

# Caspase-Glo™

## 3/7 Assay

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Homogeneous Caspase Assay

unmatched  
speed and  
sensitivity



Promega

## Description

The Caspase-Glo 3/7 Assay<sup>(a,b)</sup> is a homogeneous luminescent assay that measures caspase-3/7 activities. The assay provides a caspase-3/7 DEVD-aminoluciferin substrate and a patented thermostable luciferase in a reagent optimized for caspase-3/7 activity, luciferase activity and cell lysis. The addition of Caspase-Glo 3/7 Reagent directly to cells in an "add-mix-measure" format results in cell lysis, caspase cleavage of the substrate and liberation of free aminoluciferin that is consumed by luciferase, generating a "glow-type" luminescent signal (Figure 1). The signal produced is proportional to caspase-3/7 activity (Figures 3 and 4). The Caspase-Glo assay uses a patented stabilized luciferase and a proprietary buffer that improves performance across a wide range of assay conditions, and is less likely to be affected by compound interference than other luminescent-, fluorescent- or colorimetric-based assays. The Caspase-Glo 3/7 Assay is designed for use in multiwell plate formats using either purified enzyme or cultured cells and can be used for applications such as apoptosis determination, caspase-3/7 activity determination, and caspase-3/7 inhibitor or activator screening.

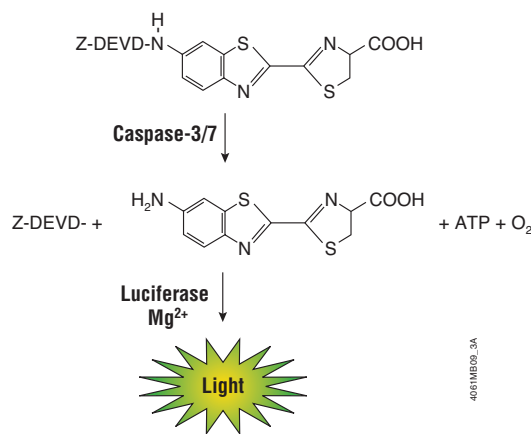


Figure 1. Cleavage of the pro-luminogenic substrate containing the DEVD sequence by caspase-3 and -7 and generation of a "glow-type" luminescent signal.

## Simple, Exquisitely Sensitive, and Fast

- Simplify Apoptosis or Caspase Detection:** The homogeneous "add-mix-measure" protocol makes the Caspase-Glo Assay easy to automate; simply add an equal volume of reagent to your sample (Figure 2). The assay has been automated on Beckman Coulter's Biomek® 2000 and FX automated workstations in both 96- and 384-well formats with cells or purified enzyme. (methods available at: [www.promega.com/automethods](http://www.promega.com/automethods))
- Use Less Enzyme or Fewer Cells:** The luminescent Caspase-Glo Assay is more sensitive than fluorescence-based caspase assays, with a detection limit of 0.1pg recombinant caspase-3 enzyme. There is little background luminescence and therefore, excellent signal-to-noise ratios. This means you can use fewer cells when monitoring apoptosis, and can use less recombinant caspase when screening for caspase inhibitors (Figures 3 and 4).
- Decrease Assay Time:** No sample preparation or manipulation required, and no extended incubation times are necessary. Achieve maximum sensitivity in as little as 0.5–1 hour.

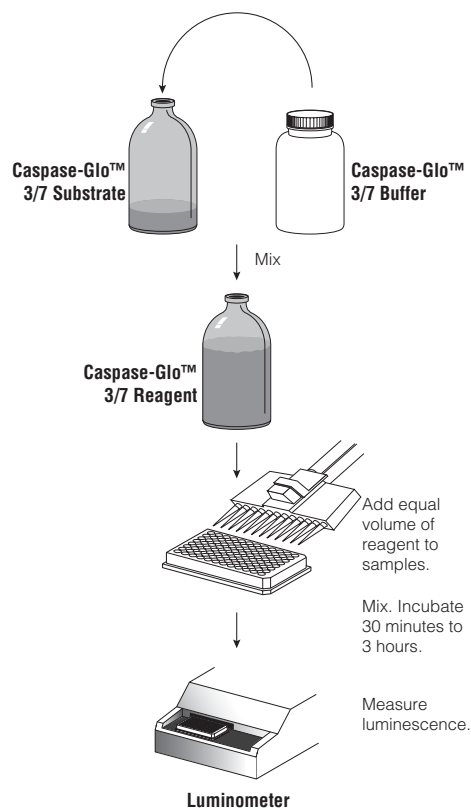


Figure 2. Schematic diagram of the Caspase-Glo 3/7 Assay protocol.

# 3/7 Assay

## Reliable and Flexible

- Rely on a Performance-tested Assay:** The Caspase-Glo Assay delivers excellent Z'-factors in cell and purified enzyme models (Figure 5). Z'-factor is a statistical value that compares the dynamic range of an assay to data variation in order to assess assay quality. Z'-factor values greater than 0.5 indicate excellent assay quality. The assay is also useful for determining IC<sub>50</sub> and EC<sub>50</sub> values (Figure 6).
- Process Plates in Batch mode:** The extended-glow signal allows data recording from plates over a 3-hour period for batch processing; reagent injectors are not required.
- Select for Caspase-3 and -7 Activity:** The Caspase-Glo 3/7 Assay uses an optimized buffer and luminogenic substrate containing the DEVD sequence, which has been shown to be selective for caspase-3 and -7.

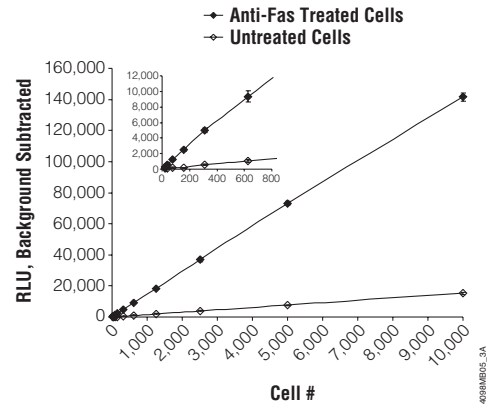


Figure 3. The Caspase-Glo 3/7 Assay provides superior sensitivity and linearity over a broad range of cell numbers.

*The luminescent Caspase-Glo 3/7 Assay is significantly faster and more sensitive than fluorescence-based caspase assays and provides excellent signal-to-noise ratios and Z'-factor values.*

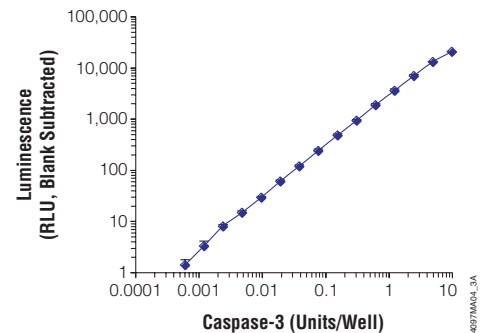


Figure 4. The Caspase-Glo 3/7 Assay has linearity over 4 orders of magnitude of caspase concentration. (One unit of caspase, 0.07ng protein, is the amount of enzyme required to cleave 1pmol of substrate hydrolyzed/minute at 30°C, per the manufacturer's unit definition.)

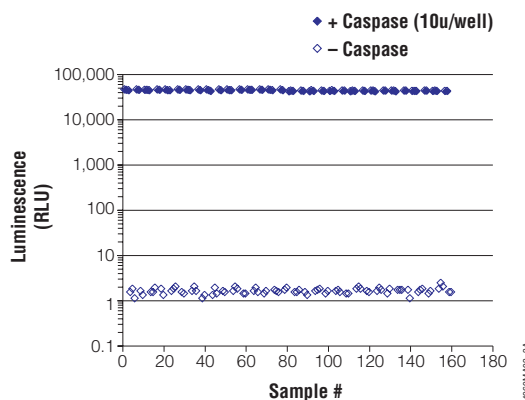


Figure 5. The Caspase-Glo 3/7 Assay can produce excellent Z'-factor values. Z'-factor =0.92 for this assay. A Z'-factor value was calculated using recombinant caspase-3 and a non-caspase control in two 96-well plates.

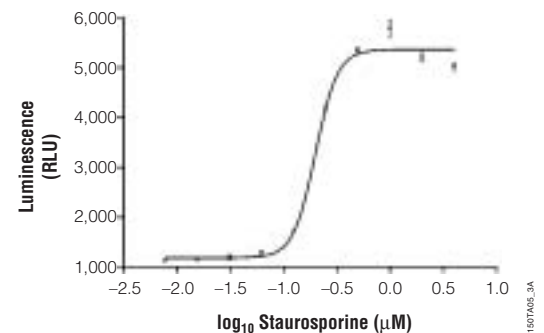


Figure 6. The Caspase-Glo 3/7 Assay can be used to obtain EC<sub>50</sub> values. HeLa cells in 96-well plates (10,000 cells/well) were treated with various concentrations of staurosporine for 4.5hrs. Caspase-Glo Reagent was added and readings were taken at 1 hour.

### Ordering Information

| Product | Size | Cat. # |
|---------|------|--------|
|---------|------|--------|

Caspase-Glo™ 3/7 Assay<sup>(a,b)</sup> 2.5ml G8090  
 Each system contains sufficient reagents for 25 assays at 100µl per assay in 96-well format or 100 assays at 25µl per assay in a 384-well format.

| Product | Size | Cat. # |
|---------|------|--------|
|---------|------|--------|

Caspase-Glo™ 3/7 Assay<sup>(a,b)</sup> 10ml G8091  
 Each system contains sufficient reagents for 100 assays at 100µl per assay in 96-well format or 400 assays at 25µl per assay in a 384-well format.

| Product | Size | Cat. # |
|---------|------|--------|
|---------|------|--------|

Caspase-Glo™ 3/7 Assay<sup>(a,b)</sup> 100ml G8092  
 Each system contains sufficient reagents for 1,000 assays at 100µl per assay in 96-well format or 4,000 assays at 25µl per assay in a 384-well format.

#### Additional Product Information

|                    |       |
|--------------------|-------|
| Technical Bulletin | TB323 |
| Automated Protocol | EP017 |
| Cell Notes         | CN006 |

### Related Products

#### Instrumentation Products

| Product                                 | Size   | Cat. # |
|---|--------|--------|
| Reporter™ Microplate Luminometer System | 1 each | E2701  |

#### Apoptosis Products

| Product  | Size         | Cat. # |
|--|--------------|--------|
| Apo-ONE® Homogeneous Caspase-3/7 Assay <sup>(c)</sup> (Fluorometric) | 1ml          | G7792  |
| Caspase Inhibitor Ac-DEVD-CHO  | 100 µl       | G5961  |
| Caspase Inhibitor Z-VAD-FMK  | 50µl         | G7231  |
| CaspACE™ Assay System, Colorimetric                                  | 50 assays    | G7351  |
| CaspACE™ Assay System, Fluorometric                                  | 160 assays   | G3540  |
| DeadEnd™ Colorimetric TUNEL System*                                  | 40 reactions | G7130  |
| DeadEnd™ Fluorometric TUNEL System <sup>(d)</sup>                    | 60 reactions | G3250  |
| CaspACE™ FITC-VAD-FMK In Situ Marker                                 | 50µl         | G7461  |

#### Cell Viability Assays

| Product   | Size           | Cat. # |
|---|----------------|--------|
| CellTiter-Glo® Luminescent Cell Viability Assay <sup>(a,b)</sup>  | 10ml           | G7570  |
| CellTiter-Blue™ Cell Viability Assay (Fluorometric, resazurin-based assay)                                  | 20ml           | G8080  |
| CellTiter 96® AQueous One Solution Cell Proliferation Assay <sup>(e)*</sup> (Colorimetric, MTS-based assay) | 200 assays     | G3582  |
| CytoTox-ONE™ Homogeneous Membrane Integrity Assay <sup>(a)</sup> (Fluorometric, LDH release)                | 200-800 assays | G7890  |
| CytoTox 96® Non-Radioactive Cytotoxicity Assay* (Colorimetric, LDH release)                                 | 1,000 assays   | G1780  |

\*For Laboratory Use

<sup>(a)</sup> Patent Pending.

<sup>(b)</sup> The method of recombinant expression of Coleoptera luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

<sup>(c)</sup> This product is covered by U.S. Pat. Nos. 4,557,862 and 4,640,893 and is sold for research use only. All other uses, including but not limited to use as a clinical diagnostic or therapeutic, require a separate license. Please contact Promega Corporation for details relating to obtaining a license for such other use.

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<sup>(e)</sup> The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida and is licensed exclusively to Promega Corporation.

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.

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