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2.2 Comprehensive Testing:

Table 5 Concentrations Tested and Cell Viability Table

Agonist:plate1

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0009	$1.00 \times 10^{+1}$	1
	2.00×10^0	1
	4.00×10^{-1}	1
	8.00×10^{-2}	1
	1.60×10^{-2}	1
	3.20×10^{-3}	1
	6.40×10^{-4}	1
	1.28×10^{-4}	1
	2.56×10^{-5}	1
	5.12×10^{-6}	1
	1.02×10^{-7}	1
	H0010	$1.00 \times 10^{+1}$
2.00×10^0		1
4.00×10^{-1}		1
8.00×10^{-2}		1
1.60×10^{-2}		1
3.20×10^{-3}		1
6.40×10^{-4}		1
1.28×10^{-4}		1
2.56×10^{-5}		1
5.12×10^{-6}		1
1.02×10^{-7}		1

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Antagonist;plate2

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0011	1.00×10^{-4}	1
	2.00×10^{-5}	1
	4.00×10^{-6}	1
	8.00×10^{-7}	1
	1.60×10^{-7}	1
	3.20×10^{-8}	1
	6.40×10^{-9}	1
	1.28×10^{-9}	1
	2.56×10^{-10}	1
	5.12×10^{-11}	1
	1.02×10^{-11}	1
H0012	$1.00 \times 10^{+2}$	1
	$2.00 \times 10^{+1}$	1
	4.00×10^0	1
	8.00×10^{-1}	1
	1.60×10^{-1}	1
	3.20×10^{-2}	1
	6.40×10^{-3}	1
	1.28×10^{-3}	1
	2.56×10^{-4}	1
	5.12×10^{-5}	1
	1.02×10^{-5}	1

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Agonist;plate3

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0013	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×10^0	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1
H0014	$1.00 \times 10^{+2}$	1
	$2.00 \times 10^{+1}$	1
	4.00×10^0	1
	8.00×10^{-1}	1
	1.60×10^{-1}	1
	3.20×10^{-2}	1
	6.40×10^{-3}	1
	1.28×10^{-3}	1
	2.56×10^{-4}	1
	5.12×10^{-5}	1
	1.02×10^{-5}	1

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Agonist;plate4

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0015	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×10^0	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1
H0016	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×10^0	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1

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Antagonist;plate1

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0017	$1.00 \times 10^{+1}$	1
	5.00×10^0	1
	2.50×10^0	1
	1.25×10^0	1
	6.25×10^{-1}	1
	3.13×10^{-1}	1
	1.56×10^{-1}	1
	7.81×10^{-2}	1
	3.91×10^{-2}	1
	1.95×10^{-2}	1
	9.77×10^{-3}	1
H0018	$1.00 \times 10^{+1}$	1
	5.00×10^0	1
	2.50×10^0	1
	1.25×10^0	1
	6.25×10^{-1}	1
	3.13×10^{-1}	1
	1.56×10^{-1}	1
	7.81×10^{-2}	1
	3.91×10^{-2}	1
	1.95×10^{-2}	1
	9.77×10^{-3}	1

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Antagonist;plate2

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0019	$1.00 \times 10^{+1}$	1
	5.00×10^0	1
	2.50×10^0	1
	1.25×10^0	1
	6.25×10^{-1}	1
	3.13×10^{-1}	1
	1.56×10^{-1}	1
	7.81×10^{-2}	1
	3.91×10^{-2}	1
	1.95×10^{-2}	1
	9.77×10^{-3}	1
H0020	$1.00 \times 10^{+1}$	1
	5.00×10^0	1
	2.50×10^0	1
	1.25×10^0	1
	6.25×10^{-1}	1
	3.13×10^{-1}	1
	1.56×10^{-1}	1
	7.81×10^{-2}	1
	3.91×10^{-2}	1
	1.95×10^{-2}	1
	9.77×10^{-3}	1

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Antagonist;plate3

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0021	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×100	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1
H0022	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×100	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1

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Antagonist;plate4

Substance Code	Concentrations Tested (Final) ($\mu\text{g/ml}$)	Cell Viability Results
H0023	$1.00 \times 10^{+1}$	1
	5.00×10^0	1
	2.50×10^0	1
	1.25×10^0	1
	6.25×10^{-1}	1
	3.13×10^{-1}	1
	1.56×10^{-1}	1
	7.81×10^{-2}	1
	3.91×10^{-2}	1
	1.95×10^{-2}	1
	9.77×10^{-3}	1
H0024	$1.00 \times 10^{+2}$	1
	$5.00 \times 10^{+1}$	1
	$2.50 \times 10^{+1}$	1
	$1.25 \times 10^{+1}$	1
	6.25×10^0	1
	3.13×100	1
	1.56×10^0	1
	7.81×10^{-1}	1
	3.91×10^{-1}	1
	1.95×10^{-1}	1
	9.77×10^{-2}	1

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Table 6.1 Summary of Experiments Template: Comprehensive Testing

Experiments: Phase IIb Comprehensive Testing; Agonist						
Experiment I.D.	Substance Code	Date	Plate Induction	EC ₅₀ (µg/mL)	Experiment Used for Data Analysis or Repeated?	Rationale for Unacceptability
CT 1-1	H0009	07-Nov-08	3.5	0.83	Used	Acceptable
	H0010			0.27	Used	Acceptable
CT 2-1	H0011	07-Nov-08	1.6	1.7x10 ⁻⁶	Repeated	Fail ¹
	H0012			NA	Repeated	Fail ¹
CT 3-1	H0013	07-Nov-08	4.1	1.1x10 ⁺¹	Used	Acceptable
	H0014			0.051 ²	Used	Acceptable
CT 4-1	H0015	07-Nov-08	2.2	21	Repeated	Fail ²
	H0016			0.80	Repeated	Fail ²
CT 1-2	H0009	21-Nov-08	4.3	0.99	Used	Acceptable
	H0010			0.26	Used	Acceptable
CT 2-2	H0011	21-Nov-08	4.9	3.0x10 ⁻⁶	Used	Acceptable
	H0012			0.49 ²	Used	Acceptable
CT 3-2	H0013	21-Nov-08	4.5	1.9x10 ⁺¹	Repeated	Pass
	H0014			0.12	Repeated	Fail ⁴
CT 4-2	H0015	21-Nov-08	4.2	Positive ⁵	Used	Acceptable
	H0016			1.4 ²	Used	Acceptable
CT 1-3	H0009	04-Dec-08	3.1	0.43	Used	Acceptable
	H0010			0.21	Used	Acceptable
CT 2-3	H0011	04-Dec-08	3.1	1.9x10 ⁻⁶	Used	Acceptable
	H0012			0.79 ²	Used	Acceptable
CT 3-3	H0013	04-Dec-08	3.0	1.5	Repeated	Fail ⁶
	H0014			0.0050	Repeated	Fail ⁶
CT 4-3	H0015	04-Dec-08	3.0	Negative	Used	Acceptable
	H0016			1.3 ²	Used	Acceptable
CT 2-4	H0011	19-Dec-08	4.7	2.6x10 ⁻⁶	Used	Acceptable
	H0012			0.41 ²	Used	Acceptable
CT 3-4	H0013	19-Dec-08	4.8	1.9x10 ⁺¹	Used	Acceptable
	H0014			0.16 ²	Used	Acceptable
CT 4-4	H0015	19-Dec-08	3.3	Negative	Used	Acceptable
	H0016			8.2 ²	Used	Acceptable

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Experiments: Phase IIb Comprehensive Testing; Agonist						
Experiment I.D.	Substance Code	Date	Plate Induction	EC ₅₀ (µg/mL)	Experiment Used for Data Analysis or Repeated?	Rationale for Unacceptability
CT 3-5	H0013	25-Dec-08	3.3	2.5x10 ⁺¹	Used	Acceptable
	H0014			0.14 ²	Used	Acceptable

NA = Not applicable, EC₅₀ values are not calculated for substances tested on plates not meeting acceptance criteria.

¹The reason for the failure is because 1) DMSO Control / E2 Reference Standard / Methoxychlor did not meet acceptance criteria and 2) Induction is lower than the criteria.

²Deleted some points for calculating EC50. The deleted points are shown in Figure 3.

³ The reason for the failure is because 1) DMSO Control / Methoxychlor is higher than the criteria and 2) Induction is lower than the criteria

⁴ The reason for the failure is because E2 Reference Standard curve did not show sigmoid shape.

⁵Positive for agonism at 25 x 10⁰ µg/mL

⁶ The reason for the failure is because DMSO Control is higher than the criteria.

Table 6.2 Summary of Experiments Template: Comprehensive Testing

Experiments: Phase IIb Comprehensive Testing; Antagonist						
Experiment I.D.	Substance Code	Date	Plate Reduction	IC ₅₀ (µg/mL)	Experiment Used for Data Analysis or Repeated?	Rationale for Unacceptability
CT 1-1	H0017	14-Nov-08	9.8	positive ¹	Used	Acceptable
	H0018			positive ²	Used	Acceptable
CT 2-1	H0019	14-Nov-08	8.8	negative	Used	Acceptable
	H0020			negative	Used	Acceptable
CT 3-1	H0021	14-Nov-08	13	550	Used	Acceptable
	H0022			25	Used	Acceptable
CT 4-1	H0023	14-Nov-08	13	negative	Used	Acceptable
	H0024			positive ³	Used	Acceptable
CT 1-2	H0017	29-Nov-08	8.2	negative	Repeated	Pass
	H0018			negative	Repeated	Fail ⁴
CT 2-2	H0019	29-Nov-08	10	negative	Repeated	Fail ⁴
	H0020			positive ⁵	Repeated	Fail ⁴

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Experiments: Phase IIb Comprehensive Testing; Antagonist						
Experiment I.D.	Substance Code	Date	Plate Reduction	IC ₅₀ (µg/mL)	Experiment Used for Data Analysis or Repeated?	Rationale for Unacceptability
CT 3-2	H0021	29-Nov-08	9.7	28	Used	Acceptable
	H0022			26	Used	Acceptable
CT 4-2	H0023	29-Nov-08	12	negative	Used	Acceptable
	H0024			positive ²	Used	Acceptable
CT 1-3	H0017	12-Dec-08	10	negative	Used	Acceptable
	H0018			negative	Used	Acceptable
CT 2-3	H0019	12-Dec-08	11	negative	Used	Acceptable
	H0020			positive ⁵	Used	Acceptable
CT 3-3	H0021	12-Dec-08	9.6	18	Used	Acceptable
	H0022			27	Used	Acceptable
CT 4-3	H0023	12-Dec-08	12	negative	Used	Acceptable
	H0024			positive ¹	Used	Acceptable
CT 1-4	H0017	19-Dec-08	8.4	positive ¹	Used	Acceptable
	H0018			positive ⁶	Used	Acceptable
CT 2-4	H0019	19-Dec-08	8.8	negative	Used	Acceptable
	H0020			positive ⁵	Used	Acceptable

¹ positive for antagonism at 1.0*10+1µg/mL & 5.0*10+0µg/mL

² positive for antagonism at 1.0*10+1µg/mL & 5.0*10+0µg/mL & 2.5*10+0µg/mL

³ positive for antagonism at 1.0*10+1µg/mL & 5.0*10+0µg/mL & 2.5*10+0µg/mL & 1.25*10+0µg/ml & 6.3*10-1µg/ml

⁴ for the failure is because Raloxifene/E2 Reference Standard IC50 is lower than the criteria.

⁵ positive for antagonism at 1.0*10+1µg/mL

⁶ positive for antagonism at 5.0*10+0µg/mL

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Figure 3.1 Agonist Comprehensive Testing for H0009¹

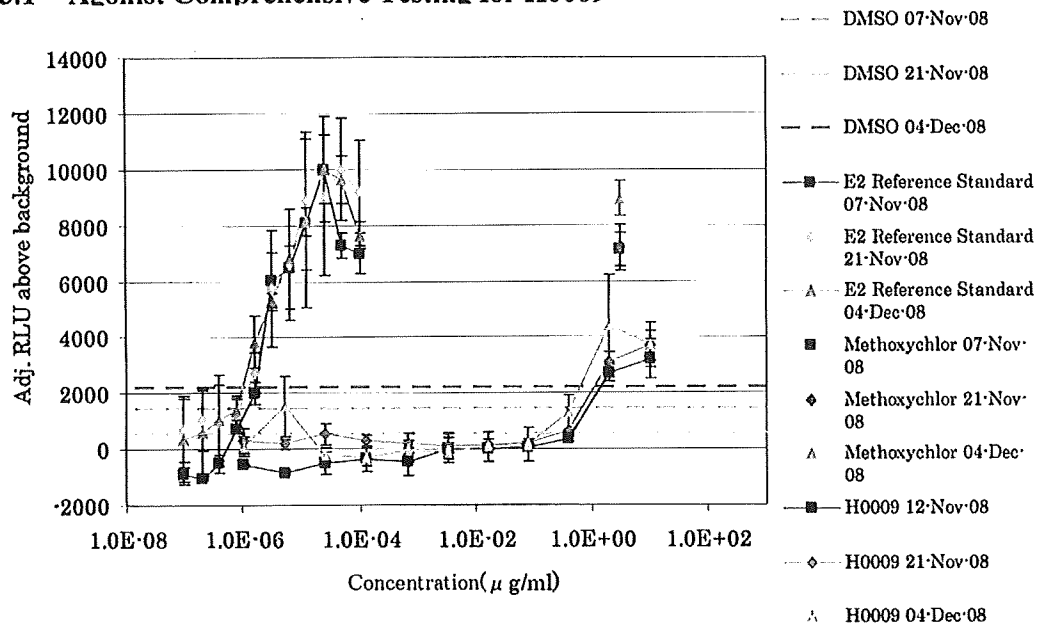
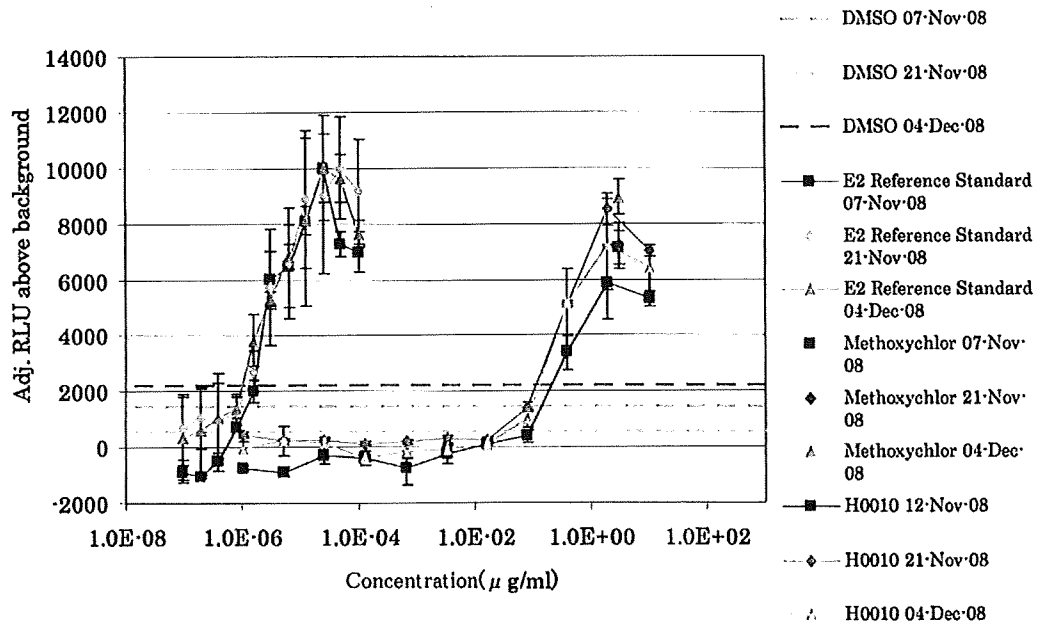


Figure 3.2 Agonist Comprehensive Testing for H0010¹



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

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Figure 3.3 Agonist Comprehensive Testing for H0011¹

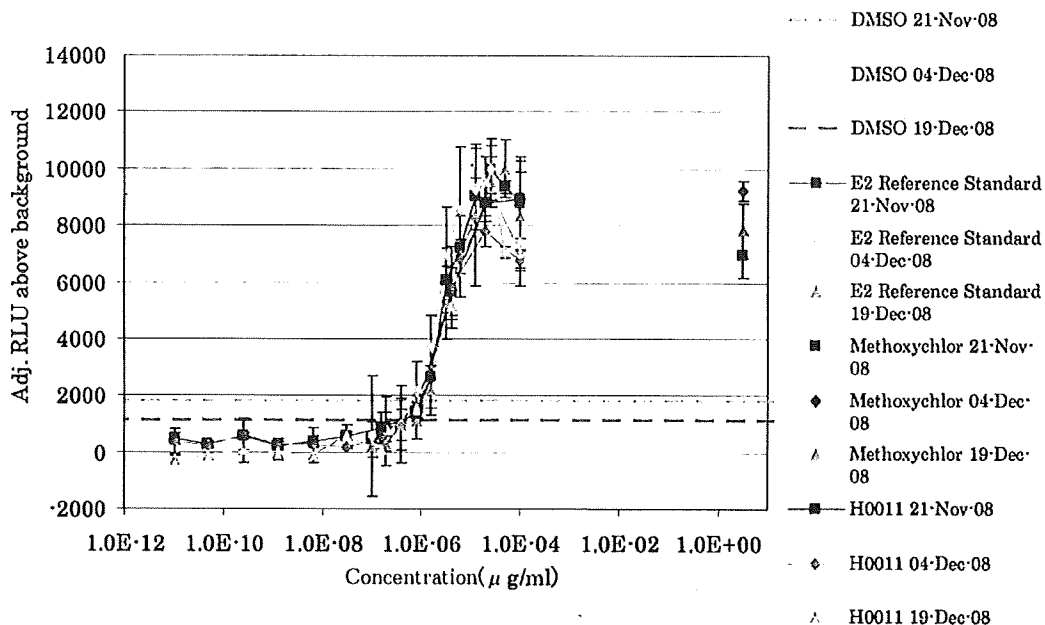
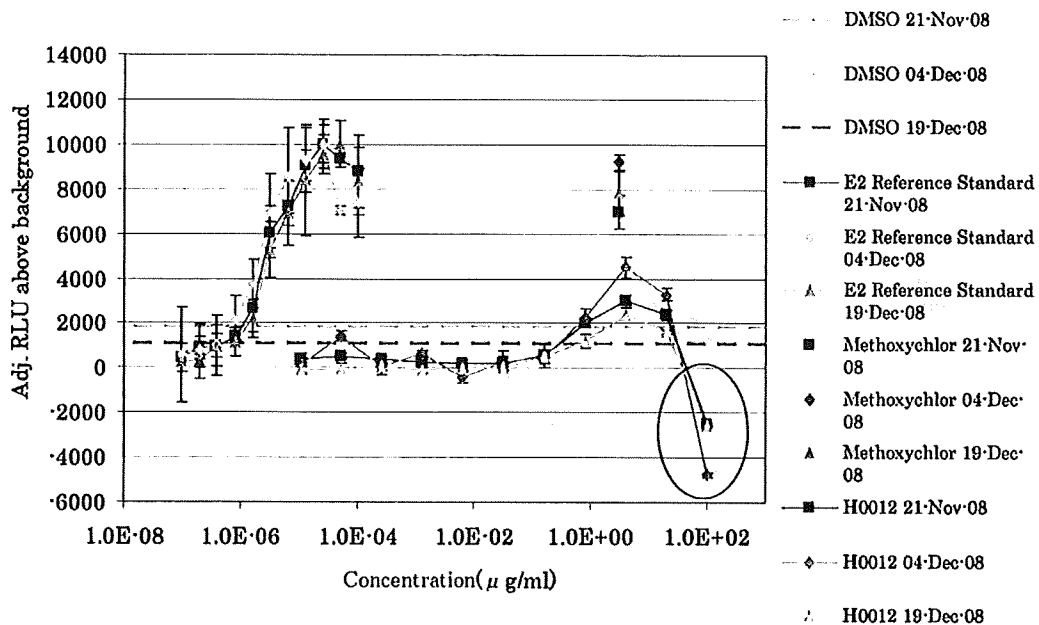


Figure 3.4 Agonist Comprehensive Testing for H0012¹



○ : Deleted 1 high concentration points to calculate EC₅₀ of H0012.

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

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Figure 3.5 Agonist Comprehensive Testing for H0013¹

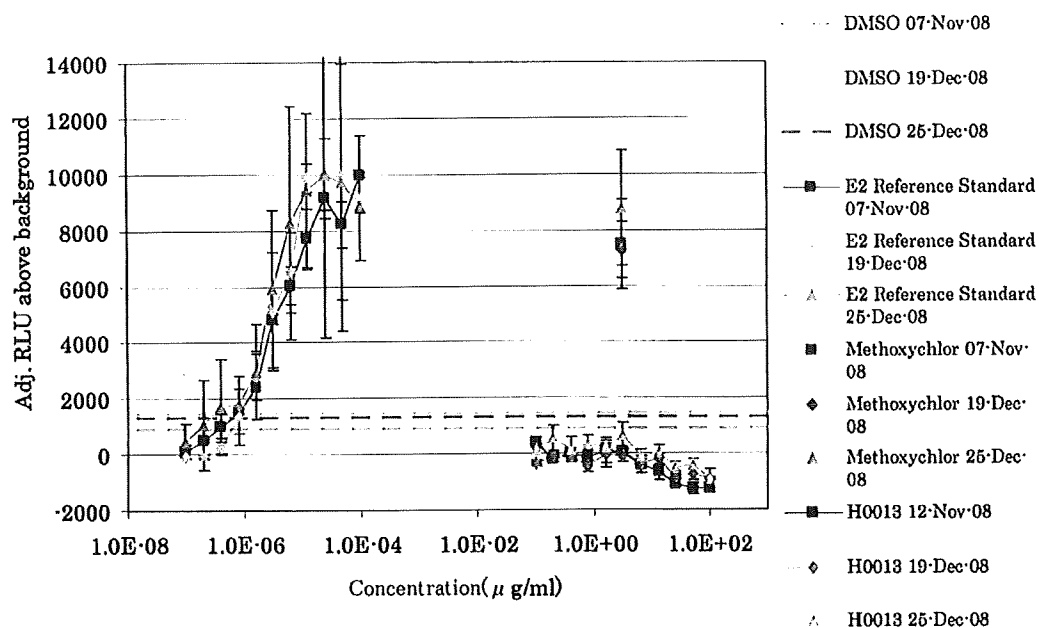
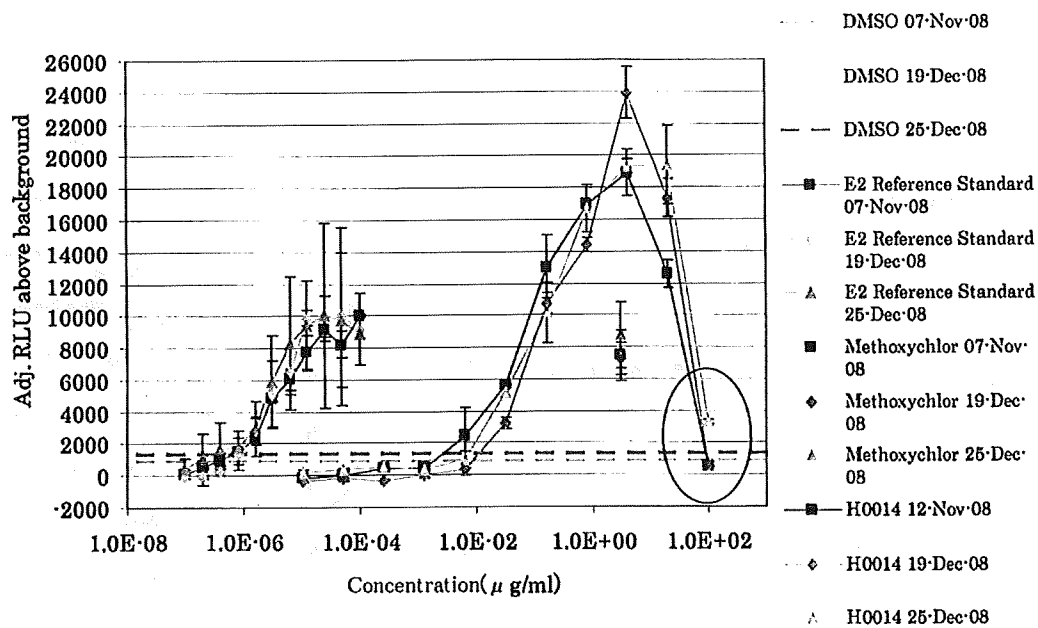


Figure 3.6 Agonist Comprehensive Testing for H0014¹



○ : Deleted 1 high concentration points to calculate EC₅₀ of H0014.

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

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Figure 3.7 Agonist Comprehensive Testing for H0015¹

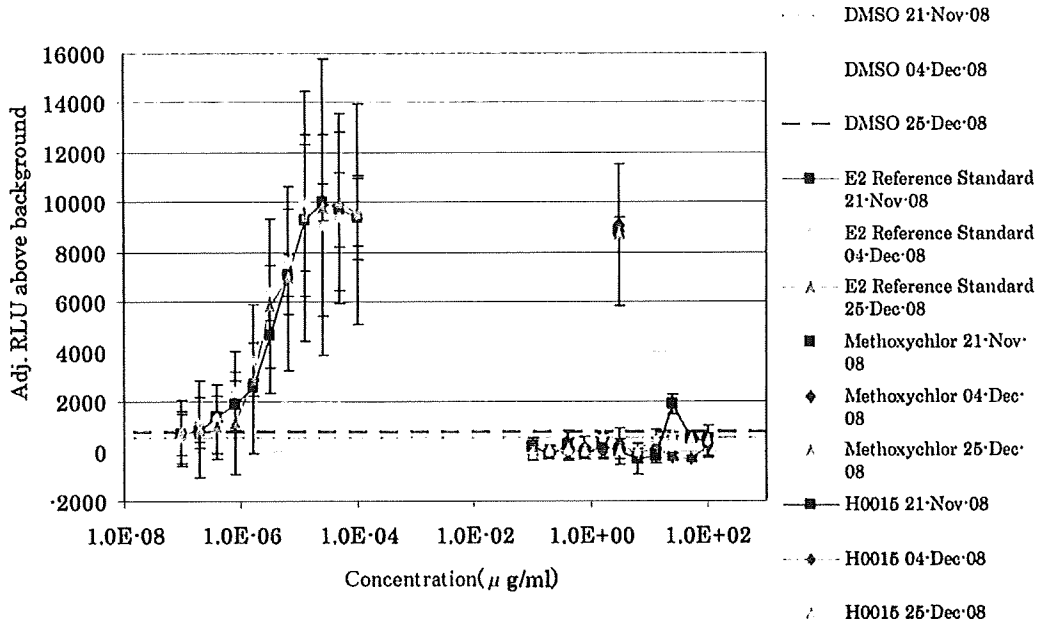
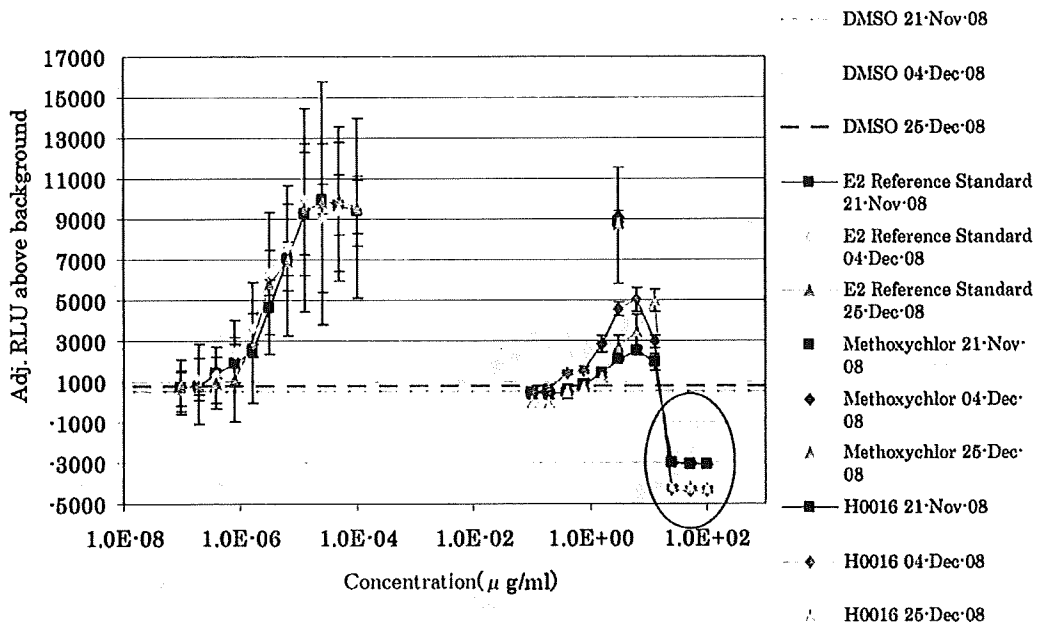


Figure 3.8 Agonist Comprehensive Testing for H0016¹



○ : Deleted 3 high concentration points to calculate EC₅₀ of H0016

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

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Figure 3.9 Antagonist Comprehensive Testing for H0017¹

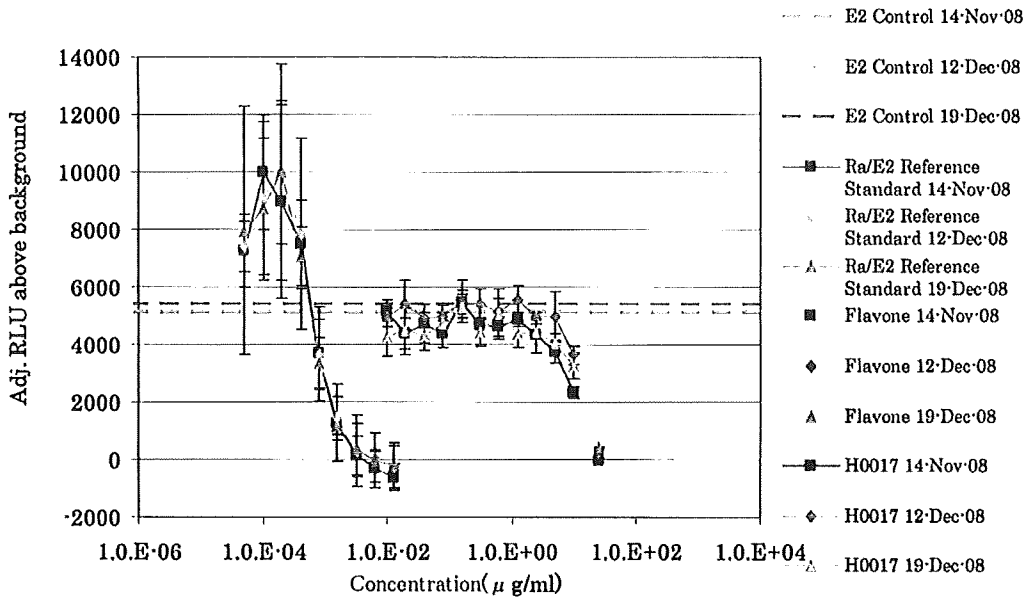
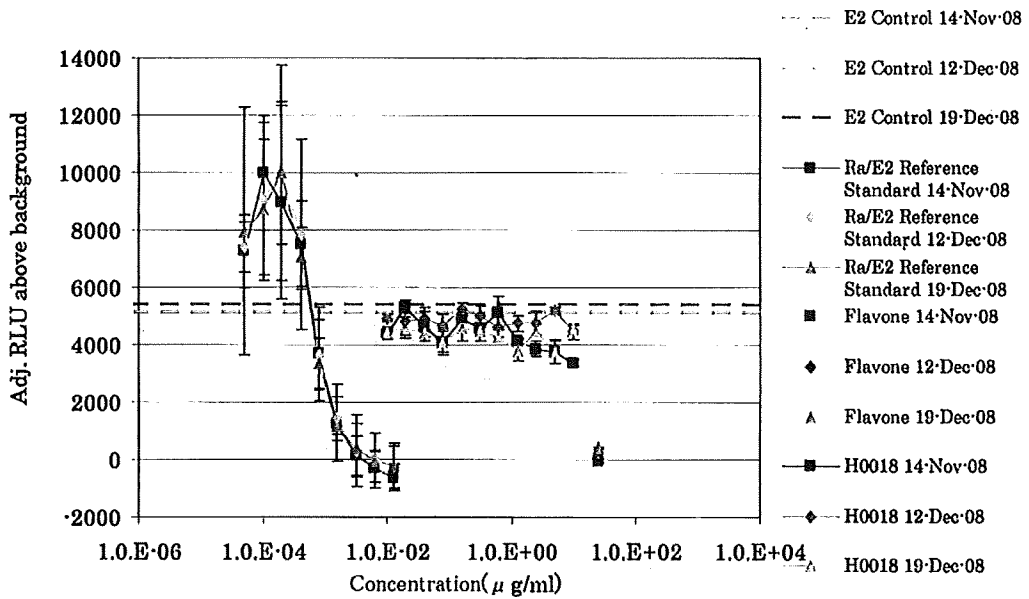


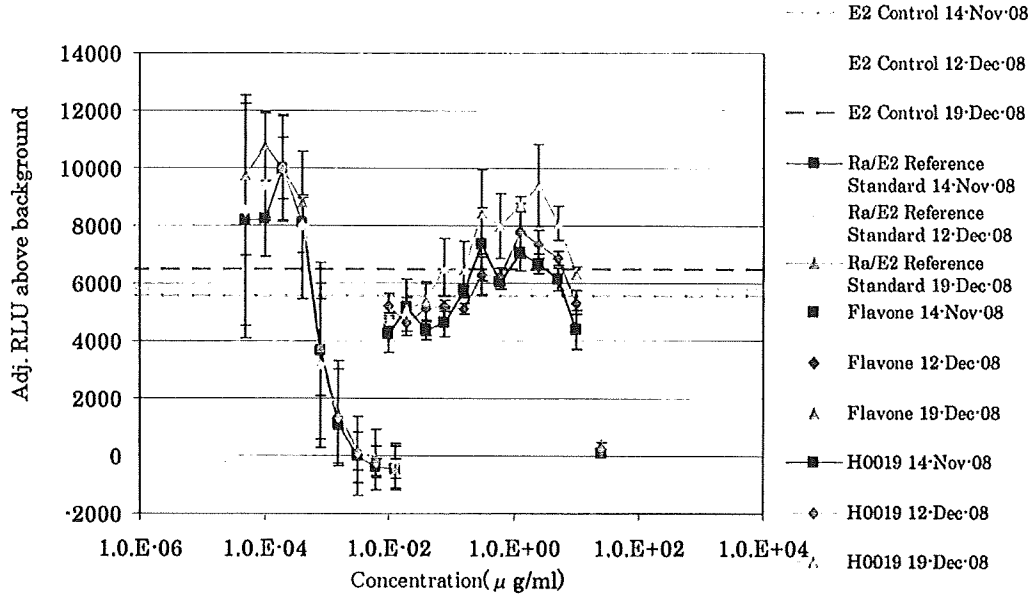
Figure 3.10 Antagonist Comprehensive Testing for H0018¹



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

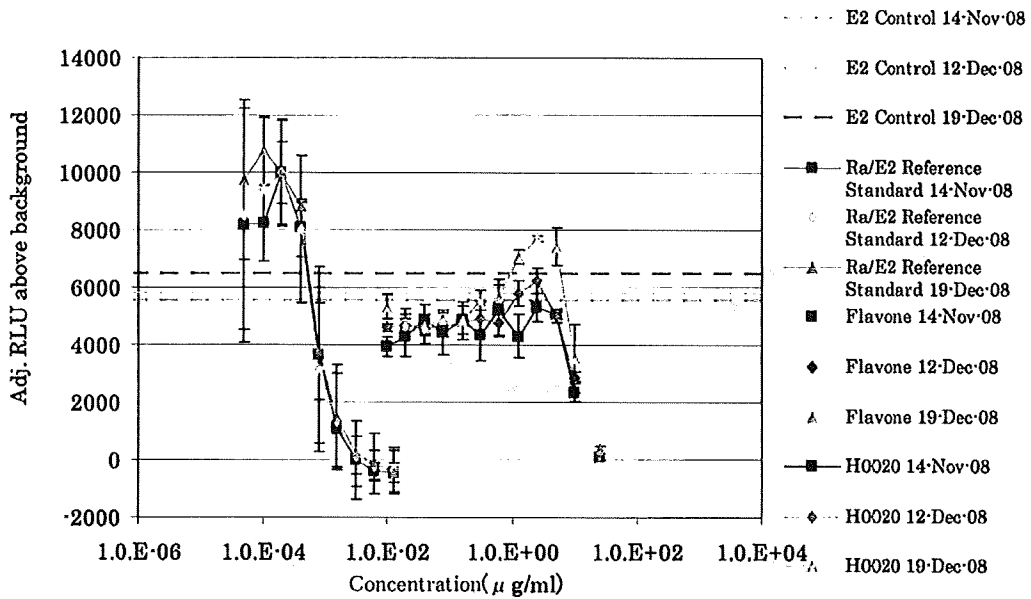
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Figure 3.11 Antagonist Comprehensive Testing for H0019¹



Deleted 5 low concentration points to calculate EC₅₀ of H0005.

Figure 3.12 Antagonist Comprehensive Testing for H0020¹



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

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Figure 3.13 Antagonist Comprehensive Testing for H0021¹

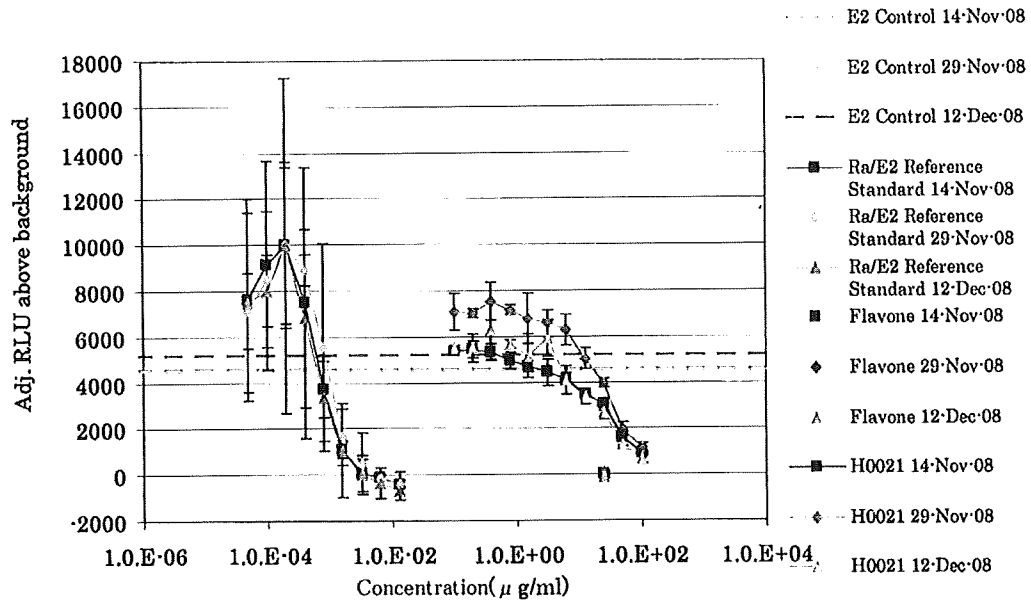
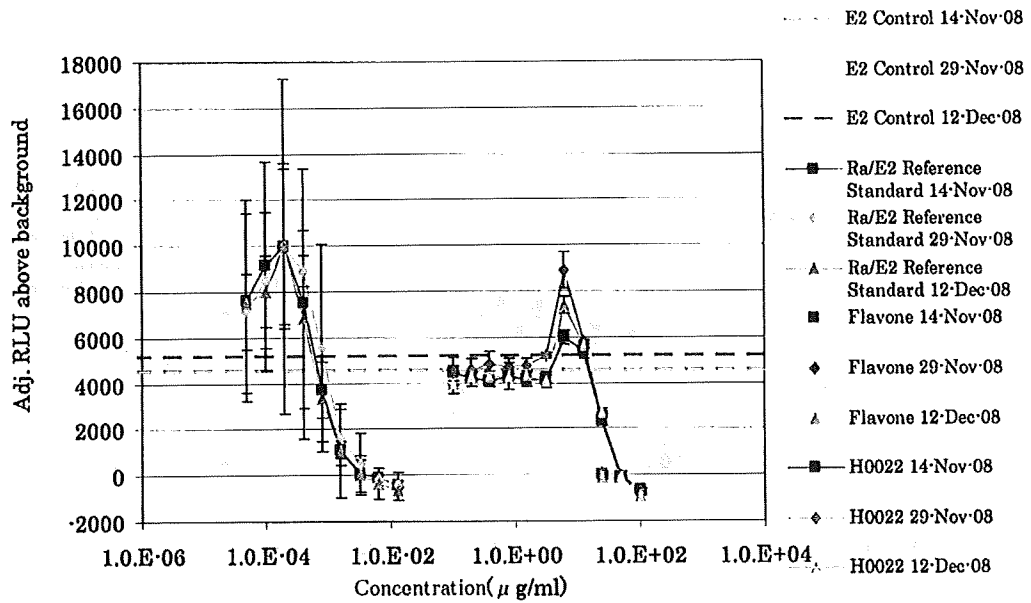


Figure 3.14 Antagonist Comprehensive Testing for H0022¹



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

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Figure 3.15 Antagonist Comprehensive Testing for H0023¹

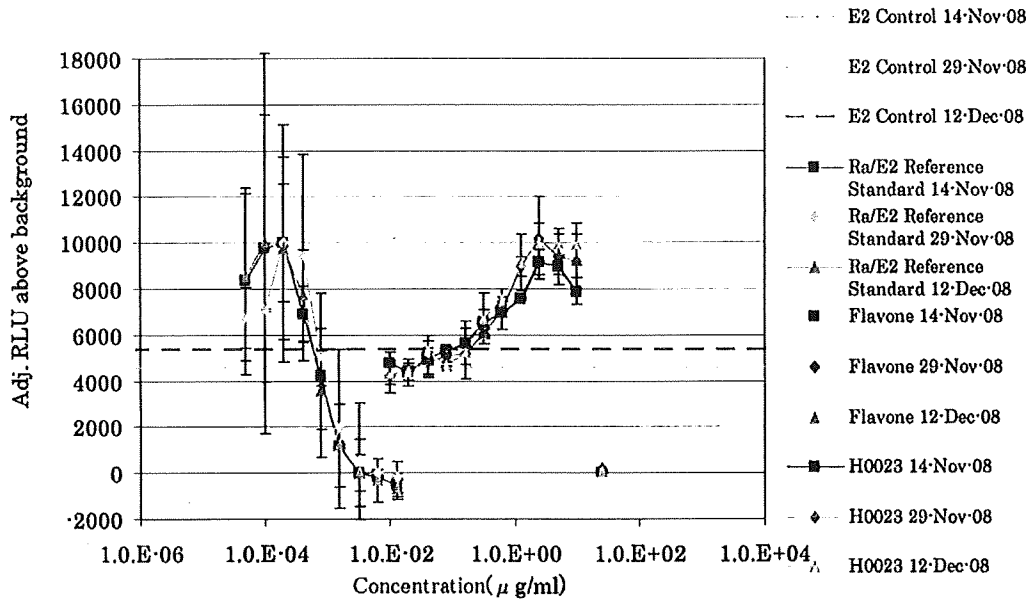
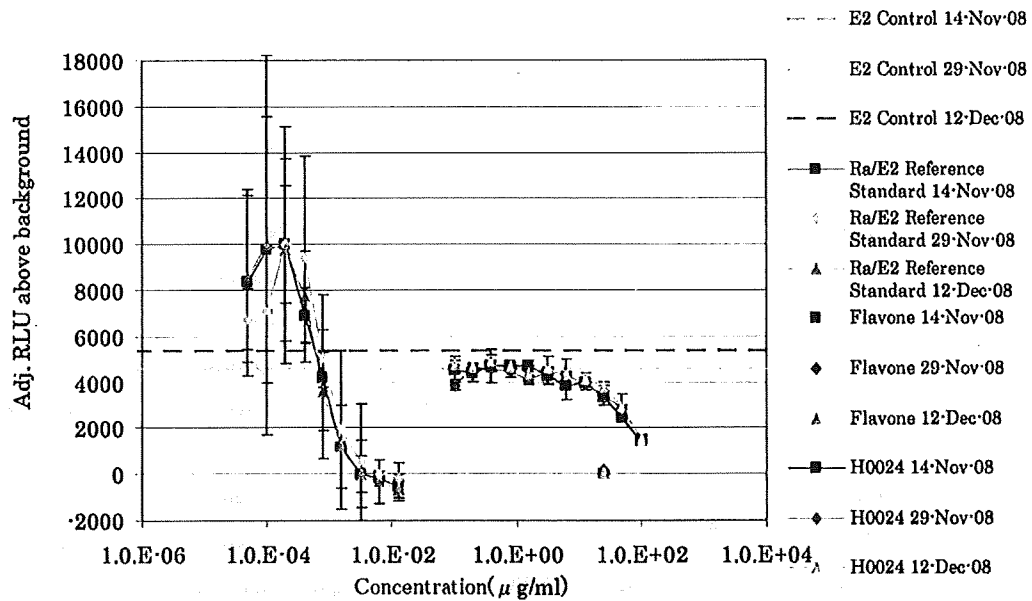


Figure 3.16 Antagonist Comprehensive Testing for H0024¹



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

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2.3 Retest(Comprehensive Testing)

Test chemicals were resent (H0019 and H0023) due to possibility of contamination of distributed samples. Range finder test were not performed and triplicate Comprehensive test were done starting from maximum amount of 100 micrograms/mL (1% DMSO/culture media).

Table 7 Materials Received for Antagonist Testing(Retest)

Sponsor Identification Number	Physical Description	Storage Conditions	Receipt Date	Received By	Comments
H0019	Liquid	Room Temp	13-Apr-09	Masafumi Nakamura	
H0023	Liquid	-4°C	13-Apr-09	Masafumi Nakamura	

Table 8 Summary of Experiments Template: Comprehensive Testing(Retest)

Experiments: Phase IIb Comprehensive Testing;Antagonist(Retest)						
Experiment I.D.	Substance Code	Date	Plate Induction	EC ₅₀ (µg/mL)	Experiment Used for Data Analysis or Repeated?	Rationale for Unacceptability
CT 5-1	H0019	23-Apr-09	11.3	12	Used	Acceptable
	H0023			0.21	Used	Acceptable
CT 5-2	H0019	01-May-09	1.6	15	Repeated	Fail ¹
	H0023			0.51	Repeated	Fail ¹
CT 5-3	H0019	12-May-09	4.1	14	Repeated	Fail ²
	H0023			0.39	Repeated	Fail ²
CT 5-4	H0019	15-May-09	2.2	18	Repeated	Fail ²
	H0023			26	Repeated	Fail ²
CT 5-5	H0019	22-May-09	4.3	26* ³	Used	Acceptable
	H0023			0.10 ³	Used	Acceptable
CT 5-6	H0019	29-May-09	4.9	15	Used	Acceptable
	H0023			0.40	Used	Acceptable

¹ The reason for the failure is because 1) DMSO Control is higher than criteria & 2) Raloxifene/E2 Reference Standard IC50 is lower than the criteria.

² The reason for the failure is because 1) DMSO Control is higher than the criteria.

³ IC₅₀ were calculated including score1-4.