TECHNICAL MANUAL

Mitochondrial ToxGlo™ Assay

Instructions for Use of Products
G8000 and G8001

Revised 4/15
TM357

Mitochondrial ToxGlo™ Assay

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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Mitochondrial ToxGlo™ Assay[a,b] is a cell-based assay method that employs a sequential-addition, multiplexed chemistry for predicting potential mitochondrial dysfunction as a result of xenobiotic exposure. The assay is based on the differential measurement of biomarkers associated with changes in cell membrane integrity and cellular ATP levels relative to vehicle-treated control cells during short exposure periods*. Cell membrane integrity is first assessed by measuring the presence or absence of a distinct protease activity associated with necrosis using a fluorogenic peptide substrate (bis-AAF-R110) to measure "dead cell protease activity" (1). The bis-AAF-R110 Substrate cannot cross the intact membrane of live cells and therefore gives insignificant signal with viable cells relative to non-viable cells. Next ATP is measured by adding the ATP Detection Reagent, resulting in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The ATP Detection Reagent consists of an optimized formulation for ATP detection containing luciferin, ATPase inhibitors and thermostable Ultra-Glo™ luciferase. The two sets of data can be combined to produce profiles representative of mitochondrial dysfunction or non-mitochondrial related cytotoxic mechanisms.

*Mammalian cells generate ATP by mitochondrial (oxidative phosphorylation) and non-mitochondrial (glycolysis) methods. It may be necessary to refine cell culture conditions to achieve optimal mitochondrial responsiveness. Replacing glucose-supplemented medium with galactose-containing medium may increase cellular oxygen consumption and augment mitochondrial susceptibility to mitotoxicants (2,3).

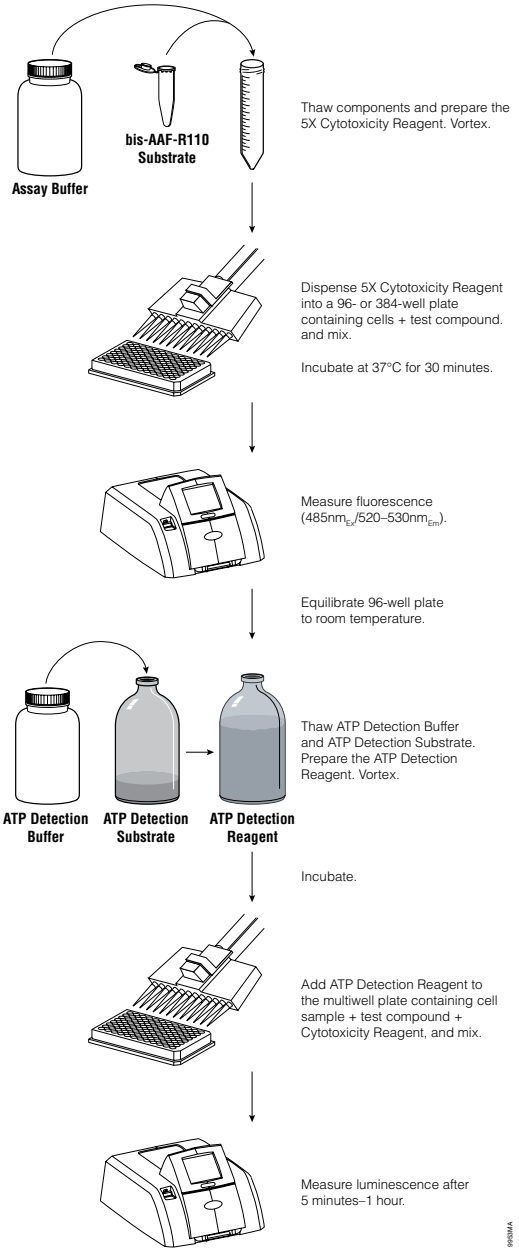

Figure 1. Schematic of the Mitochondrial ToxGlo™ Assay.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Mitochondrial ToxGlo™ Assay	**10ml**	**G8000**

Cat.# G8000 contains sufficient reagents for 100 assays at 100µl/assay in a 96-well plate format or 400 assays at 25µl/assay in a 384-well format. Includes:

- 10ml Assay Buffer
- 10µl bis-AAF-R110 Substrate
- 10ml ATP Detection Buffer
- 1 vial ATP Detection Substrate

PRODUCT	SIZE	CAT.#
Mitochondrial ToxGlo™ Assay	**100ml**	**G8001**

Cat.# G8001 contains sufficient reagents for 1,000 assays at 100µl/assay in a 96-well plate format or 4,000 assays at 25µl/assay in a 384-well format. Includes:

- 50ml Assay Buffer
- 2 × 50µl bis-AAF-R110 Substrate
- 100ml ATP Detection Buffer
- 1 vial ATP Detection Substrate

Storage Conditions: Store the Mitochondrial ToxGlo™ Assay components at –20°C.

3. Reagent Preparation and Storage

Membrane Integrity Chemistry and Reagent Preparation

1. Thaw the Cytotoxicity Assay components (Assay Buffer and bis-AAF-R110 Substrate) in a 37°C water bath. Remove the components from the bath after thawing to minimize the incubation time at 37°C.
2. Transfer 2ml of Assay Buffer from the 10ml container into a clean conical tube (for Cat.# G8001, transfer 20ml of Assay Buffer into a clean container).

   Note: Do not transfer the entire Assay Buffer volume contained in the bottle to the conical tube or add substrate directly to the Assay Buffer bottle. Using a larger volume of Assay Buffer will result in a 2X Cytotoxicity Reagent, which cannot be conveniently multiplexed because of well volume restrictions.
3. Transfer 10µl of bis-AAF-R110 Substrate into the conical tube containing 2ml of Assay Buffer to prepare a 5X Cytotoxicity Reagent (for Cat.# G8001, transfer 100µl of bis-AAF-R110 Substrate into the 20ml of Assay Buffer). A pulsed centrifugation may be necessary to recover the full volume of bis-AAF-R110 Substrate.
4. Mix the Cytotoxicity Reagent by vortexing the contents until the substrate is thoroughly dissolved. The Cytotoxicity Reagent should be used with in 24 hours if it is stored at room temperature.

   Note: Discard the remainder of the Assay Buffer.

3. Reagent Preparation and Storage (continued)

ATP Detection Chemistry and Reagent Preparation

1. Thaw the ATP Detection Buffer in a 37°C water bath. Place the ATP Detection Substrate at room temperature. Remove ATP Detection Buffer from the bath after thawing to minimize the incubation time at 37°C.
2. Transfer 10ml of ATP Detection Buffer to the amber bottle containing the ATP Detection Substrate to reconstitute the lyophilized enzyme/substrate mixture (for Cat.# G8001, add 100ml of ATP Detection Buffer to the amber bottle containing the ATP Detection Substrate). This is the 2X ATP Detection Reagent.
3. Mix the ATP Detection Reagent by vortexing or swirling the contents to obtain a homogeneous solution. Equilibrate the ATP Detection Reagent to room temperature prior to use.

   Note: The ATP Detection Reagent should be used within 24 hours if stored at room temperature. The Reagent can be stored at 4°C for 48 hours with ~5% loss of activity, at 4°C for 4 days with ~20% loss of activity or at –20°C for 21 weeks with ~3% loss of activity.

4. Protocols for the Mitochondrial ToxGlo™ Assay

Materials to Be Supplied by the User

- 96- or 384-well opaque-walled, white tissue culture plates compatible with fluorescent and luminescent measurements (clear or solid bottoms)
- multichannel pipettor or liquid handler
- reagent reservoirs
- fluorescence plate reader with rhodamine 110 filter set ($485nm_{Ex}$/520–$530nm_{Em}$)
- luminometer plate reader
- orbital plate shaker
- vortex
- positive control toxicity compound (Digitonin, 20mg/ml in DMSO; Cat.# G9441)
- positive control mitochondrial toxin (antimycin A, Sigma A8674; oligomycin, Sigma O5126; CCCP, Sigma C2759; sodium azide, Sigma S8032)
- cell-appropriate serum-free, glucose-containing culture medium (RPMI, DMEM, etc.) and culture medium prepared with galactose (without glucose). Please refer to Section 5 for more guidance.

4.A. Model Qualification: Determining Mitochondrial Susceptibility

Cells used in the Mitochondrial ToxGlo™ Assay must be maintained according to well-established standard operating procedures to reduce variability in day-to-day performance. Culture medium should be replenished, or cells passaged, regularly to maintain high cell viability (>95%) and reduce metabolic stress.

For cells growing in suspension, proceed to Step 2.

1. Harvest adherent cells (by trypsinization, etc.), wash with fresh medium (to remove residual trypsin) and resuspend in fresh medium. Resuspend the cells by pipetting until the cells are evenly dispersed.

   Note: Cell clumping may adversely affect assay results and produce variation in replicate wells.

2. Determine the number of viable cells by trypan blue exclusion, then adjust the density by dilution to 200,000 cells/ml using the standard glucose-supplemented medium and in a formulation containing no glucose but supplemented with galactose (96-well format). Cells may need to be more concentrated for higher density formats.

   Note: Centrifugation at 200 × *g* for 10 minutes may be necessary if the cell suspension is less than 200,000 cells/ml. If cells are pelleted, resuspend them by pipetting until they are evenly dispersed.

3. Add 50µl of cells at 200,000 cells/ml to a 96-well culture plate. Scale accordingly for higher density formats (e.g., 10µl of cells to 384-well plate). Stir or swirl the cell stock frequently to ensure that reproducible cell numbers are delivered across the plate. Allow attachment-dependent cells to adhere.

4. Prepare a stock solution(s) of control mitochondrial toxin at 100mM in DMSO.

5. Prepare a working stock of control mitochondrial toxin at a concentration of 1–100µM in standard glucose-containing and galactose-supplemented culture medium. Neither medium should contain serum, which is a substantial source of glucose. Prepare matched vehicle controls using DMSO in respective culture medium.

   Note: Model mitochondrial toxins vary in potency between different cell types. The 1–100µM concentration range is typically a good starting point.

6. In a separate 96-well plate prepare the stock plate containing dilutions of all the controls. Add 100µl of glucose-containing culture medium to columns 2–12, rows A–C and G. Add 100µl of galactose-containing culture medium to columns 2–12, rows D–F and H. Neither medium should contain serum, which is a substantial source of glucose. Add 100µl of mitochondrial toxin control compound prepared in glucose to columns 1 and 2, rows A–C. Add 100µl of mito-toxicant control compound prepared in galactose to columns 1 and 2, rows D–F. Add matched vehicle control solutions to columns 1 and 2, rows G and H, respectively. Refer to Table 1 for suggested plate layout.

7. Using a multichannel pipettor, mix the replicate drug dilutions in column 2 by aspirating and dispensing 100µl volumes (5 mixing cycles each recommended) prior to transferring 100µl to column 3. Repeat twofold serial dilutions in each column through column 11, transfer and discard final 100µl volume. Column 12, rows A–F, will serve as untreated medium control for ATP chemistry. Refer to Table 1.

8. Dilute Digitonin to 800µg/ml in medium and add in 10µl volumes to column 12, rows G and H. These wells will serve as a positive control (Tox Control) for the membrane integrity assay. Refer to Table 1.

4.A. Model Qualification: Determining Mitochondrial Susceptibility (continued)

Table 1. Diagram of 96-Well Cell Model Qualification Layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Glucose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
B	Glucose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
C	Glucose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
D	Galactose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
E	Galactose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
F	Galactose	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Untreated Control
G	Glucose Vehicle	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Tox Control
H	Galactose Vehicle	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Tox Control

9. Place the plate on an orbital shaker at 500–700rpm for 1 minute.

10. Using a multichannel pipettor, transfer 50µl of diluted compounds from the stock plate to the cells in culture (prepared in Steps 1–3). Change tips for each model compound and the vehicle control to prevent cross-contamination of compounds. Mix again by placing the plate on an orbital shaker at 500–700rpm for 1 minute.

    Note: The effective concentration of the compounds will now be half of the stock solution delivered to column 1 as it has been diluted 1:2 with the culture volume in the well.

11. Incubate the plate at 37°C in a humidified and CO_2-supplemented incubator for 90 minutes.

12. Prepare Cytotoxicity and ATP Detection Reagents as described in Section 3.

13. Using a multichannel pipettor, add 20µl of the 5X Cytotoxicity Reagent to each well. Mix briefly (1 minute) by orbital shaking (500–700rpm) to ensure that the Reagent and sample are well mixed and to mitigate possible partitioning of the Reagent and sample. Incubate at 37°C for 30 minutes.

    Note: Avoid stacking plates as this may create thermal gradients during the incubation period. Thermal-jacketed plate incubators (e.g., MeCour, etc.) may reduce possible edge effects produced by thermal gradients.

14. Measure fluorescence at $485nm_{Ex}/520–530nm_{Em}$. If you are using an adjustable fluorometer, set the PD gain using the positive control signal from column 12, rows G and H. The signal should be 60–90% of detector saturation for optimal results.

15. Equilibrate the assay plate to room temperature (5–10 minutes). Add 100µl of ATP Detection Reagent to each well. Mix by orbital shaking (500–700rpm) for 1–5 minutes.

16. Measure luminescence. If you are using an adjustable luminometer, set the gain using the untreated medium control signal from column 12, rows A–F. The signal should be 60–90% of detector saturation for optimal results.

17. Calculate the average of control wells from column 12, rows A–F, for both fluorescent and luminescent measurements.

    For the Cytotoxicity chemistry control, compare the average of the untreated media controls, column 12, rows A–F, to rows G and H. The average signal from rows G and H should be at least twofold higher than rows A–F for a valid test.

    Examine both sets of data for vehicle-only effects.

18. Calculate the percent response of each test well by dividing the measured value of each test well by the average values obtained in column 12, rows A–C and D–F.

19. Plot the percent response for each compound and vehicle control for each assay versus the log concentration of the compound using GraphPad Prism®, SigmaPlot® or other analysis program. See Figure 2.

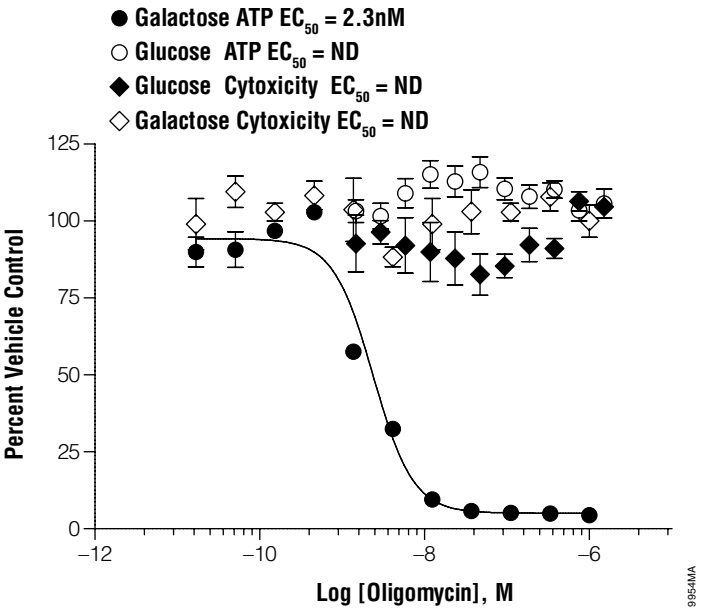

Figure 2. Mitochondrial responsiveness to a model mitochondrial toxin in the presence of galactose or glucose. Some cells treated in the presence of glucose may preferentially rely on glycolysis to meet bioenergetic needs and are therefore relatively unresponsive to mitochondrial toxins (Glucose ATP). Cells treated in the presence of galactose must use oxidative phosphorylation to generate ATP and are therefore more responsive to mitochondrial perturbation (Galactose ATP). Oligomycin treatment did not cause changes in membrane integrity in either formulation of medium (Galactose Cytotoxicity and Glucose Cytotoxicity). Shown are data from K562 cells at 10,000 cells/well in a white Costar® 96-well plate. The cells were exposed to oligomycin for 2 hours.

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4.B. Mitochondrial Toxicity Testing

Cells cultured for use in the Mitochondrial ToxGlo™ Assay must be maintained according to well-established standard operating procedures to reduce variability and day-to-day performance. Culture medium should be replenished, or cells passaged regularly, to maintain high cell viability (>95%) and reduce unnecessary metabolic stress. Cells may be grown in glucose-containing medium, but the culture medium should be removed and the cells washed with galactose-containing medium if galactose-containing medium was previously identified to produce the best mitochondrial response (Section 4.A).

For cells growing in suspension, proceed to Step 2.

1. Harvest adherent cells (by trypsinization, etc.), wash with fresh medium (to remove residual trypsin) and resuspend in fresh medium. Resuspend the cells by pipetting until the cells are evenly dispersed.

   Note: Cell clumping may adversely affect assay results and produce variation in replicate wells.

2. Determine the number of viable cells by trypan blue exclusion, then adjust the density by dilution to 200,000 cells/ml using fresh medium (96-well format). Cells may need to be more concentrated for higher density formats.

   Note: Centrifugation at 200 × *g* for 10 minutes may be necessary if the cell suspension is less than 200,000 cells/ml. If cells are pelleted, resuspend them by pipetting until they are evenly dispersed.

3. Add 50µl of cells at 200,000 cells/ml to a 96-well culture plate. Scale accordingly for higher density formats (e.g., 10 µl cells to 384-well plate). Stir or swirl cell stock frequently to ensure that reproducible cell numbers are delivered across the plate. Allow attachment-dependent cells to adhere.

4. Prepare a working stock of control mitochondrial toxin at a concentration of 1–100µM in culture medium starting at 2X the highest concentration that demonstrated maximal ATP depletion without causing membrane integrity changes (from Section 4.A). Prepare test compounds in a similar manner starting at 20µM. Prepare a matched vehicle control using DMSO (or the appropriate vehicle solvent) in culture medium.

   Note: A vehicle control may be unnecessary if matched solvent concentrations have been shown previously not to affect assay results. DMSO concentrations of 0.1% or less are typically inert to the assay chemistry.

5. In a separate 96-well plate, prepare dilutions of the mitochondrial toxin, vehicle controls and test compounds. Add 100µl of culture medium to all wells of a 96-well plate except for row A, columns 1–4. Add 100µl of control mitochondrial toxin to columns 1–3, rows A and B. Add 100µl of test compound #1 to columns 4–6, rows A and B. Add 100µl of test compound #2 to columns 7–9, rows A and B. Add 100µl of vehicle control to columns 10–12, rows A and B.

   Note: Additional test compounds can be added to columns 10–12, rows A and B, if the vehicle control has been deemed unnecessary.

6. Using a multichannel pipettor, mix the replicate drug dilutions in row B by aspirating and dispensing 100µl volumes (5 cycles each recommended). After mixing, add 100µl of the volumes in row B to row C. Repeat twofold serial dilutions in each row through G, and transfer and discard the final 100µl volume. Row H, columns 4–12, will serve as untreated medium control for ATP chemistry. See Table 2.

7. Dilute Digitonin to 800µg/ml in medium and add in 10µl volumes to row H, columns 1–3. These wells will serve as a positive control for the membrane integrity assay. See Table 2.

Table 2. Diagram of a 96-well plate layout for mitochondrial toxicity testing. UT = Untreated medium control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Mito-Toxicant	Mito-Toxicant	Mito-Toxicant	Test #1	Test #1	Test #1	Test #2	Test #2	Test #2	Vehicle Control	Vehicle Control	Vehicle Control
B	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2
C	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4	1:4
D	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8	1:8
E	1:16	1:16	1:16	1:16	1:16	1:16	1:16	1:16	1:16	1:16	1:16	1:16
F	1:32	1:32	1:32	1:32	1:32	1:32	1:32	1:32	1:32	1:32	1:32	1:32
G	1:64	1:64	1:64	1:64	1:64	1:64	1:64	1:64	1:64	1:64	1:64	1:64
H	Tox Control	Tox Control	Tox Control	UT	UT	UT	UT	UT	UT	UT	UT	UT

8. Place the plate on an orbital shaker at 500–700rpm for 1 minute.

   Note: For cells grown in suspension, proceed to Step 10.

9. For adherent cells grown in glucose-containing medium, carefully remove growth medium using a multichannel pipettor. Wash residual growth medium away by adding a 100µl volume of galactose dosing medium and removing again with a multichannel pipettor. Finally, add 50µl of galactose dosing medium to all wells.

   Notes:

   1. Exercise care when washing cells so as to not damage the monolayer. Medium removal and addition can be best achieved by placing plates on a 45° angle and using the sides of the wells as the tip guide.
   2. For cells grown in suspension, centrifuge briefly to collect the cells. Remove the glucose-containing medium and resuspend the cells in galactose-containing medium.

10. Using a multichannel pipettor, transfer 50µl of diluted compounds from the stock plate to cells in culture (prepared in Steps 1–3). Change tips for each model compound and the vehicle control to prevent cross-contamination of compounds. Mix again by placing the plate on an orbital shaker at 500–700rpm for 1 minute.

    Note: The effective concentration of the compounds will now be ½ of the stock solution delivered to column 1 due to 1:2 dilution with cell culture volume.

11. Incubate the plate at 37°C in a humidified and CO_2-supplemented incubator for 90 minutes.

12. Prepare Cytotoxicity and ATP Detection Reagents as described in Section 3.

4.B. Mitochondrial Toxicity Testing (continued)

13. Using a multichannel pipettor, add 20µl of 5X Cytotoxicity Reagent to each well. Mix briefly (1 minute) by orbital shaking (500–700rpm) to ensure Reagent/sample homogeneity and to mitigate possible Reagent/sample partitioning. Incubate at 37°C for 30 minutes.

    Note: Avoid stacking plates because this may create thermal gradients during the incubation period. Thermal-jacketed plate incubators (e.g., MeCour, etc.) may reduce possible edge-effects produced by thermal gradients.

14. Measure fluorescence at $485nm_{Ex}/520–530nm_{Em}$. If using an adjustable fluorometer, set PMT gain using positive control signal from row H, columns 1–3. The signal should be 60–90% of detector saturation for optimal results.

15. Equilibrate the assay plate to room temperature (5–10 minutes). Add 100µl of ATP Detection Reagent to each well. Mix by orbital shaking (500–700rpm) for 1–5 minutes.

16. Measure luminescence. If using an adjustable luminometer, set detector gain according to the signal from the untreated medium control column 12, rows A–F. The signal should be 60–90% of detector saturation for optimal results.

17. Calculate the average of untreated media control wells from row H, columns 4–12, for both fluorescent and luminescent measurements.

    For the Cytotoxicity Reagent chemistry control, compare the average of the cytotoxicity control, row H, columns 1–3, to the untreated media control, row H, columns 4–12. The average signal from columns 1–3 should be at least twofold higher than row H, columns 4–12, for a valid test.

    Examine both sets of data for vehicle-only effects.

18. Calculate the percent response of each test well by dividing the measured value of each test well by the average value obtained in the vehicle control, row A, columns 10–12.

19. Plot percent response for each compound and vehicle control for each assay versus log concentration of compound using GraphPad Prism®, SigmaPlot® or other analysis program.

20. **Data Analysis:** Compare the profiles generated in this section to the representative profiles in Figure 3.

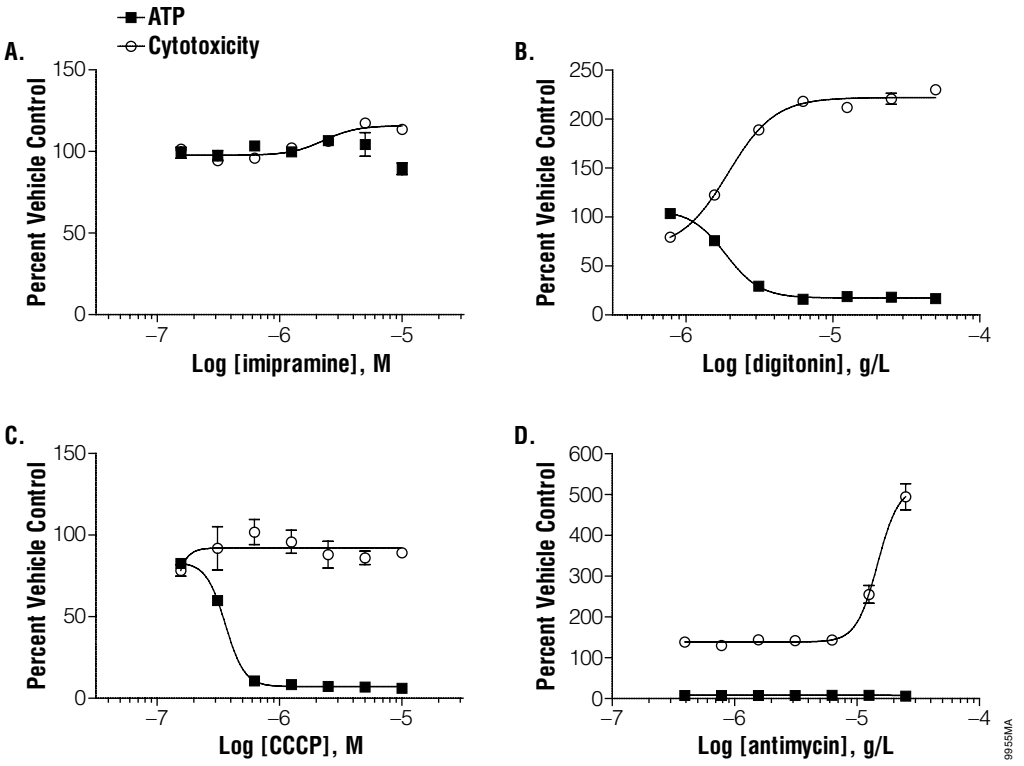

Figure 3. Representative profiles of mitochondrial toxicity with the Mitochondrial ToxGlo™ Assay. K562 cells were plated at 10,000 cells/well in white 96-well plates (Costar®) and treated with serial dilutions of compounds resuspended in glucose-free (galactose-supplemented) RPMI 1640 media for 2 hours. **Panel A.** shows no changes in ATP or membrane integrity (MI), which indicates that the compound is not a mitochondrial toxin. **Panel B.** The reduction in ATP with commensurate MI changes indicate that the compound is not a mitochondrial toxin, instead primary necrosis is taking place. **Panel C.** The reduction in ATP with no changes in MI indicates that the compound is a mitochondrial toxin. **Panel D.** The reduction in ATP with discordant changes in MI indicate that the compound is a mitochondrial toxin. **Note:** If a decrease in fluorescence, or fluorescence and luminescence is observed, it is typically due to color quenching interferences adversely affecting assay measures. If the cells are dosed in glucose-containing medium, compounds producing ATP-depletion affects should be counter-screened in galactose-containing medium to rule out inhibition of glycolysis.

5. General Considerations

Medium

Cell lines propagated in standard glucose-containing culture media tend to use glycolysis to meet ATP needs despite having fully functional mitochondria. This phenomenon is known as the Crabtree Effect (4). Production of ATP via glycolysis using galactose-containing media yields no net ATP and is bioenergetically untenable for cell survival. Therefore, cells cultured or dosed in galactose-containing medium are typically more responsive to mitochondrial insults because they primarily use mitochondrial oxidative phosphorylation to produce ATP. However, primary or terminally differentiated cell lines may preferentially use oxidative phosphorylation in culture. Therefore, we recommend using glucose and galactose to test mitochondrial susceptibility in each model cell system before performing any experiments.

Glucose-free media can be obtained from Sigma Aldrich (DMEM, Sigma Cat.# D5030; RPMI, Sigma Cat.# R1383) and supplemented with 5–10mM galactose (Sigma Cat.# G5388). Media should be supplemented with bicarbonate or HEPES and pH adjusted if necessary. Filter sterilize media before use.

If cells are fastidious and don't readily adapt to galactose growth medium, they can be grown in standard medium, washed and dosed in serum-free, galactose-containing medium.

Temperature

Both the membrane integrity and ATP measures are dependent upon enzymatic reactions. The dead-cell protease activity is best measured after incubation at 37°C for at least 30 minutes. The luciferase measurement should be conducted at room temperature for optimal signal stability. Measures should be taken to reduce thermal gradients leading to edge effects.

Single Concentration Screens

Dose-dependent evaluations of mitochondrial toxicity are the preferred method for screening because they are typically more conclusive and less susceptible to assay artifacts than single-concentration screens. However, the Mitochondrial ToxGlo™ Assay can be conducted in single-compound concentration screens if replicate wells are used and positive results are followed up using dose-dependent secondary screens.

Data Analysis

Replicate wells improve intrinsic variation and the predictive nature of the multiplexed assay. In general, a compound can be considered a potential mitochondrial toxin if it decreases the ATP measure by greater than 20% of the average of control wells with less than a 20% increase in cytotoxicity. Dose-dependent data indicating mitotoxicity should be scrutinized for data set confidence intervals and goodness of fit rather than relying solely on software curve-fitting values. Mitotoxicity should be confirmed using orthogonal methods.

Concordant decreases in ATP and increases in cytotoxicity should be considered non-mitochondrial toxicities because these results are indicative of primary necrosis.

Assay interference should be explored if the ATP measure decreases and cytotoxicity measures also decrease.

6. References

1. Niles, A.L. *et al.* (2007) A homogeneous assay to measure live and dead cells in the same sample by detecting different protease biomarkers. *Anal Biochem.* **366**, 197–206.
2. Marroquin, L. D. *et al.* (2007) Circumventing the Crabtree effect: Replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. *Toxicol. Sci.* **97**, 539–47.
3. Rossignol, R. *et al.* (2004) Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells. *Cancer Res.* **64**, 985–93.
4. Rodriguez-Enriquez, S. *et al.* (2001) Multisite control of the Crabtree effect in ascites hepatoma cells. *Eur. J. Biochem.* **268**, 2512–9.

7. Related Products

Product	Size	Cat.#
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260
	5 × 10ml	G9261
	2 × 50ml	G9262
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
Caspase-Glo® 3/7 Assay	2.5ml	G8090
	10ml	G8091
	100ml	G8092
	10 × 10ml	G8093
Caspase-Glo® 2 Assay	10ml	G0940
Caspase-Glo® 6 Assay	10ml	G0970
	50ml	G0971
Caspase-Glo® 8 Assay	2.5ml	G8200
	10ml	G8201
	100ml	G8202
Caspase-Glo® 9 Assay	2.5ml	G8210
	10ml	G8211
	100ml	G8212
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
	5 × 10ml	G6081
	2 × 50ml	G6082

7. Related Products (continued)

Product	Size	Cat.#
ApoTox-Glo™ Triplex Assay	10ml	G6320
	5 × 10ml	G6321
ApoLive-Glo™ Multiplex Assay	10ml	G6410
	5 × 10ml	G6411
GSH-Glo™ Glutathione Assay	10ml	V6911
	50ml	V6912
GSH/GSSG-Glo™ Assay	10ml	V6611
	50ml	V6612
Digitonin	40µl	G9441

Detection Instrumentation

Product	Size	Cat.#
GloMax® 96 Microplate Luminometer	1 each	E6501
GloMax® 96 Microplate Luminometer w/Single Injector	1 each	E6511
GloMax® 96 Microplate Luminometer w/Dual Injectors	1 each	E6521
GloMax®-Multi+ Detection System with Instinct® Software: Base Instrument with Shaking	1 each	E8032
GloMax®-Multi+ Detection System with Instinct® Software: Base Instrument with Heating and Shaking	1 each	E9032
GloMax®-Multi+ Luminescence Module	1 each	E8041
GloMax®-Multi+ Fluorescence Module	1 each	E8051
GloMax®-Multi+ Visible Absorbance Module	1 each	E8061
GloMax®-Multi+ UV-Visible Absorbance Module	1 each	E9061

8. Summary of Changes

The following changes were made to the 4/15 revision of this document:

1. The patent/license statements were updated.
2. The document design was updated.

[a]U.S. Pat. Nos. 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

[b]U.S. Pat. Nos. 6,602,677, 7,241,584 and 8,030,017, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

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