

Appendix A

Experiments Conducted During the BG1LUC ER TA Protocol Standardization Effort

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Table A-1 Experiments Conducted During Agonist Plate Design

Experiment I.D.¹	Substance Code²	Date	Plate Induction³	EC₅₀ (µg/mL)⁴	Rationale for Unacceptability⁵
Temp1	E2	09/16/05	not calculated	not calculated	Acceptable
Temp1a	E2	09/16/05	not calculated	not calculated	Acceptable
Temp2	E2	09/16/05	not calculated	not calculated	Acceptable
ICCVAM New Plate	E2	09/23/05	not calculated	not calculated	Acceptable
ICCVAM Old Plate	E2	09/23/05	12.6	not calculated	Acceptable
ICCVAM Template QC Test	E2	09/29/05	7.4	not calculated	Acceptable
Temp CM	E2	09/30/05	8.6	not calculated	Acceptable
new compound test	E2	10/06/05	6.5	not calculated	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate induction was not calculated during early plate design experiments

⁴ EC₅₀ values for the E2 reference standard were not calculated during the plate design phase.

⁵ Acceptability of plate design experiments was determined by comparison of the shape of the E2 reference standard curve to historical laboratory curves

Table A-2 Experiments Conducted During Compilation of the Agonist Historical Database

Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL)	Rationale for Unacceptability
AG 1	E2	10/12/05	8.9	2.58 x 10 ⁻⁵	Acceptable
AG 2	E2	10/12/05	9.9	2.90 x 10 ⁻⁵	Acceptable
AG 3	E2	10/12/05	8.2	2.80 x 10 ⁻⁵	Acceptable
AG 4	E2	10/15/05	10.8	2.53 x 10 ⁻⁵	Acceptable
AG 5	E2	10/15/05	11.7	2.26 x 10 ⁻⁵	Acceptable
AG 6	E2	10/19/05	9.3	2.27 x 10 ⁻⁵	Acceptable
AG 7	E2	10/19/05	8.0	2.09 x 10 ⁻⁵	Acceptable
AG 8	E2	10/21/05	10.5	2.58 x 10 ⁻⁵	Acceptable
AG 9	E2	10/21/05	7.1	2.09 x 10 ⁻⁵	Acceptable
AG 10	E2	10/24/05	7.0	2.14 x 10 ⁻⁵	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate induction values are calculated as the averaged highest non-adjusted E2 values divided by the averaged non-adjusted DMSO values for each experiment.

Table A-3 Agonist Range Finder Experiments

Experiment I.D.	Substance Code	Date	Plate Induction	EC ₅₀ (µg/mL) ⁴	Rationale for Unacceptability
N0001 - N0004	N0001 - N0004	02/14/06	3.3	not calculated	Abnormal Morphology
N0005 - N0008	N0005 - N0008	02/14/06	2.9	not calculated	Low Induction; Abnormal Morphology
N0001 - N0004	N0001 - N0004	02/27/06	2.7	not calculated	Low Induction; Abnormal Morphology
N0005 - N0008	N0005 - N0008	02/27/06	3.0	not calculated	Abnormal Morphology
N1-N4 Range	N0001 - N0004	03/28/06	3.4	6.82 x 10 ⁻⁶	Acceptable
N5-N8 Range	N0005 - N0008	03/28/06	3.4	1.05 x 10 ⁻⁵	Acceptable
N0008 Range trip ⁵	N0008	05/09/06	3.4	8.57 x 10 ⁻⁶	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate induction values are calculated as the averaged highest non-adjusted E2 values divided by the averaged non-adjusted DMSO values for each experiment.

⁴ EC₅₀ values for the E2 reference standard were not calculated for plates that did not meet acceptance criteria

⁵ A separate range finder experiment was conducted for N0008 – flavone, to determine whether the biphasic concentration-response curve observed during initial range finder testing was repeatable.

Table A-4 Agonist Comprehensive Testing Experiments

Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL)	Rationale for Unacceptability
N0002-ag1	N0002	04/01/06	9.3	3.35 x 10 ⁻⁵	Acceptable
N0001-ag1	N0001	04/03/06	3.8	1.66 x 10 ⁻⁵	Acceptable
N0002-ag1	N0002	04/03/06	3.8	1.52 x 10 ⁻⁵	Acceptable
N0003-ag1	N0003	04/03/06	3.2	1.99 x 10 ⁻⁵	Acceptable
N0004-ag1	N0004	04/03/06	3.1	1.72 x 10 ⁻⁵	Acceptable
N0001 ag2	N0001	04/04/06	5.4	1.92 x 10 ⁻⁵	Acceptable
N0002 ag2	N0002	04/04/06	4.0	2.26 x 10 ⁻⁵	Acceptable
N0003 ag2	N0003	04/04/06	4.2	3.09 x 10 ⁻⁵	Acceptable
N0004 ag2	N0004	04/04/06	4.6	1.82 x 10 ⁻⁵	Acceptable
N0001 ag3	N0001	04/05/06	6.4	2.84 x 10 ⁻⁵	Acceptable
N0002 ag3	N0002	04/05/06	6.7	2.74 x 10 ⁻⁵	Acceptable
N0003 ag3	N0003	04/05/06	5.4	2.89 x 10 ⁻⁵	Acceptable
N0004 ag3	N0004	04/08/06	3.6	1.71 x 10 ⁻⁵	Acceptable
N0005 ag1	N0005	04/08/06	3.9	1.73 x 10 ⁻⁵	Acceptable
N0006 ag1	N0006	04/08/06	3.9	1.84 x 10 ⁻⁵	Acceptable
N0007 ag1	N0007	04/08/06	4.0	1.73 x 10 ⁻⁵	Acceptable
N0008 ag1	N0008	04/08/06	3.5	2.15 x 10 ⁻⁵	Acceptable
N0006 ag1	N0006	04/11/06	3.9	1.03 x 10 ⁻⁵	Acceptable
N0007 ag1	N0007	04/11/06	3.9	2.12 x 10 ⁻⁶	Acceptable
N0008 ag1	N0008	04/11/06	3.9	2.62 x 10 ⁻⁵	Acceptable
N0005 ag2	N0005	04/14/06	3.7	1.18 x 10 ⁻⁵	Acceptable
N0006 ag2	N0006	04/14/06	3.0	1.28 x 10 ⁻⁵	Acceptable
N0007 ag2	N0007	04/14/06	5.0	8.77 x 10 ⁻⁶	Acceptable

Experiment I.D.¹	Substance Code²	Date	Plate Induction³	EC₅₀ (µg/mL)	Rationale for Unacceptability
N0005 ag3	N0005	04/17/06	3.6	1.96 x 10 ⁻⁵	Acceptable
N0006 ag3	N0006	04/17/06	3.0	2.52 x 10 ⁻⁵	Acceptable
N0007 ag3	N0007	04/17/06	4.5	1.96 x 10 ⁻⁵	Acceptable
N0007 ag3	N0007	05/08/06	3.4	7.86 x 10 ⁻⁶	Acceptable
N0008 ag3	N0008	05/11/06	3.9	8.86 x 10 ⁻⁶	Acceptable
N0008 ag4	N0008	05/13/06	3.2	1.34 x 10 ⁻⁵	Acceptable
N0008 ag5	N0008	05/16/06	5.0	1.17 x 10 ⁻⁵	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate induction values are calculated as the averaged highest non-adjusted E2 values divided by the averaged non-adjusted DMSO values for each experiment.

Table A-5 Experiments Conducted During Antagonist Plate Design

Experiment I.D.¹	Substance Code²	Date	Plate Reduction³	IC₅₀ (µg/mL)⁴	Rationale for Unacceptability⁵
Ral x3	Raloxifene	10/06/05	5.6	not calculated	Acceptable
Antagonist Test	Raloxifene	10/07/05	3.7	not calculated	Acceptable
Ral x3	Raloxifene	10/10/05	7.6	not calculated	Acceptable
Ral x3	Raloxifene	10/13/05	3.4	not calculated	Acceptable
AN 1	Raloxifene	10/22/05	10.2	not calculated	Acceptable
AN 2	Raloxifene	10/22/05	9.0	not calculated	Acceptable
AN 3	Raloxifene	10/22/05	6.8	not calculated	Acceptable
AN6r	Raloxifene	12/02/05	7.2	not calculated	Acceptable
AN7r	Raloxifene	12/02/05	5.8	not calculated	Acceptable
AN8r	Raloxifene	12/04/05	4.7	not calculated	Acceptable
AN9	Raloxifene	12/06/05	6.1	not calculated	Acceptable
AN10	Raloxifene	12/06/05	6.2	not calculated	Acceptable
AN12	Raloxifene	12/06/05	6.2	not calculated	Acceptable
AN13	Raloxifene	12/09/05	6.0	not calculated	Acceptable
AN14	Raloxifene	12/09/05	5.8	not calculated	Acceptable
New Curve Design	Raloxifene	12/09/05	6.8	not calculated	Acceptable
AN15	Raloxifene	12/12/05	5.6	not calculated	Acceptable
New Curve Design - 4 A	Raloxifene	12/14/05	8.1	not calculated	Acceptable
New Curve Design - 4 B	Raloxifene	12/14/05	9.0	not calculated	Acceptable
AN New 3 - BE - 1	Raloxifene	12/27/05	11.1	not calculated	Acceptable
AN New 3 - BE - 2	Raloxifene	12/27/05	9.5	not calculated	Acceptable
AN New 3 - BE - 4	Raloxifene	12/29/05	11.2	not calculated	Acceptable
AN New 3 - BE - 5	Raloxifene	12/29/05	10.6	not calculated	Acceptable

Experiment I.D.¹	Substance Code²	Date	Plate Reduction³	IC₅₀ (µg/mL)⁴	Rationale for Unacceptability⁵
AN New 3 - BE - 1b	Raloxifene	12/30/05	10.6	not calculated	Acceptable
AN New 3 - BE - 2b	Raloxifene	12/30/05	11.3	not calculated	Acceptable
AN New 3 - BE - 3b	Raloxifene	12/30/05	10.3	not calculated	Acceptable
AN New 3 - BE - 4b	Raloxifene	12/30/05	9.5	not calculated	Acceptable
AN New 3 - BE - 5b	Raloxifene	12/30/05	10.3	not calculated	Acceptable
New 3-1b, Estradiol Test	Raloxifene	01/03/06	5.1	not calculated	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate reduction was calculated as the averaged highest non-adjusted Ral/E2 values divided by the averaged non-adjusted Ral/E2 values for each experiment.

⁴ IC₅₀ values for the ral/E2 reference standard were not calculated during the plate design phase.

⁵ Acceptability of antagonist plate design experiments was determined by calculation of reduction, and by observation of the shape of the ral/E2 reference standard curve.

Table A-6 Experiments Conducted During Compilation of the Antagonist Historical Database

Experiment I.D.¹	Substance Code²	Date	Plate Reduction³	IC₅₀ (µg/mL)	Rationale for Unacceptability
New 3A	Raloxifene	12/17/05	6.4	8.20 x 10 ⁻⁴	Acceptable
New 3B	Raloxifene	12/17/05	5.5	1.50 x 10 ⁻³	Acceptable
New 3C	Raloxifene	12/20/05	9.0	1.10 x 10 ⁻³	Acceptable
New 3D	Raloxifene	12/20/05	6.1	7.20 x 10 ⁻⁴	Acceptable
New 3E	Raloxifene	12/20/05	8.0	1.30 x 10 ⁻³	Acceptable
New 3F	Raloxifene	12/23/05	8.6	9.50 x 10 ⁻⁴	Acceptable
New 3G	Raloxifene	12/23/05	7.1	1.70 x 10 ⁻³	Acceptable
New 3H	Raloxifene	12/27/05	7.2	6.50 x 10 ⁻⁴	Acceptable
New 3I	Raloxifene	12/27/05	7.1	1.70 x 10 ⁻³	Acceptable
New 3J	Raloxifene	12/27/05	6.9	1.40 x 10 ⁻³	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate reduction was calculated as the averaged highest non-adjusted Ral/E2 values divided by the averaged non-adjusted Ral/E2 values for each experiment.

Table A-7 Experiments Conducted During Redesign of the Ral/E2 Reference Standard

Experiment I.D. ¹	Substance Code ²	Date	Plate Reduction ³	IC ₅₀ (µg/mL)	Rationale for Unacceptability
Ral serial test	Raloxifene	04/01/06	6.9	not calculated	Acceptable
Ral 1-2A	Raloxifene	04/04/06	9.8	1.51 x 10 ⁻³	Acceptable
Ral 1-2B	Raloxifene	04/04/06	9.1	1.49 x 10 ⁻³	Acceptable
Ral 1-2C	Raloxifene	04/04/06	9.7	1.53 x 10 ⁻³	Acceptable
Ral 1-2D	Raloxifene	04/04/06	9.4	1.50 x 10 ⁻³	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate reduction was calculated as the averaged highest non-adjusted Ral/E2 values divided by the averaged non-adjusted Ral/E2 values for each experiment.

Table A-8 Antagonist Range Finder Experiments

Experiment I.D. ¹	Substance Code ²	Date	Plate Reduction ³	IC ₅₀ (µg/mL)	Rationale for Unacceptability
N9 - N12	N0009 - N0012	04/06/06	8.2	1.33 x 10 ⁻³	Acceptable
N13 - N16	N0013 - N0016	04/06/06	7.8	1.40 x 10 ⁻³	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate reduction was calculated as the averaged highest non-adjusted Ral/E2 values divided by the averaged non-adjusted Ral/E2 values for each experiment.

Table A-9 Antagonist Comprehensive Testing Experiments

Experiment I.D.¹	Substance Code²	Date	Plate Reduction³	IC₅₀ (µg/mL)	Rationale for Unacceptability
N0009 ant1	N0009	04/12/06	4.4	9.55 x 10 ⁻⁴	Acceptable
N0010 ant1	N0010	04/12/06	5.1	7.93 x 10 ⁻⁴	Acceptable
N0011 ant1	N0011	04/12/06	5.4	7.96 x 10 ⁻⁴	Acceptable
N0012 ant1	N0012	04/12/06	5.7	9.07 x 10 ⁻⁴	Acceptable
N0009 ant2	N0009	04/15/06	6.4	1.18 x 10 ⁻³	Acceptable
N0010 ant2	N0010	04/15/06	6.2	1.01 x 10 ⁻³	Acceptable
N0011 ant2	N0010	04/15/06	6.3	1.10 x 10 ⁻³	Acceptable
N0012 ant2	N0012	04/15/06	5.8	1.12 x 10 ⁻³	Acceptable
N0013 ant1	N0013	04/15/06	5.3	1.27 x 10 ⁻³	Acceptable
N0014 ant1	N0014	04/15/06	5.3	1.43 x 10 ⁻³	Acceptable
N0009ant3	N0009	04/18/06	5.4	9.74 x 10 ⁻⁴	Acceptable
N0010 ant3	N0010	04/18/06	4.8	1.21 x 10 ⁻³	Acceptable
N0011 ant3	N0011	04/18/06	6.1	8.73 x 10 ⁻⁴	Acceptable
N0012 ant3	N0012	04/18/06	4.5	1.10 x 10 ⁻³	Acceptable
N0013 ant2	N0013	04/20/06	6.6	1.29 x 10 ⁻³	Acceptable
N0014 ant2	N0014	04/20/06	6.5	1.18 x 10 ⁻³	Acceptable
N0015 ant1	N0015	04/20/06	6.2	1.15 x 10 ⁻³	Acceptable
N0016 ant1	N0016	04/20/06	6.4	1.29 x 10 ⁻³	Acceptable
N0013 ant3	N0013	05/01/06	6.3	1.12 x 10 ⁻³	Acceptable
N0015 ant2	N0015	05/01/06	5.8	1.07 x 10 ⁻³	Acceptable
N0016 ant2	N0016	05/01/06	6.1	1.65 x 10 ⁻³	Acceptable
N0014 ant3	N0014	05/05/06	5.7	9.73 x 10 ⁻⁴	Acceptable
N0015 ant3	N0015	05/05/06	5.6	1.14 x 10 ⁻³	Acceptable

Experiment I.D.¹	Substance Code²	Date	Plate Reduction³	IC₅₀ (µg/mL)	Rationale for Unacceptability
N0016 ant3	N0016	05/05/06	5.7	1.13 x 10 ⁻³	Acceptable
N0016 ant4	N0016	05/09/06	5.1	1.09 x 10 ⁻³	Acceptable
N0016 ant5	N0016	05/11/06	10.8	1.30 x 10 ⁻³	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate reduction was calculated as the averaged highest non-adjusted Ral/E2 values divided by the averaged non-adjusted Ral/E2 values for each experiment.

Table A-10 CellTiter-Blue™ Experiments Conducted During Protocol Standardization

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
Via Test 5 Blue	Raloxifene	12-8-05	No color developed
Via Test 7 Blue	Raloxifene	12-14-05	No color developed

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

Table A-11 CellTiter-Glo® Experiments Conducted During Protocol Standardization

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
Via b	Raloxifene and E2	10/28/05	Acceptable
Via Test 2	Raloxifene and E2	12/04/05	Acceptable
Via Test 3	E2	12/08/05	Acceptable
Via Test 4 Glo	Raloxifene and E2	12/08/05	Acceptable
Via Test 6 Glo	Raloxifene and E2	12/14/05	Acceptable
Ag Via 1	E2	12/28/05	Acceptable
Ag Via 2	E2	12/28/05	Acceptable
AG Via 3	E2	01/03/06	Acceptable
AG Via 4	E2	01/03/06	Acceptable
Flav VIABILITY	Flavone	01/31/06	Acceptable
N1 - N4 Via	N1 - N4	02/14/06	Acceptable
N5 - N8 Via	N5 - N8	02/14/06	Acceptable
N0001 - N0004 Via b	N1 - N4	02/27/06	Acceptable
N0005 - N0008 Via b	N5 - N8	02/27/06	Acceptable
Agonist N0001 - N0004 costar plate Via	N1 - N4	03/06/06	Acceptable
Agonist N0001 - N0004 new plate Via	N1 - N4	03/06/06	Acceptable
Agonist N0005 - N0008 Costar sealed plate via	N5 - N8	03/06/06	Acceptable
Agonist N0001 - N0004 Viability	N1 - N4	03/08/06	Acceptable
Agonist N0005 - N0008 Viability	N5 - N8	03/08/06	Acceptable
Agonist N0001 - N0004 Viability	N1 - N4	03/09/06	Acceptable

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
bg1, 11-12-03, G418 QC via	G418	03/12/06	Acceptable
bg1, 11-12-03, G418 treated cell via	G418	03/12/06	Acceptable
111203 agonist,antagonist corning flasks via	E2	03/12/06	Acceptable
111203 agonist,antagonist falcon flasks via	E2	03/12/06	Acceptable
bg1-DB agonist,antagonist, via QC	E2	03/12/06	Acceptable
N0001 Via	N0001	03/18/06	Acceptable
N0002 Via	N0002	03/18/06	Acceptable
bg1 3801, Denisons DMEM&FBS(A19)	E2	03/19/06	Acceptable
bg1 3801, Denisons DMEM&FBS(A20)	E2	03/19/06	Acceptable
N0001 Via	N0001	03/20/06	Acceptable
N0002 Via	N0002	03/20/06	Acceptable
N0002-ag1 via	N0002	04/01/06	Acceptable
N0001-ag1 via	N0001	04/03/06	Acceptable
N0002-ag1 via	N0002	04/03/06	Acceptable
N0003-ag1 via	N0003	04/03/06	Acceptable
N0004-ag1 via	N0004	04/03/06	Acceptable
N0001 ag2 via	N0001	04/04/06	Acceptable
N0002 ag2 via	N0002	04/04/06	Acceptable
N0003 ag2 via	N0003	04/04/06	Acceptable
N0004 ag2 via	N0004	04/04/06	Acceptable
N0001 ag3 via	N0001	04/05/06	Acceptable

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
N0002 ag3 via	N0002	04/05/06	Acceptable
N0003 ag3 via	N0003	04/05/06	Acceptable
N0009 - N00012 via	N0009 - N00012	04/06/06	Acceptable
N0013 - N0016 via	N0013 - N0016	04/06/06	Acceptable
N0004 ag3 via	N0004	04/08/06	Acceptable
N0005 ag1 via	N0005	04/08/06	Acceptable
N0006 ag1 via	N0006	04/08/06	Acceptable
N0007 ag1 via	N0007	04/08/06	Acceptable
N0008 ag1 via	N0008	04/08/06	Acceptable
N0006 ag1 via	N0006	04/11/06	Acceptable
N0007 ag1 via	N0007	04/11/06	Acceptable
N0008 ag1 via	N0008	04/11/06	Acceptable
N0005 ag2 via	N0005	04/14/06	Acceptable
N0006 ag2 via	N0006	04/14/06	Acceptable
N0007 ag2 via	N0007	04/14/06	Acceptable
N0005 ag3 via	N0005	04/17/06	Acceptable
N0006 ag3 via	N0006	04/17/06	Acceptable
N0007 ag3 via	N0007	04/17/06	Acceptable
N0009 ant1 via	N0009	04/12/06	Acceptable
N0010 ant1 via	N0010	04/12/06	Acceptable
N0011 ant1 via	N0011	04/12/06	Acceptable
N0012 ant1 via	N0012	04/12/06	Acceptable
N0009 ant2 via	N0009	04/15/06	Acceptable
N0010 ant2 via	N0010	04/15/06	Acceptable

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
N0011 ant2 via	N0011	04/15/06	Acceptable
N0012 ant2 via	N0012	04/15/06	Acceptable
N0013 ant1 via	N0013	04/15/06	Acceptable
N0014 ant1 via	N0014	04/15/06	Acceptable
N0009ant3 via	N0009	04/18/06	Acceptable
N0010 ant3 via	N0010	04/18/06	Acceptable
N0011 ant3 via	N0011	04/18/06	Acceptable
N0012 ant3 via	N0012	04/18/06	Acceptable
N0013 ant2 via	N0013	04/20/06	Acceptable
N0014 ant2 via	N0014	04/20/06	Acceptable
N0015 ant1 via	N0015	04/20/06	Acceptable
N0016 ant1 via	N0016	04/20/06	Acceptable
N0013 ant3 via	N0013	05/01/06	Acceptable
N0015 ant2 via	N0015	05/01/06	Acceptable
N0014 ant3 via	N0014	05/05/06	Acceptable
N0015 ant3 via	N0015	05/05/06	Acceptable
N0016 ant2 via	N0016	05/01/06	Acceptable
N0016 ant3 via	N0016	05/05/06	Acceptable
N0007 ag3 via	N0007	05/08/06	Acceptable
N0016 ant4 via	N0016	05/09/06	Acceptable
N0008 Range Trip via	N0008	05/09/06	Acceptable
N0016 ant5 via	N0016	05/11/06	Acceptable
N0008 ag3 via	N0008	05/11/06	Acceptable
N0008 ag4 via	N0008	05/13/06	Acceptable

Experiment I.D.¹	Substance Code²	Date	Rationale for Unacceptability
N0008 ag5 via	N0008	05/16/06	Acceptable

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

Table A-12 Experiments Conducted to Evaluate Abnormal Responses and/or Decreased Viability

Quality Testing of Materials							
Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL) ⁴	Plate Reduction ⁵	IC ₅₀ (µg/mL) ⁶	Rationale for Unacceptability
BE Test Mick BE, John BE	E2	01/05/06	2.6	NC	N/A	N/A	Low Induction; Abnormal Morphology
Time Course DMSO	Ral	01/21/06	N/A	N/A	6.1	NC	Abnormal Morphology
Time Course EtOH	Ral	01/21/06	N/A	N/A	4.3	NC	Abnormal Morphology
Time Course Mix 1 and 2	Ral	01/21/06	N/A	N/A	11.1	NC	Abnormal Morphology
DMSO Test	E2	01/31/06	2.9	NC	N/A	N/A	Low Induction; Abnormal Morphology
Rinsed vs non Rinsed	E2	01/31/06	2.0	NC	N/A	N/A	Low Induction; Abnormal Morphology
Old Flasks	E2	02/03/06	4.9	NC	N/A	N/A	Abnormal Morphology
berthold vs lucy1	E2	02/16/06	3.3	NC	N/A	N/A	Abnormal Morphology
NOB-F - Testing Stripped FBS and DMSO	E2	02/22/06	4.7	NC	N/A	N/A	Abnormal Morphology
NOB-R - Testing Stripped FBS and DMSO	E2	02/22/06	6.4	NC	N/A	N/A	Abnormal Morphology
OB-F - Testing Stripped FBS and DMSO	E2	02/22/06	4.1	NC	N/A	N/A	Abnormal Morphology

Quality Testing of Materials							
Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL) ⁴	Plate Reduction ⁵	IC ₅₀ (µg/mL) ⁶	Rationale for Unacceptability
OB-R - Testing Stripped FBS and DMSO	E2	02/22/06	4.7	NC	N/A	N/A	Abnormal Morphology
no G418, RPMI, lot	E2	02/23/06	4.2	NC	N/A	N/A	Abnormal Morphology
NO G418 vs G418, DMEM	E2	02/25/06	4.3	NC	N/A	N/A	Abnormal Morphology
bg1, 110ul G418 RPMI, DMEM new vs bad	E2	03/01/06	3.0	NC	N/A	N/A	Abnormal Morphology
bg1, 220ul G418 RPMI, DMEM new vs bad	E2	03/01/06	3.4	NC	N/A	N/A	Abnormal Morphology
bg1, new plate, rinsed vs non-rinsed tubes	E2	03/01/06	6.5	NC	N/A	N/A	Abnormal Morphology
Agonist N0005 - N0008 derws cells new plate	N0005 - N0008	03/05/06	4.4	NC	N/A	N/A	Abnormal Morphology
Agonist N0001 - N0004 Costar plate	N0001 - N0004	03/05/06	3.4	NC	N/A	N/A	Abnormal Morphology
Agonist N0001 - N0004 new plate	N0001 - N0004	03/06/06	1.3	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1, agonist, antagonist QC	E2	03/07/06	4.9	NC	10.9	N/A	Abnormal Morphology

Quality Testing of Materials							
Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL) ⁴	Plate Reduction ⁵	IC ₅₀ (µg/mL) ⁶	Rationale for Unacceptability
Agonist N0001 - N0004	N0001 - N0004	03/08/06	1.9	NC	N/A	N/A	Low Induction; Abnormal Morphology
Agonist N0005 - N0008	N0005 - N0008	03/08/06	1.9	NC	N/A	N/A	Low Induction; Abnormal Morphology
Agonist N0001 - N0004	N0001 - N0004	03/09/06	1.4	NC	N/A	N/A	Low Induction; Abnormal Morphology
Beta tests	E2	03/09/06	1.4	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1, 11-12-03, Media QC, G418 treated cells	E2	03/12/06	1.9	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1, 11-12-03, Media QC, Non-G418 treated cells	E2	03/12/06	1.7	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1,111203 agonist,antagonist corning flasks	E2	03/12/06	1.8	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1,111203 agonist,antagonist falcon flasks	E2	03/12/06	1.8	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1-DB agonist,antagonist, via QC	E2	03/12/06	1.2	NC	N/A	N/A	Low Induction; Abnormal Morphology
N0001 Trip	E2	03/18/06	1.6	NC	N/A	N/A	Abnormal Morphology

Quality Testing of Materials							
Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL) ⁴	Plate Reduction ⁵	IC ₅₀ (µg/mL) ⁶	Rationale for Unacceptability
N0002 Trip	E2	03/18/06	1.5	NC	N/A	N/A	Abnormal Morphology
bg13801,Denisons background experiment 24hrs	E2	03/21/06	2.5	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg13801,Denisons background experiment 48hrs	E2	03/21/06	2.0	NC	N/A	N/A	Low Induction; Abnormal Morphology
bg1-3801 f-cfc, cell supply QC	E2	03/24/06	6.2	NC	N/A	N/A	Acceptable
BG-1 3-8-01	E2	03/24/06	6.2	3.02 x 10 ⁻⁵	N/A	N/A	Acceptable
DB 2-7-06	E2	03/26/06	4.5	1.38 x 10 ⁻⁵	N/A	N/A	Acceptable
bg1DB3706 F25 2 days,luc and via QC	E2	03/26/06	4.5	NC	N/A	N/A	Acceptable
Test No Falcon	E2	03/27/06	7.5	1.57 x 10 ⁻⁵	N/A	N/A	Acceptable
bgMERE	E2	03/29/06	4.4	1.40 x 10 ⁻⁵	N/A	N/A	Acceptable
bg1DB3706 RPMI no phenol red luc and via QC	E2	03/29/06	2.9	NC	N/A	N/A	Low Induction; Abnormal Morphology
bgMERE 11203 luc and via QC	E2	03/29/06	7.0	NC	N/A	N/A	Acceptable

Quality Testing of Materials							
Experiment I.D. ¹	Substance Code ²	Date	Plate Induction ³	EC ₅₀ (µg/mL) ⁴	Plate Reduction ⁵	IC ₅₀ (µg/mL) ⁶	Rationale for Unacceptability
bg1 N0008 RF R&D	E2	04/08/06	1.7	NC	N/A	N/A	Low Induction; Abnormal Morphology
N0005 ag-2 fails induction	E2	04/08/06	1.8	NC	N/A	N/A	Low Induction; Abnormal Morphology
DMSO Test, 3 point BE	E2	04/14/06	3.7	NC	N/A	N/A	Abnormal Morphology

Abbreviations: N/A = Not Applicable; NC = Not Calculated; Ral = Raloxifene

¹ Experiment I.D. is the name assigned by the laboratory to each test plate (1 test plate = 1 experiment)

² The substance code is used by the laboratory to track the main substance being tested during the experiment (controls are not reflected in the substance code)

³ Plate induction values are calculated as the averaged highest non-adjusted E2 values divided by the averaged non-adjusted DMSO values for each experiment.

⁴ EC₅₀ values for the E2 reference standard were not calculated for plates that did not meet acceptance criteria

⁵ Plate reduction was calculated as the averaged highest non-adjusted ral/E2 values divided by the averaged non-adjusted ral/E2 values for each experiment.

⁶ IC₅₀ values for the ral/E2 reference standard were not calculated for plates that did not meet acceptance criteria