FINAL REPORT

Study Title

GreenScreen HC (GADD45α-GFP) Assay

Test Article

GB67B and GB594

Authors

Kamala Pant, M.S.
Shannon Wilson Bruce, M.F.S.

Study Completion Date

03 April 2008

Testing Facility

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AC13JU-JV.801.BTL

Sponsor

Emory University
1515 Dickey Drive
Atlanta, GA 30322

GreenScreen Human Cell Genotoxicity Assay

STUDY INFORMATION

Sponsor:	**Emory University** **1515 Dickey Drive** **Atlanta, GA 30322**
Authorized Representative:	**Gregory Bluemling**
Testing Facility:	**BioReliance** **9630 Medical Center Drive** **Rockville, Maryland 20850**
Storage Conditions:	**Refrigerated (2 to 8°C) in the dark with desiccant**
Test Article Receipt and Login:	**04 March 2008**
Study Initiation:	**12 March 2008**
Experimental Start Date:	**12 March 2008**
Experimental Completion Date:	**20 March 2008**
Laboratory Manager	**Shannon Wilson Bruce, M.F.S.**

Test Article ID	BioReliance Study No.	Test Article Description	Test Article Purity
GB67B	AC13JU.801.BTL	white powder	99%
GB594	AC13JV.801.BTL	white powder	99%

Study Director: Kamala Pant 03 Apr 2008

Kamala Pant, M.S. Date

EXPERIMENTAL DESIGN AND METHODOLOGY

Assay Description

The assay uses the GFP reporter to show the transcription of GADD45α gene, an indication of the genotoxic potential of a test article. GADD45α is a human protein that is involved in many cellular processes: DNA damage and repair, apoptosis, cell cycle control, etc. Following exposure to genotoxic stress, the GADD45α gene is transcriptionally induced and GFP is produced (Hastwell *et al.*, 2006).

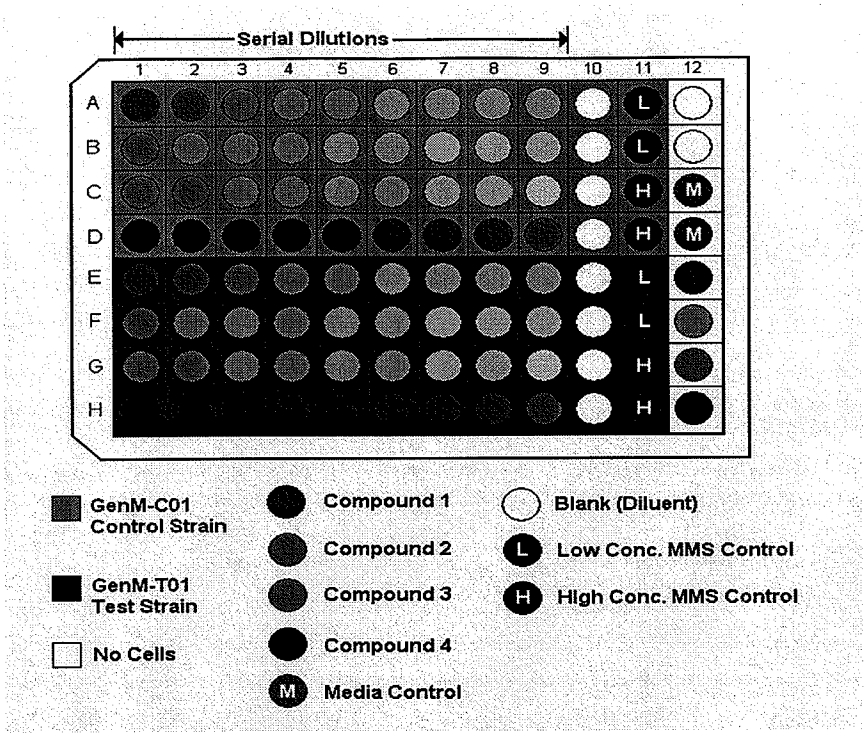
GreenScreen HC assay can only be used to test compounds in the absence of metabolic activation at the present time. Research is underway to develop a GreenScreen HC assay using metabolic activation (Hastwell *et al.*, 2006).

Test System

Two human cell lines, GenM-C01 and GenM-T01 (Gentronix©, Manchester, UK) were used in the assay. The reporter cell line (GenM-T01) consists of TK6 cells transfected by electroporation with an episomally replicating Epstein-Barr virus-based plasmid bearing the upstream promoter region and regulatory gene sequences of the human Growth Arrest and DNA Damage (GADD45α) gene operatively linked to a human codon optimized green fluorescent protein (GFP) gene (EGFP). The control cell line (GenM-C01) consists of TK6 cells transfected with an identical plasmid except that 4 base pairs have been removed at the start of the EGFP gene, such that a functional GFP protein is not produced. The plasmids are stably maintained in TK6 cells by addition of 200 µg/mL hygromycin B to the cultures.

Experimental Design

The test system was exposed to the test article via a microplate format described by Hastwell *et al.* (2006). The test article was tested at nine serially diluted levels. In the 96-well microplate, rows A, B, C, and D (columns 1-11) were treated with GenM-C01 cell suspension and rows E, F, G, and H (columns 1-11) were treated with GenM-T01 cell suspension. Column 12 was used for diluent, media and test article sample contamination controls. Once all the treatment solutions, positive controls, negative controls, sterility control, blank control (diluent) and the cells have been added to the 96-well microplate, the microplate was covered with a breathable membrane. The microplate was incubated in a humidified, 5% CO_2 incubator set at 37 ± 1 °C. Following is the plate diagram showing the assay details:



Data Collection

The GFP-reporter fluorescence and cell culture absorbance data were collected from the microplates approximately 24 and 48 hours after treatment. In addition, fluorescence polarization data were collected and used only if needed. The fluorescence polarization is performed if the test article is significantly fluorescent and causes interference in both control and test strains. The data were inserted into an Excel spreadsheet template obtained from Gentronix (Manchester, UK). Individual data sheets are given in Appendix I.

Data Analysis

Relative Cell Density Calculation

Green fluorescent protein induction was evidenced by an increase in the fluorescence level of the GreenScreen Test Strain (GenM-T01) as compared to the GreenScreen Control Strain (GenM-C01). In addition, relative cell density was determined. The relative cell density results were calculated as follows:

$$\text{Relative Cell Density} = \frac{\text{Absorbance of the Test Well} - \text{Absorbance of the Media Blank}}{\text{Absorbance of the Respective UC} - \text{Absorbance of the Media Blank}} \times 100$$

where the "Media Blank" is an average reading from the wells filled with diluted assay medium alone (C12 and D12), and a "Respective UC" is an average reading from wells containing the relevant strain exposed to diluent only, i.e. the untreated control.

Fluorescence Induction Calculations

Fluorescence data values are divided by absorbance data to give 'brightness units', the measure of the average GFP induction per cell. These data are then normalized to the untreated control (=1). In order to correct for induced cellular auto-fluorescence and intrinsic test article fluorescence, the brightness value for the GenM-C01 cell line was subtracted from those of GenM-T01 cell line.

Fluorescence data values are divided by absorbance data to give 'brightness units', the measure of the average GFP induction per cell. These data are then normalized to the untreated control (=1). In order to correct for induced cellular auto-fluorescence and intrinsic test article fluorescence, the brightness value for the GenM-C01 cell line was subtracted from those of GenM-T01 cell line.

The "Brightness" values (normalized fluorescence) for both GenM-C01 and GenM-T01 test wells are calculated thus:

$$\textbf{Brightness} = \frac{\textbf{Fluorescence of the Test Well - Fluorescence of the Media Blank}}{\textbf{Absorbance of the Test Well - Absorbance of the Media Blank}}$$

The relative GFP induction of both GenM-C01 and GenM-T01 test wells (presented in the "Raw Data for Individual Strains" graph) are calculated thus:

$$\textbf{Induction} = \frac{\textbf{Brightness of the Test Well}}{\textbf{Average Brightness of GenM - T01 Untreated Control}}$$

Where the GenM-T01 untreated control, is the GenM-T01 cell strain exposed to diluent only.

Note: that both strains are normalized to the brightness of the GenM-T01 cell strain to allow comparison of the relative brightness of the two strains.

The induction data for the "Genotoxicity Evaluation" graph, which is used for genotoxicity assessment, is that for GenM-T01 corrected for the brightness of GenM-C01 the control strain. Thus:

$$\textbf{Overall Induction} = \frac{\textbf{Brightness of GenM - T01 Test Well - Brightness of corresponding GenM - C01 Test Well}}{\textbf{Average Brightness of GenM - T01 UC - Average Brightness of GenM - C01 UC}}$$

Where UC = untreated control.

Evaluation of Test Results

A positive result for genotoxicity is observed when the relative GFP induction ratio is greater than the 1.5 threshold (i.e., greater than 3 times the standard deviation of the background brightness) (Hastwell *et al.*, 2006).

A positive result for cell growth inhibition (cytotoxicity) is observed when relative suspension growth drops below the 80% threshold (Hastwell *et al.*, 2006).

The Lowest Effective Concentration (LEC) is determined for any cytotoxicity and genotoxicity positive result. LEC is defined as the lowest concentration that produced at positive result for either growth inhibition (cytotoxicity) or GFP induction (genotoxicity).

Criteria for a Valid Test

The cell culture density used in the assay should be between 0.7×10^6 and 1.2×10^6 cells per mL on the day of the assay. If the cell density is not within this range, the cells are not used to perform the assay.

The media contamination check (absorbance ratio between media and blank) at the time of plate readings should indicate a lack of contamination. The fluorescence quality control check (fluorescence ratio between media and blank) at the time of plate readings should give a correct pass/fail determination.

The program gives a pass/fail indication for cytotoxicity determination in cell line GenM-C01 and genotoxicity determination in cell line GenM-T01 (the ratio between the two concentrations of methyl methane sulfonate).

The positive control ratio for both the cell lines should fall within the historical control data range for each time point. The negative control ratio reading should fall within the historical control data range for each time point. Historical control data are given in Appendix II.

Archives

Upon issue of the final report, all raw data for procedures performed at BioReliance will be sent to the sponsor.

Deviations

No known deviations from the protocol or assay-method SOPs occurred during the conduct of the study.

Results

Summarized results are shown in the following tables. Actual data tables are located in Appendix I.

GreenScreen Human Cell Genotoxicity Assay

RESULTS

BioReliance Test Code	**AC13JU**
Sponsor Test Article ID	**GB67B**
Maximum test article solublility in Dimethyl Sulfoxide (DMSO)	**100 mg/mL**
Maximum test article solubility in Sterile Water with 2% DMSO	**0.50 mg/mL**
Highest concentration of test article tested on microplate	**250 µg/mL**
Test article concentrations tested on microplate	**250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, and 0.98 µg/mL**
Concentrations of positive control (Methyl Methanesulfonate) tested on microplate	**50 and 10 µg/mL**
Genotoxicity Results	**Not Genotoxic**
Lowest Effective Concentration (LEC) to give positive result	**Not Applicable**
Cytotoxicity Results	**Cytotoxic**
Lowest Effective Concentration (LEC) to give positive result	**125 µg/mL**

GreenScreen Human Cell Genotoxicity Assay

RESULTS

BioReliance Test Code	**AC13JV**
Sponsor Test Article ID	**GB594**
Maximum test article solublility in Dimethyl Sulfoxide (DMSO)	**100 mg/mL**
Maximum test article solubility in Sterile Water with 2% DMSO	**2.0 mg/mL (B1), 0.08 mg/mL (B2)**
Highest concentration of test article tested on microplate	**1000 µg/mL (B1), 40 µg/mL (B2)**
Test article concentrations tested on microplate	**1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 µg/mL (B1)** **40, 20, 10, 5.0, 2.5, 1.25, 0.63, 0.31, and 0.16 µg/mL (B2)**
Concentrations of positive control (Methyl Methanesulfonate) tested on microplate	**50 and 10 µg/mL**
Genotoxicity Results (Experiment B2)	**Not Genotoxic**
Lowest Effective Concentration (LEC) to give positive result	**Not Applicable**
Cytotoxicity Results (Experiment B2)	**Not Cytotoxic**
Lowest Effective Concentration (LEC) to give positive result	**Not Applicable**

Conclusions

Test article GB67B (AC13JU) gave a negative response for gentoxicity and gave a positive response for cytotoxicity (LEC = 125 μg/mL) in this assay.

Test article GB594 (AC13JV) gave a positive response for gentoxicity (LEC = 7.81 μg/mL) and gave a positive response for cytotoxicity (LEC = 7.81 μg/mL) in the first experiment (B1) of this assay. However, the genotoxic response was seen only at excessively cytotoxic test article concentrations. In order to confirm these findings, the assay was repeated (experiment B2) at lower non cytotoxic test article concentrations.

Test article GB594 (AC13JV) gave a negative response for genotoxicity and cytotoxicity in the second experiment (B2) of this assay. Because the genotoxicity results from the first experiment (B1) were not duplicated in the second experiment (B2) and the genotoxic result was only seen at excessively cytotoxic test article dose concentrations, the test article was determined to be non genotoxic.

These assays met all criteria for a valid assay. Positive and negative controls were within expected ranges.

References

Hastwell PW, Chai LL, Roberts KJ, Webster TW, Harvey JS, Rees RW, Walmsley, RM. (2006). High-specificity and high-sensitivity genotoxicity assessment in a human cell line: validation of the GreenScreen HC GADD45α-GFP genotoxicity assay. Mutat Res. Sep 5; 607(2):160-75.

Van Gompel J, Woestenborghs F, Beerens D, Mackie C, Cahill PA, Knight AW, Billinton N, Tweats DJ, Walmsley RM. (2005). An assessment of the utility of the yeast GreenScreen assay in pharmaceutical screening. Mutagenesis Nov; 20(6):449-54.

Walsh L, Hastwell PW, Keenan PO, Knight AW, Billinton N, Walmsley RM. (2005). Genetic modification and variations in solvent increase the sensitivity of the yeast RAD54-GFP genotoxicity assay. Mutagenesis Sep; 20(5):317-27.

APPENDIX I

Individual Data Tables

TEST CONDITIONS

Operator: Shannon Bruce

Diluent: 2% DMSO / Water

Dilution Regime:

Sample Volume:	75	µl
Culture Volume:	75	µl
Serial Dilution Volume:	75	µl
Dilution Factor: 1/	2.00	

Assay ID: AC13JU-JV.801.BTL.B1

Set up Date: 3/12/08
Time: 12:30

Read Date: 3/13/08
Time 13:04

Assay run time: 24.6 HOURS

SAMPLE ID AND RESULTS		CYTOTOXICITY		GENOTOXICITY	
		Result	LEC	Result	LEC
1	2% DMSO Diluent	NEGATIVE	- ug/ml	NEGATIVE	- ug/ml
	Ref. No.				
	Concentration	ug/ml (Units)			
2		STRONG POSITIVE	- ug/ml	NEGATIVE	- ug/ml
	Ref. No.				
	Concentration	ug/ml (Units)			
3	AC13JU	NEGATIVE	-[3] ug/ml	NEGATIVE	- ug/ml
	Ref. No.	GB67B			
	Concentration	250 ug/ml (Units)			
4	AC13JV	STRONG POSITIVE	15.63 ug/ml	POSITIVE	7.81 ug/ml
	Ref. No.	GB594			
	Concentration	1000 ug/ml (Units)			

ABSORBANCE AND AUTOFLUORESCENCE CHECKS

1	2% DMSO Diluent	Not absorbing	Not auto-fluorescent
2	0	Not absorbing	Auto-fluorescent
3	AC13JU	Not absorbing	Not auto-fluorescent
4	AC13JV	Not absorbing	Not auto-fluorescent

CONTROLS

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY		RESULT
	HIGH	*LOW*	
GenM-C01	50.4	78.7	PASS

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION		RESULT
	HIGH	*LOW*	
GenM-T01	2.75	1.43	PASS

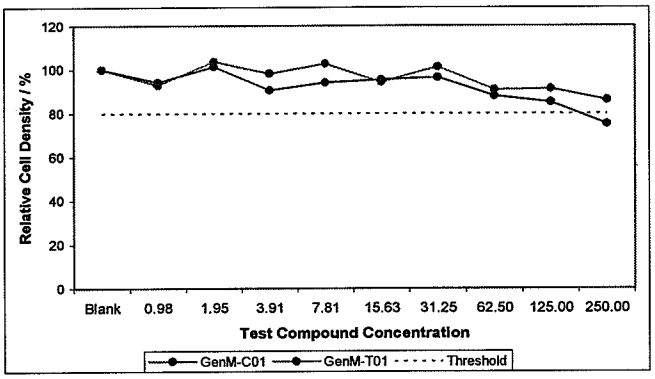
MEDIA CONTAMINATION CHECK

Abs. Ratio (Media / Blank) =	1.36
MEDIA CLEAR OF CONTAMINATION	

Fluor. Ratio (Media / Blank) =	4.74
MEDIA PASSES FLUORESCENCE QC	

Test Sample:	AC13JU	Concentration: 250.00 ug/ml	
Reference No. :	GB67B	Start Time: Day 1 Time 12:30	
Date:	3/12/2008	Read Time: Day 1 Time 13:04	
Operator:	Shannon Bruce	Run Time: 24.6 hours	
Diluent:	2% DMSO / Water		
Dilution Regime:	Sample Volume: 75 µl	Serial Dilution Volume: 75 µl	
	Culture Volume: 75 µl	Dilution Factor: 2.00	

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

NEGATIVE

LEC - ug/ml

COMPOUND ABSORBANCE CHECK

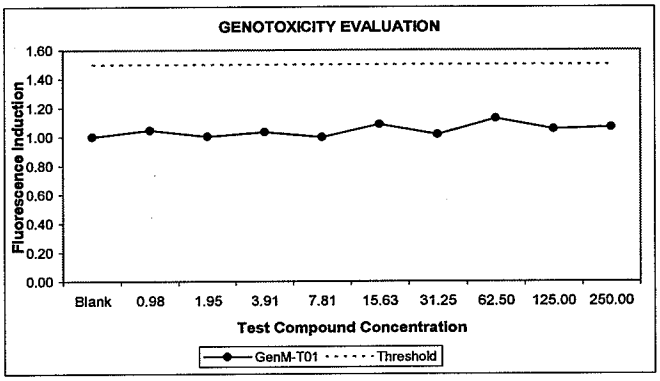
Absorbance Ratio (Control / Blank) = 1.00

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY HIGH	CELL DENSITY LOW	RESULT
GenM-C01	50.4	78.7	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

NEGATIVE

LEC - ug/ml

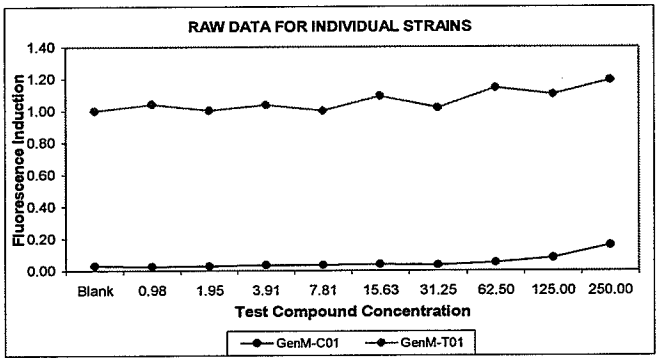
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 1.74

Not auto-fluorescent

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION HIGH	GFP INDUCTION LOW	RESULT
GenM-T01	2.75	1.43	PASS

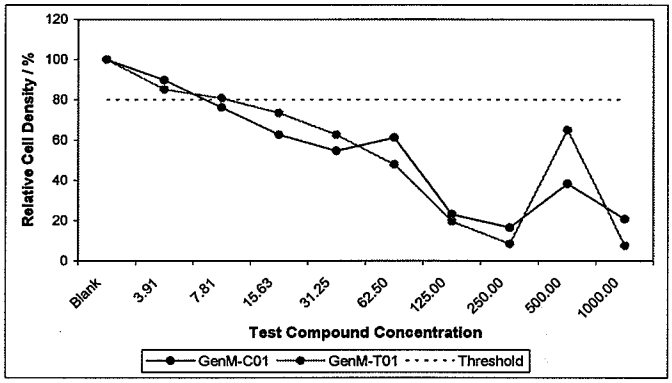


NOTES:
None

Test Sample:	AC13JV	Concentration: 1000.00	Units: ug/ml
Reference No. :	GB594		
Date:	3/12/2008		
Operator:	Shannon Bruce		
Diluent:	2% DMSO / Water		
Dilution Regime: Sample Volume:	75 µl	Serial Dilution Volume:	75 µl
Culture Volume:	75 µl	Dilution Factor:	2.00

	Day	Time
Start Time:	1	12:30
Read Time:	1	13:04
Run Time:	24.6	hours

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

STRONG POSITIVE

LEC: 15.63 ug/ml

COMPOUND ABSORBANCE CHECK

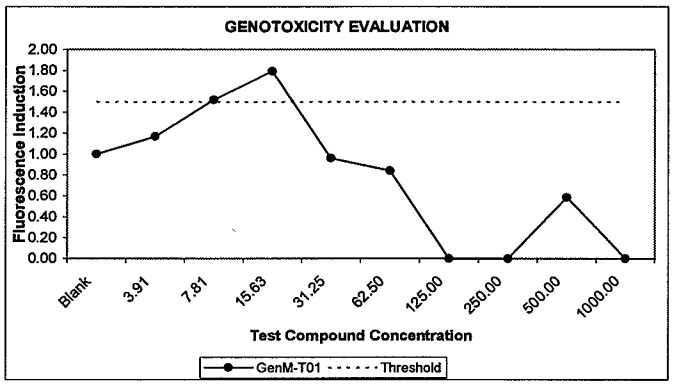
Absorbance Ratio (Control / Blank) = 1.02

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY HIGH	CELL DENSITY LOW	RESULT
GenM-C01	50.4	78.7	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

POSITIVE

LEC: 7.81 ug/ml

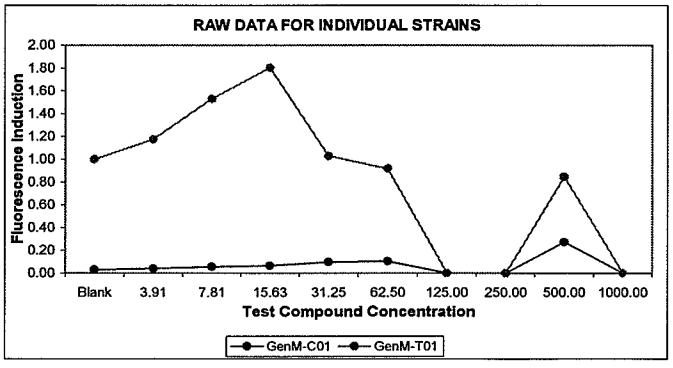
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 1.22

Not auto-fluorescent

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION HIGH	GFP INDUCTION LOW	RESULT
GenM-T01	2.75	1.43	PASS



NOTES:
None

TEST CONDITIONS

Operator:	Shannon Bruce		Assay ID:	AC13JU-JV.801.BTL.B1
Diluent:	2% DMSO / Water		Set up Date:	3/12/08
			Time:	12:30
Dilution Regime:	Sample Volume:	75 µl	Read Date:	3/14/08
	Culture Volume:	75 µl	Time	12:32
	Serial Dilution Volume:	75 µl	Assay run time:	48.0 HOURS
	Dilution Factor:	2.00		

SAMPLE ID AND RESULTS		CYTOTOXICITY		GENOTOXICITY	
1	2% DMSO Diluent	Result: NEGATIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.: 0				
	Concentration: 0 ug/ml (Units)				
2	0	Result: STRONG POSITIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.: 0				
	Concentration: 0 ug/ml (Units)				
3	AC13JU	Result: POSITIVE	LEC: 125.00 ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.: GB67B				
	Concentration: 250 ug/ml (Units)				
4	AC13JV	Result: STRONG POSITIVE	LEC: 7.81 ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.: GB594				
	Concentration: 1000 ug/ml (Units)				

ABSORBANCE AND AUTOFLUORESCENCE CHECKS

1	2% DMSO Diluent	Not absorbing	Not auto-fluorescent
2	0	Not absorbing	Not auto-fluorescent
3	AC13JU	Not absorbing	Auto-fluorescent
4	AC13JV	Not absorbing	Not auto-fluorescent

CONTROLS

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY		RESULT
	HIGH	*LOW*	
GenM-C01	35.0	66.3	PASS

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION		RESULT
	HIGH	*LOW*	
GenM-T01	2.23	1.43	PASS

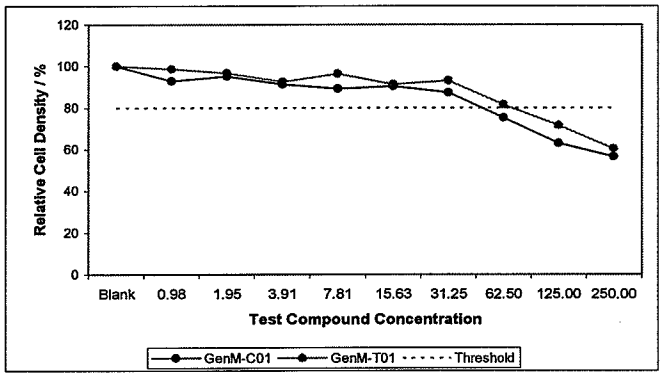
MEDIA CONTAMINATION CHECK

Abs. Ratio (Media / Blank) =	1.39
MEDIA CLEAR OF CONTAMINATION	

Fluor. Ratio (Media / Blank) =	4.52
MEDIA PASSES FLUORESCENCE QC	

Test Sample:	AC13JU	Concentration: 250.00	Units: ug/ml
Reference No. :	GB67B		
Date:	3/12/2008	Start Time: Day 1	Time 12:30
Operator:	Shannon Bruce	Read Time: Day 2	Time 12:32
Diluent:	2% DMSO / Water	Run Time:	48.0 hours
Dilution Regime: Sample Volume:	75 µl	Serial Dilution Volume:	75 µl
Culture Volume:	75 µl	Dilution Factor:	2.00

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

POSITIVE

LEC 125.00 ug/ml

COMPOUND ABSORBANCE CHECK

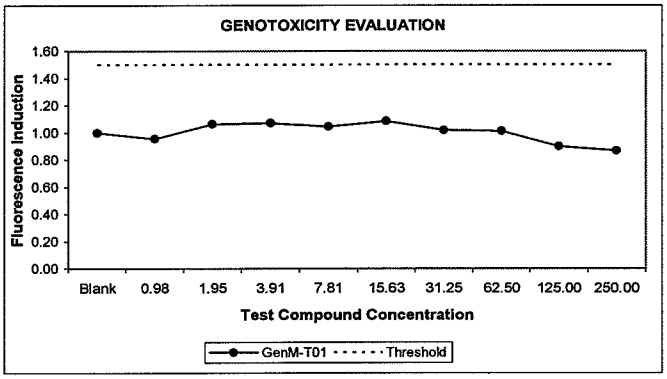
Absorbance Ratio (Control / Blank) = 0.98

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY HIGH	CELL DENSITY LOW	RESULT
GenM-C01	35.0	66.3	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

NEGATIVE

LEC - ug/ml

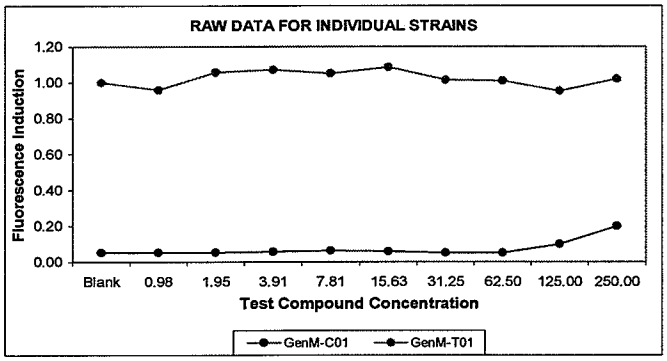
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 2.28

Auto-fluorescent

GENOTOXIC CONTROLS

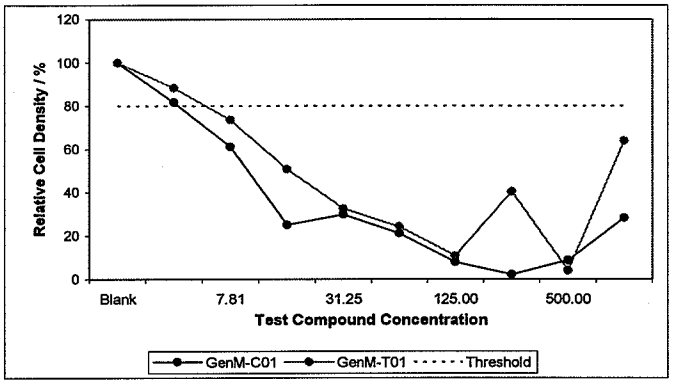
CELL LINE	GFP INDUCTION HIGH	GFP INDUCTION LOW	RESULT
GenM-T01	2.23	1.43	PASS



NOTES:

Test Sample:	AC13JV	Concentration: 1000.00	Units: ug/ml
Reference No. :	GB594		
Date:	3/12/2008	Start Time: Day 1	12:30
Operator:	Shannon Bruce	Read Time: Day 2	12:32
Diluent:	2% DMSO / Water	Run Time:	48.0 hours
Dilution Regime: Sample Volume:	75 µl	Serial Dilution Volume:	75 µl
Culture Volume:	75 µl	Dilution Factor:	2.00

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

STRONG POSITIVE

LEC: 7.81 ug/ml

COMPOUND ABSORBANCE CHECK

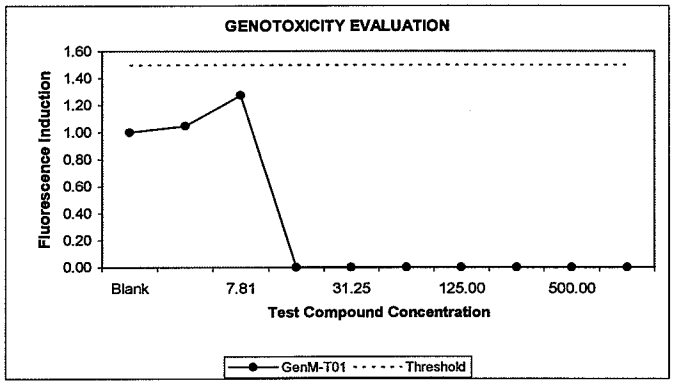
Absorbance Ratio (Control / Blank) = 1.05

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY HIGH	CELL DENSITY LOW	RESULT
GenM-C01	35.0	66.3	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

NEGATIVE

LEC: - ug/ml

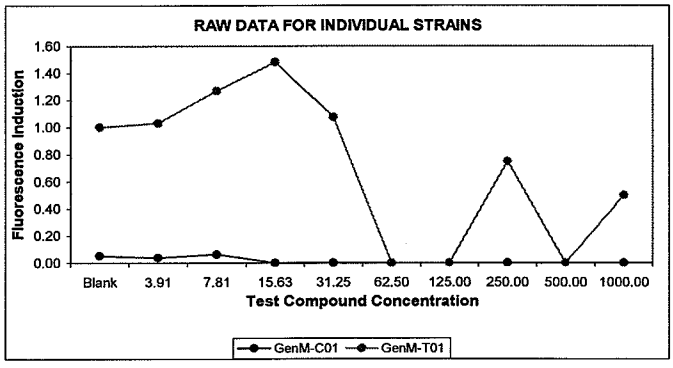
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 1.38

Not auto-fluorescent

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION HIGH	GFP INDUCTION LOW	RESULT
GenM-T01	2.23	1.43	PASS



NOTES:
None

TEST CONDITIONS

Operator:	Shannon Bruce		Assay ID:	AC13JV.801.BTL.B2
Diluent:	2% DMSO / Water		Set up Date:	3/18/08
			Time:	10:45
Dilution Regime:	Sample Volume:	75 µl	Read Date:	3/19/08
	Culture Volume:	75 µl	Time	10:41
	Serial Dilution Volume:	75 µl	Assay run time:	23.9 HOURS
	Dilution Factor: 1/	2.00		

SAMPLE ID AND RESULTS		CYTOTOXICITY		GENOTOXICITY	
1	2% DMSO Diluent	Result: NEGATIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.				
	Concentration	ug/ml (Units)			
2		Result: STRONG POSITIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.				
	Concentration	ug/ml (Units)			
3		Result: STRONG POSITIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.				
	Concentration	ug/ml (Units)			
4	AC13JV	Result: NEGATIVE	LEC: - ug/ml	Result: NEGATIVE	LEC: - ug/ml
	Ref. No.	GB594			
	Concentration	40 ug/ml (Units)			

ABSORBANCE AND AUTOFLUORESCENCE CHECKS

1	2% DMSO Diluent	Not absorbing	Not auto-fluorescent
2	0	Not absorbing	Not auto-fluorescent
3	0	Absorbing	Not auto-fluorescent
4	AC13JV	Not absorbing	Not auto-fluorescent

CONTROLS

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY		RESULT
	HIGH	*LOW*	
GenM-C01	50.1	81.1	PASS

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION		RESULT
	HIGH	*LOW*	
GenM-T01	2.69	1.40	PASS

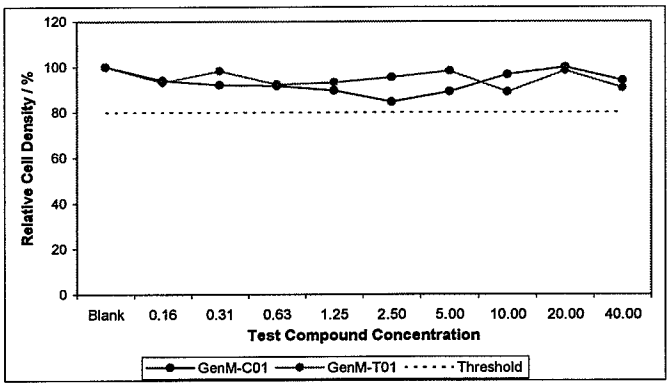
MEDIA CONTAMINATION CHECK

Abs. Ratio (Media / Blank) =	1.43
MEDIA CLEAR OF CONTAMINATION	

Fluor. Ratio (Media / Blank) =	5.17
MEDIA PASSES FLUORESCENCE QC	

Test Sample:	AC13JV	Concentration: 40.00 ug/ml	
Reference No. :	GB594	Start Time: Day 1, Time 10:45	
Date:	3/18/2008	Read Time: Day 1, Time 10:41	
Operator:	Shannon Bruce	Run Time: 23.9 hours	
Diluent:	2% DMSO / Water		
Dilution Regime:	Sample Volume: 75 µl	Serial Dilution Volume: 75 µl	
	Culture Volume: 75 µl	Dilution Factor: 2.00	

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

NEGATIVE

LEC	-	ug/ml

COMPOUND ABSORBANCE CHECK

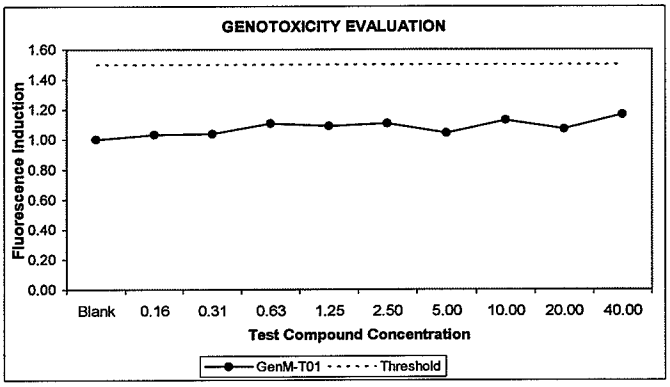
Absorbance Ratio (Control / Blank) = 1.01

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY *HIGH*	CELL DENSITY *LOW*	RESULT
GenM-C01	50.1	81.1	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

NEGATIVE

LEC	-	ug/ml

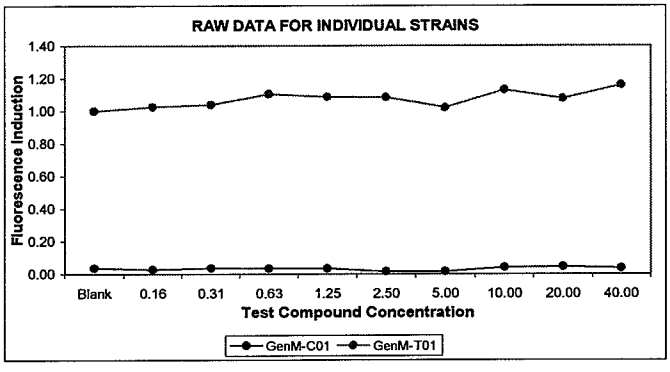
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 1.07

Not auto-fluorescent

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION *HIGH*	GFP INDUCTION *LOW*	RESULT
GenM-T01	2.69	1.40	PASS



NOTES:
None

TEST CONDITIONS

Operator:	Shannon Bruce		Assay ID:	AC13JV.801.BTL.B2
Diluent:	2% DMSO / Water		Set up Date:	3/18/08
			Time:	10:45
Dilution Regime:	Sample Volume:	75 µl	Read Date:	3/20/08
	Culture Volume:	75 µl	Time	10:48
	Serial Dilution Volume:	75 µl	Assay run time:	48.1 HOURS
	Dilution Factor:	2.00		

SAMPLE ID AND RESULTS		CYTOTOXICITY		GENOTOXICITY	
		Result	LEC	Result	LEC
1 2% DMSO Diluent		NEGATIVE	- ug/ml	NEGATIVE	- ug/ml
Ref. No.	0				
Concentration	0 ug/ml (Units)				
2	0	STRONG POSITIVE	- ug/ml	NEGATIVE	- ug/ml
Ref. No.	0				
Concentration	0 ug/ml (Units)				
3	0	STRONG POSITIVE	- ug/ml	NEGATIVE	- ug/ml
Ref. No.	0				
Concentration	0 ug/ml (Units)				
4 AC13JV		NEGATIVE	- ug/ml	NEGATIVE	- ug/ml
Ref. No.	GB594				
Concentration	40 ug/ml (Units)				

ABSORBANCE AND AUTOFLUORESCENCE CHECKS

1	2% DMSO Diluent	Not absorbing	Not auto-fluorescent
2	0	Not absorbing	Not auto-fluorescent
3	0	Absorbing	Not auto-fluorescent
4	AC13JV	Not absorbing	Not auto-fluorescent

CONTROLS

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY		RESULT
	HIGH	*LOW*	
GenM-C01	36.7	68.7	PASS

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION		RESULT
	HIGH	*LOW*	
GenM-T01	2.19	1.45	PASS

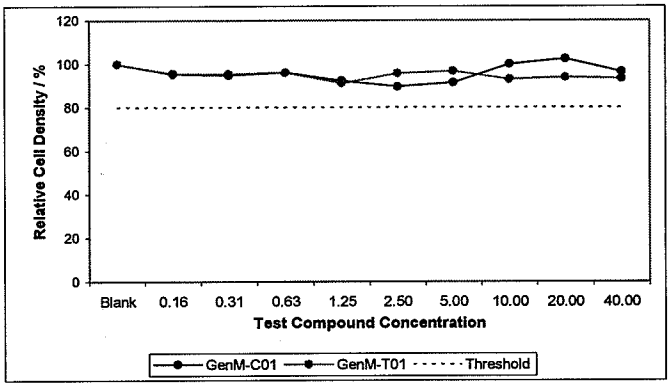
MEDIA CONTAMINATION CHECK

Abs. Ratio (Media / Blank) =	1.39
MEDIA CLEAR OF CONTAMINATION	

Fluor. Ratio (Media / Blank) =	5.59
MEDIA PASSES FLUORESCENCE QC	

Test Sample:	AC13JV	Concentration: 40.00 ug/ml	
Reference No. :	GB594	Start Time: Day 1, Time 10:45	
Date:	3/18/2008	Read Time: Day 2, Time 10:48	
Operator:	Shannon Bruce	Run Time: 48.1 hours	
Diluent:	2% DMSO / Water		
Dilution Regime:	Sample Volume: 75 µl	Serial Dilution Volume: 75 µl	
	Culture Volume: 75 µl	Dilution Factor: 2.00	

CYTOTOXICITY RESULTS



CYTOTOXICITY RESULT

NEGATIVE

LEC - ug/ml

COMPOUND ABSORBANCE CHECK

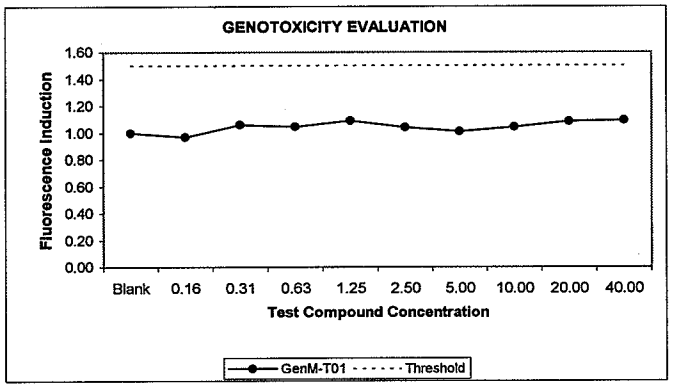
Absorbance Ratio (Control / Blank) = 0.98

Not absorbing

CYTOTOXIC CONTROLS

CELL LINE	CELL DENSITY HIGH	CELL DENSITY LOW	RESULT
GenM-C01	36.7	68.7	PASS

GENOTOXICITY RESULTS



GENOTOXICITY RESULT

NEGATIVE

LEC - ug/ml

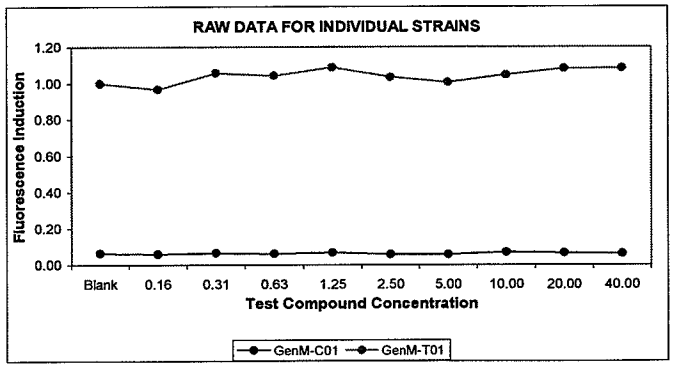
COMPOUND AUTO-FLUORESCENCE CHECK

Fluorescence Ratio (Control / Blank) = 1.09

Not auto-fluorescent

GENOTOXIC CONTROLS

CELL LINE	GFP INDUCTION HIGH	GFP INDUCTION LOW	RESULT
GenM-T01	2.19	1.45	PASS



NOTES:
None

APPENDIX II

Historical Control Data

GREENSCREEN CYTOTOXICITY AND GENOTOXICITY ASSAY

GREENSCREEN TEST STRAIN (T01)

	Media Control		MMS HIGH		MMS LOW	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Mean	5.77	6.09	3.18	3.03	1.70	1.83
Minimum	4.50	4.52	2.05	1.83	1.23	1.18
Maximum	8.51	8.58	27.92	31.18	16.17	19.23

GREENSCREEN CONTROL STRAIN (C01)

	Media Control		MMS HIGH		MMS LOW	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Mean	1.33	1.35	54.2	36.9	80.8	64.2
Minimum	1.15	1.05	37.9	18.6	56.9	35.0
Maximum	1.49	1.49	66.4	61.8	92.4	84.8

Media control = Media control wells compared to diluent only wells for GFP induction
MMS HIGH and MMS LOW = GFP induction of pertinent cell line
MMS = Methyl Methanesulfonate
MMS HIGH = 50 µg/mL
MMS LOW = 10 µg/mL