TECHNICAL BULLETIN

MultiTox-Glo Multiplex Cytotoxicity Assay

Instructions for Use of Products
G9270, G9271 and G9272

Revised 1/16
TB358

Promega

MultiTox-Glo Multiplex Cytotoxicity Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. **Description** 2
2. **Product Components and Storage Conditions** 7
3. **Reagent Preparation and Storage** 8
4. **Protocols** 8
   - 4.A. Determining Assay Sensitivity, Method 1 9
   - 4.B. Determining Assay Sensitivity, Method 2 11
   - 4.C. Example Viability and Cytotoxicity Assay Protocol 13
   - 4.D. Recommended Controls 14
5. **General Considerations** 15
6. **References** 17
7. **Related Products** 18
8. **Summary of Changes** 19

Promega

1. Description

The MultiTox-Glo Multiplex Cytotoxicity Assay[(a–b)] is a sequential-reagent-addition fluorescent and luminescent assay that measures the relative number of live and dead cells in cell populations (Figure 1). The MultiTox-Glo Multiplex Cytotoxicity Assay gives ratiometric, inversely correlated measures of cell viability and cytotoxicity, which correlate well with established methods for measuring viability and cytotoxicity (Figure 2). The ratio of viable cells to dead cells is independent of cell number and, therefore, can be used to normalize data. Having complementary cell viability and cytotoxicity measurements reduces errors associated with pipetting and cell clumping. Assays can be subject to chemical interference by test compounds or media components and can give false-positive or false-negative results. Independent cell viability and cytotoxicity assays serve as internal controls and allow identification of errors resulting from chemical interference from test compounds or media components.

The MultiTox-Glo Assay sequentially measures two protease activities; one is a marker of cell viability, and the other is a marker of cytotoxicity (Figures 3 and 4). The live-cell protease activity is restricted to intact viable cells and is measured using a fluorogenic, cell-permeant, peptide substrate (glycyl-phenylalanyl-aminofluorocoumarin, GF-AFC) (1). This substrate enters intact cells, where it is cleaved by the live-cell protease activity to release AFC and generate a fluorescent signal that is proportional to the number of living cells. This live-cell protease becomes inactive upon loss of cell membrane integrity and leakage into the surrounding culture medium. A second, luminogenic cell-impermeant peptide substrate (alanyl-alanyl-phenylalanyl-aminoluciferin; AAF-Glo™ Substrate) is used to measure dead-cell protease activity, which is released from cells that have lost membrane integrity. The AAF-Glo™ Substrate is not cell-permeant; therefore, essentially no signal is generated from this substrate by intact, viable cells. The liberated AFC fluorophore is conveniently measured by fluorescence excitation and emission intensity. The liberated aminoluciferin product is measured as luminescence generated by recombinant luciferase provided in the AAF-Glo™ Reagent (Figure 5). This luciferase is the proprietary thermostable Ultra-Glo™ Recombinant Luciferase, which uses aminoluciferin as a substrate to generate a stable "glow-type" luminescent signal and is formulated to improve performance across a wide range of assay conditions. A schematic diagram showing the MultiTox-Glo Assay protocol is shown in Figure 6.

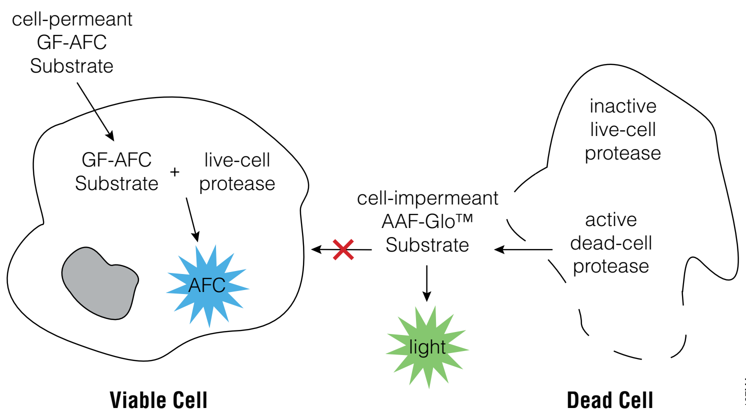


Figure 1. The biology of the MultiTox-Glo Multiplex Cytotoxicity Assay. The fluorogenic GF-AFC Substrate can enter live cells, where it is cleaved by the live-cell protease to release AFC. The luminogenic AAF-Glo™ Substrate cannot enter live cells but can be cleaved by the protease activity released by dead cells to generate aminoluciferin, which is detected in a luminescent reaction

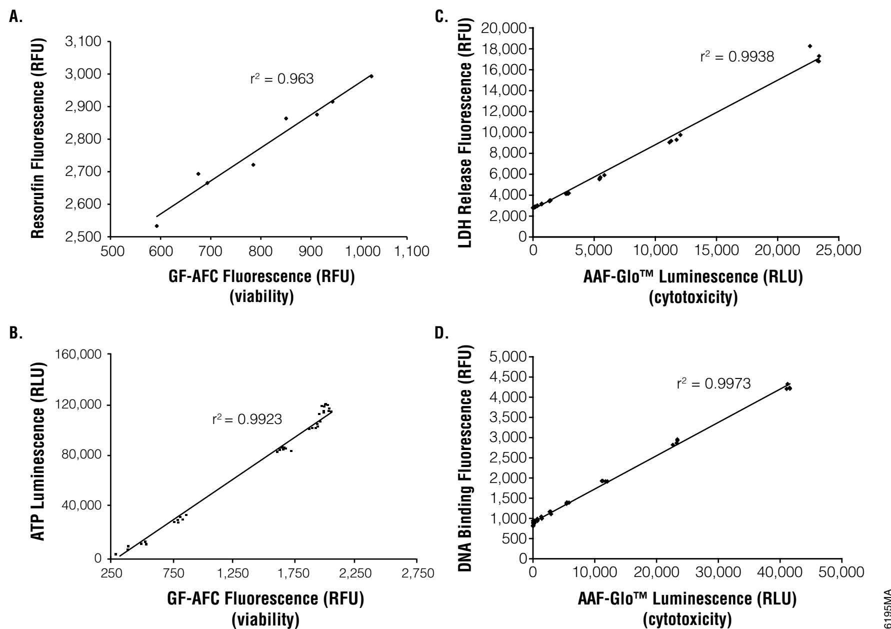


Figure 2. Live- and dead-cell protease activities detected by the MultiTox-Glo Assay show strong correlation with established methods for measuring viability and cytotoxicity. Panel A. GF-AFC signal (viability) plotted against CellTiter-Blue® Cell Viability Assay (resazurin reduction). **Panel B**. GF-AFC signal (viability) plotted against CellTiter-Glo® Luminescent Cell Viability Assay (ATP content). **Panel C**. AAF-Glo™ signal (cytotoxicity) plotted against CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH-release). **Panel D**. AAF-Glo™ signal (cytotoxicity) plotted against ethidium homodimer (DNA quantitation). These data were generated using serial twofold dilutions of live and dead cells.

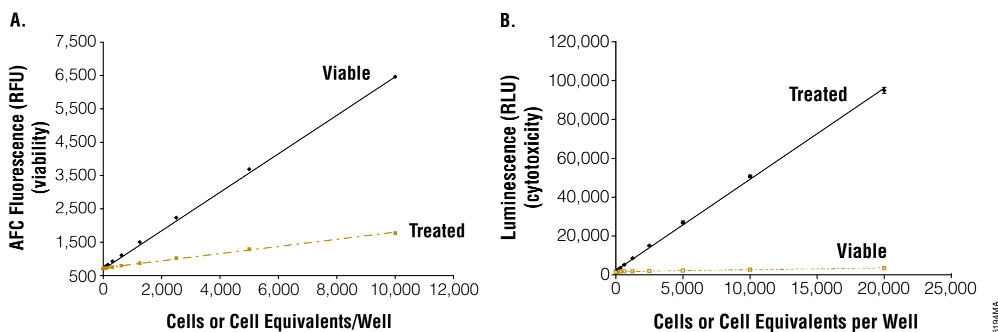


Figure 3. The MultiTox-Glo Assay measures distinct and differential protease activities. The signals derived from viable cells (untreated) or cells lysed by sonication (treated) are proportional to cell number. **Panel A**. GF-AFC Substrate, the live-cell substrate. **Panel B**. AAF-Glo™ Substrate, the dead-cell substrate. Note that the Y-axis scales in Panels A and B are different. Results are provided in relative fluorescence units (RFU) or relative light units (RLU), and the scales reflect the difference in signal output of fluorescence and luminescence.

Promega

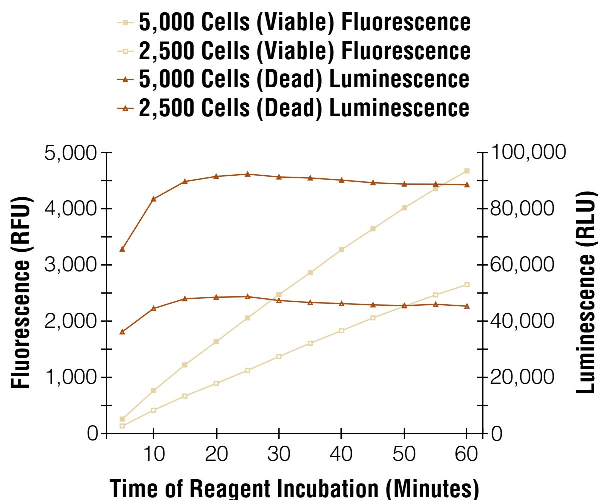


Figure 4. The signals derived from the live- and dead-cell measures are fundamentally different with respect to assay kinetics. The fluorescent cleavage product of the live-cell response (AFC) accumulates in the sample wells as a function of time and is proportional to the number of live cells. Results with 2,500 and 5,000 live cells are shown. The dead-cell protease activity generates aminoluciferin, which is used to produce photon emission from the luminescence reaction chemistry. The luminescent signal is also proportional to the number of dead cells (2,500 and 5,000 dead cells) and is typically stable for greater than 1 hour after reaching reaction steady-state.

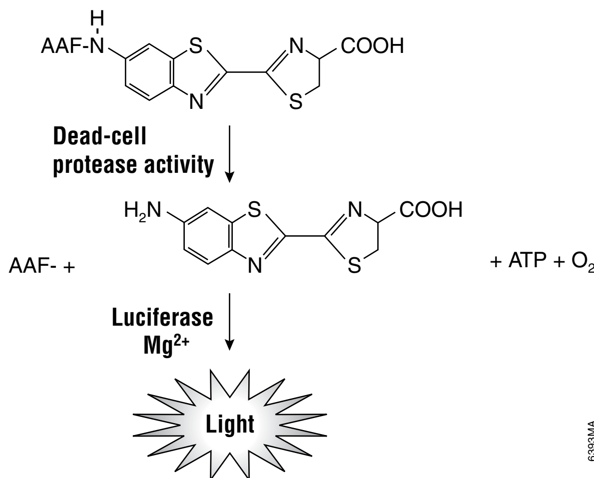
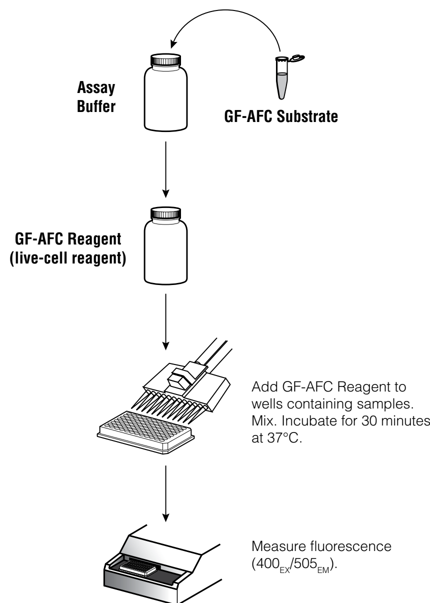
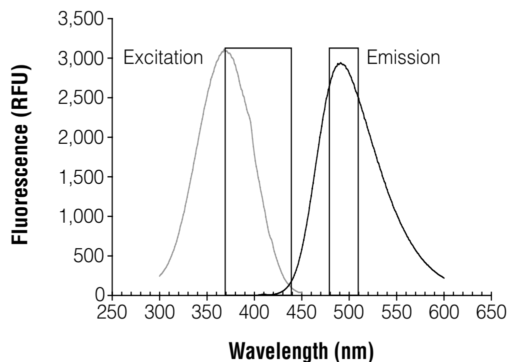


Figure 5. Cleavage of the luminogenic AAF-Glo™ Substrate by dead-cell protease activity. Following cleavage, a substrate for luciferase (aminoluciferin) is released, resulting in the luciferase-mediated production of light.





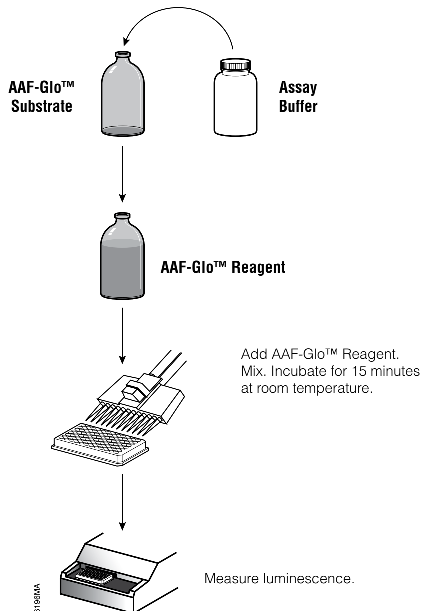


Figure 6. Schematic diagram of the MultiTox-Glo Multiplex Cytotoxicity Assay. Live-cell fluorescence is measured at $400nm_{Ex}/505nm_{Em}$; dead-cell luminescence is a measure of photon emission. Inset shows the excitation and emission spectra for AFC.

Promega

Assay Advantages

Measure the Number of Live Cells and the Number of Dead Cells in Culture: Measure viability and cytotoxicity with a sequential-reagent-addition, homogeneous, "add-mix-measure" protocol.

Normalize Data with a Built-In Internal Control: The ratio of the number of live cells/number of dead cells is independent of cell number and can be used to normalize data. This normalization makes results more comparable well-to-well, plate-to-plate and day-to-day.

Identify More False Positives and False Negatives Immediately: Independent cell viability and cytotoxicity measurements serve as controls for each other (Figure 7). If test compounds interfere with one assay chemistry, the other serves as an internal control.

Improve Your Data: Reduce statistical probability of false positives (or negatives), and eliminate fluorescence interference issues with a luminescence readout.

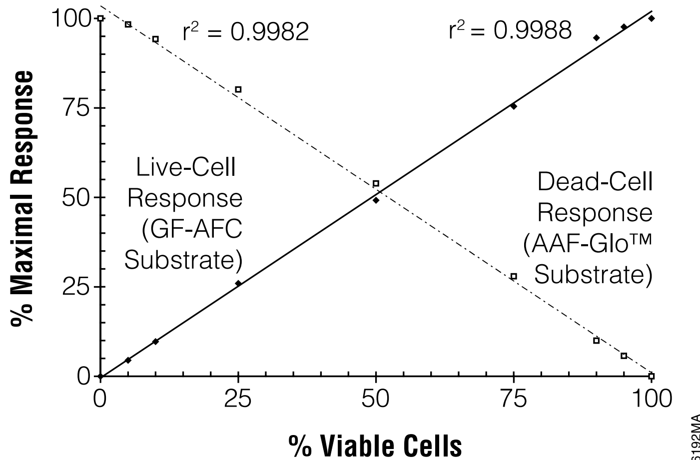


Figure 7. Viability and cytotoxicity measurements are inversely correlated and ratiometric. When viability is high, the live-cell fluorescent signal is highest, and the dead-cell luminescent signal is lowest. When viability is low, the live-cell signal is lowest, and the dead-cell signal is highest. Solid line, live-cell signal; dotted line, dead-cell signal.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MultiTox-Glo Multiplex Cytotoxicity Assay	**10ml**	**G9270**

G9270 contains sufficient reagents for 100 assays at 100µl per assay in a 96-well plate format or 400 assays at 25µl per assay in a 384-well format. Includes:

- 1 × 10µl GF-AFC Substrate (100mM in DMSO)
- 1 bottle AAF-Glo™ Substrate
- 2 × 5ml Assay Buffer

PRODUCT	SIZE	CAT.#
MultiTox-Glo Multiplex Cytotoxicity Assay	**5 × 10ml**	**G9271**

G9271 contains sufficient reagents for 500 assays at 100µl per assay in a 96-well plate format or 2,000 assays at 25µl per well in a 384-well format. Includes:

- 5 × 10µl GF-AFC Substrate (100mM in DMSO)
- 5 bottles AAF-Glo™ Substrate
- 10 × 5ml Assay Buffer

PRODUCT	SIZE	CAT.#
MultiTox-Glo Multiplex Cytotoxicity Assay	**2 × 50ml**	**G9272**

G9272 contains sufficient reagents for 1,000 assays at 100µl per assay in a 96-well plate format or 4,000 assays at 25µl per well in a 384-well format. Includes:

- 2 × 50µl GF-AFC Substrate (100mM in DMSO)
- 2 bottles AAF-Glo™ Substrate
- 4 × 25ml Assay Buffer

Storage Conditions: Store the MultiTox-Glo Multiplex Cytotoxicity Assay components at –20°C, protected from light. See product label for expiration date information.

3. Reagent Preparation and Storage

1. Thaw the MultiTox-Glo Multiplex Cytotoxicity Assay components in a 37°C water bath.
2. Mix the Assay Buffer and GF-AFC Substrate to ensure homogeneity.
3. Transfer the contents of one bottle of Assay Buffer to the AAF-Glo™ Substrate bottle to create the AAF-Glo™ Reagent. Mix by vortexing to ensure homogeneity. This is the dead-cell reagent.

   Note: The appearance of the AAF-Glo™ Substrate prior to addition of Assay Buffer may vary from lot to lot. This variation does not affect assay performance.
4. Transfer the contents of the GF-AFC Substrate vial (10µl for Cat.# G9270 and G9271 or 50µl for Cat.# G9272) to a second bottle of Assay Buffer to create the GF-AFC Reagent. This reagent may appear opaque or "milky" initially. Mix by vortexing the contents until the substrate is thoroughly dissolved. This is the live-cell reagent.

   Note: The concentration of each reagent prepared in this manner is 4X, and each reagent should be added in a 1:2 ratio relative to the cell culture volume (50µl of reagent per 100µl cell sample).

Storage: The MultiTox-Glo Multiplex Cytotoxicity Assay reagents should be used within 12 hours if stored at room temperature. We recommend storing any undiluted GF-AFC Substrate and Assay Buffer at –20°C. However, these components and the prepared GF-AFC and AAF-Glo™ Reagents can also be stored at 4°C for up to 7 days with no appreciable loss of performance.

4. Protocols

Materials to be Supplied by the User

- 96-, 384- or 1536-well, white-walled tissue culture plates compatible with luminometer and fluorometer (clear or solid bottom)
- multichannel pipettor or liquid-dispensing robot
- reagent reservoirs
- fluorescence plate reader equipped with filter sets of excitation ~400nm and emission ~505nm or monochrometer, and luminometer or multimode microplate reader
- orbital plate shaker
- digitonin (Calbiochem Cat.# 300410, at 20mg/ml in DMSO)

If you have not performed this assay with your cell line previously, we strongly recommend that you determine the assay sensitivity for your cells using one of the two methods described below (Section 4.A or 4.B). Use Method 1 to determine the linear range of the assay for your cell type. Use Method 2 to determine the practical sensitivity once you have already chosen the number of cells to use in the assay. If you do not need to determine assay sensitivity for your cells, proceed to Section 4.C.

4.A. Determining Assay Sensitivity, Method 1

1. Harvest adherent cells (by trypsinization, etc.), wash with fresh medium to remove residual trypsin and resuspend in fresh medium.

   Note: For cells growing in suspension, proceed directly to Step 2.

2. Determine the number of viable cells by trypan blue exclusion using a hemacytometer, then dilute to 100,000 viable cells/ml in at least 3.0ml of fresh medium.

   Note: Concentrate cells by centrifugation if the cell suspension is less dense than 100,000 cells/ml.

3. Add 100µl of the 100,000 cells/ml suspension (10,000 cells/well) to all wells of rows A and B in a 96-well plate.

4. Add 100µl of fresh medium to all wells in rows B–H.

5. Using a multichannel pipettor, mix the cell suspension in row B by pipetting, being careful not to create foam or bubbles. Transfer 100µl from each well of row B to row C. Repeat mixing, and transfer 100µl from row C to row D. Continue this process to row G. After mixing the diluted suspension at row G, aspirate 100µl from the wells, and discard. This procedure creates dilutions of 10,000 cells/well in row A to 156 cells/well in row G. Row H will serve as no-cell background control (Table 1).

Table 1. Schematic Diagram of 96-Well Plate Layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10,000 Cells/Well											
B	5,000 Cells/Well											
C	2,500 Cells/Well											
D	1,250 Cells/Well											
E	625 Cells/Well											
F	313 Cells/Well											
G	156 Cells/Well											
H	0 Cells/Well											
	untreated						treated					

6395MA

Promega

4.A. Determining Assay Sensitivity, Method 1 (continued)

6. Dilute digitonin to 300µg/ml in water. Using a multichannel pipettor, carefully add 10µl to all wells of columns 7–12 to lyse cells; these are the treated samples. Add 10µl of water to all wells of columns 1–6 so that the volume in all wells is equal; these are the untreated cells.
7. Add 50µl of the GF-AFC Reagent to all wells, mix briefly by orbital shaking to ensure homogeneity and incubate at 37°C for at least 30 minutes. Protect plates from light.

   Notes:

   1. Longer incubations may improve assay sensitivity and dynamic range. However, do not incubate longer than 3 hours.
   2. If you intend to compare results from more than one plate, be sure to incubate the plates for the same amount of time since the GF-AFC signal increases with time (Figure 4).
8. Measure the resulting live-cell fluorescence at ~400nm_{Ex}/~505nm_{Em}.

   Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy). Failure to use the optimal optical filters will affect assay sensitivity and performance.
9. Add 50µl of AAF-Glo™ Reagent to all wells. Mix briefly by orbital shaking, and incubate for 15 minutes at room temperature. Protect the plates from ambient light sources.
10. Measure the resulting dead-cell luminescence.

    Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy).
11. Calculate the signal-to-noise ratios to determine practical sensitivity for your cell type for each dilution of cells (10,000 cells/well; 5,000 cells/well; etc.).

$$\text{Viability S:N} = \frac{\text{(Average Untreated RFU – Average Treated RFU)}}{\text{S.D. of No-cell Background Controls (H1 through H12)}}$$

$$\text{Cytotoxicity S:N} = \frac{\text{(Average Treated RLU – Average Untreated RLU)}}{\text{S.D. of No-cell Background Controls (H1 through H12)}}$$

Note: The practical level of assay sensitivity for either assay is a signal-to-noise ratio of greater than 3 standard deviations (derived from reference 2).

Promega

4.B. Determining Assay Sensitivity, Method 2

1. Harvest adherent cells (by trypsinization, etc.), wash with fresh medium to remove residual trypsin and resuspend in fresh medium.

   Note: For cells growing in suspension, proceed directly to Step 2.

2. Determine the number of viable cells by trypan blue exclusion using a hemacytometer, then dilute to 100,000 viable cells/ml in at least 20ml of fresh medium.

   Note: Concentrate the cells by centrifugation if the cell suspension is less dense than 100,000 cells/ml.

3. Divide the volume of diluted cells into two separate tubes. Subject one tube to "moderate" sonication, which is empirically determined by post-sonication morphological examination, to disrupt cell membrane integrity and to simulate a 100% population of dead cells. The second tube of untreated cells will serve as the maximum viable population.

4. Create a spectrum of viability by blending sonicated and untreated populations in 1.5ml tubes as described in Table 2.

Table 2. Spectrum of Viability from Blending Sonicated and Untreated Cells.

Percent Viability	Volume of Sonicated Cells (µl)	Volume of Untreated cells (µl)
100	0	1,000
95	50	950
90	100	900
75	250	750
50	500	500
25	750	250
10	900	100
5	950	50
0	1,000	0

5. After mixing each blend of sonicated and untreated cells by gentle vortexing, pipet 100µl of each blend into eight replicate wells of a 96-well plate. Add the 100% viable cell sample to column 1, 95% viable to column 2, etc. Add 100µl of cell culture medium only to column 10 to serve as the no-cell background control.

4.B. Determining Assay Sensitivity, Method 2 (continued)

6. Add 50µl of the GF-AFC Reagent to all wells, mix briefly by orbital shaking to ensure homogeneity, then incubate at 37°C for at least 30 minutes. Protect plates from light.

   Notes:

   1. Longer incubations may improve assay sensitivity and dynamic range. However, do not incubate longer than 3 hours.
   2. If you intend to compare results from more than one plate, be sure to incubate the plates for the same amount of time since the GF-AFC-generated signal increases with time (Figure 4).

7. Measure the resulting live-cell fluorescence at ~$400nm_{Ex}$/~$505nm_{Em}$.

   Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy). Failure to use the optimal optical filters will affect assay sensitivity and performance.

8. Add 50µl of the AAF-Glo™ Reagent to each well. Mix by orbital shaking, and incubate for 15 minutes at room temperature. Protect the plate from ambient light sources.

9. Measure the resulting dead-cell luminescence.

   Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy).

10. Calculate the signal-to-noise (S:N) ratios to determine the practical sensitivity for your cell type for each blend of cell viability (X = 95%, 90%, etc.)

    Note: The practical level of assay sensitivity for either assay is a signal-to-noise ratio of greater than 3 standard deviations (derived from reference 2).

$$\text{Viability S:N} = \frac{\text{Average fluorescence for X\% viable cells} - \text{Average fluorescence for 0\% viable cells}}{\text{S.D. of 0\% viable cells}}$$

$$\text{Cytotoxicity S:N} = \frac{\text{Average luminescence for X\% viable cells} - \text{Average luminescence for 100\% viable cells}}{\text{S.D. of 100\% viable cells}}$$

4.C. Example Viability and Cytotoxicity Assay Protocol

1. Set up 96-well assay plates containing cells in culture medium at the desired density.
2. Add test compounds and vehicle controls to appropriate wells so that the final volume is 100µl in each well (25µl for a 384-well plate).
3. Culture cells for the desired test exposure period.

   Note: All enzymatic markers for cytotoxicity have finite activity half-lives (Figure 8). Although the protease marker(s) measured in this assay demonstrate an improved stability profile compared to other enzymatic markers under most circumstances, we recommend exposing the cells to the test compound for 24 hours or less to ensure that cytotoxicity is not underestimated. If longer exposures are desired, reducing the initial compound concentration may influence the kinetics of cytotoxicity and benefit the assessment of cell death.
4. Add 50µl of the GF-AFC Reagent to all wells (12.5µl for a 384-well plate). Mix briefly by orbital shaking to ensure homogeneity, and incubate at 37°C for at least 30 minutes. Protect plates from light.

   Notes:

   1. Longer incubations may improve assay sensitivity and dynamic range. However, do not incubate longer than 3 hours.
   2. If you intend to compare results from more than one plate, be sure to incubate the plates for the same amount of time since the GF-AFC-generated signal increases with time (Figure 4).
5. Measure the resulting live-cell fluorescence at ~400nm_{Ex}/~505nm_{Em}.

   Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy). Failure to use the optimal optical filters will affect assay sensitivity and performance.
6. Add 50µl of AAF-Glo™ Reagent to all wells (12.5µl for a 384-well plate). Mix by orbital shaking, and incubate for 15 minutes at room temperature. Protect the plate from ambient light sources.
7. Measure the resulting dead-cell luminescence.

   Note: You may need to adjust instrument gain setting (applied photomultiplier tube energy). Data are collected as either relative fluorescence units (RFU) or relative light units (RLU); therefore, the scales for fluorescence and luminescence will differ because of the difference in signal output (Figure 4).

Promega

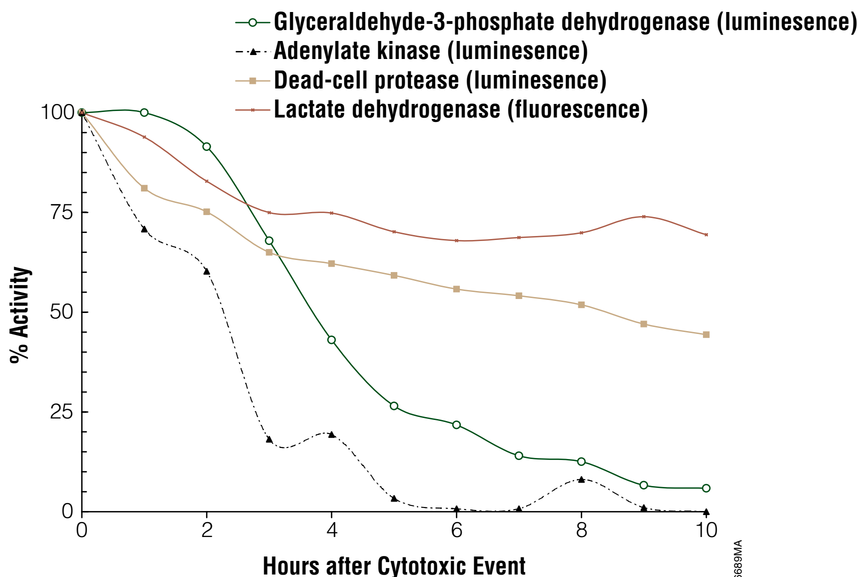


Figure 8. Half-life of enzymatic markers for cytotoxicity. Jurkat cells were plated in white-walled plates in 100µl volumes at a density of 10,000 cells per well in RPMI 1640 medium + 10%FBS. Digitonin was added to appropriate replicate wells in 10µl volumes (30µg/ml final concentration) every hour for 10 hours to facilitate maximal cytotoxicity. The plate was incubated at 37°C during this period. AAF-Glo™ Reagent and reagents to measure glyceraldehyde-3-phosphate dehydrogenase and adenylate kinase activity were prepared and added as directed by the manufacturer. Luminescence was measured after 15 minutes using a BMG POLARstar luminometer. CytoTox-ONE™ Reagent was prepared and added as directed. Fluorescence was measured using a Fluoroskan Ascent. All data were background subtracted and plotted as a percentage of the last lysis time point.

4.D. Recommended Controls

No-Cell Background Control: Prepare triplicate wells without cells to serve as the negative control to determine background signal.

Untreated Cells Control: Prepare triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds to the untreated-cells control wells.

Test Compound Control: Prepare triplicate wells without cells and containing the vehicle and test compound to test for possible interference with the MultiTox-Glo Assay chemistries.

Positive Control for Cytotoxicity: Prepare triplicate wells containing cells treated with a compound known to be toxic to the cells used in your model system (staurosporine, ionomycin, etc).

Promega

5. General Considerations

Background Luminescence and Inherent Serum Activity

Tissue culture medium supplemented with animal serum may contain detectable levels of the protease marker used for dead-cell measurement. The level of this protease activity can vary between different lots of serum. To correct for variability, background luminescence should be determined using samples that contain medium plus serum without cells (i.e., the no-cell background control).

Assay Controls

In addition to a no-cell background control to establish background fluorescence, we recommend including an untreated-cell control to measure maximum viability and a positive control to measure maximum cytotoxicity in the experimental design. The maximum viability control is established by adding the vehicle used to deliver the test compound to test wells. In most cases, this consists of a buffer system or medium and the equivalent amount of solvent used to deliver the test compound. Maximum cytotoxicity can be determined using a compound that causes cytotoxicity or a lytic reagent added to compromise the cell membrane (i.e., digitonin at 30µg/ml final concentration). See Section 4.A.

Temperature

The generation of fluorescent and luminogenic products is proportional to the protease activity of the markers associated with cell viability and cytotoxicity. The activities of these proteases and the luciferase detection chemisty are influenced by temperature. For best results, we recommend incubating reactions at a constant temperature to ensure uniformity across the plate. After adding the GF-AFC Reagent and mixing briefly, we suggest one of two methods:

1. Incubate at 37°C in a water-jacketed incubation module (Me'Cour, etc.).

   Note: Incubation at 37°C in a CO_2 culture cabinet may lead to edge effects resulting from thermal gradients.

2. Incubate at room temperature with or without orbital shaking.

   Note: Assays performed at room temperature may require more than 30 minutes of incubation. However, do not incubate longer than 3 hours.

After adding AAF-Glo™ Reagent and mixing briefly, we suggest a room-temperature incubation with or without orbital shaking.

5. General Considerations (continued)

Optical Filters, Instrumentation and Signal Separation Useful for Multiplexing

Fluorescence mode: Fluorogenic dyes exhibit distinct absorption (excitation) and emission profiles when a light energy source is applied. In multiplexed formats, there is often a modest overlap in these profiles beyond their optimal peaks. Most fluorometers or multimode instruments contain optical band-pass filters that restrict the wavelengths of light used to excite a fluorophore and the wavelengths passing through to the detector. Note that deviation from the optimal filter set recommendations may adversely affect assay sensitivity, signal separation and performance.

Luminescence mode: Luminescence chemistry does not require an external light source to generate photons but generally collects unrestricted/unfiltered light output.

Cytotoxicity Marker Half-Life

The activity of the protease marker released from dead cells has a half-life estimated to be greater than 10 hours (Figure 8). In situations where cytotoxicity occurs very rapidly such as necrosis and the incubation time is greater than 24 hours, the degree of cytotoxicity may be underestimated. When using extended incubation times, adding a lytic detergent such as digitonin may be useful to determine the total cytotoxicity marker activity remaining (from remaining live cells).

Luminescent Signal Half-Life

The luminescent dead-cell assay uses the AAF-Glo™ Substrate in conjunction with a thermostable, recombinant luciferase to generate a "glow-type" luminescent signal that is proportional to the number of dead cells in the sample. After a short signal ramping period (0–15 minutes), the signal reaches a plateau and is relatively stable for a period of approximately 1 hour (Figure 9). Although the signal half-life is greater than 5 hours in a 96-well format and about 2 hours in a 1536-well format, we suggest measuring the luminescent signal shortly after steady-state has been reached (after about 15 minutes).

Light Sensitivity

The MultiTox-Glo Multiplex Cytotoxicity Assay uses a fluorogenic peptide substrate to measure the number of live cells. Although the substrate demonstrates good general photostability, the liberated fluor (AFC) or aminoluciferin can degrade with prolonged exposure to ambient light sources. We recommend shielding the plates from ambient light at all times.

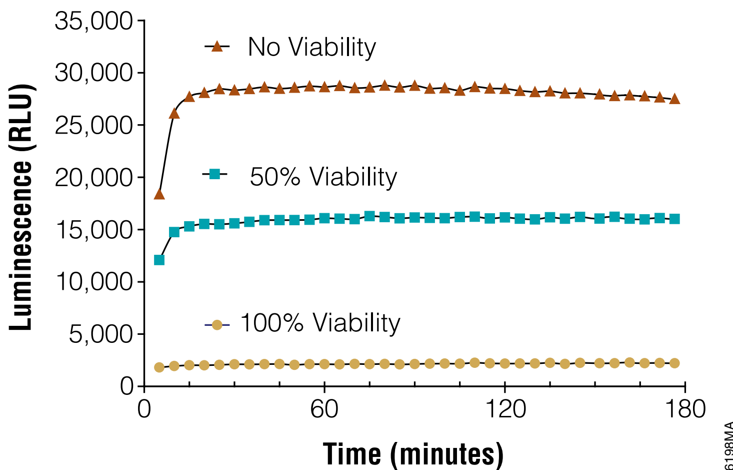


Figure 9. AAF-Glo™ Reagent luminescent signal.

Cell Culture Medium

The GF-AFC and AAF-Glo™ Substrates are introduced into the test well using an optimized buffer system that mitigates differences in pH from treatment with your test compound. In addition, the buffer system supports protease activity in a host of different culture media with varying osmolarity. With the exception of medium formulations with either high serum content or phenol red indicator, no substantial performance differences should be observed among media.

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 markers. *Anal. Biochem.* **366**, 197–206.

2. Zhang, J.H., Chung, T.D. and Oldenburg, K. (1999) A simple statistical parameter for use in evaluation and validation of high-throughput screening assays. *J. Bio. Mol. Screen.* **4**, 67–73.

7. Related Products

Cell Viability and Cytotoxicity Assays

Product	Size	Cat.#
CytoTox-Glo™ Cytotoxicity Assay	10ml	G9290
CytoTox-Fluor™ Cytotoxicity Assay	10ml	G9260
MultiTox-Fluor Multiplex Cyotoxicity Assay	10ml	G9200
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
CellTiter-Fluor™ Viability Assay	10ml	G6080
CytoTox-ONE™ Homogeneous Membrane Integrity Assay (fluorescent)	1,000–4,000 assays	G7891
CellTiter-Blue® Cell Viability Assay (fluorescent)	20ml	G8080

Additional Sizes Available.

Apoptosis Assays

Product	Size	Cat.#
Apo-ONE® Homogeneous Caspase-3/7 Assay (fluorescent)	10ml	G7790
Caspase-Glo® 3/7 Assay	10ml	G8091
Caspase-Glo® 8 Assay	10ml	G8201
Caspase-Glo® 9 Assay	10ml	G8211

Additional Sizes Available.

Reporter Gene Assays

Product	Size	Cat.#
Bright-Glo™ Luciferase Assay System	10ml	E2610
Steady-Glo® Luciferase Assay System	10ml	E2510

Additional Sizes Available.

Protease Assays

Product	Size	Cat.#
Calpain-Glo™ Protease Assay	10ml	G8501
DPPIV-Glo™ Protease Assay	10ml	G8350
Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay	10ml	G8660
Proteasome-Glo™ Trypsin-Like Cell-Based Assay	10ml	G8760
Proteasome-Glo™ Caspase-Like Cell-Based Assay	10ml	G8860
Proteasome-Glo™ 3-Substrate Cell-Based Assay	10ml	G1180

Protease Assays (continued)

Product	Size	Cat.#
Proteasome-Glo™ 3-Substrate System	10ml	G8531
Proteasome-Glo™ Caspase-Like Assay	10ml	G8641
Proteasome-Glo™ Chymotrypsin-Like Assay	10ml	G8621
Proteasome-Glo™ Trypsin-Like Assay	10ml	G8631

Additional Sizes Available.

cAMP Assay

Product	Size	Cat.#
cAMP-Glo™ Assay	300 assays	V1501

Additional Sizes Available.

ADME Assays

Product	Size	Cat.#
P450-Glo™ CYP1A1 Assay	50ml	V8752
P450-Glo™ CYP1B1 Assay	50ml	V8762
P450-Glo™ CYP1A2 Assay	50ml	V8772
P450-Glo™ CYP2C8 Assay	50ml	V8782
P450-Glo™ CYP2C9 Assay	50ml	V8792
P450-Glo™ CYP3A4 Assay	50ml	V8802
P450-Glo™ CYP3A7 Assay	50ml	V8812
P450-Glo™ CYP2D6 Assay	50ml	V8892
P450-Glo™ CYP2C19 Assay	50ml	V8882
Pgp-Glo™ Assay System	10ml	V3591
Pgp-Glo™ Assay System with P-glycoprotein	10ml	V3601
MAO-Glo™ Assay	50ml	V1402

Additional Sizes Available

8. Summary of Changes

The following changes were made to the 1/16 revision of this document:

1. The patent information was updated to remove expired statements.
2. The document design was updated.

[a]U.S. Pat. Nos. 7,416,854, 7,553,632 and other patents pending.

[b]U.S. Pat. Nos. 6,602,677, 7,241,584, 8,030,017 and 8,822,170, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

© 2007–2016 Promega Corporation. All Rights Reserved.

Apo-ONE, Caspase-Glo, CellTiter-Blue, CellTiter-Glo and Steady-Glo are registered trademarks of Promega Corporation. AAF-Glo, Bright-Glo, Calpain-Glo, cAMP-Glo, CellTiter-Fluor, CytoTox-ONE, CytoTox-Fluor, CytoTox-Glo, DPPIV-Glo, MAO-Glo, P450-Glo, Pgp-Glo, Proteasome-Glo and Ultra-Glo are trademarks of Promega Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.