

25 Jan 2009

Figure 3.17 Antagonist Comprehensive Testing for H0019¹(Retest)

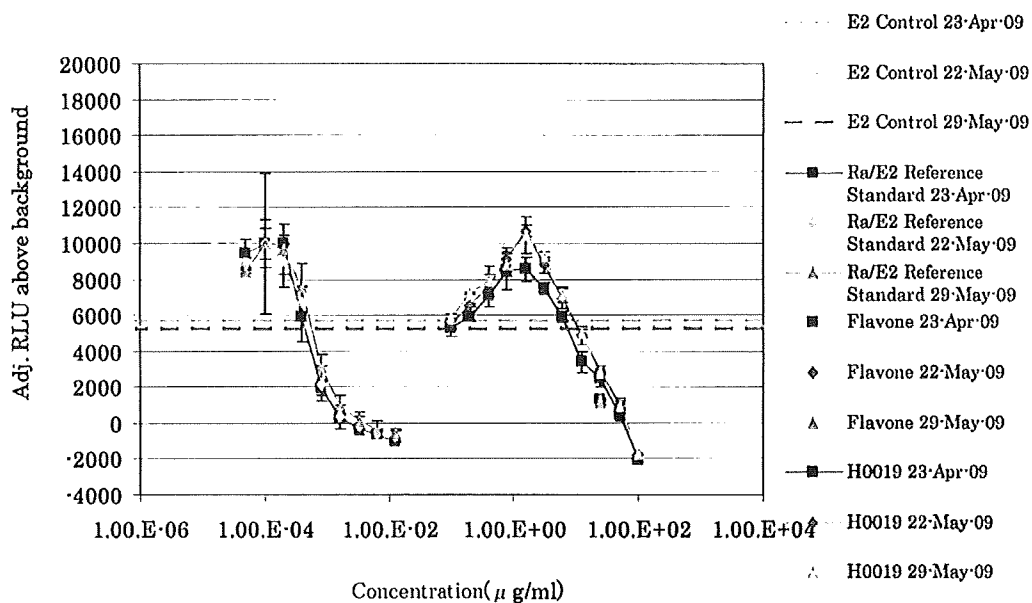
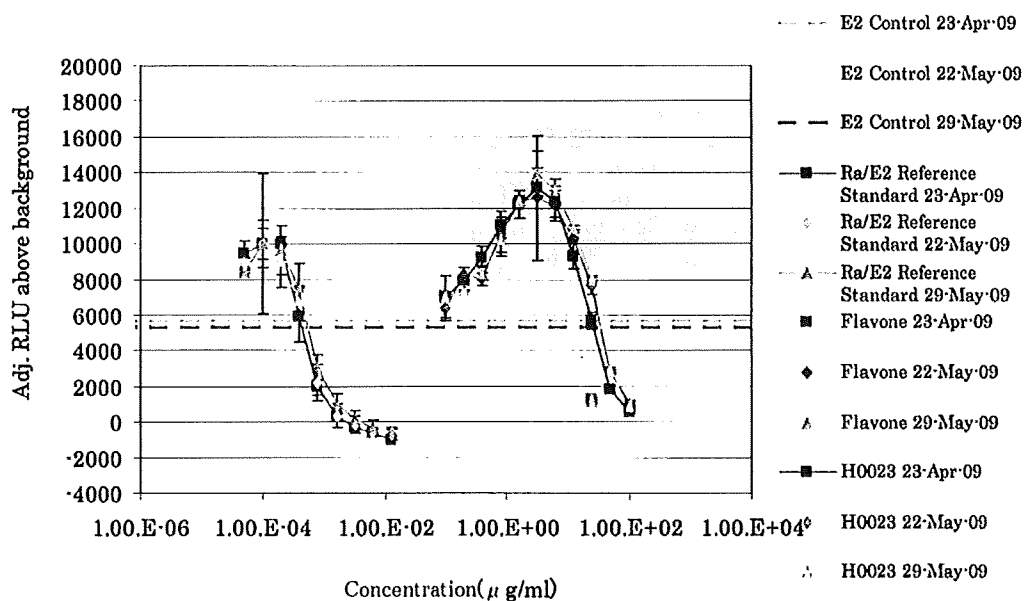


Figure 3.18 Antagonist Comprehensive Testing for H0023¹(Retest)



¹ Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

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3.0 PROBLEMS ENCOUNTERED

- The agonist test failed since 4 plates out of 16 plates did not pass the criteria. The Antagonist test failed since 2 plates out of 14 plates did not pass the criteria. The criteria for Phase IIb are shown above. The major reason for the failures was because of DMSO control and some of the well-created excessive activity, which lead to the result above the criteria. For Agonist test, excessive DMSO control will lead to judging the failure for E2 Reference Standard EC50 / Induction and for Antagonist test, same will lead to judging the failure for Ral/E2 Reference Standard IC50. It might be necessary to use less subculture cell even if it was less than 50 times.
- Following shows the criteria for Phase Ib. Usually the criteria are set for each phase but it is necessary to set and maintain common criteria value among multiple institutes.

Table 9 List of Criteria in Phase II b

Agonist	Phase IIb, Criteria
Methoxychlor Control	>DMSO mean+3S.D.
E2 Reference Standard EC50	7.1E-7-4.7E-6
DMSO Control	428-8119
Induction	>3times

Antagonist	Phase II b
Flavone/E2 Control	<E2 Control mean-3S.D.
E2 Control	3333-8399
Ral/E2 Reference Standard IC50	2.9E-4-9.8E-4
DMSO Control	428-8119
Reduction	>3times

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- Below chart shows triplicate EC₅₀ and IC₅₀ result of test substances.

Table 10 List of triplicate EC₅₀ and IC₅₀ result of test substances

Code	1st	2nd	3rd	Ave.	S.D.	C.V.
H0009	0.83	0.99	0.43	0.75	0.29	38
H0010	0.27	0.26	0.21	0.25	0.032	13
H0011	3.0E-06	1.9E-06	2.6E-06	2.5E-06	5.9E-07	24
H0012	0.49	0.79	0.41	0.56	0.20	36
H0013	11	18	25	18	7.3	40
H0014	0.051	0.16	0.14	0.12	0.058	49
H0015	positive	negative	negative	-	-	-
H0016	1.4	1.3	8.1	3.6	3.95	110
H0017	positive	negative	positive	-	-	-
H0018	positive	negative	positive	-	-	-
H0019	negative	negative	negative	-	-	-
H0020	negative	positive	positive	-	-	-
H0021	550	28	18	200	300	150
H0022	25	26	27	26	0.9	3.5
H0023	negative	negative	negative	-	-	-
H0024	positive	positive	positive	-	-	-
H0019*	12	26	15	18	6.0	34
H0023*	0.21	0.10	0.40	0.24	0.12	52

* Redistributed chemicals

We need to decide up to which level of variability (C.V.) is good and acceptable. I also felt that we need protocols to confirm that the result from Graph pad prism is appropriate, and the determination of the data deleted.

4.0 MATERIALS AND EQUIPMENT USED

- For luminometer, we used Centro LB 960, BERTHOLD TECHNOLOGIES, Germany
- Used Lot:5-1 #30th.~#43th., Lot:6 #16th.~#21th. subculture cell
- List of chemicals used are shown in Table 1,2,7 as Phase II b

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Table 11 List of chemicals used in Phase II b

Item	Chemical Name	Product Name	Manufacture	Code No.
RPMI Adjusted Media	RPMI Media	RPMI 1640 medium, with L-glutamine	NAKARAI	Code 30264-85
	FBS	Fetal Bovine Serum, UCDA Qualified	Mediatech, Inc	Cat. No. 35-010-CU
	P/S Solution	Penicillin/streptomycin solution, 5000 I.U. Penicillin, 5000 µg/ml	NAKARAI	Code 26252-94
DMEM Adjusted Media	DMEM Media	Dulbecco's Modification of Eagle's Medium (DMEM), (1×Solution) without L-Glutamine, phenol red.	MP Biomedicals inc	CAT NO: 16427749
	Carbon Stripped FBS	Fetal Bovine Serum, Charcoal/Dextran Treated, triple 0.1 µm sterile filtered	HYCLONE	Cat No. SH30068.03
	P/S Solution	Penicillin/streptomycin solution, 5000 I.U. Penicillin, 5000 µg/ml	MP Biomedicals inc	Code 26252-94
	L-Glutamine	Glutamine for Sterilized freeze dry for tissue cultivation	Nissui Pharmaceutical Co., Ltd.	Code 05908
Control	DMSO	Dimethyl sulfoxide 99.9%, HPLC grade	SIGMA ALDRICH	Cat#: D8418
Culture	PBS	Dulbecco's Phosphate Buffered saline(-) without Calcium and Magnesium	NAKARAI	Code 14249-95
	G418	G418	INVIVOGEN	#ant-gn-1
	Trypsin	0.25% Trypsin Colorless liquid, Filtration sterilization completion	NAKARAI	Code 3555-54
Assay	Lysis Buf.	Cell Culture Lysis Reagent 5X	Promega	Cat. #E153A
	Substances	Luciferase Assay System	Promega	Cat. #E1501

5.0 DEVIATIONS FROM PROTOCOL

Protocol was revised in Phase IIb and it became slightly complicated but there seems to be no problem using it. We hope to continue Phase I I I with the same protocol.

6.0 DISCUSSION

Three times repeated result of Agonist H0009 to H0016 and Antagonist H0017 to H0024 were good. I felt it is necessary to set clear criteria for omitting data or using Hill function for sample solution.

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PHASE II b .REPORT APPROVAL

This report has been reviewed and approved by the following:

Sponsor Representative

Atsushi Ono _____

小野 敦

23.2.2010

Co-chairficer

Signature

Date

JaCVAM

Study Director

The study will be conducted to the standards of.

Masafumi Nakamura _____

中村 昌文

23.2.2010

Study Director

Signature

Date

Hiyoshi Corporation

Review

Hajime Kojima _____

小島 肇

23.2.2010

Quality Assurance Officer

Signature

Date

JaCVAM

DRAFT REPORT :**LUMI-CELL ER VALIDATION STUDY – PHASE III****Author:Masafumi Nakamura****Testing Facility:** Hiyoshi Corporation / 908 Kitanoshocho Omihachiman Shiga,Japan**Experimental Start Date** : 20-Oct-08**Experimental End Date** : 06-Nov-09**Archive Location:** Hiyoshi Corporation / 908, Kitanoshocho Omihachiman, Shiga, Japan**Study Director:** Masafumi Nakamura, Hiyoshi Corporation**Key Personnel:**International Study Management TeamNICEATM

- Dr. William Stokes (NICEATM/NIEHS) – Co-Chair/Project Officer
- Dr. Raymond Tice (NICEATM/NIEHS) – Co-Chair
- Dr. David Allen (NICEATM/ILS) – NICEATM Principal Investigator
- Mr. Frank Deal (NICEATM/ILS) – Project Coordinator
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ECVAM

- Dr. Susanne Bremer

JaCVAM

- Dr. Hajime Kojima
- Dr. Atsushi Ono

Laboratory Personnel

- Mr. Hiroshi Murata – Facility Management
- Mr. Masafumi Nakamura – Study Director/Safety Officer
- Dr. Hajime Kojima – Director of Quality Assurance
- Mr. Tsukasa Yamamoto – Consultant
- Mr. Hiroshi Handa – Laboratory Technician

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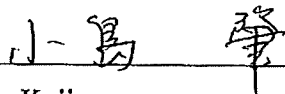
QUALITY ASSURANCE STATEMENT

DRAFT REPORT 1: LUMI-CELL ER VALIDATION STUDY – PHASE III

Listed below are the Phase III procedures performed by Hiyoshi Corporation that were inspected and audited by the Quality Assurance Unit during the study described in the report. Findings were reported to the study director and management periodically.

Procedures	Inspection/ Audit Date	Date Study Director Notified
Agonist testing for Phase III	18, 9, 2009	16, 12, 2009
Antagonist testing for Phase III	18, 9, 2009	16, 12, 2009

The results presented in this audited final report accurately reflect the raw data.



Hajime Kojima,
Quality Assurance Officer

23 2, 2010
Date

EXECUTIVE SUMMARY

Hiyoshi has completed Phase III of international validation study of an estrogen receptor (ER) transcriptional activation (TA) assay (LUMI-CELL® ER assay) for the detection of ER agonists and antagonists designed by NICEATM, ECVAM and JaCVAM. In the Phase III, various independent tests were done for reference standards, controls, and test substances (40 Agonist test substances and 40 Antagonist test substances) using common protocol to evaluate ability of LUMI-CELL® ER. The result of reference standards, controls, and test substances tests will be used for establishing Phase III protocol and the historical database. The test result will be sent to Validation Study Project Coordinator and intralaboratory repeatability and intra- and inter-laboratory reproducibility will be evaluated.

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1.0 METHODS

The sponsor provided the BG1Luc4E2 (BG-1) cells for cell viability, agonist, and antagonist testing.

1.1 Agonist Materials

The sponsor provided the following test materials:

- H0025~H0051, H0053~H0065

Two substances of the above-mentioned test materials were exposed in one plate.

Table 1 Materials Received for Agonist Testing

Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments
H0025	Solid	Room temp	20-Oct-08	M.Nakamura	
H0026	Solid	Store under nitrogen, room temp	05-Nov-08	M.Nakamura	
H0027	Solid	Room temp	17-Nov-08	M.Nakamura	
H0028	Solid	Room temp	17-Nov-08	M.Nakamura	
H0029	Solid	Room temp	20-Oct-08	M.Nakamura	
H0030	Solid	Room temp	20-Oct-08	M.Nakamura	
H0031	Solid	Room temp	05-Nov-08	M.Nakamura	
H0032	Solid	Room temp	05-Nov-08	M.Nakamura	
H0033	Solid	Room temp	02-Feb-09	M.Nakamura	
H0034	Solid	Store at 4 degrees C	17-Nov-08	M.Nakamura	
H0035	Solid	Room temp	13-Jan-09	M.Nakamura	
H0036	Solid	Room temp	27-Oct-08	M.Nakamura	
H0037	Solid	Room temp	13-Jan-09	M.Nakamura	
H0038	Solid	Room temp	13-Jan-09	M.Nakamura	
H0039	Solid	Room temp	20-Oct-08	M.Nakamura	
H0040	Solid	Room temp, in dark	21-Nov-08	M.Nakamura	
H0041	Solid	Room temp	08-Nov-08	M.Nakamura	
H0042	Solid	Room temp	20-Oct-08	M.Nakamura	
H0043	Solid	Room temp	17-Nov-08	M.Nakamura	
H0044	Solid	Room temp	13-Jan-09	M.Nakamura	
H0045	Solid	Store at -20 degrees C	08-Nov-08	M.Nakamura	
H0046	Solid	Room temp	17-Nov-08	M.Nakamura	
H0047	Solid	Room temp	20-Oct-08	M.Nakamura	
H0048	Solid	Store at 4 degrees C	02-Feb-09	M.Nakamura	
H0049	Solid	Store at 4 degrees C	05-Nov-08	M.Nakamura	
H0050	Solid	Room temp	27-Oct-08	M.Nakamura	

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Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments
H0051	Solid	Store at -20 degrees C	21-Nov-08	M.Nakamura	
H0053	Solid	Store at 4 degrees C	05-Nov-08	M.Nakamura	
H0054	Solid	Room temp	27-Oct-08	M.Nakamura	
H0055	Solid	Room temp	02-Fev-09	M.Nakamura	
H0056	Solid	Room temp	02-Fev-09	M.Nakamura	
H0057	Solid	Room temp	02-Fev-09	M.Nakamura	
H0058	Solid	Room temp	05-Nov-08	M.Nakamura	
H0059	Solid	Store at -20 degrees C	17-Nov-08	M.Nakamura	
H0060	Solid	Room temp	21-Nov-08	M.Nakamura	
H0061	Solid	Room temp	21-Nov-08	M.Nakamura	
H0062	Solid	Room temp	27-Oct-08	M.Nakamura	
H0063	Liquid	Room temp	27-Oct-08	M.Nakamura	
H0064	Solid	Store at 4 degrees C	27-Oct-08	M.Nakamura	
H0065	Solid	Store at 4 degrees C	21-Nov-08	M.Nakamura	

1.2 Antagonist Materials

The sponsor provided the following test materials:

- H0034, H0043, H0048, H0056, H0059
- H0066~H0093, H0095~H0106

Two substances of the above-mentioned test materials were exposed in one plate.

Table 2 Materials Received for Antagonist Testing

Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments
H0034	Solid	Store at 4 degrees C	Received for Agonist test		
H0043	Solid	Room temp	Received for Agonist test		
H0048	Solid	Store at 4 degrees C	Received for Agonist test		
H0056	Solid	Room temp	Received for Agonist test		
H0059	Solid	Store at -20 degrees C	Received for Agonist test		
H0066	Solid	Room temp	20-Oct-08	M.Nakamura	
H0067	Solid	Store under nitrogen, room temp	05-Nov-08	M.Nakamura	
H0068	Solid	Room temp	17-Nov-08	M.Nakamura	
H0069	Solid	Room temp	17-Nov-08	M.Nakamura	
H0070	Solid	Room temp	20-Oct-08	M.Nakamura	

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Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments
H0071	Solid	Room temp	20-Oct-08	M.Nakamura	
H0072	Solid	Room temp	08-Nov-08	M.Nakamura	
H0073	Solid	Room temp	08-Nov-08	M.Nakamura	
H0074	Solid	Room temp	05-Nov-08	M.Nakamura	
H0075	Solid	Room temp	05-Nov-08	M.Nakamura	
H0076	Solid	Room temp	02-Fev-09	M.Nakamura	
H0078	Solid	Room temp	13-Jan-09	M.Nakamura	
H0079	Solid	Room temp	27-Oct-08	M.Nakamura	
H0080	Solid	Room temp	13-Jan-09	M.Nakamura	
H0081	Solid	Room temp	13-Jan-09	M.Nakamura	
H0082	Solid	Room temp	20-Oct-08	M.Nakamura	
H0084	Solid	Room temp, in dark	08-Nov-08	M.Nakamura	
H0085	Solid	Room temp	08-Nov-08	M.Nakamura	
H0086	Solid	Room temp	20-Oct-08	M.Nakamura	
H0088	Solid	Room temp	13-Jan-09	M.Nakamura	
H0089	Solid	Room temp	17-Nov-08	M.Nakamura	
H0090	Solid	Room temp	20-Oct-08	M.Nakamura	
H0091	Solid	Room temp	21-Nov-08	M.Nakamura	
H0092	Solid	Store at 4 degrees C	05-Nov-08	M.Nakamura	
H0093	Solid	Room temp	27-Oct-08	M.Nakamura	
H0095	Solid	Store at 4 degrees C	05-Nov-08	M.Nakamura	
H0096	Solid	Room temp	08-Nov-08	M.Nakamura	
H0097	Solid	Room temp	02-Fev-09	M.Nakamura	
H0099	Solid	Room temp	02-Fev-09	M.Nakamura	
H0100	Solid	Room temp	05-Nov-08	M.Nakamura	
H0102	Solid	Room temp	21-Nov-08	M.Nakamura	
H0103	Solid	Room temp	21-Nov-08	M.Nakamura	
H0104	Solid	Room temp	27-Oct-08	M.Nakamura	
H0105	Liquid	Room temp	27-Oct-08	M.Nakamura	
H0106	Solid	Store at 4 degrees C	27-Oct-08	M.Nakamura	

The laboratory purchased all other materials specified in the agonist and antagonist protocols and quality tested as specified in the protocols.

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2.0 RESULTS

2.1 Range Finding Testing:

- The result of the Substance solubility was as follows;

Agonist

Results 081022

- H0025 100mg/ml in DMSO, 1mg/ml in DMSO/aqueous cell culture media
- H0029 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0030 100mg/ml in DMSO, 1mg/ml in DMSO/aqueous cell culture media
- H0039 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0042 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0047 100mg/ml in DMSO, 1mg/ml in DMSO/aqueous cell culture media

Results 081031

- H0036 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0050 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0054 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0062 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0063 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0064 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

Results 090124

- H0026 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0031 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0032 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0049 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0053 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0058 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

Results 090129-1

- H0041 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0045 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media

Results 090129-2

- H0027 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0028 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0034 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0043 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0046 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media

Results 090124

- H0040 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0051 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0060 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0061 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0065 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

Results 090214

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- H0035 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0037 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0038 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0044 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

Results 090219

- H0033 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0048 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0055 100mg/ml in DMSO, 1mg/ml in DMSO/aqueous cell culture media
- H0056 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0057 10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0059 1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

Antagonist

Results 081031

- H0079 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0093 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0104 200mg/ml in DMSO, 2mg/ml in DMSO/aqueous cell culture media
- H0105 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0106 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media

Results 090124

- H0067 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0074 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0075 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0092 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0095 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0100 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media

Results 090129-1

- H0072 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0073 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0084 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0085 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0096 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media

Results 090129-2

- H0034 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0043 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0068 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0069 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0089 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media

Results 090205

- H0091 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0102 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0103 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media

Results 090214

- H0078 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media

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- H0080 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
 - H0081 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
 - H0088 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- Results 090219
- H0045 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
 - H0056 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
 - H0059 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
 - H0076 2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture med
 - H0097 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
 - H0099 20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media

Table 3 Table for Range Finding Concentrations Tested and Cell Viability

Agonist

Recorded only Cell Viability Results score was above 2

Substance Code	Concentrations Tested (final) (µg/mL)	Cell Viability Results
AgRF 1 (H0030)	$1.00 \times 10^{+3}$	3
	$1.00 \times 10^{+2}$	1
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
AgRF 2 (H0036)	$1.00 \times 10^{+2}$	3
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
AgRF 3 (H0026)	$1.00 \times 10^{+2}$	3
	$1.00 \times 10^{+1}$	1

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Substance Code	Concentrations Tested (final) ($\mu\text{g/mL}$)	Cell Viability Results
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
AgRF 5 (H0034)	$1.00 \times 10^{+1}$	4
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AgRF 6 (H0061)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AgRF 6 (H0065)	$1.00 \times 10^{+1}$	4
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AgRF 7	$1.00 \times 10^{+2}$	2

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Substance Code	Concentrations Tested (final) ($\mu\text{g/mL}$)	Cell Viability Results
(H0048)	$1.00 \times 10^{+1}$	2
	1.00×10^0	2
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
AgRF 7 (H0055)	$1.00 \times 10^{+3}$	2
	$1.00 \times 10^{+2}$	1
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1

Antagonist

Recorded only Cell Viability Results score was above 2.

Substance Code	Concentrations Tested (final) ($\mu\text{g/mL}$)	Cell Viability Results
AntRF 1 (H0066)	$1.00 \times 10^{+3}$	3
	$1.00 \times 10^{+2}$	1
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
AntRF 1 (H0071)	$1.00 \times 10^{+3}$	3
	$1.00 \times 10^{+2}$	1
	$1.00 \times 10^{+1}$	1

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Substance Code	Concentrations Tested(final) ($\mu\text{g/mL}$)	Cell Viability Results
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
AntRF 2 (H00104)	$1.00 \times 10^{+3}$	3
	$1.00 \times 10^{+2}$	1
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
AntRF 3 (H0067)	$1.00 \times 10^{+2}$	4
	$1.00 \times 10^{+1}$	1
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
AntRF 4 (H0084)	$1.00 \times 10^{+1}$	4
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 4 (H0085)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1

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Substance Code	Concentrations Tested(final) ($\mu\text{g/mL}$)	Cell Viability Results
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 5 (H0068)	$1.00 \times 10^{+1}$	4
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 5 (H0069)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 6 (H0102)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 6	$1.00 \times 10^{+1}$	2

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Substance Code	Concentrations Tested(final) ($\mu\text{g/mL}$)	Cell Viability Results
(H0103)	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 7 (H0080)	$1.00 \times 10^{+1}$	4
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
AntRF 7 (H0081)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1
AntRF 8 (H0059)	$1.00 \times 10^{+1}$	2
	1.00×10^0	1
	1.00×10^{-1}	1
	1.00×10^{-2}	1
	1.00×10^{-3}	1
	1.00×10^{-4}	1
	1.00×10^{-5}	1