Annex K4

Phase 2b Experiments

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Table A-1 Phase IIb Agonist Range Finder Plates Tested at XDS

Experiment I.D.	Date	Induction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
XIIbAgRFX9-X12v3	17-Sep-08	8.12	845	Yes	
XIIbAgRFX13-X16v3	17-Sep-08	11.02	559	Yes	

[1]Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-2 Phase IIb Agonist Range Finder Plates Tested at ECVAM

Experiment I.D.	Date	Induction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
AgRf1 V0005 V0008	02-Oct-08	17.61	2326	Yes	
AgRf1 V0009 V0012	02-Oct-08	7.86	5864	Yes	
AgRf2 V0009 V0012	15-Oct-08	6.17	18357	No	Failed DMSO Control
AgRf3 V0009 V0012	22-Oct-08	5.20	15037	No	Failed DMSO Control

[1]Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-3 Phase IIb Agonist Range Finder Plates Tested at Hiyoshi

Experiment I.D.	Date	Induction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
AgRF080923-01	23-Sep-08	8.43	2824	Yes	
AgRF080923-02	23-Sep-08	5.92	3343	Yes	

[1]Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-4 Phase IIb Antagonist Range Finder Plates Tested at XDS

Experiment I.D.	Date	Reduction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
XIIbAntRFX17-X20v3	17-Sep-08	7.96	852	Yes	
XIIbAntRFX21-X24	18-Sep-08	17.08	676	Yes	

[1]Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-5 Phase IIb Antagonist Range Finder Plates Tested at ECVAM

Experiment I.D.	Date	Reduction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
AntaRf4 V0083 V0086	12-Nov-08	6.30	3280	Yes	
AntaRf3 V0087 V0090	12-NOv-08	6.26	2155	Yes	

[1]Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-6 Phase IIb Antagonist Range Finder Plates Tested at Hiyoshi

Experiment I.D.	Date	Reduction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
AntRF080923-01	23-Sep-08	8.72	4662	Yes	
AntRF080923-02	23-Sep-08	8.40	4577	Yes	

[1]Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-7 Phase IIb Agonist Comprehensive Test Plates Tested at XDS

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	29-Oct-08	1117	8.83	2.89×10^{-6}	5751	1:2	Positive but EC_{50} value could not be calculated	Yes		XIIb0015-X0016Ag1
ATZ	04-Nov-08	1307	9.85	4.03×10^{-6}	6074	1:2	Negative	Yes		XIIb0015-X0016Ag2
ATZ	06-Nov-08	4007	5.71	2.07×10^{-6}	7743	1:2	Negative	Yes		XIIb0015-X0016Ag3
BBP	29-Oct-08	1125	6.50	1.81×10^{-6}	6131	1:5	2.43×10^{-1}	Yes		XIIb0013-X0014Ag1
BBP	04-Nov-08	1084	9.65	2.52×10^{-6}	5703	1:5	4.58×10^{-1}	Yes		XIIb0013-X0014Ag2
BBP	06-Nov-08	3330	6.63	3.05×10^{-6}	6320	1:5	4.01×10^{-1}	Yes		XIIb0013-X0014Ag3
DDT	29-Oct-08	628	10.32	3.11×10^{-6}	4705	1:2	2.89×10^{-2}	Yes		XIIb0011-X0012CTAg1
DDT	04-Nov-08	1179	9.13	2.64×10^{-6}	7595	1:2	2.03×10^{-2}	Yes		XIIb0011-X0012CTAg2
DDT	06-Nov-08	4059	5.56	3.32×10^{-6}	6573	1:2	1.59×10^{-2}	Yes		XIIb0011-X0012CTAg3
EE	29-Oct-08	1117	8.83	2.89×10^{-6}	5751	1:5	Positive but EC_{50} value could not be calculated	Yes	Wrong Test Concentrations	XIIb0015-X0016Ag1
EE	04-Nov-08	1307	9.85	4.03×10^{-6}	6074	1:5	2.83×10^{-6}	Yes		XIIb0015-X0016Ag2
EE	06-Nov-08	4007	5.71	2.07×10^{-6}	7743	1:5	1.32×10^{-6}	Yes		XIIb0015-X0016Ag3
EE	13-Nov-08	3043	5.02	1.72×10^{-6}	6507	1:5	2.15×10^{-6}	Yes		XIIb0016Ag4
FLA	29-Oct-08	1125	6.50	1.81×10^{-6}	6131	1:2	Positive but EC_{50} value could not be calculated	Yes		XIIb0013-X0014Ag1
FLA	04-Nov-08	1084	9.65	2.52×10^{-6}	5703	1:2	Positive but EC_{50} value could not be calculated	Yes		XIIb0013-X0014Ag2
FLA	06-Nov-08	3330	6.63	3.05×10^{-6}	6320	1:2	Positive but EC_{50} value could not be calculated	Yes		XIIb0013-X0014Ag3

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
GEN	29-Oct-08	628	10.32	3.11×10^{-6}	4705	1:5	4.54×10^{-3}	Yes		XIIb0011-X0012CTAg1
GEN	04-Nov-08	1179	9.13	2.64×10^{-6}	7595	1:5	4.91×10^{-3}	Yes		XIIb0011-X0012CTAg2
GEN	06-Nov-08	4059	5.56	3.32×10^{-6}	6573	1:5	7.52×10^{-3}	Yes		XIIb0011-X0012CTAg3
NON	29-Oct-08	816	7.31	2.74×10^{-6}	6658	1:5	3.81×10^{-1}	Yes		XIIb0009-X0010CTAg1
NON	04-Nov-08	1744	4.15	3.68×10^{-6}	4818	1:5	4.10×10^{-1}	Yes		XIIb0009-X0010CTAg2
NON	06-Nov-08	3641	6.20	2.50×10^{-6}	5057	1:5	3.87×10^{-1}	Yes		XIIb0009-X0010CTAg3
VIN	29-Oct-08	816	7.31	2.74×10^{-6}	6658	1:5	Negative	Yes		XIIb0009-X0010CTAg1
VIN	04-Nov-08	1744	4.15	3.68×10^{-6}	4818	1:5	Negative	Yes		XIIb0009-X0010CTAg2
VIN	06-Nov-08	3641	6.20	2.50×10^{-6}	5057	1:5	Negative	Yes		XIIb0009-X0010CTAg3

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC_{50} = half-maximal effective concentration; EE = Ethinyl estradiol E2 = 17ß-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN =- Vinclozolin

[1]Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-8 Phase IIb Agonist Comprehensive Test Plates Tested at ECVAM

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	12-Nov-08	3805	4.84	5.26×10^{-7}	6894	1:2	2.63×10^{0}	Yes		Ag CT1 V0005 V0006
ATZ	27-Nov-08	6100	3.22	2.32×10^{-6}	7905	1:2	Positive but EC_{50} could not be calculated	Yes		Ag CT2 V0005 V0006
ATZ	01-Dec-08	14386	1.71	3.13×10^{-6}	6425	1:2	Negative	No	Failed DMSO and Induction	Ag CT3 V0005 V0006
ATZ	13-Dec-08	3872	5.61	1.75×10^{-6}	8453	1:2	2.91×10^{0}	Yes		Ag CT4 V0005 V0006
BBP	16-Nov-08	3735	4.71	2.78×10^{-6}	7245	1:2	7.86×10^{-1}	Yes		Ag CT1 V0011 V0012
BBP	27-Nov-08	6209	3.12	2.06×10^{-6}	8322	1:2	3.29×10^{-1}	Yes		Ag CT2 V0011 V0012
BBP	01-Dec-08	4973	5.81	5.0×10^{-6}	7832	1:2	9.20×10^{-1}	Yes		Ag CT3 V0011 V0012
DDT	16-Nov-08	3256	5.51	3.43×10^{-6}	6875	1:5	1.59×10^{-1}	Yes		Ag CT1 V0009 V0010
DDT	27-Nov-08	4260	4.32	1.06×10^{-6}	8941	1:5	1.65×10^{-1}	Yes		Ag CT2 V0009 V00010
DDT	01-Dec-08	8411	3.25	1.85×10^{-6}	8673	1:5	1.33×10^{-1}	No	Failed DMSO	Ag CT3 V0009 V0010
DDT	13-Dec-08	4260	0.72	Negative	-12758 7	1:5	Negative	No	Experimenter error.	Ag CT4 V0009 V0010
DDT	17-Dec-08	3535	3.86	1.04×10^{-6}	8566	1:5	1.25×10^{-1}	Yes		Ag CT6 V0009 V0010
EE	16-Nov-08	3735	4.71	2.78×10^{-6}	7245	1:5	2.04×10^{-6}	Yes		Ag C1 V0011 V0012
EE	27-Nov-08	6209	3.12	2.06×10^{-6}	8322	1:5	1.25×10^{-6}	Yes		Ag CT2 V0011 V0012
EE	01-Dec-08	4973	5.81	5.0×10^{-6}	7832	1:5	1.91×10^{-6}	Yes		Ag CT3 V0011 V0012
FLA	16-Nov-08	3640	4.40	3.29×10^{-6}	8260	1:2	1.75×10^{0}	Yes		Ag CT1 V0007 V0008
FLA	27-Nov-08	7980	2.59	2.69×10^{-6}	9220	1:2	9.23×10^{-2}	No	Failed DMSO and Induction	Ag CT2 V0007 V0008

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
FLA	01-Dec-08	11137	2.47	1.78×10^{-6}	7819	1:2	Negative	No	Failed DMSO and Induction	Ag CT3 V0007 V0008
FLA	13-Dec-08	4609	4.33	1.48×10^{-6}	8823	1:2	1.36×10^{0}	Yes		Ag CT4 V0007 V0008
FLA	17-Dec-08	3743	5.22	8.29×10^{-7}	9285	1:2	1.59×10^{0}	Yes		Ag CT5 V0007 V0008
GEN	16-Nov-08	3640	4.40	3.29×10^{-6}	8260	1:5	8.33×10^{-2}	Yes		Ag CT1 V0007 V0008
GEN	27-Nov-08	7980	2.59	2.69×10^{-6}	9220	1:5	Negative	No	Failed DMSO and Induction	Ag CT2 V0007 V0008
GEN	01-Dec-08	11137	2.47	1.78×10^{-6}	7819	1:5	10.82×10^{-2}	No	Failed DMSO and Induction	Ag CT3 V0007 V0008
GEN	13-Dec-08	4609	4.33	1.48×10^{-6}	8823	1:2	8.85×10^{-2}	Yes		Ag CT4 V0007 V0008
GEN	17-Dec-08	3743	5.22	8.29×10^{-7}	9285	1:2	7.14×10^{-2}	Yes		Ag CT5 V0007V0008
NON	16-Nov-08	3256	5.51	3.43×10^{-6}	6875	1:2	7.81×10^{-1}	Yes		Ag CT1 V0009 V0010
NON	27-Nov-08	4260	4.32	1.06×10^{-6}	8941	1:2	3.13×10^{-1}	Yes		Ag CT2 V0009 V00010
NON	01-Dec-08	8411	3.25	1.85×10^{-6}	8673	1:2	7.22×10^{-1}	No	Failed DMSO	Ag CT3 V0009 V0010
NON	13-Dec-08	4260	0.72	Negative	-12758 7	1:5	Negative	No	Experimenter error.	Ag CT4 V0009 V0010
NON	17-Dec-08	3535	3.86	1.04×10^{-6}	8566	1:5	5.57×10^{-1}	Yes		Ag CT6 V0009 V0010
VIN	12-Nov-08	3805	4.84	5.26×10^{-7}	6894	1:2	5.03×10^{-1}	Yes		Ag CT1 V0005 V0006
VIN	27-Nov-08	6100	3.22	2.32×10^{-6}	7905	1:2	24.30×10^{-1}	Yes		Ag CT2 V0005 V0006
VIN	01-Dec-08	14386	1.71	3.13×10^{-6}	6425	1:2	Positive but EC_{50} could not be calculated	No	Failed DMSO and Induction	Ag CT3 V0005 V0006
VIN	13-Dec-08	3872	5.61	1.75×10^{-6}	8453	1:2	8.86×10^{-1}	Yes		Ag CT4 V0005 V0006

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC_{50} = half-maximal effective concentration; EE = Ethinyl estradiol E2 = 17ß-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN =- Vinclozolin

[1]Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-9 Phase IIb Agonist Comprehensive Test Plates Tested at Hiyoshi

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC50 (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	07-Nov-08	7922	2.27	2.07×10^{-6}	6706	1:2	Negative	No	Failed Induction	AgCTT081107 -Ag4-1(fail)
ATZ	21-Nov-08	5735	4.32	3.75×10^{-6}	8839	1:2	Positive but an EC_{50} could not be calculated	Yes		AgCTT081121 -Ag4-2
ATZ	04-Dec-08	8078	3.27	2.31×10^{-6}	9121	1:2	Negative	Yes		AgCTT081204 -Ag4-3
ATZ	25-Dec-08	7009	3.44	3.09×10^{-6}	7291	1:2	Negative	Yes		AgCTT081225 -Ag4-4
BBP	07-Nov-08	6886	3.46	2.12×10^{-6}	7139	1:5	8.30×10^{-1}	Yes		AgCTT081107 -Ag1-1
BBP	21-Nov-08	5971	4.64	3.15×10^{-6}	7212	1:5	9.93×10^{-1}	Yes		AgCTT081121 -Ag1-2
BBP	04-Dec-08	7410	3.06	2.46×10^{-6}	8949	1:5	Positive but an EC_{50} could not be calculated	Yes		AgCTT081204 Ag1-3
DDT	07-Nov-08	6886	3.46	2.12×10^{-6}	7139	1:5	2.70×10^{-1}	Yes		AgCTT081107 -Ag1-1
DDT	21-Nov-08	5971	4.64	3.15×10^{-6}	7212	1:5	2.62×10^{-1}	Yes		AgCTT081121 -Ag1-2
DDT	04-Dec-08	7410	3.06	2.46×10^{-6}	8949	1:5	2.10×10^{-1}	Yes		AgCTT081204 -Ag1-3
EE	07-Nov-08	13265	1.61	2.43×10^{-6}	5821	1:5	Positive but an EC_{50} could not be calculated	No	Failed DMSO and Induction	AgCTT081107 -Ag2-1
EE	21-Nov-08	5761	4.64	2.73×10^{-6}	6909	1:5	3.02×10^{-6}	Yes		AgCTT081121 -Ag2-2
EE	04-Dec-08	7897	3.06	1.79×10^{-6}	9229	1:5	1.85×10^{-6}	Yes		AgCTT081204 -Ag2-3
EE	19-Dec-08	4955	4.86	3.09×10^{-6}	7838	1:5	2.58×10^{-6}	Yes		AgCTT081219 -Ag2-4
FLA	07-Nov-08	13265	1.61	2.43×10^{-6}	5821	1:5	Negative	No	Failed DMSO and Induction	AgCTT081107 -Ag2-1
FLA	21-Nov-08	5761	4.64	2.73×10^{-6}	6909	1:5	Positive but an EC_{50} could not be calculated	Yes		AgCTT081121 -Ag2-2

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC50 (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
FLA	04-Dec-08	7897	3.06	1.79×10^{-6}	9229	1:5	Positive but an EC_{50} could not be calculated	Yes		AgCTT081204 -Ag2-3
FLA	19-Dec-08	4955	4.86	3.09×10^{-6}	7838	1:5	Positive but an EC_{50} could not be calculated	Yes		AgCTT081219 -Ag2-4
GEN	07-Nov-08	4182	4.43	3.23×10^{-6}	7482	1:5	5.14×10^{-2}	Yes		AgCTT081107 -Ag3-1
GEN	21-Nov-08	5942	4.62	2.49×10^{-6}	7176	1:5	12.13×10^{-2}	Yes		AgCTT081121 -Ag3-2
GEN	04-Dec-08	8836	3.04	2.60×10^{-6}	10409	1:5	14.10×10^{-2}	No	Failed DMSO Acceptance	AgCTT081204 -AG3-3(fail)
GEN	19-Dec-08	5053	4.82	3.02×10^{-6}	7265	1:5	16.10×10^{-2}	Yes		AgCTT081219 -Ag3-4
GEN	25-Dec-08	6665	3.48	2.72×10^{-6}	7748	1:5	14.03×10^{-2}	Yes		AgCTT081225 -Ag3-5
NON	07-Nov-08	7922	2.27	2.07×10^{-6}	6706	1:2	Negative	No	Failed Induction	AgCTT081107 -Ag4-1(fail)
NON	21-Nov-08	5735	4.32	3.75×10^{-6}	8839	1:2	1.33×10^{0}	Yes		AgCTT081121 -Ag4-2
NON	04-Dec-08	8078	3.27	2.31×10^{-6}	9121	1:2	1.24×10^{0}	Yes		AgCTT081204 -Ag4-3
NON	25-Dec-08	7009	3.44	3.09×10^{-6}	7291	1:2	Positive but an EC_{50} could not be calculated	Yes		AgCTT081225 -Ag4-4
VIN	07-Nov-08	4182	4.43	3.23×10^{-6}	7482	1:2	Negative	Yes		AgCTT081107 -Ag3-1
VIN	21-Nov-08	5942	4.62	2.49×10^{-6}	7176	1:2	Positive but an EC_{50} could not be calculated	Yes		AgCTT081121 -Ag3-2
VIN	04-Dec-08	8836	3.04	2.60×10^{-6}	10409	1:2	Positive but an EC_{50} could not be calculated	No	Failed DMSO Acceptance	AgCTT081204 -AG3-3(fail)
VIN	19-Dec-08	5053	4.82	3.02×10^{-6}	7265	1:2	Negative	Yes		AgCTT081219 -Ag3-4
VIN	25-Dec-08	6665	3.48	2.72×10^{-6}	7748	1:2	Negative	Yes		AgCTT081225 -Ag3-5

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC_{50} = half-maximal effective concentration; EE = Ethinyl estradiol E2 = 17ß-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN =- Vinclozolin

[1]Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-10 Phase IIb Antagonist Plates Tested at XDS

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
API	16-Nov-08	1733	16.36	6.28×10^{-4}	7154	1608	1:2	Negative	Yes		XIIbX0023-X0024CTAnt1
API	18-Nov-08	930	8.77	4.56×10^{-4}	9047	4631	1:2	Negative	Yes		XIIbX0023-X0024CTAnt2
API	19-Nov-08	1749	14.43	5.60×10^{-4}	8042	2891	1:2	Negative	Yes		XIIbX0023-X0024CTAnt3
ATZ	16-Nov-08	1733	16.36	6.28×10^{-4}	7154	1608	1:2	Negative	Yes		XIIbX0023-X0024CTAnt1
ATZ	18-Nov-08	930	8.77	4.56×10^{-4}	9047	4631	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0023-X0024CTAnt2
ATZ	19-Nov-08	1749	14.43	5.60×10^{-4}	8042	2891	1:2	Negative	Yes		XIIbX0023-X0024CTAnt3
BBP	14-Nov-08	2635	12.50	5.23×10^{-4}	8147	3314	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt1
BBP	16-Nov-08	1542	11.88	6.32×10^{-4}	8364	2180	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt2
BBP	18-Nov-08	964	9.18	3.37×10^{-4}	9795	5993	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt3
CORT	14-Nov-08	2635	12.50	5.23×10^{-4}	8147	3314	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt1
CORT	16-Nov-08	1542	11.88	6.32×10^{-4}	8364	2180	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt2
CORT	18-Nov-08	964	9.18	3.37×10^{-4}	9795	5993	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0021-X0022CTAnt3
DDT	13-Nov-08	2714	8.88	4.41×10^{-4}	7701	3349	1:2	Negative	Yes		XIIbX0017-X0018CTAnt1
DDT	14-Nov-08	1639	8.90	5.50×10^{-4}	7826	3132	1:2	Negative	Yes		XIIbX0017-X0018CTAnt2
DDT	16-Nov-08	1781	10.57	6.69×10^{-4}	7814	1601	1:2	Negative	Yes		XIIbX0017-X0018CTAnt3

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
FLA	13-Nov-08	2773	11.92	5.53×10^{-4}	8316	3635	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0019-X0020CTAnt1
FLA	14-Nov-08	1941	10.29	5.51×10^{-4}	8955	3598	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0019-X0020CTAnt2
FLA	16-Nov-08	2516	13.16	6.34×10^{-4}	7945	2042	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0019-X0020CTAnt3
GEN	13-Nov-08	2773	11.92	5.53×10^{-4}	8316	3635	1:2	Negative	Yes		XIIbX0019-X0020CTAnt1
GEN	14-Nov-08	1941	10.29	5.51×10^{-4}	8955	3598	1:2	Negative	Yes		XIIbX0019-X0020CTAnt2
GEN	16-Nov-08	2516	13.16	6.34×10^{-4}	7945	2042	1:2	Positive but an IC_{50} value could not be calculated	Yes		XIIbX0019-X0020CTAnt3
RES	13-Nov-08	2714	8.88	4.41×10^{-4}	7701	3349	1:2	Negative	Yes		XIIbX0017-X0018CTAnt1
RES	14-Nov-08	1639	8.90	5.50×10^{-4}	7826	3132	1:2	Negative	Yes		XIIbX0017-X0018CTAnt2
RES	16-Nov-08	1781	10.57	6.69×10^{-4}	7814	1601	1:2	Negative	Yes		XIIbX0017-X0018CTAnt3

Abbreviations: API = Apigenin; ATZ = Atrazine; BBP = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17ß-estradiol; Fla = Flavone; GEN = Genistein; IC_{50} = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

[1]Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

Table A-11 Phase IIb Antagonist Plates Tested at ECVAM

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
API	20-Nov-08	3675	7.51	1.44×10^{-3}	8597	1199	1:2	Negative	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0089 V0090
API	05-Dec-08	10088	6.40	6.42×10^{-4}	10047	-1607	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0089 V0090
API	09-Dec-08	4134	6.31	7.88×10^{-4}	9141	1264	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT3 V0089 V0090
API	13-Dec-08	4147	5.37	6.18×10^{-4}	10707	1600	1:2	Negative	Yes		Anta CT4 V0089 V0090
API	17-Dec-08	5449	6.40	6.16×10^{-4}	7938	4s84	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT5 V0089 V0090
ATZ	20-Nov-08	3118	7.72	1.37×10^{-3}	8818	860	1:2	Positive but an IC_{50} could not be calculated	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0087 V0088
ATZ	05-Dec-08	9946	5.81	4.23×10^{-4}	7025	-635	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0087 V0088
ATZ	09-Dec-08	3212	7.72	5.86×10^{-4}	9221	1075	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT3 V0087 V0088
ATZ	13-Dec-08	4427	5.87	6.03×10^{-4}	9053	1577	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT4 V0087 V0088
ATZ	17-Dec-08	5053	6.90	6.46×10^{-4}	8435	419	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT5 V0087 V0088
BBP	20-Nov-08	2522	6.07	2.93×10^{-4}	10399	1321	1:2	Negative	Yes		Anta CT1 V0085 V0086
BBP	05-Dec-08	14111	5.41	4.70×10^{-4}	11625	-8081	1:2	Positive but an IC_{50} could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0085 V0086
BBP	09-Dec-08	3217	7.05	5.92×10^{-4}	9226	755	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT3 V0085 V0086
BBP	13-Dec-08	5577	6.40	6.00×10^{-4}	10010	603	1:2	Negative	Yes		Anta CT4 V0085 V0086

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
CORT	20-Nov-08	2522	6.07	2.93×10^{-4}	10399	1321	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT1 V0085 V0086
CORT	05-Dec-08	14111	5.41	4.70×10^{-4}	11625	-8081	1:2	$4.79 \times 10^{+1}$	No	Failed DMSO Acceptance	Anta CT2 V0085 V0086
CORT	09-Dec-08	3217	7.05	5.92×10^{-4}	9226	755	1:2	$3.98 \times 10^{+1}$	Yes		Anta CT3 V0085 V0086
CORT	13-Dec-08	5577	6.40	6.00×10^{-4}	10010	603	1:2	$5.15 \times 10^{+1}$	Yes		Anta CT4 V0085 V0086
DDT	20-Nov-08	2654	7.16	3.74×10^{-4}	10319	694	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT1 V0083 V0084
DDT	05-Dec-08	14948	5.89	4.37×10^{-4}	9685	-16714	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0083 V0084
DDT	09-Dec-08	5398	7.02	6.80×10^{-4}	9454	-286	1:2	Negative	Yes		Anta CT3 V0083 V0084
DDT	13-Dec-08	4794	6.58	5.59×10^{-4}	8373	185	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT4 V0083 V0084
FLA	20-Nov-08	3675	7.51	1.44×10^{-3}	8597	1199	1:2	Positive but an IC_{50} could not be calculated	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0089 V0090
FLA	05-Dec-08	10088	6.40	6.42×10^{-4}	10047	-1607	1:2	Positive but an IC_{50} could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0089 V0090
FLA	09-Dec-08	4134	6.31	7.88×10^{-4}	9141	1264	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT3 V0089 V0090
FLA	13-Dec-08	4147	5.37	6.18×10^{-4}	10707	1600	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT4 V0089 V0090
FLA	17-Dec-08	5449	6.40	6.16×10^{-4}	7938	484	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT5 V0089 V0090
GEN	20-Nov-08	2654	7.16	3.74×10^{-4}	10319	694	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT1 V0083 V0084
GEN	05-Dec-08	14948	5.89	4.37×10^{-4}	9685	-16714	1:2	Positive but an IC_{50} could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0083 V0084

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
GEN	09-Dec-08	5398	7.02	6.80×10^{-4}	9454	-286	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT3 V0083 V0084
GEN	13-Dec-08	4794	6.58	5.59×10^{-4}	8373	185	1:2	Positive but an IC_{50} could not be calculated	Yes		Anta CT4 V0083 V0084
RES	20-Nov-08	3118	7.72	1.37×10^{-3}	8818	860	1:2	1.42×10^{1}	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0087 V0088
RES	05-Dec-08	9946	5.81	4.23×10^{-4}	7025	-635	1:2	1.26×10^{1}	No	Failed DMSO Acceptance	Anta CT2 V0087 V0088
RES	09-Dec-08	3212	7.72	5.86×10^{-4}	9221	1075	1:2	1.37×10^{1}	Yes		Anta CT3 V0087 V0088
RES	13-Dec-08	4427	5.87	6.03×10^{-4}	9053	1577	1:2	1.70×10^{1}	Yes		Anta CT4 V0087 V0088
RES	17-Dec-08	5053	6.90	6.46×10^{-4}	8435	419	1:2	1.53×10^{1}	Yes		Anta CT5 V0087 V0088

Abbreviations: API = Apigenin; ATZ = Atrazine; BBP = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17ß-estradiol; Fla = Flavone; GEN = Genistein; IC_{50} = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

[1]Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

Table A-12 Phase IIb Antagonist Plates Tested at Hiyoshi

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
API	14-Nov-08	6047	8.81	7.42×10^{-4}	5575	99	1:2	Negative	Yes		AntCTT081114-Ant2-1
API	29-Nov-08	7139	11.27	7.80×10^{-4}	5105	4	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081129-Ant2-2
API	12-Dec-08	5814	11.13	6.58×10^{-4}	5790	174	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081212-Ant2-3
API	19-Dec-08	5602	9.41	6.83×10^{-4}	6018	344	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081219-Ant2-4
ATZ	14-Nov-08	5231	12.90	7.24×10^{-4}	5379	22	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081114-Ant4-1
ATZ	29-Nov-08	6421	11.60	7.92×10^{-4}	4569	189	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081129-Ant4-2
ATZ	12-Dec-08	7053	7.92	6.97×10^{-4}	5361	90	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081212-Ant4-3
BBP	14-Nov-08	6338	9.82	7.43×10^{-4}	5135	-26	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081114-Ant1-1
BBP	29-Nov-08	7789	8.79	7.80×10^{-4}	5939	-336	1:2	Negative	Yes		AntCTT081129-Ant1-2
BBP	12-Dec-08	5143	11.37	7.20×10^{-4}	5181	223	1:2	Negative	Yes		AntCTT081212-Ant1-3
BBP	19-Dec-08	5250	9.58	6.65×10^{-4}	5409	422	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081219-Ant1-4
CORT	14-Nov-08	4633	14.31	7.21×10^{-4}	4603	30	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081114-Ant3-1
CORT	29-Nov-08	7336	11.15	9.78×10^{-4}	4522	94	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081129-Ant3-2

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
CORT	12-Dec-08	7992	11.46	7.13×10^{-4}	5197	-113	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081212-Ant3-3
DDT	14-Nov-08	6338	9.82	7.43×10^{-4}	5135	-26	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081114-Ant1-1
DDT	29-Nov-08	7789	8.79	7.80×10^{-4}	5939	-336	1:2	Negative	Yes		AntCTT081129-Ant1-2
DDT	12-Dec-08	5143	11.37	7.20×10^{-4}	5181	223	1:2	Negative	Yes		AntCTT081212-Ant1-3
DDT	19-Dec-08	5250	9.58	6.65×10^{-4}	5409	422	1:2	Positive but IC_{50} could not be calculated	Yes		AntCTT081219-Ant1-4
FLA	14-Nov-08	6047	8.81	7.42×10^{-4}	5575	99	1:2	Negative	Yes		AntCTT081114-Ant2-1
FLA	29-Nov-08	7139	11.27	7.80×10^{-4}	5105	4	1:2	Negative	Yes		AntCTT081129-Ant2-2
FLA	12-Dec-08	5814	11.13	6.58×10^{-4}	5790	174	1:2	Negative	Yes		AntCTT081212-Ant2-3
FLA	19-Dec-08	5602	9.41	6.83×10^{-4}	6018	344	1:2	Negative	Yes		AntCTT081219-Ant2-4
GEN	14-Nov-08	5231	12.90	7.24×10^{-4}	5379	22	1:2	Negative	Yes		AntCTT081114-Ant4-1
GEN	29-Nov-08	6421	11.60	7.92×10^{-4}	4569	189	1:2	Negative	Yes		AntCTT081129-Ant4-2
GEN	12-Dec-08	7053	7.92	6.97×10^{-4}	5361	90	1:2	Negative	Yes		AntCTT081212-Ant4-3
RES	14-Nov-08	4633	14.31	7.21×10^{-4}	4603	30	1:2	2.49×10^{1}	Yes		AntCTT081114-Ant3-1
RES	29-Nov-08	7336	11.15	9.78×10^{-4}	4522	94	1:2	2.32×10^{1}	Yes		AntCTT081129-Ant3-2
RES	12-Dec-08	7992	11.46	7.13×10^{-4}	5197	-113	1:2	2.60×10^{1}	Yes		AntCTT081212-Ant3-3

Abbreviations: API = Apigenin; ATZ = Atrazine; BB = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17ß-estradiol; Fla = Flavone; GEN = Genistein; IC_{50} = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

[1]Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

Appendix K4-1

Phase 2b Repeat Testing and XDS New Personnel Proficiency Testing

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98 **Table A- Phase IIb Repeat Testing at ECVAM - Agonist Comprehensive Test Plates**

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	20-May-09	1917	14.46	1.83×10^{-6}	5798	1:5	Positive, but an EC_{50} could not be calculated	Yes		Ag CT1 V0027 V2001
ATZ	27-May-09	2599	10.77	1.68×10^{-6}	7592	1:5	$5.65 \times 10^{=1}$	Yes		Ag CT2 V2001 CT1 V0045
ATZ	10-Jun-09	3340	8.29	2.94×10^{-6}	8196	1:5	Positive, but an EC_{50} could not be calculated	Yes		Ag CT3 V2001 E2 control old DMSO
ATZ	27-Jun-09	2382	12.20	1.31×10^{-6}	7166	1:5	2.06×10^{0}	Yes		Ag CT1 V2001 V2002 NEW
ATZ	30-Sep-09	1795	11.71	5.54×10^{-7}	6698	1:5	Positive, but an EC_{50} could not be calculated	Yes		Ag CT2 V2001 V2002 NEW
ATZ	07-Oct-09	32761	0.97	Positive, but an EC_{50} could not be calculated	11761	1:5	Positive, but an EC_{50} could not be calculated	No	Failed DMSO Control, Induction, E2 Reference Standard	Ag CT3 V2001 V2002 NEW
ATZ	14-Oct-09	3196	7.72	3.15×10^{-6}	8076	1:5	Positive, but an EC_{50} could not be calculated	Yes		Ag CT4 V2001 V2002 NEW
VIN	27-Jun-09	2382	12.20	1.31×10^{-6}	7166	1:2	Positive, but an EC_{50} could not be calculated	Yes		Ag CT1 V2001 V2002 NEW
VIN	30-Sep-09	1795	11.71	5.54×10^{-7}	6698	1:2	Positive, but an EC_{50} could not be calculated	Yes		Ag CT2 V2001 V2002 NEW
VIN	07-Oct-09	32761	0.97	Positive, but an EC_{50} could not be calculated	11761	1:2	Negative	No	Failed DMSO Control, Induction, E2 Reference Standard	Ag CT3 V2001 V2002 NEW

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
VIN	14-Oct-09	3196	7.72	3.15×10^{-6}	8076	1:2	Negative	Yes		Ag CT4 V2001 V2002 NEW

Table A-1 Phase IIb Repeat Testing at Hiyoshi – Antagonist Comprehensive Test Plates

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
FLA	01-Apr-09	6047	8.81	7.42×10^{-4}	5575	99	1:2	Negative	Yes[2]	Used wrong concentrations of FLA	AntCTT081101-AntAdd-1
FLA	23-Apr-09	3730	11.34	$4.93 \times 10^{=4}$	5660	387	1:2	Positive, but an IC_{50} could not be calculated	Yes		AntCTT081114-AntAdd-2
FLA	23-Apr-09	9806	10.40	1.30×10^{-3}	3767	1094	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-3
FLA	12-May-09	12103	8.62	6.11×10^{-4}	4695	377	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-4
FLA	15-May-09	8857	7.27	7.13×10^{-4}	4935	1011	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-5
FLA	22-May-09	4248	10.15	5.50×10^{-4}	5363	1293	1:2	Positive but an IC_{50} value could not be calculated	Yes		AntCTT081114-AntAdd-6
FLA	29-May-09	4983	9.49	6.43×10^{-4}	5247	1187	1:2	Positive but an IC_{50} value could not be calculated	Yes		AntCTT081101-AntAdd-7

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
GEN	01-Apr-09	6047	8.81	7.42×10^{-4}	5575	99	1:2	Negative	Yes[2]	Used wrong concentrations of GEN	AntCTT081101-AntAdd-1
GEN	23-Apr-09	3730	11.34	4.93×10^{-4}	5660	387	1:2	2.56×10^{1}	Yes		AntCTT081114-AntAdd-2
GEN	23-Apr-09	9806	10.40	1.30×10^{-3}	3767	1094	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-3
GEN	12-May-09	12103	8.62	6.11×10^{-4}	4695	377	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-4
GEN	15-May-09	8857	7.27	7.13×10^{-4}	4935	1011	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-5
GEN	22-May-09	4248	10.15	5.50×10^{-4}	5363	1293	1:2	Positive but an IC_{50} value could not be calculated	Yes		AntCTT081114-AntAdd-6
GEN	29-May-09	4983	9.49	6.43×10^{-4}	5247	1187	1:2	Positive but an IC_{50} value could not be calculated	Yes		AntCTT081101-AntAdd-7

Abbreviations: Cont = Control; DMSO = Dimethyl sulfoxide; E2 = 17ß-estradiol; Fla = Flavone; GEN = Genistein; IC_{50} = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl

[1]Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

[2]Abnormal test substance response during this experiment led to the shipment of replacement coded test substance to Hiyoshi

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Table A-1 New Experimenter Proficiency Testing at XDS – Agonist Range Finder

Experiment I.D.	Date	Induction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
lumi agonist 1 27 2009	27-Jan-09	3.33	1618	Yes	

[1]Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-2 New Experimenter Proficiency Testing at XDS – Antagonist Range Finder

Experiment I.D.	Date	Reduction[1]	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
lumi antagonist 1 27 2009	27 Jun 09	4.32	2295	Yes	

[1]Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A-4 New Experimenter Proficiency Testing at XDS – Agonist Comprehensive Test Plates

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	06-Mar-09	13029	1.09	Negative	35581	1:5	Negative	No	Failed DMSO, E2 Reference Standard, Induction	AgCT_X0010_X0015 030609
ATZ	31-Mar-09	1499	3.24	1.39×10^{-5}	3413	1:5	5.28×10^{-2}	No	Abnormal cell growth	AgCT_X0015_X009 033109
ATZ	11-Apr-09	642	11.25	3.03×10^{-6}	4437	1:5	4.32×10^{-5}	Yes		Lumi agonist 4 11 2009_X0015_X0016
ATZ	01-May-09	572	5.40	3.14×10^{-6}	7557	1:5	Positive but an EC_{50} value could not be	Yes		XPTX0010X0023AGCT090501

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	07-May-09	1049	3.89	4.22×10^{-6}	5300	1:5	Positive but an EC_{50} value could not be calculated	Yes		XPTX0023AGCT090507
BPA	06-Mar-09	12823	1.22	Positive but an EC_{50} value could not be calculated	-6995	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0094_X009 030609
BPA	16-Apr-09	1152	3.17	2.28×10^{-6}	2809	1:5	4.11×10^{-2}	Yes		XPTX0085X0094AGCT090416
BPA	30-Apr-09	7755	1.82	1.83×10^{-6}	3314	1:5	Positive but an EC_{50} value could not be calculated	No	Failed Induction	XPTX0094X0085AGCT090430
BPA	01-May-09	647	5.02	3.50×10^{-6}	5214	1:5	7.23×10^{-2}	Yes		XPTX0085X0094AGCT090501
DES	06-Mar-09	13510	1.02	Negative	-74511	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0085_X0016 030609
DES	16-Apr-09	1152	3.17	2.28×10^{-6}	2809	1:5	6.34×10^{-7}	Yes		XPTX0085X0094AGCT090416
DES	30-Apr-09	7755	1.82	1.83×10^{-6}	3314	1:5	2.86×10^{-6}	No	Failed Induction	XPTX0094X0085AGCT090430
DES	01-May-09	647	5.02	3.50×10^{-6}	5214	1:5	2.59×10^{-5}	Yes		XPTX0085X0094AGCT090501
DES	07-May-09	2962	3.18	3.75×10^{-6}	4650	1:5	1.66×10^{-5}	Yes		XPTX0085X0010AGCT090507
EE	06-Mar-09	13510	1.02	Negative	-74511	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0085_X0016 030609
EE	11-Apr-09	642	11.25	3.03×10^{-6}	4437	1:5	2.71×10^{-6}	Yes		Lumi agonist 4 11 2009_X0015_X0016
EE	18-Apr-08	1228	3.00	7.20×10^{-7}	5288	1:5	Positive but an EC_{50} value could not be calculated	No	Failed Induction	Lumi agonist 4 18 2009_X0010_X0016

Test Substance	Date	DMSO	Induction[1]	E2 Reference Standard EC_{50} (µg/mL)	MET	Test Substance Dilution	Test Substance EC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
EE	07-May-09	2584	3.35	3.35×10^{-6}	5973	1:5	1.25×10^{-6}	Yes		XPTX0016X001 0AGCT090507
NON	01-May-09	572	5.40	3.14×10^{-6}	7557	1:5	Positive but an EC_{50} value could not be	Yes		XPTX0010X002 3AGCT090501
NON	06-Mar-09	13029	1.09	Negative	35581	1:5	Positive but an EC_{50} value could not be calculated	No	Failed DMSO, E2 Reference Standard, Induction	AgCT_X0010_X 0015 030609
NON	11-Mar-09	206	9.07	2.68×10^{-6}	5216	1:5	Positive but an EC_{50} value could not be calculated		Yes	AgCT_X0009_X 0010 031109
NON	18-Apr-08	1228	3.00	7.20×10^{-7}	5288	1:5	Positive but an EC_{50} value could not be calculated	No	Failed Induction	Lumi agonist 4 18 2009_X0010_X0 016
NON	07-May-09	2584	3.35	3.35×10^{-6}	5973	1:5	Positive but an EC_{50} value could not be calculated	Yes		XPTX0016X001 0AGCT090507
NON	07-May-09	2962	3.18	3.75×10^{-6}	4650	1:5	Positive but an EC_{50} value could not be calculated	Yes		XPTX0085X001 0AGCT090507
VIN	06-Mar-09	12823	1.22	Positive but an EC_{50} value could not be calculated	-6995	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0094_X 009 030609
VIN	11-Mar-09	206	9.07	2.68×10^{-6}	5216	1:5	Positive but an EC_{50} value could not be calculated		Yes	AgCT_X0009_X 0010 031109
VIN	31-Mar-09	1499	3.24	1.39×10^{-5}	3413	1:5	Positive but an EC_{50} value could not be calculated	No	Abnormal cell growth	AgCT_X0015_X 009 033109

Abbreviations: ATZ = Atrazine; BPA = Bisphenol A; DES = Diethylstilbestrol; DMSO = Dimethyl sulfoxide; EC_{50} = half maximal effective concentration; E2 = 17ß-estradiol; EE = 17α-ethinyl estradiol; I.D. = Identification; NON = Nonylphenol; Vin = Vinclozolin

[1]Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Table A- New Experimenter Proficiency Testing (and Phase IIb Retests) at XDS – Antagonist Comprehensive Test Plates

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
API	03-May-09	3253	6.98	7.94×10^{-4}	6447	2705	1:2	2.56×10^{1}	Yes		XPTX0011X0054ANTCT090503_1
API	03-May-09	3133	4.27	7.34×10^{-4}	6995	6096	1:2	2.48×10^{1}	Yes		XPTX0011X0054ANTCT090503_2
API	07-May-09	3224	8.10	7.20×10^{-4}	6575	2768	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0011X0054ANTCT090507
ATZ	07-May-09	2489	6.10	5.94×10^{-4}	7893	2799	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0023X0017ANTCT090507
ATZ	22-May-09	1943	6.39	7.45×10^{-4}	8366	5097	1:2	Negative	Yes		XPTX0019X0023CT090522
ATZ	22-May-09	5231	9.37	8.75×10^{-4}	5811	-836	1:2	Negative	Yes		XPTX0019X0023CT090522_2
ATZ	09-Jun-09	912	3.83	1.16×10^{-3}	-3995	237690	1:2	Negative	No	Failed E2, FLA\E2	XPTX0019X0023ANTCT090609
ATZ	26-Jun-09	1245	6.06	7.70×10^{-4}	9355	3563	1:2	Negative	No	Failed E2. FLA\E2	XPTX0019X0023ANTCT090630
ATZ	24-Nov-09	5009	5.18	4.64×10^{-4}	8575	1410	1:2	Positive but an IC_{50} value could not be calculated	Yes		2009-11-24XPIIIAntCTX0082X0023
ATZ	05-Nov-09	926	13.02	3.13×10^{-4}	8387	3465	1:2	Negative	Yes	Experimenter error	2009-11-4-XPIIIANTCTX0023X0065

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
CORT	30-Apr-09	11983	7.92	1.12×10^{-3}	6558	-4711	1:2	Negative	No	Failed E2. FLA\E2	XPTX0020X0021A NTCT090430
CORT	01-May-09	661	5.38	7.49×10^{-4}	6792	3784	1:2	Negative	Yes		XPTX0020X0021A NTCT090501
CORT	03-May-09	2935	4.56	6.96×10^{-4}	7333	4273	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0020X0021A NTCT090503
CORT	07-May-09	2718	4.91	7.39×10^{-4}	6888	3036	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0021X0048A NTCT090507
DBA	03-May-09	3772	4.64	6.86×10^{-4}	8862	3120	1:5	Negative	No	Failed FLA\E2	XPTX0048X0026A NTCT090503_1
DBA	03-May-09	3987	4.21	6.35×10^{-4}	6286	3591	1:5	Positive but an IC_{50} value could not be calculated	Yes		XPTX0048X0026A NTCT090503_2
DBA	07-May-09	2718	4.91	7.39×10^{-4}	6888	3036	1:5	Positive but an IC_{50} value could not be calculated	Yes		XPTX0021X0048A NTCT090507
DDT	03-May-09	3253	6.98	7.94×10^{-4}	6447	2705	1:5	Positive but an IC_{50} value could not be calculated	Yes		XPTX0011X0054A NTCT090503_1
DDT	03-May-09	3133	4.27	7.34×10^{-4}	6995	6096	1:5	Positive but an IC_{50} value could not be calculated	Yes		XPTX0011X0054A NTCT090503_2
DDT	07-May-09	3224	8.10	7.20×10^{-4}	6575	2768	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0011X0054A NTCT090507
FLA	30-Apr-09	11983	7.92	1.12×10^{-3}	6558	-4711	1:2	Positive but an IC_{50} value could not be calculated	No	Failed DMSO, FLA\E2	XPTX0020X0021A NTCT090430

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
FLA	01-May-09	661	5.38	7.49×10^{-4}	6792	3784	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0020X0021A NTCT090501
FLA	03-May-09	2935	4.56	6.96×10^{-4}	7333	4273	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0020X0021A NTCT090503
GEN	07-May-09	2765	6.54	5.93×10^{-4}	5567	2618	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0017X0019A NTCT090507
GEN	22-May-09	1943	6.39	7.45×10^{-4}	8366	5097	1:2	Negative	Yes		XPTX0019X0023C T090522
GEN	22-May-09	5231	9.37	8.75×10^{-4}	5811	-836	1:2	Negative	Yes		XPTX0019X0023C T090522_2
GEN	09-Jun-09	912	3.83	1.16×10^{-3}	-3995	237690	1:2	Negative	No	Failed E2, FLA\E2	XPTX0019X0023A NTCT090609
GEN	26-Jun-09	1245	6.06	7.70×10^{-4}	10829	3563	1:2	Negative	No	Failed E2 Control	XPTX0019X0023A NTCT090630
RES	07-May-09	2489	6.10	5.94×10^{-4}	7893	2799	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0023X0017A NTCT090507
RES	07-May-09	2765	6.54	5.93×10^{-4}	5567	2618	1:2	2.54×10^{1}	Yes		XPTX0017X0019A NTCT090507
RES	19-May-09	1283	10.63	6.42×10^{-4}	8350	2899	1:2	Positive but an IC_{50} value could not be calculated	Yes		XPTX0026X0017A NTCT090519
TAM	03-May-09	3772	4.64	6.86×10^{-4}	8862	3120	1:2	Positive but an IC_{50} value could not be calculated	No	Failed FLA\E2	XPTX0048X0026A NTCT090503_1
TAM	03-May-09	3987	4.21	6.35×10^{-4}	6286	3591	1:2	1.83×10^{-1}	Yes		XPTX0048X0026A NTCT090503_2

Test Substance	Date	DMSO	Reduction[1]	Ral/E2 Reference Standard IC_{50} (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC_{50} (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
TAM	19-May-09	1283	10.63	6.42×10^{-4}	8350	2899	1:2	2.85×10^{-1}	Yes		XPTX0026X0017A NTCT090519

Abbreviations: API = Apigenin; ATZ = Atrazine; Cont = Control; CORT = Corticosterone; DBA = Dibenzo [*a,h*]anthracene; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17ß-estradiol; Fla = Flavone; GEN = Genistein; IC_{50} = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol; TAM = Tamoxifen

[1]Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.