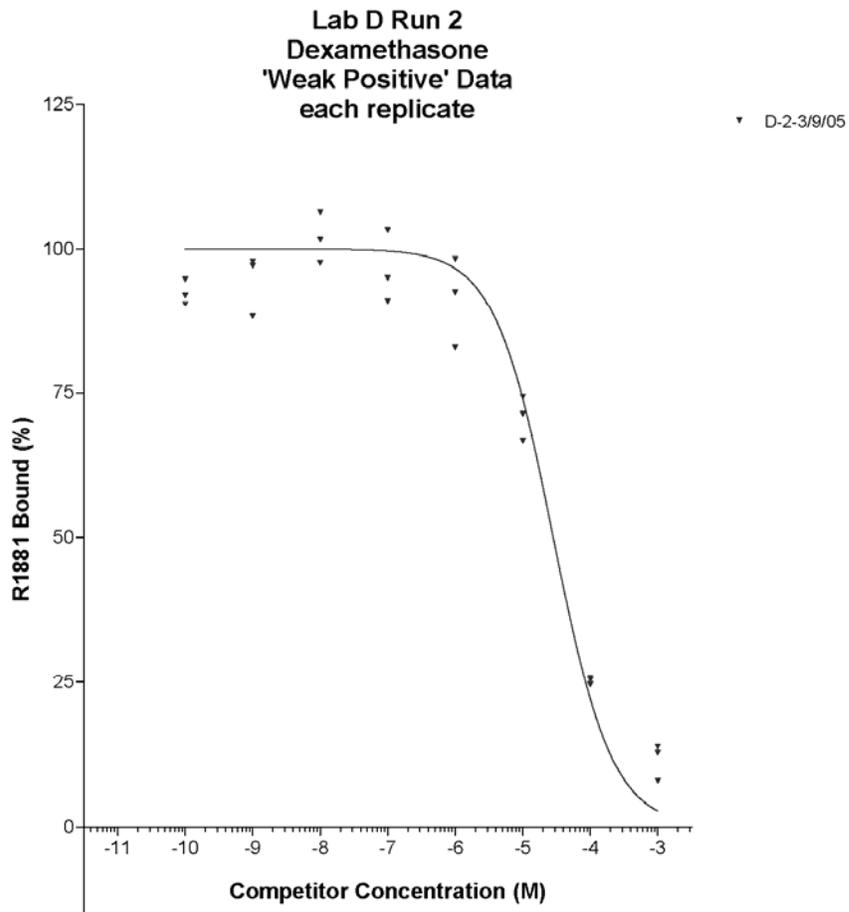
COMPETITIVE ASSAYS

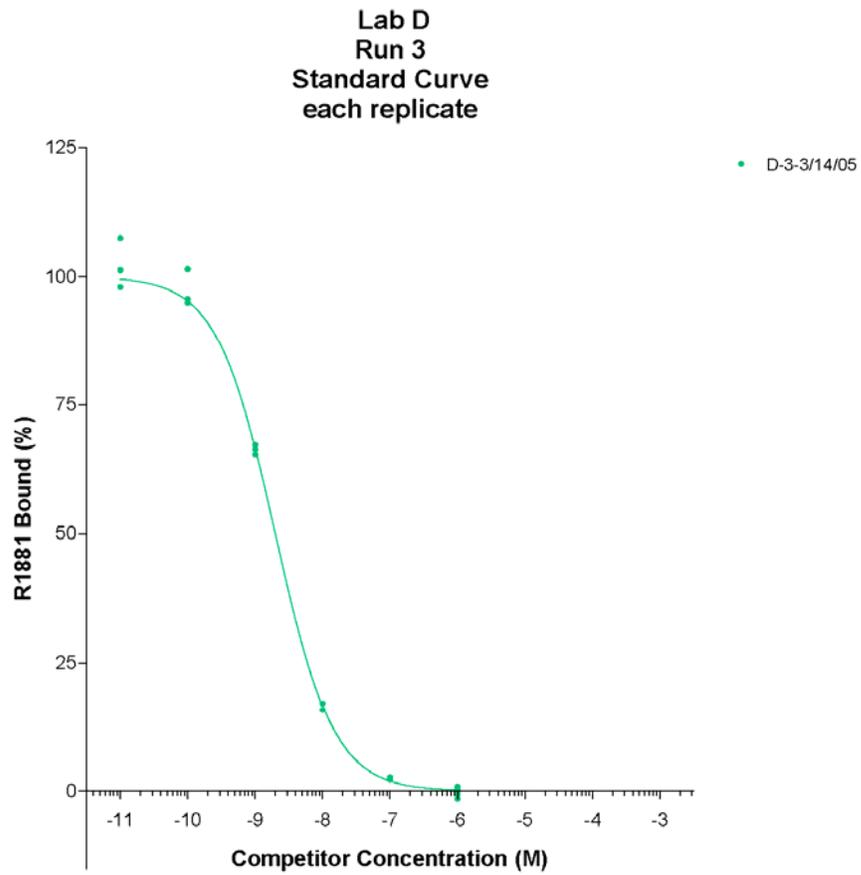


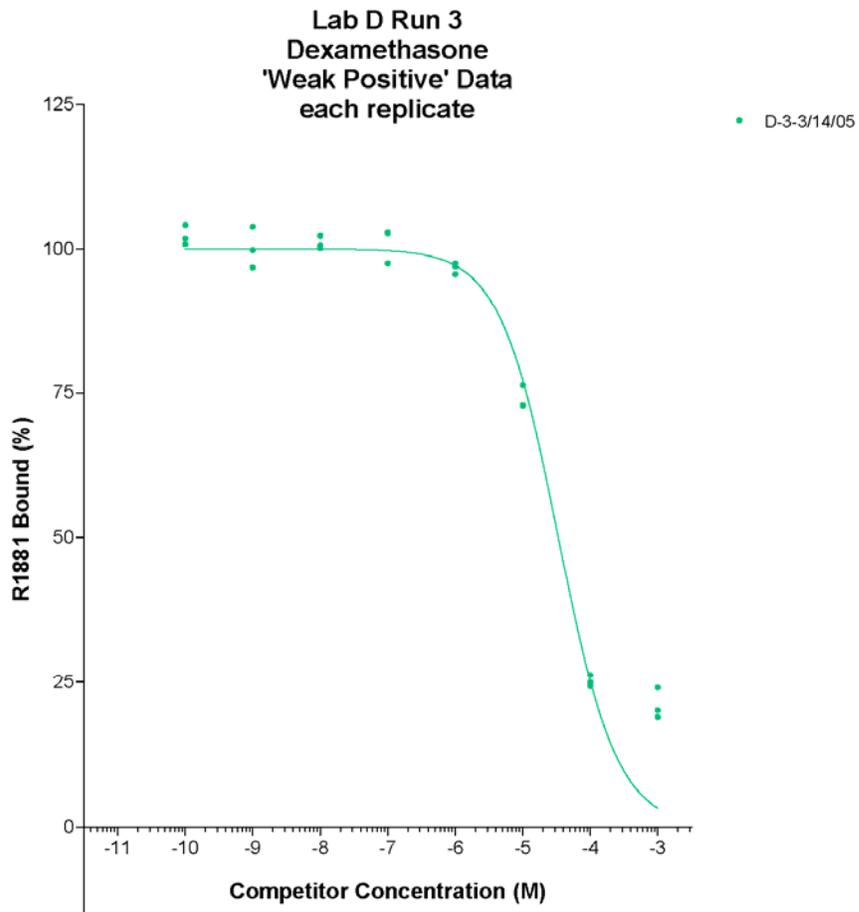


Lab D
Run 2
Standard Curve
each replicate

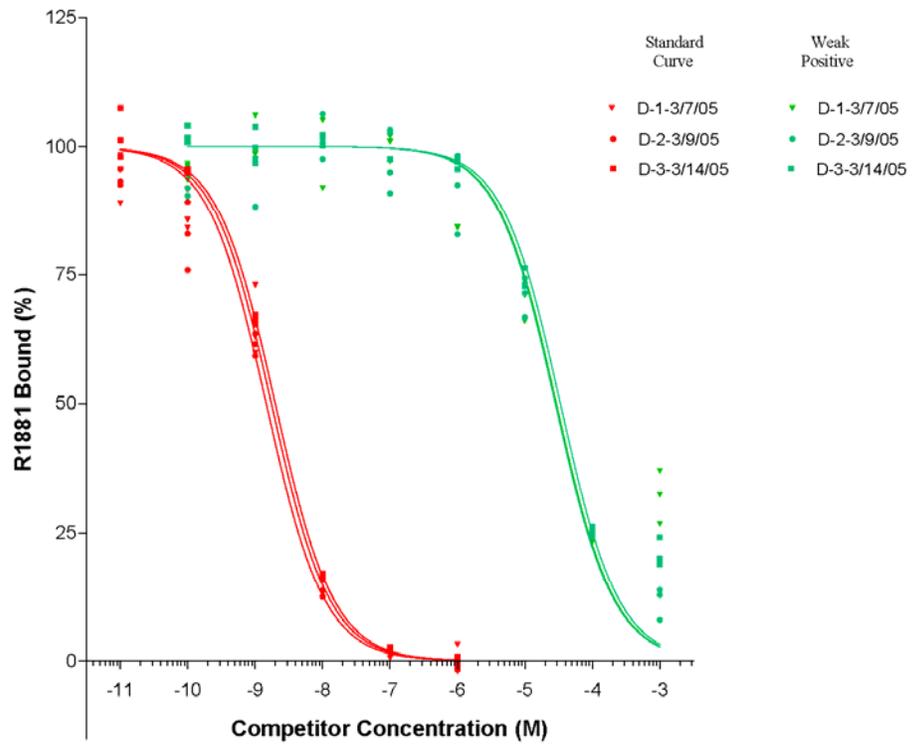








Lab D
Standard Curve and 'Weak Positive'
Protein
1.0 mg per tube



Competitive Experiment #1

Competitive Assay of a known Weak Positive 57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

include the DPM value in column O

Provide a reason in column R

the TRUE in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed by nonlinear regression software

Provide information in all blue cells in this column

Laboratory Code:	D
Run identification:	3072005
Assay start date:	3/7/2005
Tracer lot number:	3538-497
Specific activity on day of assay:	79.86 Ci/mmole
Cytosol vial or lot identification:	
Protein (cytosol):	1000 micro gram per tube
Standard Curve IC50:	1.74E-09
Weak Positive, Max Concentration:	3.00E-02 M
Weak Positive IC50:	2.94E-05
RBA:	5.93E-05

volume of ethanol counted: 2 mL
multiply DPM in sample by : 3

protocol calls for counting decanted EtOH so reflects 100ul of reaction mixture processed

Column O, Rows 10 through 13 will contain output parameters

working volume 3.1E+02 uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in set 1-1-E supplied by Battelle to laboratory "D"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triacelenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
4	1	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	100
5	2	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	100
6	3	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	100
7	1	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	100
8	2	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	100
9	3	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	100
10	1	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	100
11	2	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	100
12	3	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	100
13	1	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	100
14	2	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	100
15	3	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	100

Check the 10% rule: 19.28% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
3383.76	10489.656	TRUE	
3554.53	11019.043	TRUE	
3604.12	11172.772	TRUE	
148.31	444.93	TRUE	
174.27	522.81	TRUE	
192.44	577.32	TRUE	
181.68	563.208	TRUE	
212.89	659.959	TRUE	
201.99	626.169	TRUE	
644.33	1997.423	TRUE	
562.58	1743.998	TRUE	
657.64	2038.684	TRUE	
1990.15	6169.465	TRUE	
2087.53	6471.343	TRUE	
2393.42	7419.602	TRUE	

Competitive Experiment #1 (continued)

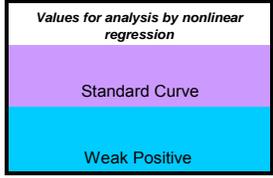
Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory by ID	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triancenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
16	1	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2782.07	8624.417	TRUE	
17	2	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2732.59	8471.029	TRUE	
18	3	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2963.86	9187.966	TRUE	
19	1	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3083.72	9559.532	TRUE	
20	2	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3170.32	9827.992	TRUE	
21	3	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2882.51	8935.781	TRUE	
22	1	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	980.36	3039.116	TRUE	
23	2	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	1293.68	4010.408	TRUE	
24	3	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	1152.04	3571.324	TRUE	
25	1	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	871.83	2702.673	TRUE	
26	2	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	940.99	2917.069	TRUE	
27	3	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	906.83	2811.173	TRUE	
28	1	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2416.06	7489.786	TRUE	
29	2	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2335.23	7239.213	TRUE	
30	3	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2182.04	6764.324	TRUE	
31	1	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2734.32	8476.392	TRUE	
32	2	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2737.31	8485.661	TRUE	
33	3	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3085.87	9566.197	TRUE	
34	1	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3279.18	10165.458	TRUE	
35	2	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3249.12	10072.272	TRUE	
36	3	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3131.81	9708.611	TRUE	
37	1	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	2972.55	9214.905	TRUE	
38	2	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3398.90	10536.59	TRUE	
39	3	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3375.34	10463.554	TRUE	
40	1	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3404.78	10554.818	TRUE	
41	2	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3176.27	9846.437	TRUE	
42	3	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3137.81	9727.211	TRUE	
43	1	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3101.69	9615.239	TRUE	
44	2	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3022.59	9370.029	TRUE	
45	3	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3118.38	9666.978	TRUE	
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3115.33	9657.523	TRUE	
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2692.09	8345.479	TRUE	
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2966.27	9195.437	TRUE	
49	1	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	102.61	307.83	TRUE	
50	2	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	114.51	343.53	TRUE	
51	3	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	271.36	814.08	TRUE	
52	1	none	Hot	—	—	—	—	30	—	—	—	—	—	52049.14	52049.14	TRUE	
53	2	none	Hot	—	—	—	—	30	—	—	—	—	—	51499.27	51499.27	TRUE	
54	3	none	Hot	—	—	—	—	30	—	—	—	—	—	52172.16	52172.16	TRUE	
55	1	none	Hot	—	—	—	—	30	—	—	—	—	—	51897.74	51897.74	TRUE	
56	2	none	Hot	—	—	—	—	30	—	—	—	—	—	51953.25	51953.25	TRUE	
57	3	none	Hot	—	—	—	—	30	—	—	—	—	—	51041.65	51041.65	TRUE	

Competitive Experiment #1 (continued)

	Summary values			
	n	Mean	SD	
EtOH	6	9980.0	1109.35	Total Binding, solvent control, tubes
Hot	6	51768.9	422.69	Total hot R1881 added to each tube
NSB	6	501.8	184.16	Nonspecific Binding
Specific EtOH	6	9478.2	1109.35	

Assay Characterization Values	
EtOH / Hot	0.19 less than 0.1?
NSB / EtOH	0.05 around 0.25 ?

upernate



	concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
		105.4	10489.66	9987.9	-1011.4	105.4	20.26248
		111.0	11019.04	10517.3	-1540.8	111.0	21.28508
		112.6	11172.77	10671.0	-1694.5	112.6	21.58203
	-6.0	-0.6	444.93	-56.8	9033.3	-0.6	0.859455
	-6.0	0.2	522.81	21.1	8955.4	0.2	1.009893
	-6.0	0.0	577.32	75.6	8900.9	0.8	1.115188
cold R1881	-7.0	0.6	563.208	61.5	8915.0	0.6	1.087928
cold R1881	-7.0	1.7	659.959	158.2	8818.3	1.7	1.274818
cold R1881	-7.0	1.3	626.169	124.4	8852.1	1.3	1.209547
cold R1881	-8.0	15.8	1997.423	1495.7	7480.8	15.8	3.858348
cold R1881	-8.0	13.1	1743.998	1242.2	7734.2	13.1	3.368816

Competitive Experiment #1 (continued)

	concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total addt - total bound)	Percent Binding (specific bound / mean specific ECH)	Ratio Total binding/ Hot
cold R1881	-8.0	16.2	2038.684	1536.9	7439.6	16.2	3.93805
cold R1881	-9.0	59.8	6169.465	5667.7	3308.8	59.8	11.91733
cold R1881	-9.0	63.0	6471.343	5969.6	3006.9	63.0	12.50045
cold R1881	-9.0	73.0	7419.602	6917.9	2058.6	73.0	14.33217
cold R1881	-10.0	85.7	8624.417	8122.7	853.8	85.7	16.65947
cold R1881	-10.0	84.1	8471.029	7969.3	1007.2	84.1	16.36317
cold R1881	-10.0	91.6	9187.966	8686.2	290.3	91.6	17.74805
cold R1881	-11.0	95.6	9559.532	9057.8	-81.3	95.6	18.46579
cold R1881	-11.0	98.4	9827.992	9326.2	-349.8	98.4	18.98437
cold R1881	-11.0	89.0	8935.781	8434.0	542.5	89.0	17.26092
Weak Positive	-3.0	26.8	3039.116	2537.4	6439.1	26.8	5.870547
Weak Positive	-3.0	37.0	4010.408	3508.7	5467.8	37.0	7.746756
Weak Positive	-3.0	32.4	3571.324	3069.6	5906.9	32.4	6.898594
Weak Positive	-4.0	23.2	2702.673	2200.9	6775.6	23.2	5.220653
Weak Positive	-4.0	25.5	2917.069	2415.3	6561.2	25.5	5.634794
Weak Positive	-4.0	24.4	2811.173	2309.4	6667.1	24.4	5.430238
Weak Positive	-5.0	73.7	7489.786	6988.0	1988.4	73.7	14.46774
Weak Positive	-5.0	71.1	7239.213	6737.5	2239.0	71.1	13.98372
Weak Positive	-5.0	66.1	6764.324	6262.6	2713.9	66.1	13.06639
Weak Positive	-6.0	84.1	8476.392	7974.6	1001.8	84.1	16.37353
Weak Positive	-6.0	84.2	8485.661	7983.9	992.6	84.2	16.39144
Weak Positive	-6.0	95.6	9566.197	9064.4	-88.0	95.6	18.47867
Weak Positive	-7.0	102.0	10165.46	9663.7	-687.2	102.0	19.63624
Weak Positive	-7.0	101.0	10072.27	9570.5	-594.0	101.0	19.45623
Weak Positive	-7.0	97.1	9708.611	9206.9	-230.4	97.1	18.75376
Weak Positive	-8.0	91.9	9214.905	8713.2	263.3	91.9	17.80009
Weak Positive	-8.0	105.9	10536.59	10034.8	-1058.4	105.9	20.35314
Weak Positive	-8.0	105.1	10463.55	9961.8	-985.3	105.1	20.21206
Weak Positive	-9.0	106.1	10554.82	10053.1	-1076.6	106.1	20.38835
Weak Positive	-9.0	98.6	9846.437	9344.7	-368.2	98.6	19.02
Weak Positive	-9.0	97.3	9727.211	9225.5	-249.0	97.3	18.78969
Weak Positive	-10.0	96.2	9615.239	9113.5	-137.0	96.2	18.5734
Weak Positive	-10.0	93.6	9370.029	8868.3	108.2	93.6	18.09974
Weak Positive	-10.0	96.7	9666.978	9165.2	-188.7	96.7	18.67334
—		96.6	9657.523	9155.8	-179.3	96.6	18.65508
—		82.8	8345.479	7843.7	1132.8	82.8	16.12065
—		91.7	9195.437	8693.7	282.8	91.7	17.76248
-6.0	-2.0	307.83		-193.9	9170.4	-2.0	0.594624
-6.0	-1.7	343.53		-158.2	9134.7	-1.7	0.663584
-6.0	3.3	814.08		312.3	8664.2	3.3	1.572528
		52049.14		51547.4			
		51499.27		50997.5			
		52172.16		51670.4			
		51897.74		51396.0			
		51953.25		51451.5			
		51041.65		50539.9			

Competitive Experiment #1 (continued)

Prism data							
standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC
	-6.0	-0.59948	0.22219		0.79730	-3.0	26.7704
-6.0	-2.04595	-1.66930	3.29523	-4.0	23.2208	25.4828	24.3655
-7.0	0.64841	1.66918	1.31268	-5.0	73.7272	71.0835	66.0732
-8.0	15.78008	13.10632	16.21540	-6.0	84.1364	84.2342	95.6343
-9.0	59.79716	62.98212	72.98671	-7.0	101.9568	100.9737	97.1369
-10.0	85.69810	84.07978	91.64381	-8.0	91.9280	105.8725	105.1019
-11.0	95.56402	98.39640	88.98314	-9.0	106.0648	98.5910	97.3331
				-10.0	96.1518	93.5647	96.6976

Standard Curve	D-1-3/7/05	Positive Control	D-1-3/7/05
One site competition		One site competition	
Best-fit values		Best-fit values	
BOTTOM	0	BOTTOM	0
TOP	100	TOP	100
LOGEC50	-8.759	LOGEC50	-4.531
EC50	1.74E-09	EC50	2.94E-05
Std. Error		Std. Error	
LOGEC50	0.04426	LOGEC50	0.1143
95% Confidence Intervals		95% Confidence Intervals	
LOGEC50	-8.851 to -8.666	LOGEC50	-4.768 to -4.295
EC50	1.4095e-009 to 2.1562	EC50	1.7063e-005 to 5.0715
Goodness of Fit		Goodness of Fit	
Degrees of Freedom	20	Degrees of Freedom	23
R ²	0.9865	R ²	0.8458
Absolute Sum of Squ:	452.3	Absolute Sum of Squ:	3275
Sy.x	4.755	Sy.x	11.93
Constraints		Constraints	
BOTTOM	BOTTOM = 0.0	BOTTOM	BOTTOM = 0.0
TOP	TOP = 100.0	TOP	TOP = 100.0
Data		Data	
Number of X values	7	Number of X values	8
Number of Y replics	3	Number of Y replics	3
Total number of val	21	Total number of val	24
Number of missing	0	Number of missing	0

Competitive Experiment #2

Competitive Assay of a known Weak Positive
57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable, include the DPM value in column O

Provide a reason in column R

the TRUE in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed by nonlinear regression software

Provide information in all blue cells in this column

Laboratory Code:	D
Run identification:	3092005
Assay start date:	3/9/2005
Tracer lot number:	3538-497
Specific activity on day of assay:	79.84 Ci/mmole
Cytosol vial or lot identification:	
Protein (cytosol):	1000 micro gram per tube
Standard Curve IC50:	1.46E-09
Weak Positive, Max Concentration:	3.00E-02 M
Weak Positive IC50:	2.84E-05
RBA:	5.15E-05

volume of ethanol counted: 2 mL
multiply DPM in sample by : 3

protocol calls for counting decanted EtOH si reflects 100ul of reaction mixture processed

Column O, Rows 10 through 13 will contain output parameters

working volume 3.1E+02 uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "D"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Irinotecanone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)
1	1	ethanol	ETOH	0			300	30	10	50	310		100
2	2	ethanol	ETOH	0			300	30	10	50	310		100
3	3	ethanol	ETOH	0			300	30	10	50	310		100
4	1	Inert R1881	NSB	D-1-S0		1.00E-05	300	30	30	50	300	1.0E-06	100
5	2	Inert R1881	NSB	D-1-S0		1.00E-05	300	30	30	50	300	1.0E-06	100
6	3	Inert R1881	NSB	D-1-S0		1.00E-05	300	30	30	50	300	1.0E-06	100
7	1	Inert R1881	S	D-1-S1		3.00E-06	300	30	10	50	310	9.7E-08	100
8	2	Inert R1881	S	D-1-S1		3.00E-06	300	30	10	50	310	9.7E-08	100
9	3	Inert R1881	S	D-1-S1		3.00E-06	300	30	10	50	310	9.7E-08	100
10	1	Inert R1881	S	D-1-S2		3.00E-07	300	30	10	50	310	9.7E-09	100
11	2	Inert R1881	S	D-1-S2		3.00E-07	300	30	10	50	310	9.7E-09	100
12	3	Inert R1881	S	D-1-S2		3.00E-07	300	30	10	50	310	9.7E-09	100
13	1	Inert R1881	S	D-1-S3		3.00E-08	300	30	10	50	310	9.7E-10	100
14	2	Inert R1881	S	D-1-S3		3.00E-08	300	30	10	50	310	9.7E-10	100
15	3	Inert R1881	S	D-1-S3		3.00E-08	300	30	10	50	310	9.7E-10	100

Check the 10% rule:	21.42%	If the ratio of ETOH / Hot is > 10% then there are problems with the assay
corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
3325.80	TRUE	
3064.89	TRUE	
3358.87	TRUE	
114.80	TRUE	
92.72	TRUE	
102.62	TRUE	
185.61	TRUE	
169.80	TRUE	
174.62	TRUE	
552.51	TRUE	
643.68	TRUE	
510.02	TRUE	
2150.15	TRUE	
2011.81	TRUE	
2085.78	TRUE	

Competitive Experiment #2 (continued)

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory ID	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triancelenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Alliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
16	1	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2770.93	8589.883	TRUE	
17	2	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2971.24	9210.844	TRUE	
18	3	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2544.74	7888.694	TRUE	
19	1	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3102.00	9616.2	TRUE	
20	2	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3179.63	9856.853	TRUE	
21	3	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3080.56	9549.736	TRUE	
22	1	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	554.41	1718.671	TRUE	
23	2	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	365.38	1132.678	TRUE	
24	3	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	521.06	1615.286	TRUE	
25	1	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	926.41	2871.871	TRUE	
26	2	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	898.79	2786.249	TRUE	
27	3	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	938.17	2908.327	TRUE	
28	1	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2251.03	6978.193	TRUE	
29	2	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2400.51	7441.581	TRUE	
30	3	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2496.39	7738.809	TRUE	
31	1	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3078.26	9542.606	TRUE	
32	2	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2768.85	8583.435	TRUE	
33	3	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3264.33	10119.423	TRUE	
34	1	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3159.14	9793.334	TRUE	
35	2	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3423.41	10612.571	TRUE	
36	3	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3028.32	9387.792	TRUE	
37	1	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3370.98	10450.038	TRUE	
38	2	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3522.78	10920.618	TRUE	
39	3	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3242.45	10051.595	TRUE	
40	1	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3249.05	10072.055	TRUE	
41	2	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3226.47	10002.057	TRUE	
42	3	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	2942.26	9121.006	TRUE	
43	1	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3060.88	9488.728	TRUE	
44	2	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3150.92	9767.852	TRUE	
45	3	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3012.10	9337.51	TRUE	
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3428.94	10629.714	TRUE	
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3236.26	10032.406	TRUE	
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3502.15	10856.665	TRUE	
49	1	Inert R1881	NSB		D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	117.27	351.81	TRUE	
50	2	Inert R1881	NSB		D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	108.36	325.08	TRUE	
51	3	Inert R1881	NSB		D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	141.28	423.84	TRUE	
52	1	none	Hot		—	—	—	30	—	—	—	—	—	48473.66	48473.66	TRUE	
53	2	none	Hot		—	—	—	30	—	—	—	—	—	48233.21	48233.21	TRUE	
54	3	none	Hot		—	—	—	30	—	—	—	—	—	48646.59	48646.59	TRUE	
55	1	none	Hot		—	—	—	30	—	—	—	—	—	48166.09	48166.09	TRUE	
56	2	none	Hot		—	—	—	30	—	—	—	—	—	46319.34	46319.34	TRUE	
57	3	none	Hot		—	—	—	30	—	—	—	—	—	48450.54	48450.54	TRUE	

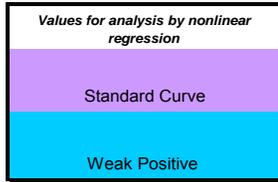
Competitive Experiment #2 (continued)

	Summary values		
	n	Mean	SD
EtOH	6	10290.4	477.61
Hot	6	48048.2	864.64
NSB	6	338.5	49.51
Specific EtOH	6	9951.9	477.61

Total Binding, solvent control, tubes
 Total hot R1881 added to each tube
 Nonspecific Binding

Assay Characterization Values	
EtOH / Hot	0.21 less than 0.1?
NSB / EtOH	0.03 around 0.25 ?

upernate



	concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
	100.2	10309.98	9971.5	-358.1	100.2	21.45756	
	92.1	9501.159	9162.6	450.7	92.1	19.77421	
	101.2	10412.5	10074.0	-460.6	101.2	21.67092	
	-6.0	0.1	344.4	5.9	9607.5	0.1	0.71678
	-6.0	-0.6	278.16	-60.4	9673.7	-0.6	0.578918
	-6.0	0.0	307.86	-30.7	9644.0	-0.3	0.640731
cold R1881	-7.0	2.4	575.391	236.9	9376.5	2.4	1.197528
cold R1881	-7.0	1.9	526.38	187.9	9425.5	1.9	1.095524
cold R1881	-7.0	2.0	541.322	202.8	9410.6	2.0	1.126622
cold R1881	-8.0	13.8	1712.781	1374.3	8239.1	13.8	3.564711
cold R1881	-8.0	16.6	1995.408	1656.9	7956.5	16.6	4.152926

Competitive Experiment #2 (continued)

concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific E(0))	Ratio Total binding/ Hor
-8.0	12.5	1581.062	1242.5	8370.8	12.5	3.290572
-9.0	63.6	6665.465	6326.9	3286.4	63.6	13.87244
-9.0	59.3	6236.611	5898.1	3715.3	59.3	12.9799
-9.0	61.6	6465.918	6127.4	3486.0	61.6	13.45714
-10.0	82.9	8589.883	8251.4	1362.0	82.9	17.87762
-10.0	89.2	9210.844	8872.3	741.0	89.2	19.16999
-10.0	75.9	7888.694	7550.2	2063.2	75.9	16.41828
-11.0	93.2	9616.2	9277.7	335.7	93.2	20.01364
-11.0	95.6	9856.853	9518.3	95.0	95.6	20.51449
-11.0	92.6	9549.736	9211.2	402.1	92.6	19.87531
-3.0	13.9	1718.671	1380.1	8233.2	13.9	3.57697
-3.0	8.0	1132.678	794.2	8819.2	8.0	2.357377
-3.0	12.8	1615.286	1276.8	8336.6	12.8	3.361801
-4.0	25.5	2871.871	2533.3	7080.0	25.5	5.977058
-4.0	24.6	2786.249	2447.7	7165.6	24.6	5.798858
-4.0	25.8	2908.327	2569.8	7043.6	25.8	6.052932
-5.0	66.7	6978.193	6639.7	2973.7	66.7	14.52331
-5.0	71.4	7441.581	7103.1	2510.3	71.4	15.48773
-5.0	74.4	7738.809	7400.3	2213.1	74.4	16.10633
-6.0	92.5	9542.606	9204.1	409.3	92.5	19.86047
-6.0	82.8	8583.435	8244.9	1368.4	82.8	17.8642
-6.0	98.3	10119.42	9780.9	-167.5	98.3	21.06097
-7.0	95.0	9793.334	9454.8	158.5	95.0	20.3823
-7.0	103.2	10612.57	10274.0	-660.7	103.2	22.08733
-7.0	90.9	9387.792	9049.3	564.1	90.9	19.53826
-8.0	101.6	10450.04	10111.5	-498.2	101.6	21.74906
-8.0	106.3	10920.62	10582.1	-968.7	106.3	22.72845
-8.0	97.6	10051.6	9713.1	-99.7	97.6	20.9198
-9.0	97.8	10072.06	9733.5	-120.2	97.8	20.96238
-9.0	97.1	10002.06	9663.5	-50.2	97.1	20.8167
-9.0	88.2	9121.006	8782.5	830.9	88.2	18.98302
-10.0	91.9	9488.728	9150.2	463.2	91.9	19.74834
-10.0	94.7	9767.852	9429.3	184.0	94.7	20.32926
-10.0	90.4	9337.51	8999.0	614.4	90.4	19.43362
---	103.4	10629.71	10291.2	-677.8	103.4	22.123
---	97.4	10032.41	9693.9	-80.5	97.4	20.87986
---	105.7	10856.67	10518.1	-904.8	105.7	22.59534
-6.0	0.1	351.81	13.3	9600.1	0.1	0.732202
-6.0	-0.1	325.08	-13.4	9626.8	-0.1	0.67657
-6.0	0.9	423.84	85.3	9528.0	0.9	0.882114
		48473.66	48135.1			
		48233.21	47894.7			
		48646.59	48308.1			
		48166.09	47827.6			
		46319.34	45980.8			
		48450.54	48112.0			

Competitive Experiment #2 (continued)

Prism data								
<i>standard curve</i>				<i>weak positive</i>				
<i>concentration (log)</i>	Y1-SC	Y2-SC	Y3-SC	<i>concentration (log)</i>	y1-PC	y2-PC	y3-PC	
	-6.0	0.05903	-0.60657		-0.30813	-3.0	13.8682	7.9799
-6.0	0.13349	-0.13510	0.85728	-4.0	25.4560	24.5956	25.8223	
-7.0	2.38011	1.88763	2.03778	-5.0	66.7177	71.3740	74.3607	
-8.0	13.80901	16.64895	12.48545	-6.0	92.4859	82.8478	98.2819	
-9.0	63.57533	59.26606	61.57022	-7.0	95.0053	103.2373	90.9302	
-10.0	82.91257	89.15220	75.86677	-8.0	101.6041	106.3326	97.6004	
-11.0	93.22536	95.64353	92.55751	-9.0	97.8060	97.1026	88.2495	
				-10.0	91.9445	94.7492	90.4250	

Standard Curve	D-2-3/9/05	Positive Control	D-2-3/9/05
One site competition		One site competition	
Best-fit values		Best-fit values	
BOTTOM	0	BOTTOM	0
TOP	100	TOP	100
LOGEC50	-8.836	LOGEC50	-4.547
EC50	1.46E-09	EC50	2.84E-05
Std. Error		Std. Error	
LOGEC50	0.04977	LOGEC50	0.06455
95% Confidence Intervals		95% Confidence Intervals	
LOGEC50	-8.939 to -8.732	LOGEC50	-4.681 to -4.414
EC50	1.1499e-009 to 1.8547	EC50	2.0845e-005 to 3.8558
Goodness of Fit		Goodness of Fit	
Degrees of Freedom	20	Degrees of Freedom	23
R ²	0.9812	R ²	0.96
Absolute Sum of Squ:	586.3	Absolute Sum of Sc	1046
Sy,x	5.414	Sy,x	6.743
Constraints		Constraints	
BOTTOM	BOTTOM = 0.0	BOTTOM	BOTTOM = 0.0
TOP	TOP = 100.0	TOP	TOP = 100.0
Data		Data	
Number of X values	7	Number of X values	8
Number of Y replic:	3	Number of Y replic:	3
Total number of val	21	Total number of val	24
Number of missing	0	Number of missing	0

Competitive Experiment #3

Competitive Assay of a known Weak Positive
57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

the TRUE in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed
by nonlinear regression software

Provide information in all blue
cells in this column

Laboratory Code:	D	
Run identification:	3142005	
Assay start date:	3/14/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.78	Ci/mmole
Cytosol vial or lot identification:		
Protein (cytosol):	1000	micro gram per tube
Standard Curve IC50:	1.99E-09	
Weak Positive, Max Concentration:	3.00E-02	M
Weak Positive IC50:	3.37E-05	
RBA:	5.91E-05	

volume of ethanol counted: 2 mL
multiply DPM in sample by : 3

protocol calls for counting decanted EtOH so
reflects 100ul of reaction mixture processed

Column O, Rows 10 through 13 will contain output parameters

working volume 3.1E+02 uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Barteite to laboratory D	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3377.94	10471.614	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3370.43	10448.333	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3398.19	10534.389	TRUE	
4	1	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	184.65	553.95	TRUE		
5	2	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	205.41	616.23	TRUE		
6	3	Inert R1881	NSB	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	161.16	483.48	TRUE		
7	1	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	239.84	743.504	TRUE		
8	2	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	254.07	787.617	TRUE		
9	3	Inert R1881	S	D-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	244.19	756.989	TRUE		
10	1	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	661.70	2051.27	TRUE		
11	2	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	659.69	2045.039	TRUE		
12	3	Inert R1881	S	D-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	698.62	2165.722	TRUE		
13	1	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2254.17	6987.927	TRUE		
14	2	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2195.13	6804.903	TRUE		
15	3	Inert R1881	S	D-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2227.29	6904.599	TRUE		

Check the 10% rule: 19.35% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Use this value? Notes to explain why "Use this value" is set to "FALSE"

Competitive Experiment #3 (continued)

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "D"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcetonone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)
16	1	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100
17	2	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100
18	3	Inert R1881	S	4	D-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100
19	1	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100
20	2	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100
21	3	Inert R1881	S	5	D-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100
22	1	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100
23	2	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100
24	3	Weak Positive	P	1	D-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100
25	1	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100
26	2	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100
27	3	Weak Positive	P	2	D-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100
28	1	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100
29	2	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100
30	3	Weak Positive	P	3	D-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100
31	1	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100
32	2	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100
33	3	Weak Positive	P	4	D-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100
34	1	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100
35	2	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100
36	3	Weak Positive	P	5	D-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100
37	1	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100
38	2	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100
39	3	Weak Positive	P	6	D-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100
40	1	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100
41	2	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100
42	3	Weak Positive	P	7	D-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100
43	1	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100
44	2	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100
45	3	Weak Positive	P	8	D-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100
49	1	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100
50	2	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100
51	3	Inert R1881	NSB	—	D-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100
52	1	none	Hot	—	—	—	—	30	—	—	—	—	—
53	2	none	Hot	—	—	—	—	30	—	—	—	—	—
54	3	none	Hot	—	—	—	—	30	—	—	—	—	—
55	1	none	Hot	—	—	—	—	30	—	—	—	—	—
56	2	none	Hot	—	—	—	—	30	—	—	—	—	—
57	3	none	Hot	—	—	—	—	30	—	—	—	—	—

DPM as sampled	corrected DPM for 2.0 mL	Check the 10% rule:	
		19.35%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay
		Use this value?	Notes to explain why "Use this value" is set to "FALSE"
3112.86	9649.866	TRUE	
3134.96	9718.376	TRUE	
3315.33	10277.523	TRUE	
3501.85	10855.735	TRUE	
3207.74	9943.994	TRUE	
3309.11	10258.241	TRUE	
758.72	2352.032	TRUE	
794.74	2463.694	TRUE	
917.66	2844.746	TRUE	
943.77	2925.687	TRUE	
924.56	2866.136	TRUE	
984.83	3052.973	TRUE	
2536.42	7862.902	TRUE	
2425.81	7520.011	TRUE	
2431.96	7539.076	TRUE	
3177.17	9849.227	TRUE	
3135.60	9720.36	TRUE	
3193.31	9899.261	TRUE	
3355.80	10402.98	TRUE	
3192.96	9898.176	TRUE	
3359.50	10414.45	TRUE	
3287.65	10191.715	TRUE	
3274.42	10150.702	TRUE	
3340.49	10355.519	TRUE	
3264.05	10118.555	TRUE	
3389.51	10507.481	TRUE	
3170.81	9829.511	TRUE	
3295.16	10214.996	TRUE	
3326.39	10311.809	TRUE	
3397.19	10531.289	TRUE	
3255.17	10091.027	TRUE	
3070.67	9519.077	TRUE	
3148.60	9760.66	TRUE	
129.28	387.84	TRUE	
183.95	551.85	TRUE	
195.16	585.48	TRUE	
52870.94	52870.94	TRUE	
51727.44	51727.44	TRUE	
52565.90	52565.90	TRUE	
52156.33	52156.33	TRUE	
52586.21	52586.21	TRUE	
52404.97	52404.97	TRUE	

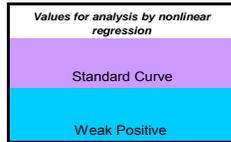
Competitive Experiment #3 (continued)

Summary values			
	n	Mean	SD
EtOH	6	10137.5	422.47
Hot	6	52385.3	398.60
NSB	6	529.8	82.39
Specific EtOH	6	9607.7	422.47

Total Binding, solvent control, tubes
Total hot R1881 added to each tube
Nonspecific Binding

Assay Characterization Values	
EtOH / Hot	0.19 less than 0.1?
NSB / EtOH	0.05 around 0.25 ?

upernate



	concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
		103.5	10471.61	9941.8	-863.9	103.5	19.9896
		103.2	10448.33	9918.5	-840.6	103.2	19.94516
		104.1	10534.39	10004.6	-926.7	104.1	20.10944
	-6.0	0.3	553.95	24.1	9053.8	0.3	1.057453
	-6.0	0.9	616.23	86.4	8991.5	0.9	1.176341
	-6.0	0.0	483.48	-46.3	9124.2	-0.5	0.922931
cold R1881	-7.0	2.2	743.504	213.7	8864.2	2.2	1.419299
cold R1881	-7.0	2.7	787.617	257.8	8820.1	2.7	1.503508
cold R1881	-7.0	2.4	756.989	227.2	8850.7	2.4	1.445041
cold R1881	-8.0	15.8	2051.27	1521.5	7556.4	15.8	3.915736
cold R1881	-8.0	15.8	2045.039	1515.2	7562.7	15.8	3.903841
cold R1881	-8.0	17.0	2165.722	1635.9	7442.0	17.0	4.134217
cold R1881	-9.0	67.2	6987.927	6458.1	2619.8	67.2	13.33948
cold R1881	-9.0	65.3	6804.903	6275.1	2802.8	65.3	12.9901
cold R1881	-9.0	66.4	6904.599	6374.8	2703.1	66.4	13.18041
cold R1881	-10.0	94.9	9649.866	9120.1	-42.2	94.9	18.42094
cold R1881	-10.0	95.6	9718.376	9188.6	-110.7	95.6	18.55172
cold R1881	-10.0	101.5	10277.52	9747.7	-669.8	101.5	19.6191
cold R1881	-11.0	107.5	10855.74	10325.9	-1248.0	107.5	20.72287
cold R1881	-11.0	98.0	9943.994	9414.2	-336.3	98.0	18.98241
cold R1881	-11.0	101.3	10258.24	9728.4	-650.5	101.3	19.58229

Competitive Experiment #3 (continued)

	concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSE)	Free DPM (mean total add - total bound)	Percent Binding (specific bound/ mean specific ETOH)	Ratio Total binding/ Hot
Weak Positive	-3.0	19.0	2352.032	1822.2	7255.7	19.0	4.48987
Weak Positive	-3.0	20.1	2463.694	1933.9	7144.0	20.1	4.703026
Weak Positive	-3.0	24.1	2844.746	2314.9	6763.0	24.1	5.430428
Weak Positive	-4.0	24.9	2925.687	2395.9	6682.0	24.9	5.584939
Weak Positive	-4.0	24.3	2866.136	2336.3	6741.6	24.3	5.47126
Weak Positive	-4.0	26.3	3052.973	2523.2	6554.7	26.3	5.827919
Weak Positive	-5.0	76.3	7862.902	7333.1	1744.8	76.3	15.00975
Weak Positive	-5.0	72.8	7520.011	6990.2	2087.7	72.8	14.35519
Weak Positive	-5.0	73.0	7539.076	7009.3	2068.6	73.0	14.39159
Weak Positive	-6.0	97.0	9849.227	9319.4	-241.5	97.0	18.80151
Weak Positive	-6.0	95.7	9720.36	9190.6	-112.6	95.7	18.55551
Weak Positive	-6.0	97.5	9899.261	9369.5	-291.5	97.5	18.89702
Weak Positive	-7.0	102.8	10402.98	9873.2	-795.3	102.8	19.85859
Weak Positive	-7.0	97.5	9898.176	9368.4	-290.5	97.5	18.89495
Weak Positive	-7.0	102.9	10414.45	9884.6	-806.7	102.9	19.88048
Weak Positive	-8.0	100.6	10191.72	9661.9	-584.0	100.6	19.4553
Weak Positive	-8.0	100.1	10150.7	9620.9	-543.0	100.1	19.37701
Weak Positive	-8.0	102.3	10355.52	9825.7	-747.8	102.3	19.76799
Weak Positive	-9.0	99.8	10118.56	9588.8	-510.8	99.8	19.31564
Weak Positive	-9.0	103.9	10507.48	9977.7	-899.8	103.9	20.05807
Weak Positive	-9.0	96.8	9829.511	9299.7	-221.8	96.8	18.76387
Weak Positive	-10.0	100.8	10215	9685.2	-607.3	100.8	19.49974
Weak Positive	-10.0	101.8	10311.81	9782.0	-704.1	101.8	19.68455
Weak Positive	-10.0	104.1	10531.29	10001.5	-923.6	104.1	20.10352
—		99.5	10091.03	9561.2	-483.3	99.5	19.26309
—		93.6	9519.077	8989.3	88.6	93.6	18.17128
—		96.1	9760.66	9230.9	-152.9	96.1	18.63244
-6.0	-1.5	387.84	-142.0	9219.9	-1.5	0.74036	
-6.0	0.2	551.85	22.0	9055.9	0.2	1.053444	
-6.0	0.6	585.48	55.7	9022.2	0.6	1.117642	
			52870.94	52341.1			
			51727.44	51197.6			
			52565.9	52036.1			
			52156.33	51626.5			
			52586.21	52056.4			
			52404.97	51875.2			

Competitive Experiment #3 (continued)

Prism data							
<i>standard curve</i>			<i>weak positive</i>				
<i>concentration (log)</i>	Y1-SC	Y2-SC	Y3-SC	<i>concentration (log)</i>	y1-PC	y2-PC	y3-PC
	Goodness of Fit				Goodness of Fit		
Degrees of Freedom	20		Degrees of Freedom	23			
R ²	0.997		R ²	0.9579			
Absolute Sum of Squares	116.4		Absolute Sum of Squares	1082			
Sy.x	2.412		Sy.x	6.86			
Constraints			Constraints				
BOTTOM	BOTTOM = 0.0		BOTTOM	BOTTOM = 0.0			
TOP	TOP = 100.0		TOP	TOP = 100.0			
Data			Data				
Number of X values	7		Number of X values	8			
Number of Y replicates	3		Number of Y replicates	3			
Total number of values	21		Total number of values	24			
Number of missing	0		Number of missing	0			

Miscellaneous Information

EPA Androgen Receptor (AR) Cytosol Planner

EPA AR Cytosol Planner

Planner Overview: The calculations on this page are presented as a guideline only and are based on experimental assumptions made in our laboratory (e.g.; 1 - cytosol protein yield/rat will be at least 20 mg, 2 - the cytosol is aliquoted such that there is minimal waste, minimal needed to repeat assays and the cytosol is stored properly, 3 - cytosol required for Saturation assay is 0.6 mg/tube yielding linear Scatchard plots and acceptable Kd and Bmax values, 4 - cytosol required for competitive assay is 1.2 mg per tube). Note: At present we are using 1.0 mg of cytosol protein to acquire acceptable data with this set of competitive assays.

NOTE: Changing any **Bold Shaded** number will change the calculated amount of rats needed. Saturation default is for 1 run @ 0.6 mg cytosol/tube. Competitive default is 1 run with a weak positive and no unknowns @ 1.2 mg cytosol/tube.

ANIMALS NEEDED	Saturation	Competitive	
Run Breakdown	Enter Number of runs (w/extras) below	Enter Number of runs (w/extras) below	
	Unknowns/run	0	
	Total Runs	3	
	Cytosol tubes/run	51	
	Total tubes	153	
	Cytosol/tube (mg)	1.2	COMBINED TOTALS
	Total Cytosol (mg)	184	299 ml
	mg Cytosol/rat	20	
	actual # of rats needed	9.2	
	Total rats/task (+20%)	12	19 rats

Cytosol Tube Breakdown

Saturation (triplicate)	number of tubes
Hots	24
Hot/Cold	24
Competitive (triplicate)	number of tubes
Zero	6
NSB	6
STDs (5)	15
WP (8 dilutions)	24
Unknown (8 dilutions)	24

order 19 rats
SD males 90-95 DO

Data Basic Information

Introduction	This workbook contains a series of Androgen Receptor binding assay data collection templates. Each template is presented as a worksheet. The worksheet names and contents are defined below. The initial rows (1 through 17 or so, depending on the number of unknowns in a competitive assay) define the assay run. The remaining rows of the template define the volumes and concentrations of the ingredients of each tube in the assay.
Recommended reading	See the extensive collection of relevant material at: http://www.graphpad.com/articles/library.cfm
Defining an assay run	Assign a unique identifier to each assay run. A run consists of a definition of contents of each assay tube and the associated DPM values obtained from the scintillation counter. Keep track of the start date of the assay run, the identification of the person performing the assay, the lot number of the cytosol and radiotracer and the purpose (saturation, competitive, Ki, etc.) of the run. There are cells provided for assay run identification in the upper rows of column O.
Adding DPM values from each tube	Most scintillation counters will produce an Excel spreadsheet as one form of electronic data. Therefore, the templates are designed so that all DPM values may be entered directly into column O by cut and paste from the electronic output of the scintillation counter. Care must be taken to insure that the DPM values from the scintillation counter are aligned correctly with the assay tube definitions in the template.
Annotating results judged to be unusable	Occasionally the laboratory procedure review or data review indicates that one or more DPM values should be discarded for cause. In this case, add an explanation in column R. The cell in column Q will automatically change to FALSE. DPM values marked "FALSE" will be ignored in the data reduction portion of the template.
10% rule	These templates facilitate checking for reasonable assay behavior. The "10% rule" (total binding DPM should not exceed 10% of total DPMs added) is indicated in column S on saturation templates and a cell in column Q on competitive templates. Values that exceed 10% are color coded. It may be acceptable for some saturation values to exceed 10% at the higher concentrations. However, there may be something wrong (usually too much protein) with a competitive assay that exceeds the 10% rule. See the Saturation Checklist and Competitive Checklist spreadsheets for additional guidance.
Competitive assay characterization values	Summary values for the competitive assay are shown in cells S4 through V13. These should be reviewed before submission of the data.
Extracting data for curve fitting	These spreadsheets provide for definition of the run, input of the DPM values and perform data reduction to the point where values are ready to be analyzed by nonlinear regression. For saturation assays, these values are presented in CF through CI, with additional variables you might wish to analysis in columns CJ through CN. For competitive assays, these values are in color coded cells in columns T and U. It is generally safest to cut and paste these values into the nonlinear curve fitting software (if it supports a spreadsheet format).
Adding summary values	The nonlinear curve fitting software will estimate parameters of the model (IC50, Kd, Bmax, etc). Use cut and paste to copy these values to the indicated cells in the upper rows of column O for competitive runs, and to the appropriate cells in column DK for saturation runs (cells other than the bmax and Kd are optional, they are not included in any analysis). For saturation runs, the conversion from molar values is done in the spreadsheet, and the converted numbers are placed automatically in column O.
Submitting results to Battelle	Fill in the blue cells in column O and submit an electronic copy of the worksheet to Battelle.

Worksheet name	Contents
Battelle Supplied Reagents Saturation	Tabulation of concentrations and codes for diluted cold R1881 and Weak Positive vials to be sent to each participating laboratory
Competitive_SC	Saturation assay tube layout for initial study using dilutions of cold R1881 supplied by Battelle.
	Competitive assay tube layout for initial study using dilutions of cold R1881 and Weak Positive supplied by Battelle.

Data Basic Information (continued)

Checklist for competitive binding

from "Fitting Models to Biological Data using Linear and Nonlinear Regression", Harvey Motulsky & Arthur Christopoulos, GraphPad Software, 2003, page 213.

Checklist for competitive binding results

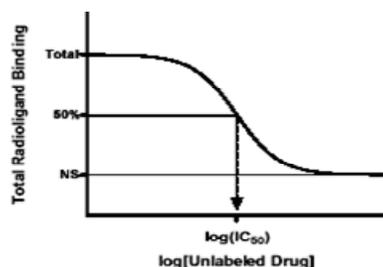
When evaluating results of competitive binding, ask yourself these questions:

Question	Comment
Is the $\log IC_{50}$ reasonable?	The IC_{50} should be near the middle of the curve, with at least several concentrations of unlabeled competitor on either side of it.
Are the standard errors too large? Are the confidence intervals too wide.	The SE of the $\log IC_{50}$ should be less than 0.5 log unit (ideally a lot less).
Are the values of <i>Top</i> and <i>Bottom</i> reasonable?	<i>Top</i> should be near the binding you observed in the absence of competitor. <i>Bottom</i> should be near the binding you observed in the presence of a maximal concentration of competitor. If the best-fit value of <i>Bottom</i> is negative, consider fixing it to a constant value equal to nonspecific binding determined in a control tube.
Has binding reached equilibrium?	Competitive binding incubations take longer to equilibrate than saturation binding incubations. You should incubate for 4-5 times the half-life for radioligand dissociation.
Does only a small fraction of the radioligand bind?	The equations are based on the assumption that the free concentration of labeled ligand is essentially identical to the concentration you added. Compare the total binding in the absence of competitor in cpm, to the amount of ligand added in cpm. If the ratio is greater than 10% at any concentration, then you've violated this assumption. Try to revise your experimental protocol, perhaps using a large incubation volume.
Does the curve have the expected steepness?	The competitive binding curve has a Hill slope (or slope factor) of -1. If your data form a curve shallower than this, see "Shallow competitive binding curves" on page 215.

Typically, the *Top* is constrained to equal 100% and the *Bottom* is constrained to 0%. See the indicated cell in column Q where this ratio is tabulated on the competitive assay worksheets.

What is a competitive binding curve?

Competitive binding experiments measure the binding of a single concentration of labeled ligand in the presence of various concentrations of unlabeled ligand. Ideally, the concentration of unlabeled ligand varies over at least six orders of magnitude.



The top of the curve is a plateau at a value equal to radioligand binding in the absence of the competing unlabeled drug. The bottom of the curve is a plateau equal to nonspecific binding. The concentration of unlabeled drug that produces radioligand binding half way between the upper and lower plateaus is called the IC_{50} (inhibitory concentration 50%) or EC_{50} (effective concentration 50%).

If the radioligand and competitor both bind reversibly to the same binding site, binding at equilibrium follows this equation (where *Top* and *Bottom* are the Y values at the top and bottom plateau of the curve).

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(N - 1) \log IC_{50}}}$$

Data Basic Information (continued)

Checklist for saturation binding

from "Fitting Models to Biological Data using Linear and Nonlinear Regression", Harvey Motulsky & Arthur Christopoulos, GraphPad Software, 2003, page 204-205.

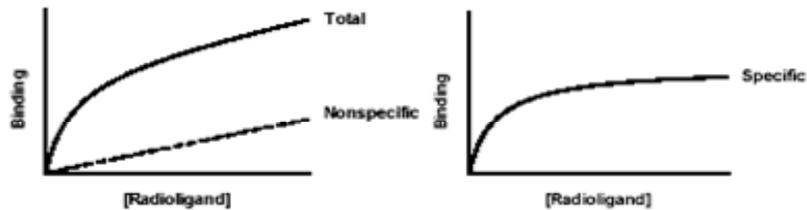
Checklist for saturation binding

When evaluating results of saturation binding analyses, ask yourself these questions:

Question	Comment
Did only a small fraction of the radioligand bind?	The analysis assumes that the free concentration is almost identical to the concentration you added. You can test this by comparing the total counts that bound to the total counts added to the tube. If more than 10% of the ligand bound (at any ligand concentration), then the standard analysis won't work. Either change the experimental protocol (increase the volume) or use a method that accounts for depletion of radioligand -- see "Analyzing saturation binding with ligand depletion" on page 208.
Did the binding equilibrate?	The tubes with the lowest concentration of radioligand take the longest to equilibrate. So test equilibration time with a low concentration of radioligand.
Did you use high enough concentrations of radioligand?	Calculate the ratio of the highest radioligand concentration you used divided by the K_d reported by the program (both in nM or pM). The highest concentration should be at least 10 times the K_d , so that occupancy exceeds 90%.
Is the B_{max} reasonable?	Typical values for B_{max} are 10-1000 fmol binding sites per milligram of membrane protein, 100-10000 sites per cell or 1 receptor per square micron of membrane. If you use cells transfected with receptor genes, then the B_{max} may be many times larger than these values.

Question	Comment
Is the K_d reasonable?	Typical values for K_d of useful radioligands range between 10 pM and 10 nM. If the K_d is much lower than 10 pM, the dissociation rate is probably very slow and it will be difficult to achieve equilibrium. If the K_d is much higher than 10 nM, the dissociation rate will probably be fast, and you may be losing binding sites during separation of bound ligand from free radioligand.
Are the standard errors too large? Are the confidence intervals too wide?	Divide the SE of the B_{max} by the B_{max} , and divide the SE of the K_d by the K_d . If either ratio is much larger than about 20%, look further to try to find out why.
Is the nonspecific binding too high?	Divide the nonspecific binding at the highest concentration of radioligand by the total binding at the highest concentration. Nonspecific binding should usually be less than 50% of the total binding.

Data Basic Information (continued)

Introduction to saturation binding experiments

Saturation radioligand binding experiments measure specific radioligand binding at equilibrium at various concentrations of the radioligand. Nonlinear regression analysis of saturation binding data allows you to determine receptor number and affinity. Because this kind of experiment used to be analyzed with linear Scatchard plots (more accurately attributed to Rosenthal), they are sometimes called "Scatchard experiments".

Analyses of saturation binding data depend on the assumption that you have allowed the incubation to proceed to equilibrium. This can take anywhere from a few minutes to many hours, depending on the ligand, receptor, temperature, and other experimental conditions. The lowest concentration of radioligand will take the longest to equilibrate. When testing equilibration time, therefore, use a low concentration of radioligand (perhaps 10-20% of the K_d).

Data Basic Information (continued)

AR Chemical Aliquots Code for Laboratory D

<i>Material</i>	<i>Initial Concentration (M)</i>	<i>Initial Concentration (nM)</i>	<i>Labels on set 1 vials</i>	<i>Labels on set 2 vials</i>	<i>Labels on set 3 vials</i>	<i>Labels on set 4 vials</i>	<i>Labels on set 5 vials</i>
Cold R-1881 for saturation, 4 vials	1.00E-05	10000.0	D-1-C1	D-2-C1			
	1.00E-06	1000.0	D-1-C2	D-2-C2			
Cold R-188 for Competitive Standard Curve, 12 vials	1.00E-05	10000.0	D-1-S0	D-2-S0			
	3.00E-06	3000.0	D-1-S1	D-2-S1			
	3.00E-07	300.0	D-1-S2	D-2-S2			
	3.00E-08	30.0	D-1-S3	D-2-S3			
	3.00E-09	3.0	D-1-S4	D-2-S4			
	3.00E-10	0.3	D-1-S5	D-2-S5			
Weak Positive (dexamethazone) 16 vials	3.00E-02	30000000.0	D-1-P1	D-2-P1			
	3.00E-03	3000000.0	D-1-P2	D-2-P2			
	3.00E-04	300000.0	D-1-P3	D-2-P3			
	3.00E-05	30000.0	D-1-P4	D-2-P4			
	3.00E-06	3000.0	D-1-P5	D-2-P5			
	3.00E-07	300.0	D-1-P6	D-2-P6			
	3.00E-08	30.0	D-1-P7	D-2-P7			
	3.00E-09	3.0	D-1-P8	D-2-P8			
Tracer Hot R-1881 for saturation and	1.00E-07	100.0	D-1-H1	D-2-H1	D-3-H1	D-4-H1	
	1.00E-08	10.0	D-1-H2	D-2-H2	D-3-H2	D-4-H2	D-5-H2

Cytosol vials will be sent on dry ice. Each container will have a unique identifier. The lot number and protein concentration will be supplied in the accompanying documentation.

Cytosol

Tracer

R1881		
lot number	3538-497	
specific activity	82 Ci/mmole	
certification date	9/16/2004	
Physical Characteristics of 3H		
	Half life	lambda
years	12.35	0.05613
days	4510.84	0.00015

APPENDIX II
PROTOCOL

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PROTOCOL

1. **Title:** Androgen Receptor Competitive Binding Protocol Using Rat Ventral Prostate Cytosol

2. **Sponsor:** Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

3. **Testing Facility:** ABC Laboratories
7200 East ABC Lane
Columbia, Missouri 65202

4. **Objective:** To provide data to establish laboratory variability among five laboratories using the same assay and common preparations of rat prostrate cytosol, R1881 and dexamethasone. This protocol is specific to the study to be conducted at ABC Laboratories.

5. **Duration:** approximately 45 days

6. **Proposed Study Dates:**

- a. Initiation of Task: November 15, 2004
- b. Completion of Task: December 31, 2004

7. **Protocol Approval:**

- a. Study Director: C. Gliser Date: Nov 17 '04
Camelia Gliser, B.S.
- b. Management: Amy Mize Date: 17 NOV 04
Amy Mize, Ph.D.
- c. Sponsor: David P. Houchens Date: Nov 15, 2004
David P. Houchens, Ph.D.

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Reviewed By:

- a. ABC QA
Representative: Michelle Haines Date: 15mar05
Michelle Haines, B.A.
- b. EDSP Battelle
QAM: Terri L. Pollock Date: 11-18-04
Terri L. Pollock, B.A.

8. **Test, control and reference substances:**

- a.1 Test Substances: Dexamethasone (CAS 50-28-2) will be prepared and supplied by Battelle.
- a.2 Reference substance: R1881 (Methyltrienolone) (CAS 965-93-5) will be supplied by Battelle.
- a.3 Marker: Radiolabeled-R1881 (³H-Methyltrienolone) (CAS 68-23-5) Tracer.
- b. Storage: Upon receipt from the supplier, ³H-R1881 will be stored at -20⁰C. Other test substances will be stored according to conditions specified by the supplier.
- c. Disposition: All quantities of the test substances which are dispensed will be documented.

9. **Test System:**

- a. Identification: The test system is rat prostrate cytosol. Each tube within the assay will be labeled as defined in the assay. Upon receipt from the supplier, cytosol will be stored at -80⁰C.
- b. Justification for selection of the test system: Rat prostate cytosol is used because of the extensive data that exist for comparison and the inability to identify appropriate recombinant system(s) without false negative and positive acting chemicals in the *in vitro* binding assay.

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- c. Source: The rat prostate cytosol will be prepared by Battelle – Richland per Battelle SOPs. The cytosol will be shipped to ABC Laboratories for use in the assays.
10. **Experimental Design:**
- a. Assay to be Performed: Saturation binding experiments and competitive binding experiments with R1881 will be run according to the updated assay protocol (see Appendix 1). Each experiment shall be conducted once each day on three separate days by the same technician. In addition, competitive binding experiments will be run with the weak AR binder dexamethasone (at this time assume 8 dilutions). Each experiment shall be conducted once each day on three separate days by the same technician.
- b. Frequency of Tests: Three tubes will be run per concentration and each assay will be run three times for the saturation and competitive binding assay. The assay is described in detail in Appendix 1.
- c. Route of administration and Reason for its choice: The test and control substances are added directly to the cytosol in assay tubes in the appropriate sequence with the other reagents in the assay. The direct application and sequence is required for this assay type.
- d. Analysis of Data: Graph pad prism will be used to calculate K_d and number of receptors for the saturation binding experiment. Also, the IC_{50} for R1881 and dexamethasone and Relative Binding Affinity (RBA) for dexamethasone as compared to R1881 from the competitive binding studies will be calculated.
- e. Method for control of bias: Replicate incubations and aliquot numbers for analysis along with simultaneous incubation of positive controls side by side with the test incubations will control experimental bias.
11. **Quality Assurance**: This study will be audited by the Quality Assurance Unit to assure adherence to Good Laboratory Practice Regulations, adherence to the study protocol and compliance with ABC Labs Standard Operating Procedures. The Quality Assurance Unit will conduct a review of the raw data for accuracy and traceability and will audit the final report.
12. **Reports**: A Report will be prepared at the completion of the study. The report will include, but not be limited to, the following:
- a. Design of the study and the results obtained.
- b. Name and address of the facility performing the study.
- c. Copy of the approved protocol, including all changes and revisions.

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- d. Date of the completed report.
 - e. Instances of ambiguity or unclear direction.
 - f. Prism or other data files.
 - g. Description of all circumstances that may have affected the quality or integrity of data.
 - h. Name, chemical structure, Chemical Abstract Service Registry Number (if known), physical nature and purity (if known) of the test, control and reference substances.
 - i. Justification for choice of solvent/vehicle if other than water or ethanol, and information to demonstrate that the solvent/vehicle, if other than an established solvent does not bind to or otherwise affect the Androgen Receptor (AR).
 - j. Type and source of AR, its isolation from tissues, protein concentration of AR preparation and method of storage.
 - k. Test conditions.
 - l. Results including extent of precipitation of test substance(s), solvent control response compared to the negative control, K_d and IC_{50} values with confidence limits for R1881, and RBA values for dexamethasone as compared to R1881.
13. **Alteration of Design:** Alterations of the protocol may be made as the study progresses. Changes will be documented as required by ABC SOPs.
14. **Data Notebooks:** All original data will be maintained in data notebooks. These will include, but not necessarily be limited to the following:
- a. The original signed protocol and all amendments.
 - b. Test system records.
 - c. Test substances receipt and use records.
 - d. Test substances preparation data.
 - e. Sample preparation data.
 - f. Scintillation counting data.
 - g. Calculations to determine final reported values.
15. **Records to be Maintained:** The protocol, any amendments, the final report and all raw data collected as a result of this study will be archived by Battelle-Richland. The associated facility records will also be archived as required by Battelle-Richland SOPs.
16. **Personnel:** *Curricula vitae* for all personnel involved in the execution of the study are on file at ABC, Columbia, Missouri.
17. **Compliance Statement:** This study will be conducted in compliance with the U.S. EPA Good Laboratory Practice Regulations as set forth in Part 160 Title 40 of the Code of Federal Regulations.

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APPENDIX 1

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APPENDIX 1

Androgen Receptor Competitive Binding Protocol Using Rat Ventral Prostate Cytosol

OP No. NHEERL-H/RTD/EB/VW/2002-03-000

1.0 Purpose and Applicability

Determine ability of compound to compete with [³H] ligand for binding in rat ventral prostate tissue homogenate.

2.0 Safety and Operating Precautions

All procedures with radioisotopes should follow the regulations and procedures as described in the Hazardous Agent Protocol and in the Radiation Safety Manual and Protocols for US EPA.

3.0 Animal Use

Follow U.S. EPA approved animal use protocols

4.0 Equipment and Materials**4.1 Equipment**

- Corning Stir/hot Plates
- Pipets
- Balance
- Polytron PT 35/10 Tissue Homogenizer
- Vacuum Concentrator
- Refrigerated General Laboratory Centrifuge
- High-Speed Refrigerated Centrifuge (up to 30,000 x g)
- pH Meter with Tris Compatible Electrode
- Scintillation Counter refrigerator
- Speed-vac
- 20° freezer

4.2 Chemicals

- Tris HCL & Tris Base
- Phenylmethylsulfonyl Fluoride (PMSF)
- Glycerol 99%+
- Sodium Molybdate
- Ethylenediaminetetraacetic acid (EDTA); Disodium salt
- Dithiothreitol (DTT)
- Potassium Chloride
- Hydroxylapatite (HAP; BIO-RAD)
- Scintillation Cocktail (Flow Scint III)
- Ethyl Alcohol, anhydrous
- Negative Control (Corticosterone)
- [³H]-R1881 (NEN; Purity >97%)
- Radioinert R1881 (NEN)

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- Triamcinolone Acetonide
- Steroids (Steraloids - recrystallized)
- scintillation cocktail (we use optifluor)

4.3 Supplies

- 20 ml Polypropylene Scintillation Vials
- 12 x 75 mm Borosilicate Glass Test Tubes
- 1 000 ml graduated cylinders
- 500 ml Erlenmeyer flasks
- pipet tips
- other glassware as appropriate

5.0 Stock Preparations

5.1. Preparation of Stock Solutions for making TEDG Buffer

5.1.1. EDTA Stock Solution: Add 7.444g disodium EDTA to 100 ml ddH₂O = 200mM. Store at 4°C. Use 750 µl/100ml TEDG buffer = 1.5 mM.

5.1.2. PMSF Stock Solution: Add 1.742 g PMSF to 100 ml ethanol = 100 mM. Store at 4°C. Use 1.00 ml/100ml TEDG buffer = 1.0 mM.

5.1.3. Sodium Molybdate Stock: Add 2.419 g sodium molybdate to 8.0 ml ddH₂O in a 10 ml volumetric flask; bring the total volume to 10 mls = 1.0 M. Store at 4°C. Use 100 µl/100ml TEDG buffer = 1.0 mM.

5.1.4. 1 M Tris Buffer: Add 147.24 g Tris-HCL + 8.0 g Tris base to 800mls ddH₂O in a volumetric flask; bring the final volume to 1.0 liter. Refrigerate to 4°C and pH (using 4°C pH standardizing solutions) the cooled solution to 7.4. Store at 4°C. Use 1.0 ml/100 ml TEDG buffer = 10mM. (50 mM Tris = 50 ml 1 M Tris/1 L ddH₂O)

5.1.5. Potassium Chloride Stock Solution: Add 298.2 g KCL to 600 ml ddH₂O in a 1000 ml volumetric flask; bring the total volume to 1000 ml = 4.0 M. Store at room temperature. Use 10.0 ml per 100 ml high-salt TEDG buffer = 0.4 M.

5.1.6. Add 15.4 mg DTT directly to 100 ml TEDG buffer the morning of the receptor isolation = 1.0 mM.

5.2. Preparation of Low-Salt TEDG Buffer (pH 7.4)

To make 100 mls of low-salt TEDG buffer add the following together in this order:

- 87.15 ml ddH₂O
- 1.0 ml 1M TRIS
- 10.0 ml glycerol
- 100 :1 1 M sodium molybdate
- 7 50 :1 200mM EDTA
- 1.0 ml 100mM PMSF
- 15.4 mg DTT (add immediately before use)

Check pH of the final solution to make sure it is 7.4 at 4°C.

5.3 Preparation of 50 mM TRIS Buffer

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Add 50.0 ml of 1.0 M TRIS to 950 ml ddH₂O. Store at 4°C. Check pH of the final solution to make sure it is 7.4 at 4°C.

5.4 Preparation of 60% Hydroxylapatite (HAP) Slurry

5.4.1. Shake BIO-RAD HT-GEL until all the HAP is in suspension (i.e., looks like milk). The evening before the receptor extraction, pour 100 ml (or an appropriate volume) into a 100 ml graduated cylinder, parafilm seal the top and place in the refrigerator for at least 2h.

5.4.2. Pour off the phosphate buffer supernatant, and bring the volume to 100 ml with 50 mM TRIS. Suspend the HAP by parafilm sealing the top of the graduated cylinder and inverting the cylinder several times. Place in the refrigerator overnight.

5.4.3. The next morning, repeat the washing steps x 2 with fresh 50 mM TRIS buffer.

5.4.4. After the last wash, add enough 50 mM TRIS to make the final solution a 60% slurry (i.e., if the volume of the settled HAP is 60 ml bring the final volume of the slurry to 100 mls with 50 mM TRIS).

5.4.5. Store at 4°C until ready for use in the extraction.

5.5 Preparation of [³H-17 α -Methyl]-R1881 Stock Solutions

Dilute the original 1.0 mCi/ml stock of [³H-17 α -methyl]-R1881 to 0.1 μ M (i.e., 1×10^{-7} M). This is most easily accomplished by pipeting 1 μ l of the stock solution for every specific activity unit (Ci/mmol) and diluting this to 10.0 mls with ethanol. Thus, if the specific activity of the stock vial is 86 Ci/mmol, then pipet 86.0 μ l into an amber colored vial (i.e., R1881 is photosensitive) and add 10.0 mls ethanol to the vial; this solution is 1×10^{-7} M.

Note: [³H-17 α -Methyl]-R1881 stock solution and dilutions should be stored at -20°C. Store stock solution in original protective vial and store dilutions in amber glass vials. This product is light-sensitive; care should be taken to minimize exposure to light.

5.6 Calculation Check and Dilutions

$$86 \mu\text{l} \times 1.0 \text{ mCi}/1000\mu\text{l} = 86 \times 10^{-3} \text{ mCi R1881} = 86 \times 10^{-6} \text{ Ci R1881}$$

$$86 \times 10^{-6} \text{ Ci} \div 86.0 \text{ Ci/mmol} = 1 \times 10^{-6} \text{ mmol R1881} = 1 \times 10^{-9} \text{ moles R1881}$$

$$1 \times 10^{-9} \text{ moles R1881} \div 0.010 \text{ liters} = 1 \times 10^{-7} \text{ moles/liter} = 0.1 \mu\text{M}$$

To prepare the 1×10^{-8} M stock simply make a 10-fold dilution of the 1×10^{-7} M stock (i.e., pipet 1.0 ml of the 1×10^{-7} M stock into a clean amber colored vial and add 9 mls ethanol = 0.01 μ M).

To prepare the 1×10^{-9} M stock simply make a 10-fold dilution of the 1×10^{-8} M stock (i.e., pipet 1.0 ml of the 1×10^{-8} M stock into a clean amber colored vial and add 9 mls ethanol = 0.001 μ M).

5.7 Preparation of 100X Radioinert R1881 Solutions

The R1881 comes as a 5.00 mg quantity. Dilute the original stock to 5.0 ml with ethanol = 3.52 mM. Take 56.82 μ l and dilute to 20 ml in an amber vial with ethanol = 1×10^{-5} M R1881. This is the 10 μ M radioinert R1881 stock.

To make the 1.0 μ M radioinert R1881 stock, pipet 2 ml of the 10 μ M stock into an amber vial and dilute to 20 ml with ethanol = 1×10^{-6} M = 1.0 μ M radioinert R1881 stock. To make the 0.10 μ M

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radioinert R1881 stock, pipet 2 ml of the 1 μ M stock into an amber vial and dilute to 20 ml with ethanol = 1×10^{-7} M = 0.10 μ M radioinert R1881 stock.

5.8 Compound Stock Preparations

5.8.1. Make stocks 30X above desired final concentration (this accounts for the use of 10 μ l stock in 300 μ l cytosol). Initial Stock of each test chemical solution will be diluted in 100% ethanol at a concentration of 3.0×10^2 M (i.e., 30 mM).

12.37mg/2ml ethanol

EXAMPLE:

4 (t) octyl phenol FW 206.33
 1M = 206.33g/L
 1mM = .20633mg/ml x 30 (30 mM desired final stock conc.) = 6.1899 mg/ml
 2 ml Stock = 6.1899 mg x 2 = 12.3798 mg

5.8.2. Prepare serial dilutions of R1881 for standard curve in ethanol (100%) to yield the Initial Concentrations as indicated in Table 1.

Standards	Initial R1881 Concentration (Molar)	*Final R1881 Concentration (Molar) in AR assay tube
Negative Control	0	
0	0 (EtOH)	0
NSB	1×10^{-5}	1×10^{-6}
S1	3×10^{-6}	1×10^{-7}
S2	3×10^{-7}	1×10^{-8}
S3	3×10^{-8}	1×10^{-9}
S4	3×10^{-9}	1×10^{-10}
S5	3×10^{-10}	1×10^{-11}

* Final concentration = 10 μ l of each standard is added to the assay tube ,except for NSB tubes which are 30 μ l.

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5.8.3. Prepare serial dilutions of the test chemicals as indicated in Table 2.

Serial Dilutions of the Test Chemical	Initial Concentration (Molar)	*Final Concentration (Molar) in AR assay tube
Concentration 1	3×10^{-3}	1×10^{-4}
Concentration 2	3×10^{-4}	1×10^{-5}
Concentration 3	3×10^{-5}	1×10^{-6}
Concentration 4	3×10^{-6}	1×10^{-7}
Concentration 5	3×10^{-7}	1×10^{-8}
Concentration 6	3×10^{-8}	1×10^{-9}
Tube 7	0 (vehicle only)	0

* Final concentration = 10 μ l of each Initial Concentration of test chemical is added to the assay tube along with 300 μ l of ventral prostate cytosol.

6.0 Tissue Homogenate Collection

- 6.1. Castrate 90 day old rats (60-90 day old acceptable; 90 day old preferred) as per laboratory animal protocols.
- 6.2. 24 hours after castration, make low salt TEDG buffer and place in an ice-water bucket.
- 6.3. Kill rat and excise ventral prostate. Tissue should be trimmed of fat, weighed and the weights recorded.
- 6.4. Add low-salt TEDG buffer at 10ml/g tissue.
- 6.5. Mince tissues with Metzenbaum scissors until all pieces are small 1-2 mm cubes. Then homogenize the tissues at 4°C with a Polytron homogenizer using 5-sec bursts of the Polytron. [Note: place probe of the Polytron in TEDG buffer in an ice-water bath to cool it down prior to its use for homogenization. Recool probe as needed.]
- 6.6. Transfer homogenates to pre-cooled centrifuge tubes, balance, and centrifuge at 30,000x g for 30 minutes (i.e., 15, 262 rpm using JA-17/JA-21 Beckman rotors).
- 6.7. The supernatant contains the low-salt cytosolic receptor. Pool the supernatant from all rats. Aliquot into 5 ml and store -80°C until needed for assay. Discard after 6 months.
- 6.8. Determine the protein content for each batch of cytosol according to the method by Bradford (1976) using the commercially available BioRad Protein Assay Kit (BioRad Chemical Division, Richmond, CA). Protein concentrations usually range from 5.5 - 8 mg/ml in undiluted cytosol.

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7.0. Assay procedure for chemicals: Day 1

- 7.1. Set up tubes: 12x75 mm glass tubes
 - 7.1.1. Label sufficient glass tubes as needed for the assay.
 - 7.1.2. Add 30µl of 0.01µM [3H] R1881 (1×10^{-8} M) and 50µl triamcinolone acetone (60µM stock) to ALL tubes
 - 7.1.3. For 3 tubes at beginning of assay and at end of assay, also add 100x inert R1881 (30µl of 10.0 µM, ie 1×10^{-6} M). These tube are for determining nonspecific binding.
 - 7.1.4. Place tubes in speed-vac and dry the tubes according to instructions. Remove when dry.
- 7.2. Add 10µl of compound stocks (see 5.8 for concentrations 1-7 in duplicate)
- 7.3. Remove aliquot of prostate cytosol and thaw on ice. Cytosol should be diluted with ice-cold low-salt TEDG buffer to give a protein concentration of 1.2 mg per 300 µl assay tube. (In our lab this is usually about a 1:1 dilution or 150 µl cytosol:150 µl TEDG buffer)
- 7.4. Add 300µl of diluted cytosol to every tube ON ICE. Gently vortex and place tubes in refrigerator overnight in rotor (20hr).
- 7.5. Before leaving for the day, prepare the first wash of the HAP slurry as described in section 5.4 above.
- 7.6. Label the HAP tubes and the scintillation vials to be used the following day - see underlines below.

8.0 Assay procedure: Day 2

- 8.1. The following morning, wash the HAP as described in section 5.4 above, dilute with 50 mM TRIS to yield a 60% slurry, and transfer contents to a 100 ml Erlenmeyer flask. Place a stir bar in the flask and place the flask into a beaker containing ice-water; stir the HAP slurry by placing the beaker on a magnetic stir plate.
- 8.2. While the HAP slurry is constantly being stirred, pipet 500 µl of the HAP slurry into clean pre-labelled 12 x 75 mm glass test tubes. Place these tubes in a rack in an ice-water bath prior to pipetting the HAP slurry and keep them in the ice-water bath for the remainder of the assay.
- 8.3. One HAP tube should be prepared for each incubation tube.
- 8.4. Take the incubation tubes from the refrigerator and place them in an ice-water bath with the HAP tubes. Pipet 100 µl from each of the incubation tubes into the appropriate pre-labelled tubes containing HAP. Repeat for all tubes. Quickly take each rack from the ice-water bath and vortex each rack of tubes using the whole-rack vortex unit. Place racks back into the ice-water bath and vortex as above every 5 minutes for 20 minutes.
- 8.5. Centrifuge the HAP tubes for 2-3 minutes at 4°C and 600 x g (1780 rpm in a Beckman GLC refrigerated centrifuge). Place the tubes back into the rack and into the ice-water bath.
- 8.6. While the tubes remain in the ice-water bath, aspirate the supernatant from each tube using a 9 inch pipet connected to an aspiration apparatus as per the radiation safety protocol.

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- 8.7. Add 2 ml of 50 mM TRIS to each tube, vortex and centrifuge at 600 x g as above. Place the tubes into decanting racks in an ice-water bath and decant the supernatant TRIS wash into the radiation safety container. Gently tap the tube openings on a clean adsorbent diaper, place the rack back in the ice-water bath and add 2 mls of 50 mM TRIS.
- 8.8. Repeat the TRIS washing procedure 3 or 4 times (to be determined empirically) keeping the tubes at 4°C at all times.
- 8.9. Following the last wash and decanting, add 2 mls of ethanol to each tube, vortex 3 times at 5 minute intervals and centrifuge the tubes at 600 x g for 10 minutes. Decant the supernatants into pre-labelled 20 ml scintillation vials. Add 14 ml of Optifluor scintillation cocktail and count samples using the single label DPM program with quench correction.

9.0. Saturation Radioligand Binding Assay

Prior to routinely conducting the AR competitive binding assays, the methods should be standardized within each laboratory. A series of saturation radioligand binding assays should be conducted to demonstrate AR specificity and saturation. Nonlinear regression analysis of these data and subsequent Scatchard plots will document AR binding affinity (K_d) and maximum specific binding number (B_{max}). Scatchard assay is to be conducted as follows:

Day 1

- 9.1. Set up tubes: 12x75 glass tubes and label for 8 concentrations in duplicate each with and without 100X inert (48 tubes total 1 through 48 below).
- 9.2. Add [3 H] R1881 from the appropriate stock solutions to tubes as listed below:
- 9.3. Place 50 μ l of 60 mM stock triamcinolone acetonide to ALL tubes.
- 9.4. An aliquot of each concentration of [3 H]R1881 should also be counted on scintillation counter to determine total counts added (tube # 49-72 below).

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Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µL)	Hot Final Concentration (nM)	Cold Initial Concentration (nM)	Cold Volume (µL)	Cold Final Concentration (nM)	Triamcelenone Acetate (µL)	Cytosol (µl)
1	1	H	10.0	7.5	0.25	—	—	—	50	300
2	2	H	10.0	7.5	0.25	—	—	—	50	300
3	3	H	10.0	7.5	0.25	—	—	—	50	300
4	1	H	10.0	15	0.50	—	—	—	50	300
5	2	H	10.0	15	0.50	—	—	—	50	300
6	3	H	10.0	15	0.50	—	—	—	50	300
7	1	H	10.0	21	0.70	—	—	—	50	300
8	2	H	10.0	21	0.70	—	—	—	50	300
9	3	H	10.0	21	0.70	—	—	—	50	300
10	1	H	10.0	30	1.00	—	—	—	50	300
11	2	H	10.0	30	1.00	—	—	—	50	300
12	3	H	10.0	30	1.00	—	—	—	50	300
13	1	H	10.0	45	1.50	—	—	—	50	300
14	2	H	10.0	45	1.50	—	—	—	50	300
15	3	H	10.0	45	1.50	—	—	—	50	300
16	1	H	100.0	7.5	2.50	—	—	—	50	300
17	2	H	100.0	7.5	2.50	—	—	—	50	300
18	3	H	100.0	7.5	2.50	—	—	—	50	300
19	1	H	100.0	15	5.00	—	—	—	50	300
20	2	H	100.0	15	5.00	—	—	—	50	300
21	3	H	100.0	15	5.00	—	—	—	50	300
22	1	H	100.0	30	10.00	—	—	—	50	300
23	2	H	100.0	30	10.00	—	—	—	50	300
24	3	H	100.0	30	10.00	—	—	—	50	300
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
28	1	HC	10.0	15	0.5	1.00	15	50	50	300
29	2	HC	10.0	15	0.5	1.00	15	50	50	300
30	3	HC	10.0	15	0.5	1.00	15	50	50	300
31	1	HC	10.0	21	0.7	1.00	21	70	50	300
32	2	HC	10.0	21	0.7	1.00	21	70	50	300
33	3	HC	10.0	21	0.7	1.00	21	70	50	300
34	1	HC	10.0	30	1	1.00	30	100	50	300
35	2	HC	10.0	30	1	1.00	30	100	50	300
36	3	HC	10.0	30	1	1.00	30	100	50	300
37	1	HC	10.0	45	1.5	1.00	45	150	50	300
38	2	HC	10.0	45	1.5	1.00	45	150	50	300

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39	3	HC	10.0	45	1.5	1.00	45	150	50	300
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
43	1	HC	100.0	15	5	10.00	15	500	50	300
44	2	HC	100.0	15	5	10.00	15	500	50	300
45	3	HC	100.0	15	5	10.00	15	500	50	300
46	1	HC	100.0	30	10	10.00	30	1000	50	300
47	2	HC	100.0	30	10	10.00	30	1000	50	300
48	3	HC	100.0	30	10	10.00	30	1000	50	300
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—
52	1	Hot	10.0	15	0.06	—	—	—	—	—
53	2	Hot	10.0	15	0.06	—	—	—	—	—
54	3	Hot	10.0	15	0.06	—	—	—	—	—
55	1	Hot	10.0	21	0.08	—	—	—	—	—
56	2	Hot	10.0	21	0.08	—	—	—	—	—
57	3	Hot	10.0	21	0.08	—	—	—	—	—
58	1	Hot	10.0	30	0.10	—	—	—	—	—
59	2	Hot	10.0	30	0.10	—	—	—	—	—
60	3	Hot	10.0	30	0.10	—	—	—	—	—
61	1	Hot	10.0	45	0.30	—	—	—	—	—
62	2	Hot	10.0	45	0.30	—	—	—	—	—
63	3	Hot	10.0	45	0.30	—	—	—	—	—
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—
67	1	Hot	100.0	15	1.00	—	—	—	—	—
68	2	Hot	100.0	15	1.00	—	—	—	—	—
69	3	Hot	100.0	15	1.00	—	—	—	—	—
70	1	Hot	100.0	30	3.00	—	—	—	—	—
71	2	Hot	100.0	30	3.00	—	—	—	—	—
72	3	Hot	100.0	30	3.00	—	—	—	—	—

- 9.5. Place tubes in speed-vac (Tubes 1-48) and dry the tubes according to instructions. Remove when dry and place on ice.
- 9.6. Cytosol should be diluted with the low salt TEDG buffer to a protein concentration of 1.2 mg per 300 µl assay (in our laboratory this was about a 1:1 dilution). Add 300 µl of diluted prostate cytosol to all tubes (1-48). Keep tubes and cytosol on ice at all times during this procedure. Gently vortex and place tubes in refrigerator overnight in rotor (20hr).
- 9.7. Before leaving for the day, prepare the first wash of the HAP slurry as described in section 5.4 above. If desired, label the HAP tubes and the scintillation vials to be used the following day.

Day 2

- 9.8. Continue as with Day 2 protocol for binding assay above in section 8.0.

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10.0 Data Processing

10.1 Free Concentration of [³H]-R1881

Multiply the DPM in the total counts tubes by 1.8047×10^{-5} . This value will yield the free concentration (i.e., nM) of [³H]-R1881 initially present in each incubation tube.

Calculation Check -

$$\frac{X \text{ DPM}}{2.22 \times 10^{-14} \text{ dpm/Ci}} = \frac{4.5045 \times 10^{-13} \text{ Ci}}{* 83.2 \text{ Ci/mmole}} = \frac{5.4141 \times 10^{-15} \text{ mmole}}{1000 \text{ mmole/mole}} = \frac{5.4141 \times 10^{-18} \text{ moles}}{0.0003 \text{ liters}}$$

$$= \frac{1.8047 \times 10^{-14} \text{ moles/liter}}{1 \times 10^{-9} \text{ moles/nmole}} = X (1.8047 \times 10^{-5}) \text{ nm}$$

*Note this value will be the Specific activity of the radioligand ([³H]R1881) used in the assay.

10.2 Calculation of Total, Nonspecific and Specific [³H]-R1881 Binding

- 10.2.1. Total binding is calculated by multiplying the DPM from the tubes that contained only radiolabelled R1881 $\times (1.6242 \times 10^{-2})$. This value will be total binding in fmoles.
- 10.2.2. Nonspecific binding is calculated by multiplying the DPM from the tubes containing radiolabelled R1881 + 100-fold molar excess of radioinert R1881 $\times (1.6242 \times 10^{-2})$. This value will be nonspecific binding in fmoles.
- 10.2.3. Specific binding is calculated by subtracting nonspecific binding from total binding i.e., fmoles total binding - fmoles nonspecific binding = specific binding in fmoles.

10.3 Graphical Presentation of the Data

- 10.3.1. Standard Curve and Test Chemical Competitive Binding Curves: Data for the standard curve and each test chemical will be plotted as the percent 3H_R1881 bound versus the molar concentration. Estimates of the IC50s will be determined using appropriate non linear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). A Scatchard Analysis may also be performed for the standard curve using R1881 to demonstrate that the assay meets acceptable QA standards.
- 10.3.2. Relative Binding Affinity: The RBA for each competitor should be calculated by dividing the IC50 for R1881 by the IC50 of the competitor and expressing as a percent (e.g., RBA for R1881 =100 %).
- 10.3.3. Maximal binding capacity (Bmax) and association/dissociation constants (Ka / Kd) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND (Munson, P.J., and Rodbard, D. (1980) Anal. Biochem. 107, 220-239).

10.4 References

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AMENDMENT TO STUDY PROTOCOL

Page 1 of 1

AMENDMENT NO.: 1	SPONSOR STUDY NO.: NA
LABORATORY: ABC Laboratories, Inc.	ABC STUDY NO.: 49063
SPONSOR: Battelle Memorial Institute	EFFECTIVE DATE: December 23, 2005
STUDY TITLE: Androgen Receptor Competitive Binding Protocol Using Rat Ventral Prostate Cytosol	

SECTIONS TO BE AMENDED

1. Section 7a

NEW PROCEDURES

1. Section 7a

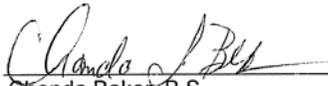
Study Director is now Chanda Baker.

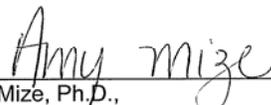
REASON: Camelia Gliser is no longer employed by ABC Laboratories.

IMPACT

Chanda Baker is fully qualified to assume the role of study director, therefore; the integrity of the study is not affected.

APPROVAL:

Study Director:  10 Jan 06
 Chanda Baker, B.S., Date
 Study Director

Management:  10 Jan 06
 Amy Mize, Ph.D., Date
 Director, DMPK and Bioanalytical Services

Sponsor:  1/11/06
 David P. Houchens, Ph.D., Date

