VALIDATION OF AN ANDROGEN RECEPTOR BINDING ASSAY

DRAFT REPORT

IITRI Project No.: 2079
IITRI Study No.: 1

Testing Facility:

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James E. Morris, Ph.D.

Study Initiation Date: **January 11, 2005**
Study Completion Date: **November 2005**

QA AUDITED
DRAFT
B. Perkins
11-25-2005

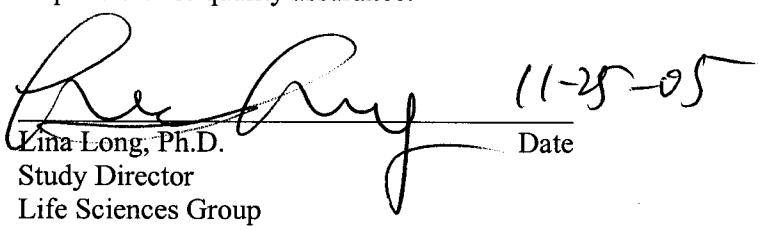
VALIDATION OF AN ANDROGEN RECEPTOR BINDING ASSAY

Study Initiation Date: January 11, 2005
Experimental Initiation Date: January 26, 2005
Experimental Completion Date: March 18, 2005

FOREWORD

This report describes a validation study of an androgen receptor binding assay, conducted by the Life Sciences Group, IIT Research Institute (IITRI), for Battelle Memorial Institute, Columbus, OH. The study was performed under IITRI Project Number 2079, Study Number (SN) 1. The Sponsor Monitor for the study was James E. Morris, Ph.D.

Lina Long, Ph.D., served as Study Director and was responsible for the overall conduct of the study. Michael J. Cwik, Ph.D., served as Principal Investigator for the study. The data analysis was conducted by the Sponsor. Glenn B. Miller, M.S., Manager, Quality Assurance Unit, was responsible for quality assurance.



11-25-05

Lina Long, Ph.D. Date
Study Director
Life Sciences Group

GLP COMPLIANCE STATEMENT

This study was 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. The Sponsor was responsible for performing all necessary chemical analyses on the test substances and positive control substance for characterization (identity, purity and stability) determinations as indicated in the included certificates of analysis.

Scientific interpretation of the data was the responsibility of the Sponsor. The test (radiolabeled and inert methyltrienolone) and weak positive control substances (dexamethasone) were provided by the Sponsor and are characterized in certificates of analysis included in this report. The study raw data have been reviewed and the information contained in this report is an accurate representation of the study data and represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria.

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Lina Long, Ph.D. Date
Study Director
Life Sciences Group

VALIDATION OF AN ANDROGEN RECEPTOR BINDING ASSAY

SUMMARY

The objective of this study was to validate the androgen receptor (AR) binding assay developed by Battelle Memorial Institute for use as a screening tool to detect substances with possible androgenic or anti-androgenic properties, and to serve as one laboratory to determine the variability of results when the assay is performed in multiple competent laboratories. The assay determines the relative binding affinities of test substances for the androgen receptor. In this study, two types of assays, saturation and competitive binding, were performed. AR saturation binding experiments measured total, non-specific and specific bindings of increasing concentrations of 17α-methyl-^{3}H-methyltrienolone (^{3}H-R1881) under conditions of equilibrium. A graph of specific ^{3}H-R1881 binding versus radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the AR with the radioligand. AR competitive binding experiments measured the binding of a single concentration of ^{3}H-R1881 in the presence of increasing concentrations of a weak positive control, dexamethasone, or inert methyltrienolone (R1881) in addition to rat prostate cytosol. A nonlinear curve fitting of ^{3}H-R1881 binding versus various concentrations of weak positive control or R1881 should each produce an EC_{50} value (effective concentration 50%), and ratio of standard curve ED_{50} (R1881 ED_{50}) to weak positive control ED_{50} indicating the relative binding affinities (RBA).

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I. INTRODUCTION

The objective of this study was to validate the androgen receptor (AR) binding assay developed by Battelle Memorial Institute, Columbus, OH, and to serve as one laboratory to determine the variability of results when the assay is performed in multiple competent laboratories.

II. MATERIALS AND METHODS

A copy of the study protocol is included as Appendix A.

A. Test, Control and Reference Substances: The test, control and reference substances were provided by the Sponsor. The test substance, dexamethasone (CAS No. 50-02-2; Lot No. P4311), a white solid, was received on January 12, 2005, in ethanol solution at various concentrations in glass vials. The control substance, Methyltrienolone, [17α-methyl-^{3}H]- (^{3}H-R1881, or Metribolone; Lot No. 3538497), was received on January 13, 2005, as an ethanol solution at concentrations of 1.00^{-7} M (4 vials x 12.5 ml) and 1.00^{-8} M (5 vials x 4 ml) in glass vials packed in ice and was stored at -20°C upon receipt. The solvent to dissolve the test and control substances was selected by the Sponsor. The reference substance, Radioinert Methyltrienolone (R1881; CAS No. 965-93-5; Lot No. 3411228), was received as an ethanol solution at various concentrations on January 12, 2005, in amber glass vials. The reagents were stored in their original containers at the protocol-specified temperatures.

Upon completion of the study, any unused test and positive control substances will be returned to the Sponsor or will be disposed of locally in accordance with all applicable local, state and federal regulations. The Sponsor was responsible for ensuring compliance with Good Laboratory Practice regulations for all test and positive control substance preparations.

B. Type and Source of Androgen Receptor: The type and source of androgen receptor (AR) were selected by the Sponsor, and the AR was prepared by the Sponsor. Rat prostate cytosol (AR) was received on January 6, 2005, as 4 ml (30 vials) and 1.8 ml (12 vials) solution packed on dry ice and was stored immediately at -75°C.

C. Experimental Design: Three sets of saturation assays and three sets of competitive assays were conducted for the study. Each set of assays was conducted once each day on three separate days.

1. Saturation Assay: The saturation assay consisted of 72 tubes: 24 tubes for eight different concentrations of total binding, 24 tubes for eight different concentrations of non-specific binding, and 24 tubes of eight different concentrations of ^{3}[H] R1881 only for verification of radioactivity counting. All tubes were in triplicate. The reaction buffer was selected by the Sponsor as low salt TEDG buffer - 50 mM Tris, 1.5 mM ethylenediaminetetraacetic acid, 1.0 mM phenylmethylsulfonyl fluoride, 1.0 mM sodium molybdate, 1.0 mM dithiothreitol and 10% glycerol. The type and concentration of reagents in each tube were designed by the Sponsor and are illustrated in Table 1.

2. Competitive Assay: The competitive assay consisted of 57 tubes: 15 tubes for five different concentrations of standard curve and 24 tubes for eight different concentrations of weak positive control, all in triplicate; six replicates for total binding with vehicle (ethanol) added, and six replicates for nonspecific binding. An additional six replicates of ^{3}H-R1881 only tubes were used for verification of radioactivity counting. The reaction buffer was selected by the Sponsor as low salt TEDG buffer - 50 mM Tris, 1.5 mM ethylenediaminetetraacetic acid, 1.0 mM phenylmethylsulfonyl fluoride, 1.0 mM sodium molybdate, 1.0 mM dithiothreitol and 10% glycerol. The type and concentration of reagents in each tube were designed by the Sponsor and are illustrated in Table 2.

D. Methods:

1. Saturation Assay:

a. Addition of Reagents: Forty-eight siliconized glass tubes (12 × 75 mm) were labeled for eight concentrations, each in triplicate, with and without inert R1881 (tube nos. 1-48 as shown in Table 1). The ^{3}H-R1881 and radio inert R1881 from the appropriate stock solutions were added to the tubes. In addition, each concentration of ^{3}H-R1881 was placed directly into scintillation vials to count for total radioactivity added (tube nos. 49-72 in Table 1). An aliquot of 50 µl 60 µM stock triamcinolone acetonide was placed into the tubes (tube nos. 1-48). The tubes were placed in a speed-vac to dry all the solution. When the solution was dry, the tubes were removed

and placed on ice. Rat prostate cytosol was diluted with the assay buffer (low salt TEDG buffer - 50 mM Tris, 1.5 mM ethylenediaminetetraacetic acid, 1.0 mM phenylmethylsulfonyl fluoride, 1.0 mM sodium molybdate, 1.0 mM dithiothreitol, 10% glycerol) to a protein concentration of 0.6 mg per 300 µl. The diluted prostate cytosol (300 µl per tube) was added to tubes 1-48. The tubes and cytosol were kept on ice at all times during this procedure.

b. Incubation of Reaction Mixture: The tubes containing all reagents were gently vortexed and placed in a rotating rotor in a refrigerator overnight (approximately 20 hours).

c. Extraction of Bound ^{3}H-R1881: Another set of 48 siliconized glass tubes (12 × 75 mm) was labeled on a tube rack as 1 to 48, and 0.5 ml cold hydroxylapatite (HAP) was placed into each tube on ice. The incubated tubes were taken out from the refrigerator and placed in an ice-water bath. An aliquot of 100 µl reaction mixture from each of the incubated tubes was placed into the corresponding pre-labeled tubes containing HAP. The tubes containing reaction mixture and HAP were quickly taken with the rack from the ice-water bath and were vortexed using the whole-rack vortex unit. The tube rack was quickly placed back into the ice-water bath and vortexed every 5 minutes for 20 minutes. The HAP tubes were centrifuged for 5 minutes at 4°C and 600 ×*g* and then were placed back into the rack and into the ice-water bath. While the tubes remained in the ice-water bath, the supernatant was transferred from each tube into a radiation waste container. A 2 ml aliquot of 50 mM TRIS buffer was added to each tube. The tubes were then vortexed and centrifuged at 600 ×*g* as described above. The HAP tubes were held on a decanting rack in an ice-water bath, and the supernatant TRIS washing solution was decanted into the radiation safety container. The tube rack was placed back into the ice-water bath, and additional 2 ml of 50 mM TRIS buffer was added. The TRIS washing procedure was repeated three times, and the tubes were kept at 4°C at all times. Following the last wash and decanting, 2 ml of ethanol was added to each tube. The tubes were vortexed three times at 5 minute intervals and centrifuged at 600 × *g* for 10 minutes.

d. Counting Bound Radioactivity and Data Analyses: An aliquot of 1 ml ethanol supernatant from each tube was transferred into pre-labeled 20 ml

scintillation vials. Aliquots of 14 ml scintillation cocktail were added to the vials (nos. 1-72) and mixed well. The vials were counted using the single label DPM program. The DPM reading from each sample was transferred into a Microsoft® Excel template provided by the Sponsor. The Excel file was sent to the Sponsor for data analysis.

2. Competitive Assay:

   a. Addition of Reagents: Fifty-one siliconized glass tubes (12 × 75 mm) were set up and labeled as shown in Table 2. An aliquot of 30 µl of 0.01 µM ^{3}H-R1881 (1×10^{-8}M) was added to all tubes. An additional six replicates of ^{3}H-R1881 (tube nos. 52-57) were added directly to the scintillation vials and counted to determine total radio activity added. The radio inert R1881 and weak positive controls were added according to the layout table (Table 2). For tubes at the beginning and end of the sequence of tubes (nos. 4-6 and 49-51), 30 µl of initial concentration at 1×10^{-5}M inert R1881 was added to determine nonspecific binding. For tubes nos. 1-3 and 46-48, 10 µl of ethanol (vehicle) was added as total binding tubes. An aliquot of 50 µl stock triamcinolone acetonide (60 µM) was placed in tube nos. 1-51. The tubes (nos. 1-51) were placed in a speed-vac and, when the solution was dried, were removed and placed on ice. Rat prostate cytosol was diluted with the assay buffer to a protein concentration of 1.0 mg per 300 µl. The diluted prostate cytosol (300 µl per tube) was added to tube nos. 1-51. The tubes and cytosol were kept on ice at all times during this procedure.

   Procedures for the incubation of reaction mixture, extraction of bound ^{3}H-R1881, counting bound radioactivity and data analyses were the same as those described for the saturation assay.

E. Archives: All raw data will be returned to the Sponsor at the time of submission of the final report. All original data will be returned to the Sponsor as bound data notebooks including the following: the original signed protocol and any amendments and/or deviations, documentation, test system records, test substance receipt and use records, and the original final report generated at IITRI.

III. RESULTS

A. Saturation Assay: Saturation run I, performed on January 26, 2005, was abandoned on Day 1 of the experiment due to the formation of white crystals caused by using a concentration of triamcinolone acetonide that was 1,000 times too high (60 mM), resulting from an error in the protocol specific method. After confirming with the Sponsor, a correct solution of 60 μM triamcinolone acetonide in ethanol was prepared and used in subsequent experiments. The saturation run IV was excluded because of unusually large replicate variation for non-specific binding tubes (tube nos. 25-48) due to improper mixing of the radio inert R1881 solution after a new technician took over the assay. No visible precipitation of test substance was observed during the study. Runs II, III and V were submitted to the Sponsor for analysis (Tables 3-5).

B. Competitive Assay: Competitive assay runs I, II and III are shown in Tables 6-8, respectively. No visible precipitation of test substance or positive control substance was observed during the study. There were fairly large variations between replicates, especially for NSB tubes across all three runs. These variations were likely due to a lack of consistency during the performance of the experiment, most likely during the extraction process.

C. Binding Affinity Analysis: Saturation and competitive assay results are summarized in Table 9. Determination of the binding affinity was performed by the Sponsor. Analysis of the binding kinetics findings was performed by the Sponsor and is presented in Appendix C. AR saturation binding experiments measured total and non-specific of increasing concentrations of 17α-methyl-^{3}H-methyltrienolone (^{3}H-R1881) and radio inert R1881 under conditions of equilibrium. The specific binding was obtained by subtracting nonspecific binding from total binding. Nonlinear curve fitting of total R1881 (M) (X axis) and specific radiolabeled binding (M) (Y axis) was performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA) to obtain K_D and B_{MAX}. AR competitive binding experiments measured the binding of a single concentration of ^{3}H-R1881 in the presence of increasing concentrations of a weak positive control, dexamethasone, and inert R1881 in addition to rat prostate cytosol. Nonlinear curve fitting of weak positive control or radioinert R1881 concentration (M) (X axis) versus % of bound radiolabeled R1881 to obtain ED_{50} (or IC_{50}). The relative binding affinities (RBA) were obtained by divided weak positive control ED_{50} with standard curve (inert R1881) ED_{50}.

IV. DISCUSSION AND CONCLUSIONS

The objective of this study was to validate the androgen receptor (AR) binding assay developed by Battelle Memorial Institute as one of the five institutes nationwide. The competitive assay results were provided by the Sponsor and are shown in Appendix C. The mean value obtained in IITRI's lab was fairly close to those of the other institutes, but the standard deviation was larger than those of the other institutes.

V. QUALITY ASSURANCE STATEMENT

Study Title: Validation of an Androgen Receptor Binding Assay.

Project Number: 2079

Study Number: 1

Study Director: Lina Long, Ph.D.

The portions of this study conducted at IITRI have been subjected to inspections, and the report has been audited by the IITRI Quality Assurance Unit in accordance with the U.S. EPA Good Laboratory Practice Regulations as set forth in Part 160 Title 40 of the Code of Federal Regulations. The report describes the methods and procedures used in the study and the reported results accurately reflect the raw data.

The following are the inspection dates and the dates inspection findings were reported:

		Date of Notification:	
Inspection Dates	Phase Inspected	Study Director	Management
January 26, 2005	Saturation Binding Assay, Assay Day 1	February 17, 2005	February 17, 2005
March 17, 2005	Competitive Binding Assay, Assay Day 2	April 25, 2005	April 27, 2005
November 14, 15, 2005	Analytical Data	November 15, 2005	
November 15, 2005	Draft Report	November 15, 2005	

QA AUDITED
DRAFT
B. Perkins
11-25-2005

Glenn B. Miller, M.S. Date
Assistant Vice-President and Manager
Quality Assurance Unit
Life Sciences Group

VI. TABLES

Table 1

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)
1	1	H	10.0	7.5	0.25	—[a]	—	—	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

[a] not applicable

Table 1 (cont.)

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)
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
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	—[a]	—	—	—	—
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	—	—	—	—	—

[a] not applicable

Table 2

Competitive Assay Tube Layout

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-A Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)
1	1	Ethanol	EtOH	0	—[a]	—	300	30	10	50	300	—
2	2	Ethanol	EtOH	0	—	—	300	30	10	50	300	—
3	3	Ethanol	EtOH	0	—	—	300	30	10	50	300	—
4	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
5	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
6	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
7	1	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	300	1.0E-07
8	2	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	300	1.0E-07
9	3	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	300	1.0E-07
10	1	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	300	1.0E-08
11	2	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	300	1.0E-08
12	3	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	300	1.0E-08
13	1	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	300	1.0E-09
14	2	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	300	1.0E-09
15	3	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	300	1.0E-09
16	1	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	300	1.0E-10
17	2	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	300	1.0E-10
18	3	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	300	1.0E-10
19	1	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	300	1.0E-11
20	2	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	300	1.0E-11
21	3	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	300	1.0E-11
22	1	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	300	1.0E-03
23	2	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	300	1.0E-03
24	3	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	300	1.0E-03
25	1	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	300	1.0E-04
26	2	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	300	1.0E-04
27	3	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	300	1.0E-04
28	1	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	300	1.0E-05
29	2	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	300	1.0E-05
30	3	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	300	1.0E-05
31	1	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	300	1.0E-06
32	2	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	300	1.0E-06
33	3	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	300	1.0E-06

[a] not applicable

Table 2 (cont.)

Competitive Assay Tube Layout

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-A Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)
34	1	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	300	1.0E-07
35	2	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	300	1.0E-07
36	3	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	300	1.0E-07
37	1	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	300	1.0E-08
38	2	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	300	1.0E-08
39	3	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	300	1.0E-08
40	1	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	300	1.0E-09
41	2	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	300	1.0E-09
42	3	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	300	1.0E-09
43	1	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	300	1.0E-10
44	2	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	300	1.0E-10
45	3	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	300	1.0E-10
46	1	Ethanol	EtOH	0	—[a]	—	300	30	10	50	300	—
47	2	Ethanol	EtOH	0	—	—	300	30	10	50	300	—
48	3	Ethanol	EtOH	0	—	—	300	30	10	50	300	—
49	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
50	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
51	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06
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	—	—	—	—

[a] not applicable

Table 3

Saturation Assay Data Set II [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µL)	Cytosol (µl)	Significant portion of label on Vial Supplied by Battelle	Full on Vials in Set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
1	1	H	10.0	7.5	0.25	— [c]	—	—	50	300	—	—	656.38
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	646.95
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	718.68
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	1187.77
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	1167.76
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	1219.70
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	1516.62
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1490.28
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1578.61
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	1804.93
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	1830.84
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	1804.11
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	2250.16
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	2279.74
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	2265.25
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2899.74
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3011.84
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3137.32
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	3944.27
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	4049.34
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	4229.48
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	5359.63
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	5585.29
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	5031.14
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	94.31
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	113.86
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	107.18
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	135.76
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	146.94
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	123.30
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	168.53
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	158.62

[a] Performed on January 27, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 3 (cont.)

Saturation Assay Data Set II[a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µL)	Cytosol (µl)	Significant portion of label on Vial Supplied by Battelle	Full on Vials in Set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	178.47
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	187.91
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	197.78
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	189.74
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	247.57
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	220.64
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	272.80
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	392.74
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	481.46
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	440.26
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	833.31
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	791.63
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	760.03
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1776.28
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1651.46
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1714.62
49	1	Hot	10.0	7.5	0.03	—[c]	—	—	—	—	—	—	11812.3
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12996.5
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12847.2
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	24670.7
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	25127.3
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	25519.6
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36114.7
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36603.4
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36900.2
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	50864.3
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	51168.0
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	51029.4
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	82469.0
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	81977.3
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	81402.6

[a] Performed on January 27, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 3 (cont.)

Saturation Assay Data Set II[a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µL)	Cytosol (µl)	Significant portion of label on Vial Supplied by Battelle	Full on Vials in Set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
64	1	Hot	100.0	7.5	0.60	—[c]	—	—	—	—	—	—	127437
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	126325
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	125503
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—	250031
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	254599
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	255763
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	535246
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	537604
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	530371

[a] Performed on January 27, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 4

Saturation Assay Data Set III [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
1	1	H	10.0	7.5	0.25	— [c]	—	—	50	300	—	—	589.60
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	549.72
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	538.10
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	871.03
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	1890.27
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	622.05
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	1214.52
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1151.17
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1255.55
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	1286.65
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	1436.86
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	1345.97
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	1790.38
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	1589.42
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	1902.10
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2239.62
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2226.46
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2353.64
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	2992.00
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	2829.55
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	2946.44
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	4894.78
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	3602.90
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	4494.04
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	83.59
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	123.22
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	85.49
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	139.60
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	79.11
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	155.57
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	122.41
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	202.12

[a] performed on February 23, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 4 (cont.)

Saturation Assay Data Set III [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	164.97
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	162.79
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	156.27
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	181.94
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	213.22
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	226.13
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	258.17
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	332.12
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	279.11
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	394.53
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	710.98
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	758.08
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	605.71
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	2730.62
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1214.56
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1105.89
49	1	Hot	10.0	7.5	0.03	— [c]	—	—	—	—	—	—	12660.0
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12261.6
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12446.7
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	23466.6
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	23381.5
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	22542.1
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	35467.1
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	34800.3
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	35790.7
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	49652.2
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	53253.8
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	50448.7
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	79844.9
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	80392.2
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	71847.0
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	125450
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	123400

[a] performed on February 23, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 4 (cont.)

Saturation Assay Data Set III [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
66	3	Hot	100.0	7.5	0.60	— [c]	—	—	—	—	—	—	126690
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—	248394
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	250958
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	250838
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	510275
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	513608
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	542147

[a] Performed on February 23, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 5

Saturation Assay Data Set V [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
1	1	H	10.0	7.5	0.25	— [c]	—	—	50	300	—	—	601.23
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	593.83
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	570.28
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	956.08
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	937.38
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	1820.34
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	1179.80
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1237.95
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1194.72
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	1581.34
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	1360.05
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	1399.08
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	1884.37
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	1754.71
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	1759.78
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2207.13
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2114.86
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	2280.79
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	2915.80
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	3055.30
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	3313.94
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	3984.37
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	4366.06
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	4553.75
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	100.26
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	95.49
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	A-1-C8	120.18
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	160.81
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	149.61
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	A-1-C7	149.96
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	173.90
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	4037.96
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	A-1-C6	223.44

[a] Performed on March 3, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 5 (cont.)

Saturation Assay Data Set V [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	309.84
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	235.13
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	A-1-C5	209.68
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	267.32
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	360.68
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	A-1-C4	365.72
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	755.98
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	654.28
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	A-1-C3	652.25
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	1010.96
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	1100.55
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	A-1-C2	1156.87
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1838.95
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	1946.76
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	A-1-C1	2842.32
49	1	Hot	10.0	7.5	0.03	— [c]	—	—	—	—	—	—	12446.1
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12229.4
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	11691.8
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	24034.4
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	24158.7
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	26572.0
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36584.2
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	35625.6
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	37223.8
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	52077.1
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	52781.7
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	51820.9
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	39909.1
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	76364.2
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	77288.9
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	121683
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	128124
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	124847

[a] Performed on March 3, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 5 (cont.)

Saturation Assay Data Set V [a, b]

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (µl)	Hot Final Concentration (nM)	Cold Initial Concentration (µM)	Cold R1881 Volume (µl)	Cold Final Concentration (nM)	Triamcelenone Acetate (µl)	Cytosol (µl)	Significant portion of Label on Vial Supplied by Battelle	Full on vials in set 1-1-A Supplied by Battelle to Laboratory "A"	DPM as Counted
67	1	Hot	100.0	15	1.00	— [c]	—	—	—	—	—	—	267383
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	241094
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	241865
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	538824
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	531484
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	538681

[a] Performed on March 3, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 6

Competitive Assay Data Set I [a, b]

Position	Replicate	Competitor	Competitor code	Concentration Code	Labels on Vials in Set 1-1-e Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (m)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor volume (µl)	Triamcelenone volume (µl)	Final volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
1	1	Ethanol	EtOH	0	— [c]	—	300	30	10	50	310	—	100	1792.75
2	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1593.40
3	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1731.05
4	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	249.93
5	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	311.18
6	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	181.81
7	1	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	361.20
8	2	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	318.49
9	3	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	321.19
10	1	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	846.20
11	2	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	487.93
12	3	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	589.19
13	1	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1150.43
14	2	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1207.16
15	3	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1287.39
16	1	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1899.79
17	2	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1854.85
18	3	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1786.88
19	1	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1777.26
20	2	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1995.61
21	3	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1829.25
22	1	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	219.37
23	2	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	230.67
24	3	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	207.10
25	1	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	435.29
26	2	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	474.92
27	3	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	831.84
28	1	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1061.29
29	2	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1483.68
30	3	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1388.49
31	1	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1791.98
32	2	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1644.26

[a] Performed on March 9, 2005

[b] Partial Excel sheet with raw data input

[c] not applicable

Table 6 (cont.)

Competitive Assay Data Set I [a, b]

Position	Replicate	Competitor	Competitor code	Concentration Code	Labels on Vials in Set 1-1-e Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (m)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor volume (µl)	Triamcelenone volume (µl)	Final volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
33	3	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1666.95
34	1	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1568.80
35	2	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1820.85
36	3	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1795.48
37	1	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1659.52
38	2	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1955.97
39	3	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1777.28
40	1	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1859.86
41	2	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1801.06
42	3	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1870.91
43	1	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1907.09
44	2	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1877.59
45	3	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1765.90
46	1	Ethanol	EtOH	0	— [c]	—	300	30	10	50	310	—	100	1582.03
47	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1907.91
48	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1668.91
49	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	322.46
50	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	267.87
51	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	254.87
52	1	none	Hot	—	—	—	—	30	—	—	—	—	—	56886.2
53	2	none	Hot	—	—	—	—	30	—	—	—	—	—	56291.9
54	3	none	Hot	—	—	—	—	30	—	—	—	—	—	57224.0
55	1	none	Hot	—	—	—	—	30	—	—	—	—	—	52031.0
56	2	none	Hot	—	—	—	—	30	—	—	—	—	—	52507.8
57	3	none	Hot	—	—	—	—	30	—	—	—	—	—	49449.1

[a] Performed on March 9, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 7

Competitive Assay Data Set II[a, b]

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-E Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
1	1	Ethanol	EtOH	0	—[c]	—	300	30	10	50	310	—	100	1927.41
2	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1771.30
3	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1794.66
4	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	630.14
5	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	510.15
6	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	486.00
7	1	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	591.97
8	2	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	486.02
9	3	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	450.51
10	1	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	687.46
11	2	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	532.70
12	3	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	643.84
13	1	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1351.11
14	2	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1308.47
15	3	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1262.13
16	1	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1638.47
17	2	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1660.14
18	3	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1546.13
19	1	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1606.14
20	2	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1818.56
21	3	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1955.66
22	1	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	545.57
23	2	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	541.16
24	3	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	579.79
25	1	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1100.07
26	2	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1044.73
27	3	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1209.95
28	1	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1783.06
29	2	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1530.01
30	3	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1496.30
31	1	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1761.45

[a] Performed on March 14, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 7 (cont.)

Competitive Assay Data Set II[a, b]

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-E Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
32	2	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1475.41
33	3	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2400.25
34	1	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	2036.66
35	2	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	2060.38
36	3	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1900.98
37	1	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1921.43
38	2	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1921.65
39	3	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1961.30
40	1	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1876.47
41	2	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1932.82
42	3	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1821.75
43	1	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1988.23
44	2	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	—
45	3	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1513.43
46	1	Ethanol	EtOH	0	—[c]	—	300	30	10	50	310	—	100	1650.58
47	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1601.99
48	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	—
49	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	683.04
50	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	471.25
51	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	352.46
52	1	none	Hot	—	—	—	—	30	—	—	—	—	—	52346.1
53	2	none	Hot	—	—	—	—	30	—	—	—	—	—	49209.9
54	3	none	Hot	—	—	—	—	30	—	—	—	—	—	48878.3
55	1	none	Hot	—	—	—	—	30	—	—	—	—	—	50499.9
56	2	none	Hot	—	—	—	—	30	—	—	—	—	—	50907.7
57	3	none	Hot	—	—	—	—	30	—	—	—	—	—	49214.5

[a] Performed on March 14, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 8

Competitive Assay Data Set III[a, b]

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-E Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
1	1	Ethanol	EtOH	0	—[c]	—	300	30	10	50	310	—	100	1619.11
2	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2326.08
3	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2288.33
4	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	234.02
5	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	177.32
6	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	192.44
7	1	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	160.42
8	2	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	251.48
9	3	Inert R1881	S	1	A-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	2535.80
10	1	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	514.80
11	2	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	457.50
12	3	Inert R1881	S	2	A-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	549.37
13	1	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1278.86
14	2	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1424.59
15	3	Inert R1881	S	3	A-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1367.24
16	1	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1742.82
17	2	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2182.79
18	3	Inert R1881	S	4	A-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2046.34
19	1	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2396.92
20	2	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2597.82
21	3	Inert R1881	S	5	A-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1967.73
22	1	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	3391.66
23	2	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	2391.00
24	3	Weak Positive	P	1	A-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	2638.19
25	1	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	659.66
26	2	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	799.14
27	3	Weak Positive	P	2	A-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	786.47
28	1	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1879.77
29	2	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1994.40
30	3	Weak Positive	P	3	A-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2075.95
31	1	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2547.87

[a] Performed on March 16, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 8 (cont.)

Competitive Assay Data Set III[a, b]

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in Set 1-1-E Supplied by Battelle to Laboratory "A"	Competitor Initial Concentration (M)	Cytosol (µl)	Tracer (Hot R1881) Volume (µl)	Competitor Volume (µl)	Triamcelenone Volume (µl)	Final Volume (µl)	Competitor Final Concentration (M)	Aliquot (µl)	DPM as Sampled
32	2	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2550.89
33	3	Weak Positive	P	4	A-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	2546.39
34	1	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	2354.07
35	2	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	2450.62
36	3	Weak Positive	P	5	A-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	2253.74
37	1	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	2293.64
38	2	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	2506.22
39	3	Weak Positive	P	6	A-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	2322.17
40	1	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	2603.70
41	2	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	2266.79
42	3	Weak Positive	P	7	A-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	2691.85
43	1	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	2435.96
44	2	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	2424.11
45	3	Weak Positive	P	8	A-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	2406.64
46	1	Ethanol	EtOH	0	—[c]	—	300	30	10	50	310	—	100	2470.47
47	2	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2409.82
48	3	Ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2428.62
49	1	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	262.13
50	2	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	301.56
51	3	Inert R1881	NSB	—	A-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	216.40
52	1	none	Hot	—	—	—	—	30	—	—	—	—	—	51448.8
53	2	none	Hot	—	—	—	—	30	—	—	—	—	—	51450.7
54	3	none	Hot	—	—	—	—	30	—	—	—	—	—	51901.5
55	1	none	Hot	—	—	—	—	30	—	—	—	—	—	52515.8
56	2	none	Hot	—	—	—	—	30	—	—	—	—	—	50494.2
57	3	none	Hot	—	—	—	—	30	—	—	—	—	—	50788.9

[a] Performed on March 16, 2005
[b] Partial Excel sheet with raw data input
[c] not applicable

Table 9

Summary of Saturation and Competitive Assay Results

Saturation Assay			
Run ID	S2-012705	S3-022305	S5-030305
K_D[a] (M)	1.44×10^{-9}	1.57×10^{-9}	1.00×10^{-9}
B_{MAX}[b] (M/100µg)	2.32×10^{-10}	1.79×10^{-10}	1.33×10^{-10}

Competitive Assay			
Run ID	C1-030905	C2-031405	C3-031605
ED_{50}[c] (M) standard curve	2.26×10^{-9}	1.58×10^{-9}	1.24×10^{-9}
ED_{50} (M) weak positive	2.33×10^{-5}	9.17×10^{-5}	4.47×10^{-5}
RBA[d]	0.0097%	0.0017%	0.0028%

[a] K_D: dissociation constants
[b] B_{MAX}: maximum binding capacity
[c] ED_{50}: 50% effective inhibition dose
[d] RBA: relative binding affinity

VII. APPENDICES

Appendix A: Protocol

Appendix A

PROTOCOL

1. **Title:** Validation of an Androgen Receptor Binding Assay

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

3. **Testing Facility:** IIT Research Institute (IITRI)
10 West 35th Street
Chicago, Illinois 60616

4. **Objective:** To serve as one laboratory to determine the variability of results when the assay is performed in multiple competent laboratories.

5. **Duration:** 6 months

6. **Proposed Study Dates:**

   a. Experimental Start: To be determined by protocol amendment
   b. Experimental Completion: To be determined by protocol amendment

7. **Protocol Approval:**

   a. Study Director: _____ Date: 1-11-05
   Lina Long, Ph.D.

   b. Director, Life Sciences: _____ Date: 1/12/05
   David L. McCormick, Ph.D., D.A.B.T.

   c. Sponsor: _____ Date: 12/20/04
   James Morris, Ph.D.

   _____ Date: 12/17/04
   David P. Houchens, Ph.D.

   _____ Date: 12-17-04
   Terri L. Pollock, B.A.

8. This protocol complies with specific requirements of the Sponsor.

9. Test, Control and Reference Substances:

a. Test, Control and Reference Substances: The test substance is dexamethasone (CAS 50-02-2). The reference substance is R1881, Radioinert Methyltrienolone, CAS 965-93-5 (Perkin-Elmer, NEN, catalog number NLP 005005MG). ^{3}H-R1881, (New England Nuclear) will be used as the marker (control substance). Radiolabeled and inert R1881 and dexamethasone will be provided by the Sponsor.

b. Hazards to Personnel: Routine procedures for handling radioactive materials and for the conduct of *in vitro* studies will be followed to insure the health and safety of personnel handling the test materials. Personnel handling the test substances have been trained in safe handling of radioactive materials by the IITRI Radiation Safety Officer.

c. Storage: Test substances will be stored according to conditions specified by the Sponsor.

d. Disposition: All quantities of the test substances which are dispensed will be documented.

e. Test Substance Return: Upon completion of the study, remaining test substances will be returned to the Sponsor.

10. Test System:

a. Identification: The test system is rat prostrate cytosol which will be supplied by Battelle. Samples will be labeled as indicated in the methods. Upon receipt from the supplier, cytosol will be stored at -80°C.

b. Justification for Selection of the Test System: Rat cytosol is used because of the extensive data that exist for comparison and the inability to identify appropriate recombinant systems without false negative and positive acting chemicals in the *in vitro* binding assay. The current study is part of a multi-center validation of the test system. The test system was developed by the Sponsor to determine competitive binding to the androgen receptor. In order to validate the test system, the same test must be performed at several laboratories. IITRI will serve as one of those laboratories and will perform the test as instructed by the Sponsor.

c. Assays to be Performed: Competitive binding of dexamethasone to the androgen receptor using rat prostate cytosol, compared to R1881 will be performed. The radio tracer for the assay will be radiolabeled R1881 tracer. The assay will be described in detail in separate protocol-specific methods (Appendix 1, IITRI Project Number 2079 Study No. 1, Protocol Specific Method No. 2, Androgen Receptor (AR) Competitive Binding Assay) based on methods provided by the Sponsor. Prior to conducting the competitive binding assay, a saturation assay will be performed to demonstrate that the assay is properly standardized. (Appendix 1, IITRI Project No. 2079 Study No.1, Protocol-Specific Method, No. 1, *In Vitro* Saturation Radioligand Binding Assay.)

d. Frequency of Tests: Three tubes will be run per concentration and each assay will be run three times on three separate days. One technician will perform all of the tests.

e. Route of Administration and Reason for its Choice, Concentrations of Test, Control and Reference Substance to be Administered, Analysis and Measurements to be Made: The cytosol is added directly to the test and reference substances in assay tubes in appropriate sequence with the other reagents in the assay. The direct application and sequence is required for this assay type. The route of administration, concentrations of test, control and reference substances, analysis and measurements to be made are provided in detail in a separate protocol specific method (Appendix 1, IITRI Project No. 2079 Study No.1, Protocol-Specific Method, No. 1).

f. Analysis of Data: Nonlinear curve fitting of unlabeled R1881 (nM) (X axis) and total radiolabeled binding (DPM) (Y axis) will be performed to obtain IC_{50} and then K_d using GraphPad program.

11. **Quality Assurance:** This study will be audited by the IITRI Quality Assurance Unit to assure adherence to Good Laboratory Practice Regulations, adherence to the study protocol and compliance with IITRI Standard Operating Procedures. The IITRI Quality Assurance Unit will conduct a 100% review of the raw data for accuracy and traceability.

12. **Reports:** Draft Reports will be submitted to the Sponsor at the completion of the study. The reports 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
d. Date of the completed report
e. Raw data
f. Instances of ambiguity or unclear direction
g. Prism or other data files
h. Description of all circumstances that may have affected the quality or integrity of data
i. Name, chemical structure, Chemical Abstract Service Registry Number (if known), physical nature and purity (if known) of the test, reference and control substances.
j. 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 AR
k. Type and source of AR, its isolation from tissues, protein concentration of AR preparation and method of storage
l. Test conditions
m. Results including extent of precipitation of test substance, K_d and IC_{50} values with confidence limits for R1881, the positive control and test substances, and RBA values for weak positive control and test substances.

13. **Alteration of Design:** Alterations of the protocol may be made as the study progresses. No changes in the protocol will be made without the specific written consent of the Sponsor.

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

Appendix A (cont.)

15. **Data Retention:** All raw data from the study will be returned to the Sponsor at the time of submission of the final report.

16. **Personnel:** *Curricula vitae* for all personnel involved in the execution of the study are on file at IITRI.

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.

Appendix B: Certificates of Analysis

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PerkinElmer Life Sciences, Inc.
549 Albany Street
Boston, MA 02118

Products You Trust NEN wallac

^{3}H Technical Data Certificate of Analysis

Caution: For Laboratory Use. A research chemical for research purposes only.

NET-590 METHYLTRIENOLONE, [17α-METHYL-^{3}H]- (R 1881*) (METRIBOLONE)

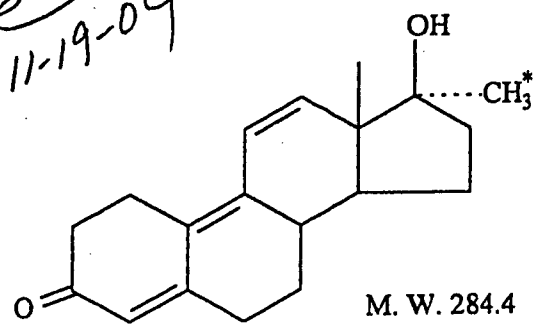
Stock Date 9-16-04

11-19-04

Lot Number: 3538 497

Specific Activity: 82.0 Ci/mmol

3.04 TBq/mmol



M. W. 284.4

PACKAGING: 1.0 mCi/ml (37 MBq/ml) in Ethanol, under argon, in a vial which protects contents from UV light.

STABILITY AND STORAGE RECOMMENDATIONS:

When methyltrienolone, [17α-methyl-^{3}H]- is stored at -20°C in its original solvent and at its original concentration, the rate of decomposition is approximately 2-3% over the first three months from date of purification. Lot to lot variation may occur and it is advisable to check purity prior to use.

- This product is very light-sensitive; care should be taken to minimize its exposure to light.

SPECIFIC ACTIVITY RANGE: 70-87 Ci/mmol (2.59-3.22 TBq/mmol)

RADIOCHEMICAL PURITY: This product initially found to be greater than 97% when determined by the following methods:

1. High pressure liquid chromatography on a Zorbax ODS column using the following mobile phase:
   acetonitrile : water, (4:6)

2. Thin layer chromatography on Silica Gel using the following solvent system:
   toluene : ethyl acetate, (4:1).

QUALITY CONTROL: The radiochemical purity of methyltrienolone, [17α-methyl-^{3}H]- is checked at appropriate intervals using the first listed chromatography method. Current purity data is available upon request.

99197-0401

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precisely.

PerkinElmer Life and Analytical Sciences
549 Albany Street, Boston, MA 02118

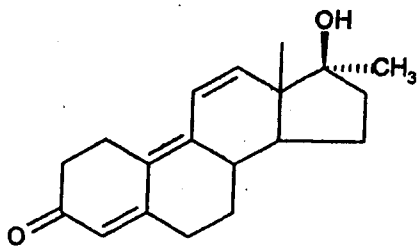
Technical Data

Caution: For Laboratory Use. A research chemical for research purposes only.

NLP-005 Methyltrienolone (R1881)

Specific Lot Data

Lot Number: 3411228



MW. 284.38

Packaging: Solid in a screw cap bottle, wrapped in foil.

Storage Recommendations:

Methyltrienolone stored at –20°C away from light

Chemical Purity:

1) M.P
2) U.V.
3) NMR
4) Thin layer chromatography on Silica Gel using the following solvent systems:

a) toluene: ethylacetate, (8:2)
b) chloroform: ethanol, (98:2)

Special Instructions:

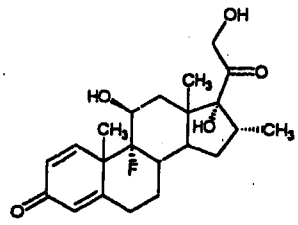
To increase the solubility of this product in aqueous solutions, pre-dissolve in a minimal amount of Ethanol DMSO.

PRODUCT DATA

PRODUCT: Dexamethasone **CAS NO:** 50-02-2

CATALOG NO.: EI-126 **LOT NO.:** P4311

STRUCTURE:



PHYSICAL APPEARANCE: White solid (m.p. 262-264°C)

MOLECULAR FORMULA: $C_{22}H_{29}FO_5$ **MOLECULAR WEIGHT:** 392.5

PURITY: 99% (TLC)

SOLUBILITY: Soluble in ethanol (25 mg/mL) and DMSO (25 mg/mL)

STORAGE: Store, as supplied, at room temperature for up to 5 years. Store solutions at -20°C for up to 3 months.

APPLICATION NOTES: Antiinflammatory glucocorticoid. Inhibits induction of NO synthase in a variety of cell lines and tissues (IC_{50} = 5nM).[1] Inhibits the production of PLA_2 inhibitory protein(lipocortin).[2] Induces apoptosis[3] in eosinophils, but inhibits it in neutrophils; both effects blocked by RU-486.[4] The product is not sterile.

REFERENCES:

1. S.Moncada *et al. Trends Pharmacol.Sci.* 1991 **12** 130
2. M.A.Clark *et al. Prostaglandins* 1986 **32** 703
3. H.O.Fearnhead *et al. Biochem.Pharmacol.* 1994 **48** 1073
4. L.C.Meagher *et al. J.Immunol.* 1996 **156** 4422

The pharmacological and toxicological properties of this product have not been fully investigated. Exercise caution in use and handling. This product must not be used in humans.

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Appendix C: Binding Kinetics Data Analysis

VALIDATION OF AN ANDROGEN RECEPTOR BINDING ASSAY

Appendix C

Table C-1

Competitive Assay Results for Five Institutes [a]

Summary Table (IC50 and RBA)

Lab A

Run identification:	C1-0309005	C2-031405	C3-031606	average	SD	coefficient of variation
Assay start date:	3/9/2005	3/14/2005	3/16/2005			
Standard Curve IC50:	2.27E-09	1.68E-09	1.24E-09	1.70E-09	5.21666E-10	30.78%
Weak Positive IC50:	2.34E-06	9.17E-05	4.47E-06	5.33E-06	3.49703E-06	65.66%
RBA:	0.0097%	0.0017%	0.0028%	0.0047%	0.0043%	91.59%
RBA of means:				0.0032%		

Lab B

Run identification:	1	3	4	average	SD	coefficient of variation
Assay start date:	2/1/2005	3/3/2005	3/14/2005			
Standard Curve IC50:	1.35E-09	1.55E-09	1.50E-09	1.46E-09	1.41916E-10	9.80%
Weak Positive IC50:	3.61E-06	3.88E-06	3.68E-06	3.69E-06	2.61276E-08	7.08%
RBA:	0.0038%	0.0040%	0.0041%	0.0039%	0.0001%	2.73%
RBA of means:				0.0039%		

Lab C

Run identification:	1 (sequence # 2	(sequence # 4	(sequence # 6150)	average	SD	coefficient of variation
Assay start date:	3/8/2005	3/10/2005	3/17/2005			
Standard Curve IC50:	1.42E-09	1.36E-09	1.29E-09	1.35E-09	6.28298E-11	4.65%
Weak Positive IC50:	3.43E-06	3.67E-06	2.96E-06	3.35E-06	3.58118E-06	10.68%
RBA:	0.0041%	0.0037%	0.0044%	0.0041%	0.0004%	8.67%
RBA of means:				0.0040%		

Lab D

Run identification:	3072005	3092005	3142005	average	SD	coefficient of variation
Assay start date:	3/7/2005	3/9/2005	3/14/2005			
Standard Curve IC50:	1.74E-09	1.46E-09	1.99E-09	1.73E-09	2.66983E-10	15.36%
Weak Positive IC50:	2.94E-06	2.84E-06	3.37E-06	3.05E-06	2.83341E-06	9.29%
RBA:	0.0059%	0.0052%	0.0059%	0.0057%	0.0004%	7.82%
RBA of means:				0.0057%		

Lab E

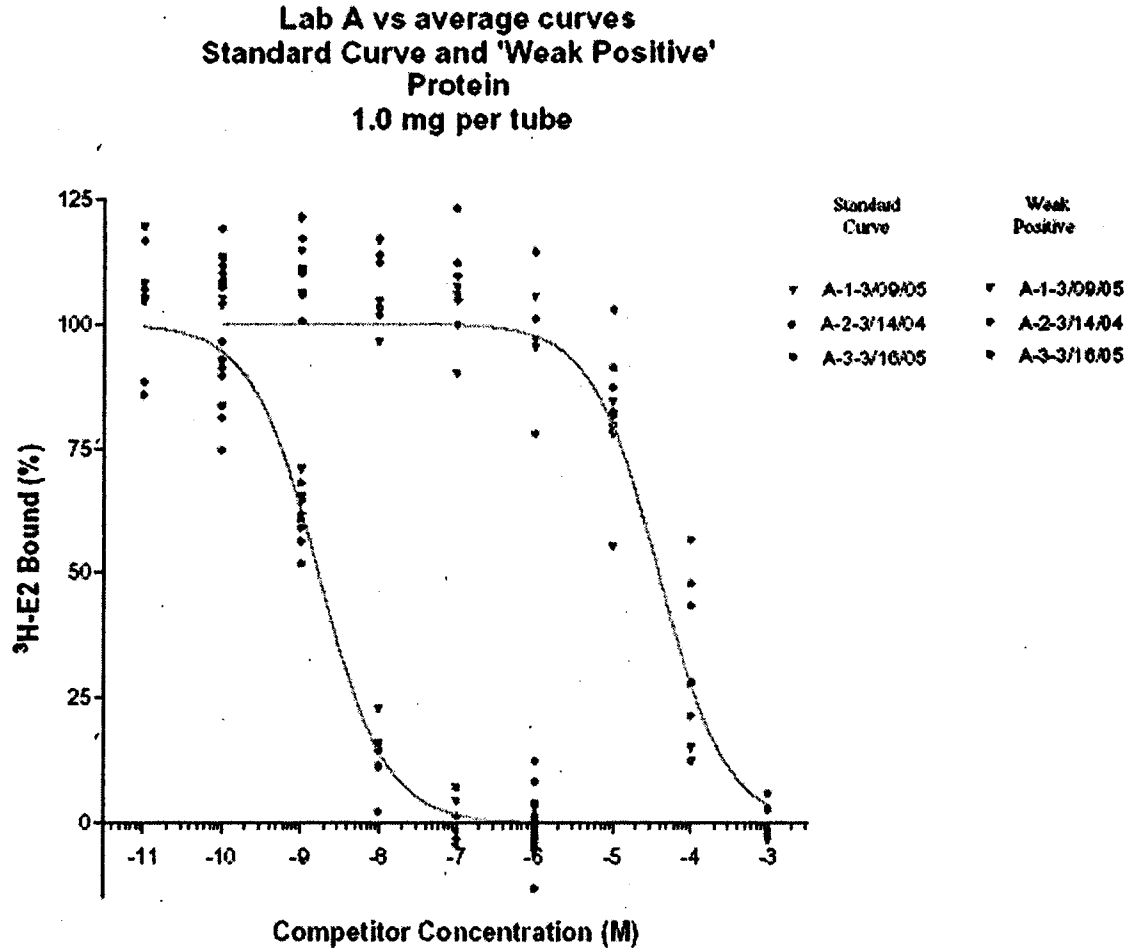
Run identification:	467	468	473	average	SD	coefficient of variation
Assay start date:	1/27/2005	2/1/2005	3/1/2005			
Standard Curve IC50:	1.98E-09	1.85E-09	1.84E-09	1.89E-09	7.84676E-11	4.16%
Weak Positive IC50:	4.24E-05	4.16E-05	4.30E-05	4.23E-05	7.13902E-07	1.69%
RBA:	0.0047%	0.0044%	0.0043%	0.0045%	0.0002%	4.49%
RBA of means:				0.0045%		

[a] IITRI served as Lab A and the Sponsor as Lab E

Appendix C (cont.)

Figure C-1

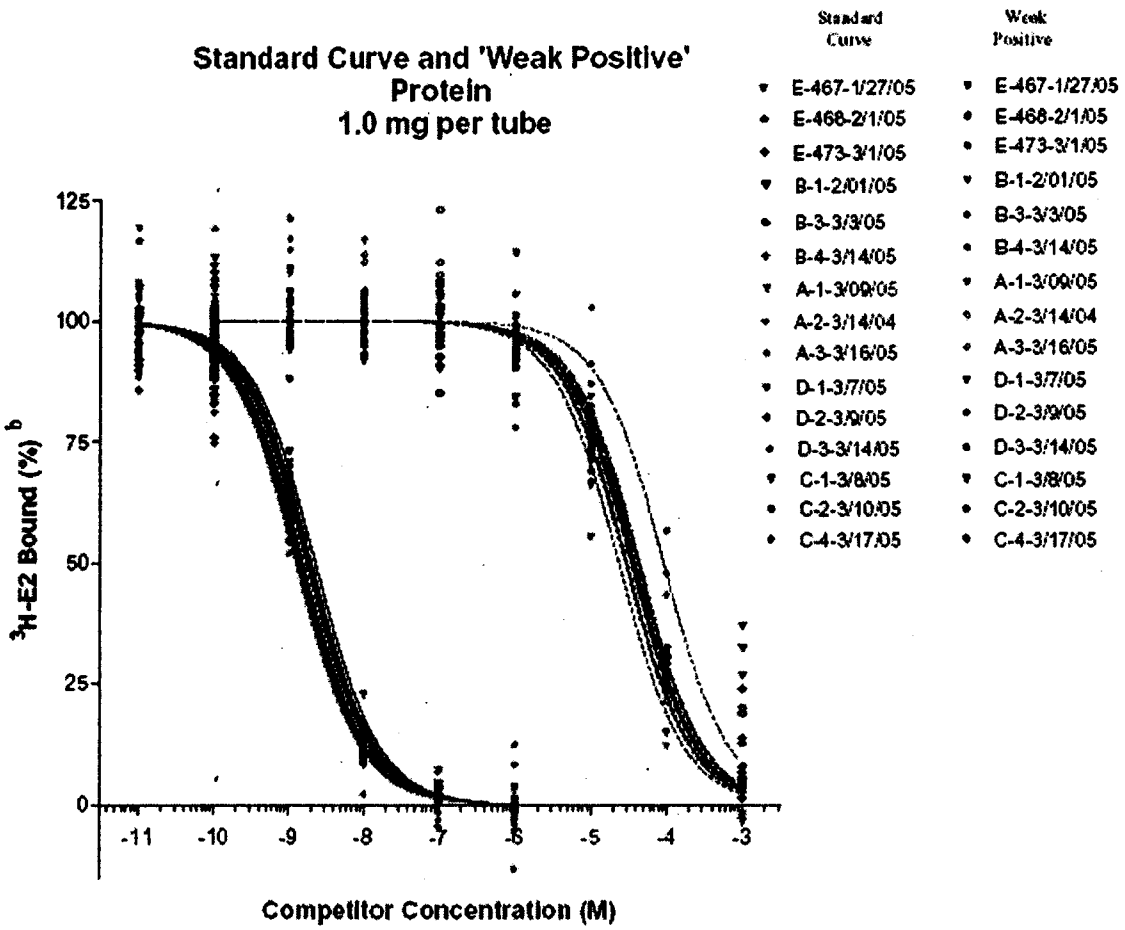
GraphPad Plot of Competitive Assay Results for IITRI (Lab A)



Appendix C (cont.)

Figure C-2

GraphPad Plot of Competitive Assay Results for Five Institutes [a]



[a] IITRI served as Lab A and the Sponsor as Lab E
[b] Y axis should be ^{3}H-R1881 (%) instead of ^{3}H-E2 (%)