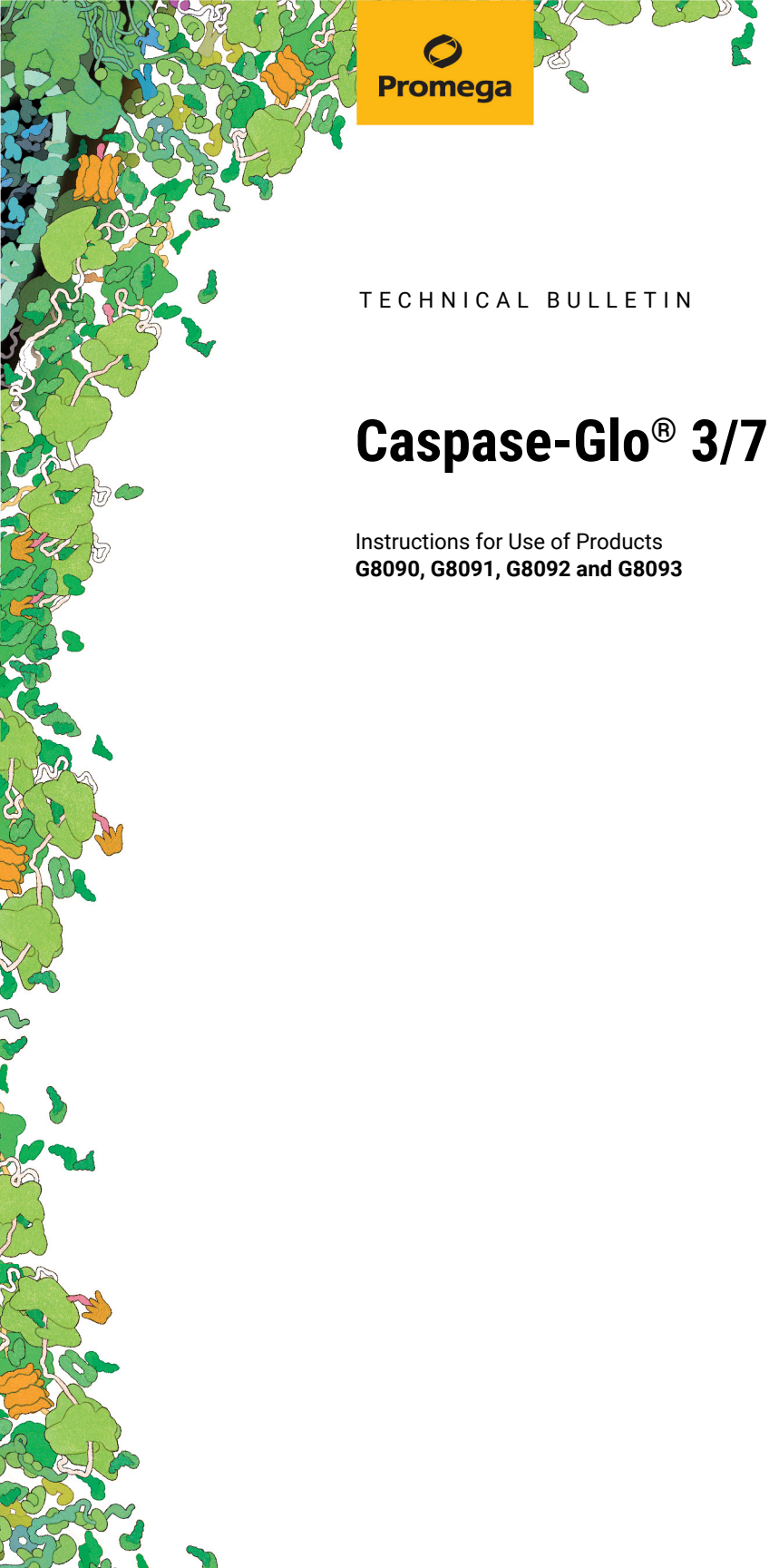

TECHNICAL BULLETIN

Caspase-Glo® 3/7 Assay

Instructions for Use of Products
G8090, G8091, G8092 and G8093

Revised 9/23
TB323

Promega

Caspase-Glo® 3/7 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

The Caspase-Glo® 3/7 Assay[a] is a homogeneous, luminescent assay that measures caspase-3 and -7 activities. The assay provides a luminogenic caspase-3/7 substrate, which contains the tetrapeptide sequence DEVD, in a reagent optimized for caspase activity, luciferase activity and cell lysis. Adding a single Caspase-Glo® 3/7 Reagent in an "add-mix-measure" format results in cell lysis, followed by caspase cleavage of the substrate and generation of a "glow-type" luminescent signal, produced by luciferase (Figure 1). Luminescence is proportional to the amount of caspase activity present (Figure 2). The Caspase-Glo® 3/7 Reagent relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which is formulated to generate a stable "glow-type" luminescent signal and improve performance across a wide range of assay conditions.

The Caspase-Glo® 3/7 Assay is designed for use with multiwell-plate formats, making it ideal for automated high-throughput screening of caspase activity or apoptosis. The assay has been automated in 96-, 384- and 1536-well formats. Cell washing, removing medium and multiple pipetting steps are not required (Figure 3). The caspase and luciferase enzyme activities reach steady state so that the luminescent signal peaks in approximately one hour and is maintained for several hours with a minimal loss of signal (Figures 5 and 6). This results in a rapid, sensitive and flexible caspase-3/7 activity assay. The assay system may be used with purified enzyme preparations (Figure 2) and has been tested on a variety of cell model systems including monolayer cultures of adherent or suspension cells (Figure 5); 3D microtissue cultures (Figure 10) and zebrafish embryos. We have tested this assay on a variety of cell lines (Jurkat, L929, HeLa, HL-60, SH-SY5Y, HepG2) that have been exposed to several different drugs and apoptosis-inducing agents (anti-Fas, TNF-α, staurosporine, clozapine, vinblastine, tamoxifen). This assay also can be multiplexed with other homogeneous assays to measure more than one parameter from a single well (Figure 8).

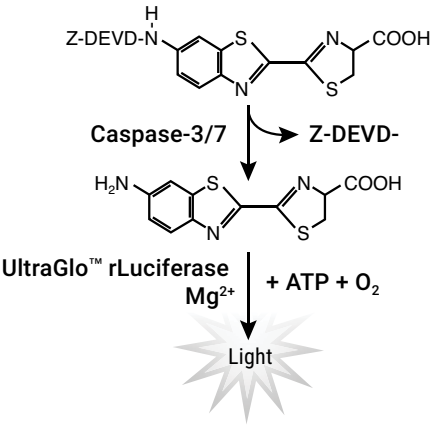

Figure 1. Schematic Diagram of the Caspase-Glo® 3/7 Assay Technology. Following caspase cleavage of the proluciferin DEVD substrate, a substrate for luciferase (aminoluciferin) is released and, in the presence of luciferase and ATP, results in the luciferase reaction and the production of light.

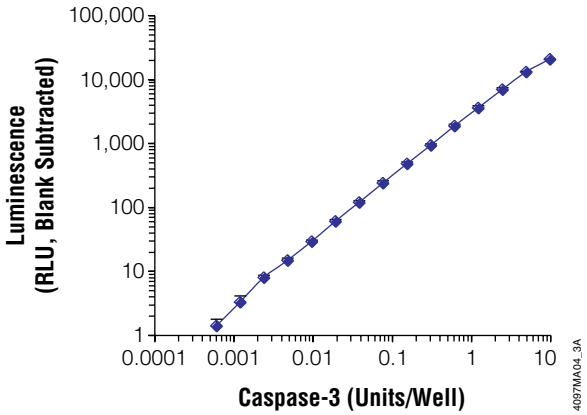

Figure 2. Luminescence is proportional to caspase-3 activity. Purified caspase-3 was titrated and assayed in a total volume of 200µl per well in a 96-well plate. Luminometer readings were taken 1 hour after adding the Caspase-Glo® 3/7 Reagent. The assay is linear over 4 orders of magnitude of caspase concentration (r^2 = 0.998, slope = 0.989)[1]. One unit caspase (0.07ng protein) is the amount of enzyme required to cleave 1pmol of substrate (Ac-DEVD-pNA) hydrolyzed/minute at 30°C per the manufacturer's unit definition[2]. Each point represents the average of 4 wells. Values are blank-subtracted (blank = no caspase).

[1]Determined by converting the log graph to a $\log_{10}$ linear plot to calculate r^2 and slope.
[2]Unit definitions of caspase-3 activity may vary between manufacturers, so the number of units may not translate directly between vendors. **Notes:** 1) Due to the extended dynamic range of of the Caspase-Glo® 3/7 Assay, data were graphed on a log scale. 2) The number of relative light units (RLU) obtained will depend on the type of luminometer used. For this reason, and because unit definitions may vary, your results may differ.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Caspase-Glo® 3/7 Assay	**2.5ml**	**G8090**

Each system contains sufficient reagents for 25 assays at 100µl per assay in a 96-well plate or 100 assays of 25µl per assay in a 384-well plate. Includes:

- 1 × 2.5ml Caspase-Glo® 3/7 Buffer
- 1 bottle Caspase-Glo® 3/7 Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Caspase-Glo® 3/7 Assay	**10ml**	**G8091**

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

- 1 × 10ml Caspase-Glo® 3/7 Buffer
- 1 bottle Caspase-Glo® 3/7 Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Caspase-Glo® 3/7 Assay	**10 × 10ml**	**G8093**

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

- 10 × 10ml Caspase-Glo® 3/7 Buffer
- 10 bottles Caspase-Glo® 3/7 Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Caspase-Glo® 3/7 Assay	**100ml**	**G8092**

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

- 1 × 100ml Caspase-Glo® 3/7 Buffer
- 1 bottle Caspase-Glo® 3/7 Substrate (lyophilized)

Storage Conditions: Store the Caspase-Glo® 3/7 Substrate and Caspase-Glo® 3/7 Buffer at –30°C to –10°C protected from light. The Caspase-Glo® 3/7 Buffer may be thawed and stored at +2°C to +10°C for 3 months or at room temperature for up to 4 days with no loss in signal. See the product label for expiration date.

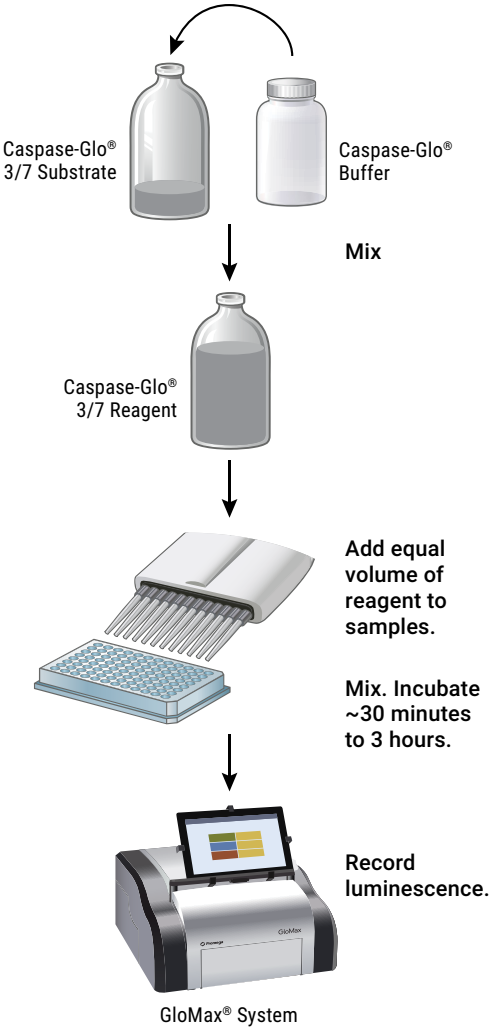

Figure 3. Schematic diagram of the Caspase-Glo® 3/7 Assay protocol.

3. Reagent Preparation and Storage

1. Equilibrate the Caspase-Glo® 3/7 Buffer and lyophilized Caspase-Glo® 3/7 Substrate to room temperature before use.
2. Transfer the contents of the Caspase-Glo® 3/7 Buffer bottle into the amber bottle containing Caspase-Glo® 3/7 Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo® 3/7 Reagent. Buffer volumes are 2.5ml for G8090, 10ml for G8091 and G8093, and 100ml for G8092.

Storage: The reconstituted Caspase-Glo® 3/7 Reagent may be stored at 4°C for up to 3 days with no loss of activity compared to that of freshly prepared reagent. Reconstituted reagent stored at 4°C for 1 week will give a signal approximately 90% of that obtained with freshly prepared reagent, while reconstituted reagent stored at 4°C for 4 weeks will give a signal approximately 75% of that obtained with freshly prepared reagent. Reconstituted reagent that has been refrozen and stored at –20°C for 1 week will give a signal approximately 75% of that of freshly prepared reagent, and refrozen reagent stored at –20°C for 4 weeks will give a signal approximately 60% of that of freshly prepared reagent.

4. Detection of Caspase-3 and -7 Activities in Cell-Based Assays

Directions are given for performing the assay in a total volume of 200µl using 96-well plates. However, the assay can be adapted to other volumes, provided the 1:1 ratio of Caspase-Glo® 3/7 Reagent volume to sample volume is used (e.g., 25µl of sample and 25µl Caspase-Glo® 3/7 Reagent in a 384-well format).

Materials to Be Supplied by the User

- white-walled multiwell plates adequate for cell culture and compatible with the luminometer being used, such as Corning® Microplates (Cat.# 3917)
- multichannel pipette or automated pipetting station
- plate shaker for mixing multiwell plates
- luminometer capable of reading multiwell plates

4.A. Assay Conditions

Prepare the following reactions to detect caspase-3 and -7 activities in cell culture. Grow cells in multiwell plates that are adequate for cell culture and compatible with the luminometer being used. “Vehicle” refers to the solvent used to dissolve the drug or protein of interest.

- Blank reaction: Caspase-Glo® 3/7 Reagent, vehicle and cell culture medium without cells
- Negative control: Caspase-Glo® 3/7 Reagent and vehicle-treated cells in medium
- Assays: Caspase-Glo® 3/7 Reagent and treated cells in medium

The blank reaction is used to measure background luminescence associated with the cell culture system and Caspase-Glo® 3/7 Reagent. Subtract the value for the blank reaction from experimental values. Negative control reactions are important for determining the basal caspase activity of the cell culture system.

Because of the sensitivity of this assay, background caspase activity can be detected in serum as well as in untreated cells. Perform positive and negative controls for each plate when processing multiple assay plates.

Due to the sensitive nature of the Caspase-Glo® 3/7 Assay, take care to avoid contamination with solutions containing caspase enzymes or luciferin.

Notes:

a. You may need to determine empirically the optimal cell number or spheroid size, apoptosis induction treatment and incubation time for the cell culture system. We recommend using <20,000 cells/well in a 96-well plate.

b. Use identical cell number and volume for the assay and the negative control samples.

c. Total incubation time for the assay depends upon the culture system, but typically peak luminescent signal will be reached in 1–2 hours. For optimal results, the maximum recommended incubation time is 3 hours. In general the luminescent signal remaining at 3 hours is greater than 70% of peak luminescence (Panel B of Figure 5 and 6).

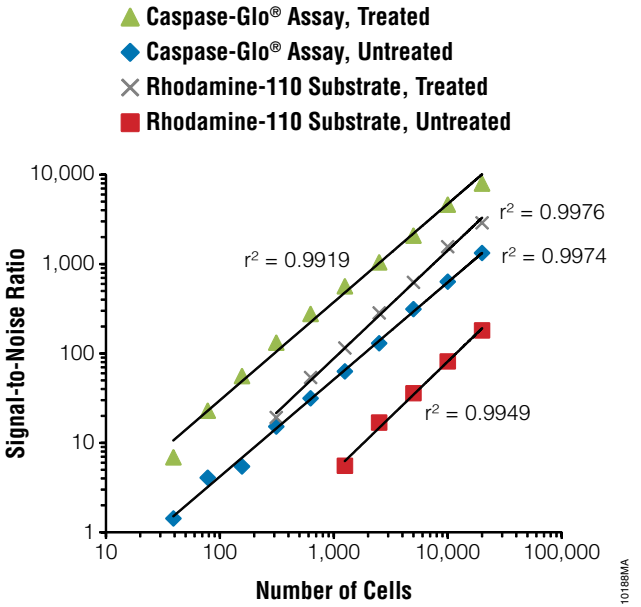

Figure 4. A comparison between bioluminescent and fluorescent caspase assays. HeLa cells were grown in OptiMEM® (Gibco) + 1% fetal bovine serum (Hyclone). Cells were plated in 96-well plates and treated with staurosporine (1µM) for 4.5 hours or left untreated. Caspase-Glo® 3/7 Reagent and Rhodamine-110 Substrate were added to wells, and luminescence and fluorescence were recorded at 1 hour on a GloMax® Multi+ System using the Blue ($490nm_{Ex}$, $510–570nm_{Em}$) optical kit. Results were plotted as signal-to-noise ratios. Background readings were determined from wells containing culture medium without cells.

4.A. Assay Conditions (continued)

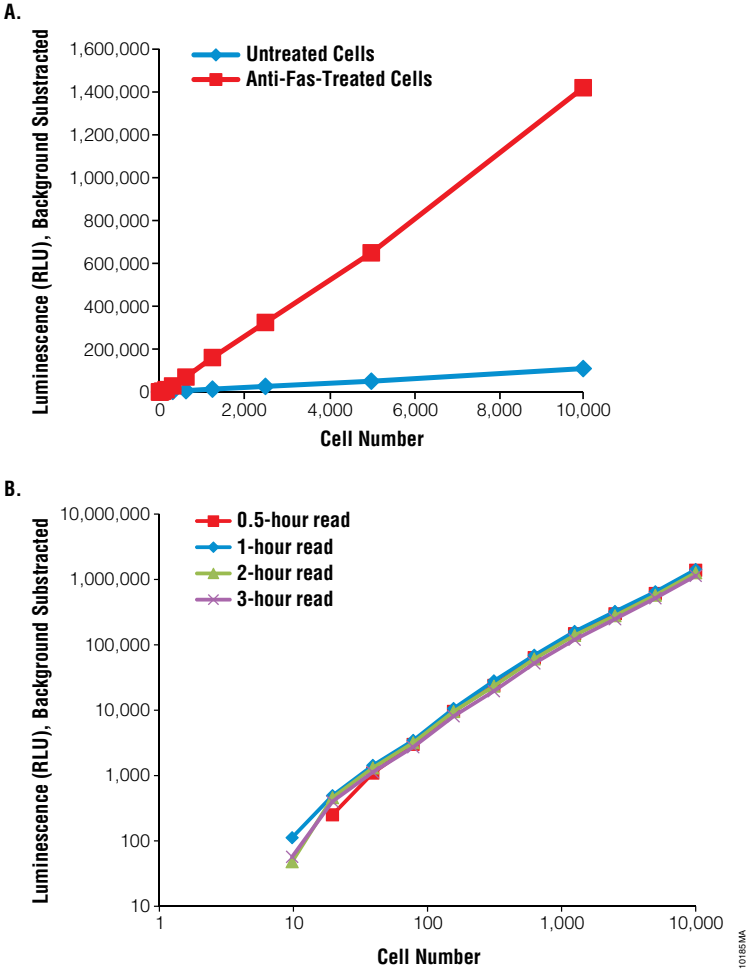

Figure 5. Sensitivity and signal stability of caspase activity in apoptotic Jurkat cells. Jurkat cells were treated with anti-Fas mAb (14,15) for 4.5 hours to induce apoptosis; an identical population of cells was left untreated. The Caspase-Glo® 3/7 Reagent was added directly to cells in 96-well plates; the final volume was 200µl per well. The assays were incubated at room temperature for various times before recording luminescence with a GloMax® Multi+ Luminometer. Each point represents the average of 4 wells. The "no-cell" blank control value has been subtracted from each point. **Panel A.** Luminometer readings were taken 1 hour after adding Caspase-Glo® 3/7 Reagent. **Panel B.** Luminometer readings were taken at various times after adding the Caspase-Glo® 3/7 Reagent. Data generated with untreated cells are not shown. **Note:** Due to the extended dynamic range of the Caspase-Glo® 3/7 Assay, the data for Panel B were graphed on a log scale.

4.B. Standard Protocol for Cells Cultured in a 96-Well Plate

1. Before starting the assay, prepare the Caspase-Glo® 3/7 Reagent (see Section 3). Allow the reagent to equilibrate to room temperature. Mix well.
2. Remove 96-well plates containing treated cells from the incubator and allow plates to equilibrate to room temperature.
3. Add 100µl of Caspase-Glo® 3/7 Reagent to each well of a white-walled 96-well plate containing 100µl of blank, negative control cells or treated cells in culture medium. Because of the sensitivity of this assay, be careful not to touch pipette tips to the wells containing samples to avoid cross-contamination. Cover the plate with a plate sealer or lid.

   Note: If you are reusing pipette tips, do not touch pipette tips to the wells containing samples to avoid cross-contamination.
4. Gently mix contents of wells using a plate shaker at 300–500rpm for 30 seconds. Incubate at room temperature for 30 minutes to 3 hours, depending upon the cell culture system. The optimal incubation period should be determined empirically.

   Note: Temperature fluctuations will affect the luminescence reading. If the room temperature fluctuates, use a constant-temperature incubator. See Section 6.
5. Measure the luminescence of each sample in a plate-reading luminometer as directed by the luminometer manufacturer.

4.C. Standard Protocol for 3D Microtissues in Corning® Spheroid Plates

1. Before starting the assay, prepare the Caspase-Glo® 3/7 Reagent (see Section 3). Allow the reagent to equilibrate to room temperature. Mix well.
2. Remove plates containing treated spheroids from the incubator and allow plates to equilibrate to room temperature.
3. Add a volume of Caspase-Glo® 3/7 Reagent equal to the volume of medium in the well.

   Note: If you are reusing pipette tips, do not touch pipette tips to the wells containing samples to avoid cross-contamination.
4. Gently mix contents of wells using a plate shaker at 300–500rpm for 5 minutes. Incubate at room temperature for 30 minutes to 3 hours, depending upon the cell culture system. The optimal incubation period should be determined empirically.

   Note: Temperature fluctuations will affect the luminescence reading. If the room temperature fluctuates, use a constant-temperature incubator. See Section 6.
5. Measure the luminescence of each sample in a plate-reading luminometer as directed by the luminometer manufacturer.

5. Detection of Caspase-3 or -7 Activity Using Purified Caspases

Directions are given for performing the assay in a total volume of 200µl using 96-well plates. However, the assay can be easily adapted to different volumes provided the 1:1 ratio of Caspase-Glo® 3/7 Reagent volume to sample volume is used (e.g., 25µl of sample and 25µl Caspase-Glo® 3/7 Reagent in a 384-well format).

Materials to Be Supplied by the User

- white-walled multiwell luminometer plates
- multichannel pipette or automated pipetting station
- plate shaker, for mixing multiwell plates
- luminometer capable of reading multiwell plates
- purified caspase-3 or -7 enzyme (e.g., ENZO Cat.# BML-SE169)

5.A. Assay Conditions

Prepare the following reactions in luminometer plates to detect caspase-3 or -7 activity or the inhibition of activity in purified enzyme preparations. "Vehicle" refers to the solvent used to dissolve the test compound.

- Blank reaction: Caspase-Glo® 3/7 Reagent and vehicle control for enzyme treatment agent or inhibitor, if used
- Positive control: Caspase-Glo® 3/7 Reagent, vehicle control and purified caspase-3 or -7 enzyme
- Experimental reactions: Caspase-Glo® 3/7 Reagent, test compound and purified caspase-3 or -7 enzyme

The blank reaction is used to measure background luminescence associated with the vehicle used to deliver the test compound in the presence of the Caspase-Glo® 3/7 Reagent. Luminescence values for the blank reaction should be subtracted from experimental values. The positive control is used to determine the maximum luminescence that can be obtained with the purified enzyme. Positive and negative controls should be performed for each plate when processing multiple assay plates.

Notes:

a. You may need to determine the optimal caspase concentration and treatment vehicle empirically. In general, we recommend <1ng of purified caspase-3 enzyme per well.

b. Use identical enzyme concentrations for the assay and positive control reactions.

c. Caspase specific activities and unit definitions can vary widely, depending on the manufacturer. You may need to optimize procedures describing the use of caspase by weight, depending on the specific activity of the caspase being used.

5.B. Standard Protocol for Purified Caspase Enzyme

1. Before starting the assay, prepare the Caspase-Glo® 3/7 Reagent (see Section 3). Allow the reagent to equilibrate to room temperature. Mix well.
2. Prepare the blank reactions, positive controls and test samples described in Section 5.A by adding 100µl of vehicle, purified enzyme with vehicle or purified enzyme with test compound to each well of a white-walled 96-well luminometer plate.

   Note: If you are reusing pipette tips, do not touch the pipette tips to the wells containing samples to avoid contamination.
3. Add 100µl of Caspase-Glo® 3/7 Reagent to each sample. Plates may be covered with a plate sealer if incubating reactions for extended periods of time (>1 hour).

   Note: Temperature fluctuations will affect the luminescence readings. If the room temperature fluctuates, use a constant-temperature incubator. See Section 6.
4. Gently mix contents of wells using a plate shaker. Incubate at room temperature.

   Notes:

   a. The maximal luminescent signal will be reached in approximately 20–60 minutes, and this signal will be stable for several hours (see Figure 6). Peak luminescence is usually achieved sooner in assays using purified enzymes rather than cells. In general the luminescent signal remaining at 2 hours after peak luminescence is approximately 70% of peak luminescence. For optimal results, the maximum recommended incubation time is 3 hours.

   b. Gentle mixing may be done using a plate shaker. Mixing is not required but may improve reproducibility between replicate samples.
5. Measure the luminescence of each sample in a plate-reading luminometer as directed by the luminometer manufacturer.

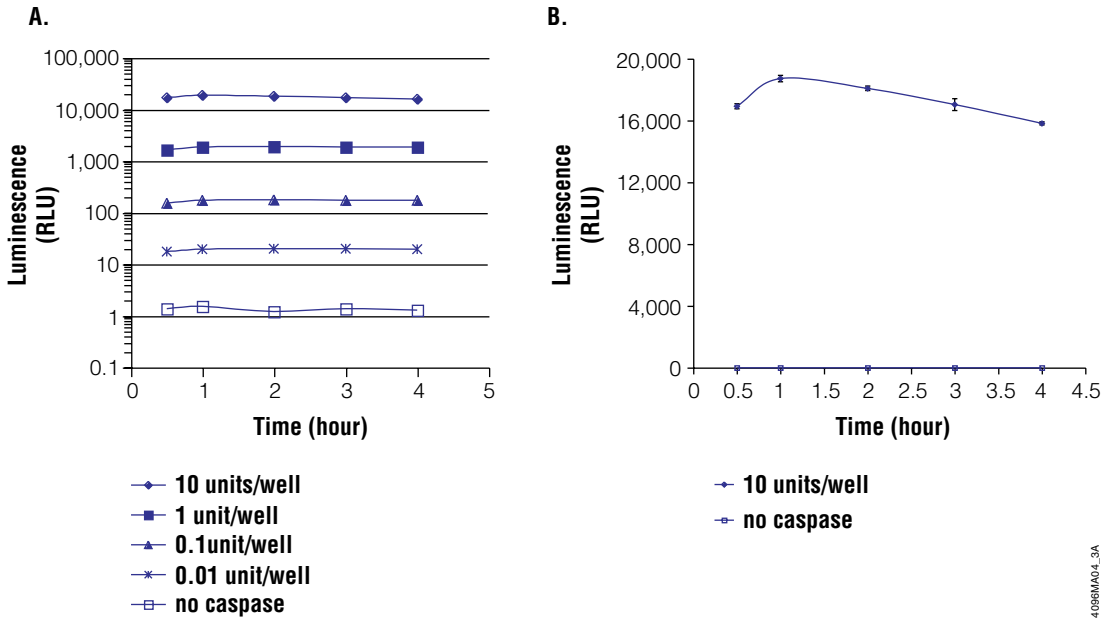

Figure 6. Signal stability using purified caspase-3 enzyme. Purified recombinant caspase-3 enzyme was titrated and assayed in a total volume of 200µl per well of a 96-well plate using the Caspase-Glo® 3/7 Assay. Caspase-3 was diluted in 10mM HEPES buffer containing 0.1% Prionex® as a carrier. One unit of caspase (0.1ng of protein) is the amount of enzyme required to cleave 1pmol of substrate (Ac-DEVD-pNA) hydrolyzed/minute at 30°C per the manufacturer's unit definition. **Panel A.** Data for each concentration of caspase-3 are graphed on a log graph. **Panel B.** Data generated using 10 units/well of caspase-3 are shown on a linear scale.

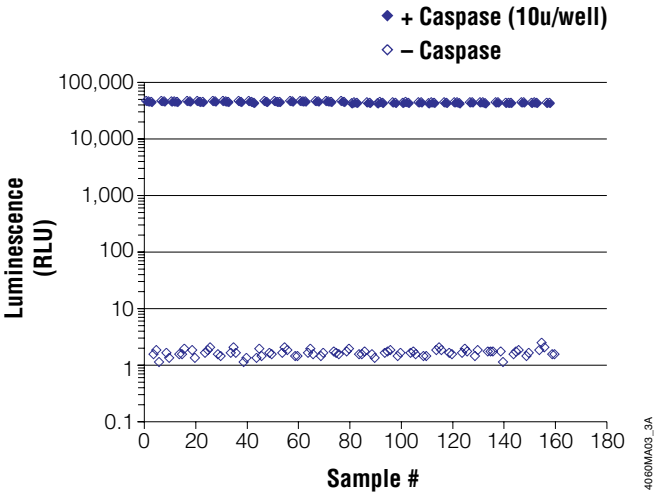

Figure 7. Z´-factor analysis. Z´-factor values (11) for the Caspase-Glo® 3/7 Assay were calculated using recombinant caspase-3 (10u/well) and a no-caspase blank. Assays were performed in a total volume of 200µl in two 96-well plates (80 wells/plate). Half of the wells of each plate contained buffer and purified caspase (+), and half of the wells contained buffer only with no purified caspase (–). Z´-factor = 0.92 for this assay [1 unit of caspase (0.1ng of protein) is the amount of enzyme required to cleave 1pmol of substrate (Ac-DEVD-pNA) hydrolyzed/minute at 30°C, per the manufacturer's unit definition].

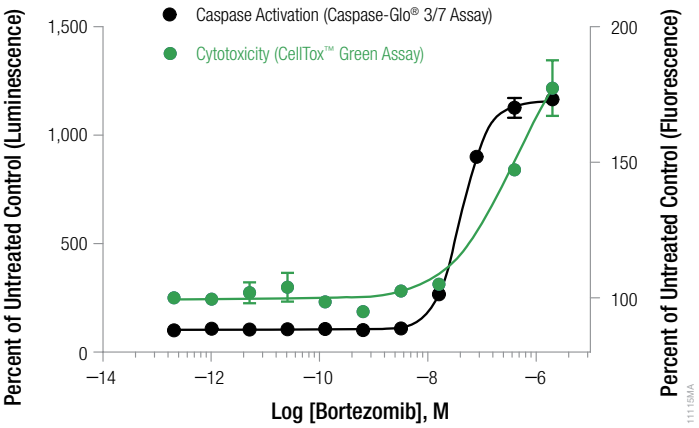

Figure 8. Multiplexing in kinetic mode to determine mechanism of toxicity. CellTox™ Green Dye was added to cells at plating, and bortezomib treatment applied. At the first indication of cytotoxicity (at 24 hours), Caspase-Glo® 3/7 Reagent was added as an indicator of apoptosis (luminescence). Increased caspase activation with cytotoxicity indicates cytotoxicity is the result of apoptosis.

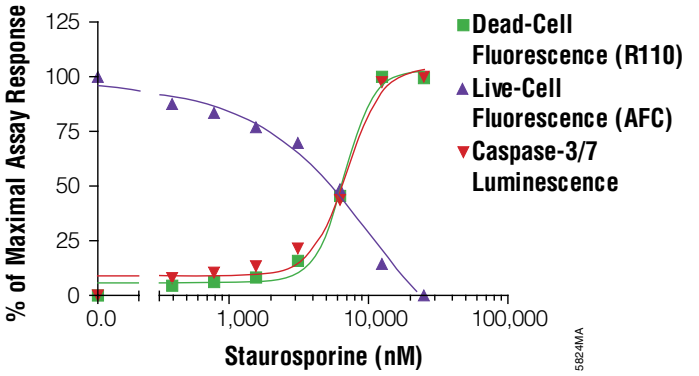

Figure 9. The Caspase-Glo® 3/7 Assay can be multiplexed with the MultiTox-Fluor Assay to confirm mechanism of cell death. LN-18 cells were plated at a density of 10,000 cells per well in 50µl volumes of MEM + 10% fetal bovine serum and allowed to attach overnight. Staurosporine was serially diluted twofold and added to wells in 50µl volumes. The plate was incubated at 37°C in 5% CO_2 for 6 hours. MultiTox-Fluor Reagent was prepared by combining 10µl of each substrate with 1ml of Assay Buffer, and 10µl was used per well. The plate was mixed and incubated for 30 minutes at 37°C. Fluorescence was measured at $400_{Ex}/505_{Em}$ and $485_{Ex}/520_{Em}$ using a BMG PolarStar plate reader. Caspase-Glo® 3/7 Reagent then was added in an additional 100µl volume, and luminescence was measured after a 10-minute incubation. The resulting signals were normalized to a percentage of maximal response and plotted using GraphPad Prism® software.

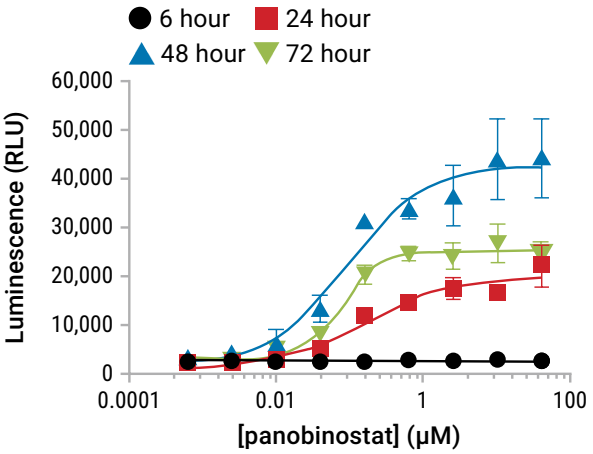

Figure 10. 3D microspheroid tissue treatment with panobinostat in a timecourse experiment. HCT116 spheroids were created using the InSphero GravityPLUS™ Hanging Drop System, and then treated with panobinostat for up to 72 hours. At indicated time points, Caspase-Glo® 3/7 Reagent was added to the wells and shaken for 30 minutes prior to reading luminescence. Separate wells were used for each time point.

6. General Considerations

Sensitivity

In fluorescence-based assays, fluorescent substrates, such as Z-DEVD-AMC, are cleaved by the protease, and the released fluor exhibits a shift in the excitation/emission wavelengths. Consequently, there can be some overlap in the emission spectra of the substrate before and after cleavage, creating substantial inherent background. The luminogenic substrate (Z-DEVD-aminoluciferin) is not a substrate for luciferase until cleaved to release aminoluciferin; hence there is insignificant inherent background (Figure 4).

The low background results in a high signal:background ratio (see Figure 7). The low background also allows a broad range of linearity for the assay, approximately four orders of magnitude of caspase concentration (see Figure 2), and allows detection of caspase activity in as few as 20 apoptotic cells (see Figure 4). Because of the high signal:background ratio and the broad range of linearity, typically, the data need to be plotted on a log-scale graph (see Figures 2–7). We recommend using cell numbers <20,000 cells/well in a 96-well plate or purified caspase-3 enzyme at <1ng/well to maintain maximum signal stability.

Because of the sensitivity of this assay, caspase-3 and -7 activities can be detected in fetal bovine, horse and calf serum. The luminescent signals generated from serum in the assay were inhibited by the caspase inhibitors Ac-DEVD-CHO and Z-VAD-FMK in a dose-dependent manner. When performing an assay using cells in medium with serum, we strongly recommend a “no-cell” control in addition to an untreated control to account for the signal generated from serum alone. The “no-cell” control signal can be subtracted from signal produced by the treated and untreated cells.

Unlike fluorescence-based assays, the maximum sensitivity of the Caspase-Glo® 3/7 Assay is not dependent upon extended incubations to accumulate cleaved product. Therefore, maximum sensitivity is achieved once the caspase and luciferase activities reach steady state. Typically this occurs within one hour. The time required for the assay to reach steady state may vary, depending upon the cell culture system used.

Temperature

The intensity and rate of decay of the luminescent signal from the Caspase-Glo® 3/7 Assay depends on the rate of decrease in caspase activity and the rate of the luciferase reaction. Environmental factors that affect the rate of the luciferase reaction also will affect the intensity of light output and the stability of the luminescent signal. Temperature can affect the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates to a constant temperature before performing the assay. For batch-mode processing of multiple assay plates, positive and negative controls should be included for each plate. Additionally, precautions should be taken to ensure complete temperature equilibration. Plates removed from a 37°C incubator and placed in tall stacks at room temperature will require longer for equilibration than plates arranged in a single layer.

Chemicals

The chemical environment of the luciferase reaction will affect the enzymatic activity and thus luminescence intensity. Differences in luminescence intensity have been observed using different types of culture media and sera. Solvents used for various chemical compounds may affect the luciferase reaction and thus the light output from the assay. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, has been tested at final concentrations of up to 10% in the assay and found to have a minimal effect on light output.

Mixing

Mixing is not required after adding the Caspase-Glo® 3/7 Reagent for assays with purified enzyme, although mixing may increase reproducibility between wells. We recommend mixing assays with cultured cells or 3D microtissues to aid in cell lysis and release of Caspase-3/7.

Luminometers

For highly sensitive luminometric assays, the luminometer model and settings greatly affect the quality of the data obtained. Luminometers from different manufacturers vary in their sensitivity and dynamic range. If you are not using a GloMax® luminometer, consult the operating manual for your luminometer to determine the optimal settings. The limits should be verified on each instrument before analysis of experimental samples. The assay should be linear in some portion of the detection range of the instrument used. Within an individual luminometer there may be different gain/sensitivity settings. We recommend that you optimize the gain/sensitivity settings.

We recommend the GloMax® product family of luminometers. These instruments do not require gain adjustments to achieve optimal sensitivity and dynamic range. The Promega GloMax® family of luminometers are preloaded with Promega product protocols for ease of use.

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7. Related Products

Apoptosis Products

Product	Size	Cat.#
Caspase-Glo® 3/7 3D Assay	10ml	G8981
	100ml	G8982
	10 x 10ml	G8983
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
ApoTox-Glo™ Triplex Assay	10ml	G6320
ApoLive-Glo™ Multiplex Assay	10ml	G6410
Caspase-Glo® 8 Assay	100ml	G8202
Caspase-Glo® 9 Assay	100ml	G8212
Apo-ONE® Homogeneous Caspase-3/7 Assay	100ml	G7791
Caspase Inhibitor Z-VAD-FMK	50µl	G7231
Caspase Inhibitor AC-DEVD-CHO	100µl	G5961
CaspACE™ FITC-VAD-FMK In Situ Marker	50µl	G7461
DeadEnd™ Colorimetric TUNEL System	20 reactions	G7360
DeadEnd™ Fluorometric TUNEL System	60 reactions	G3250
Terminal Deoxynucleotidyl Transferase, Recombinant	300u	M1871

Cell Viability Assays

Product	Size	Cat.#
RealTime-Glo™ MT Cell Viability Assay	100 reactions	G9711
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Glo® One Solution Assay	100ml	G8461
	500ml	G8462
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
	10 x 10ml	G7571
	100ml	G7572
	10 x 100ml	G7573
CellTiter-Glo® 2.0 Assay	10ml	G9241
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080

Cytotoxicity Assays

Product	Size	Cat.#
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
	50ml	J2381
CellTox™ Green Cytotoxicity Assay	10ml	G8741
CellTox™ Green Express Cytotoxicity Assay	200µl	G8731
CytoTox-Fluor™ Cytotoxicity Assay (fluorescent)	10ml	G9260
CytoTox-Glo™ Cytotoxicity Assay (luminescent)	10ml	G9290
MultiTox-Fluor Multiplex Cytotoxicity Assay (fluorescent; dual assay)	10ml	G9200
MultiTox-Glo Multiplex Cytotoxicity Assay (luminescent and fluorescent; dual assay)	10ml	G9270

Not for Medical Diagnostic Use. Additional kit formats are available

Oxidative Stress and Metabolism Assays

Product	Size	Cat.#
Cholesterol/Cholesterol Ester-Glo	5ml	J3190
Glycerol-Glo	5ml	J3150
Glucose Uptake-Glo™ Assay	5ml	J1341
Glucose-Glo™ Assay	5ml	J6021
Glutamate-Glo™ Assay	5ml	J7021
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611
Lactate-Glo™ Assay	5ml	J5021
Mitochondrial ToxGlo™ Assay	10ml	G8000
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H_2O_2 Assay	10ml	G8820
Triglyceride-Glo	5ml	J3160

Not for Medical Diagnostic Use. Additional kit formats are available.

Luminometers

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	1 each	GM3500
GloMax® Navigator System	1 each	GM2000

8. Summary of Changes

The following changes were made to the 9/23 revision of this document:

1. Updated patent statements.
2. Changed font and cover image.
3. Made minor text edits.

[a]U.S. Pat. Nos. 7,148,030, 7,384,758 and 7,666,987,and 8,071,328, Japanese Pat. No. 4451663 and other patents pending.