Promega

Technical Bulletin

Caspase-Glo® 2 Assay

INSTRUCTIONS FOR USE OF PRODUCTS G0940 AND G0941.

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1. Description

The Caspase-Glo® 2 Assay[a,b,c] is a homogeneous, luminescent assay that measures caspase-2 activity. Caspase-2 is a member of the cysteine aspartic acid-specific protease (caspase) family, a family of enzymes with well known roles in apoptosis and inflammation. Caspase-2 was one of the first caspases discovered and is one of the most conserved caspases across species, but its physiological function has been difficult to dissect and remains controversial (1–5). Caspases involved in apoptosis are divided into initiator and effector caspases. Initiator caspases function upstream within apoptotic signaling pathways and are capable of activating downstream caspases either directly or indirectly. Upon activation by an initiator caspase, effector caspases are immediate executioners of the apoptotic program, cleaving several substrates to cause controlled breakdown of the cell (6). There are several reports indicating that caspase-2 can function as an initiator caspase in diverse apoptotic pathways (3, 7–13).

The Caspase-Glo® 2 Assay provides a luminogenic caspase-2 substrate, Z-VDVAD-aminoluciferin, in a reagent optimized for caspase-2 and luciferase activity. The VDVAD pentapeptide sequence is an optimized recognition sequence for caspase-2, although this sequence is also an acceptable substrate for caspases-3 and -7 (14). A single Caspase-Glo® 2 Reagent is added to test samples, resulting in 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® 2 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 and luciferase enzyme activities reach a steady state so that the luminescent signal peaks in approximately 30 minutes and is maintained for several hours with a minimal loss of signal (Figure 3). This results in a rapid, sensitive and flexible caspase-2 activity assay. The assay system may be used with purified enzyme preparations (Figures 2–6) and is ideal for automated high-throughput screening of inhibitors (Figures 2, 5). This luminescent format significantly improves the sensitivity over comparable fluorescent assays (Figure 4).

The buffer contains a lysis reagent enabling use of the assay to measure caspase activities in cultured cells; the Z-VDVAD-aminoluciferin substrate will detect added caspase-2, as well as caspases-3 and -7 in cultured cells (14, Figures 9, 10). In addition, the proteasome also can cleave the Z-VDVAD-aminoluciferin substrate. In order to select for caspase-2 activity, the caspase-3/7 inhibitor, Ac-DEVD-CHO, and the proteasome inhibitor, MG-132, can be combined with the Caspase-Glo® 2 Assay to inhibit non-specific activities (Figures 6, Panel B, 9 and 10).

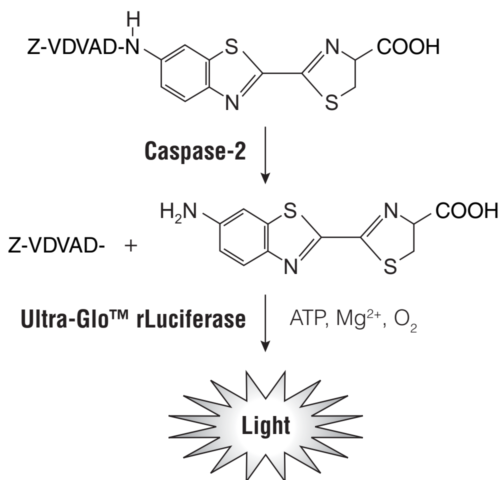


Figure 1. Caspase-2 cleavage of the luminogenic substrate containing the VDVAD sequence. Following caspase cleavage, a substrate for luciferase (aminoluciferin) is released, resulting in the production of light from the luciferase reaction.

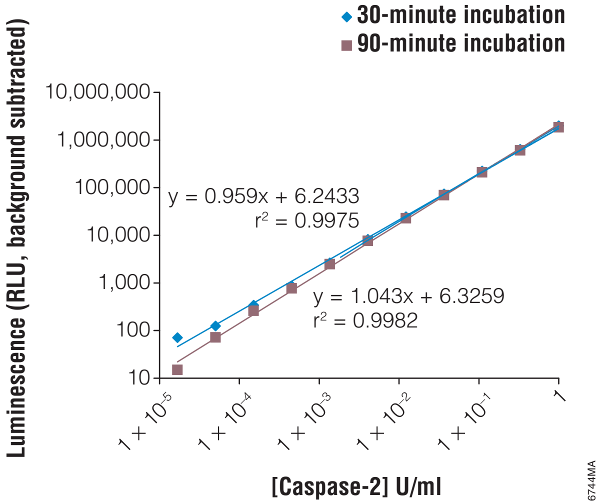
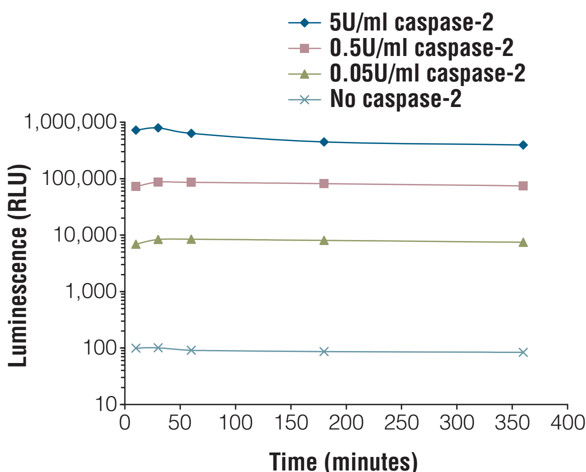


Figure 2. Titration of caspase-2 enzyme assayed in 96-well plates using the Caspase-Glo® 2 Assay. Recombinant human caspase-2 (BIOMOL International) was serially diluted in 10mM HEPES [pH 7.0], 2mM DTT. Luminescence was recorded as relative light units (RLU) on a GloMax® 96 Microplate Luminometer (Cat.# E6501) 30 and 90 minutes after adding the Caspase-Glo® 2 Reagent. The assay is linear over 4 logs of caspase-2 concentration (r^2 = 0.99, slope = 0.96–1.0). Each point represents the average of 4 wells. The no-caspase-2 control value was subtracted from each. r^2 and slope were calculated after transforming the data to a log_{10}-log_{10} plot. The specific activity for the caspase-2 was 100U/µg. One unit of caspase-2 was defined as the amount of enzyme required to cleave 1pmol of substrate/minute at 30°C using 200µM Ac-LEHD-pNA as substrate.

A.



B.

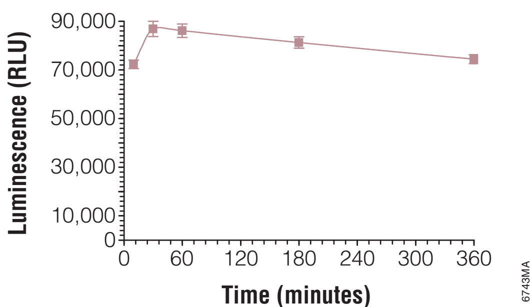


Figure 3. Signal stability of the Caspase-Glo® 2 Assay. Recombinant human caspase-2 enzyme (BIOMOL International) was titrated and assayed in 384-well plates using the Caspase-Glo® 2 Assay. Luminescence was monitored for 6 hours on a BMG PHERAstar luminometer. **Panel A.** The assay gives a stable signal for several hours over a broad range of caspase-2 concentration, as shown on a log scale. **Panel B.** The luminescent signal has a half-life greater than 6 hours, shown here for 0.5U/ml caspase-2 on a linear scale. All points represent the average of 4 wells. The specific activity for the caspase-2 was 100U/µg.

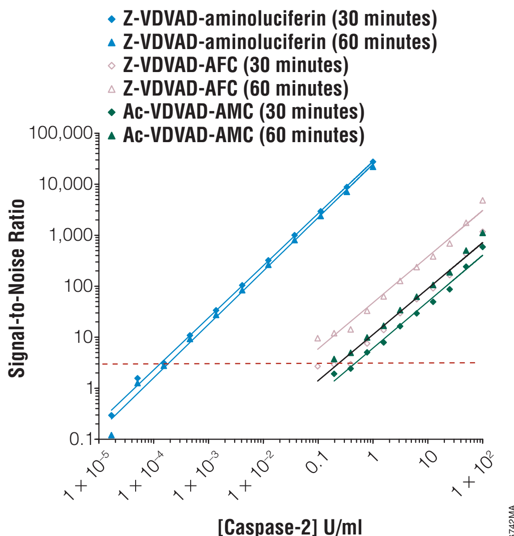


Figure 4. Sensitivity of the Caspase-Glo® 2 Assay compared to fluorescent assays. Human recombinant caspase-2 was titrated in 10mM HEPES (pH 7.0), 2mM DTT and assayed in 96-well plates using the Caspase-Glo® 2 Assay (Z-VDVAD-Glo™ Substrate) or comparable fluorogenic substrates, Z-VDVAD-AFC and Ac-VDVAD-AMC. Luminescence and fluorescence were measured at 30 and 60 minutes on a GloMax® 96 Microplate Luminometer (Cat.# E6501) or a Labsystems Fluoroskan Ascent plate reader, respectively. The results are plotted as signal-to-noise ratios. The limit of detection is defined as the amount of caspase-2 giving a signal-to-noise ratio >3 (dashed line). The signal-to-noise ratio is greater, and the limit of detection is significantly lower for the luminescence assay compared to the fluorescence assays. The specific activity for the caspase-2 was 100U/µg.

Advantages of the Caspase-Glo® 2 Assay

Simplified Method: The homogeneous "add-mix-measure" protocol makes the assay highly amenable to automation (Figure 5).

Greater Sensitivity: The Caspase-Glo® 2 Assay is more sensitive than fluorescence-based caspase assays (Figure 4), with maximum sensitivity typically reached within 30 minutes. The luminescence assay avoids interference from fluorescent signals of test compounds, providing excellent signal-to-noise ratios. The sensitivity allows you to use less purified caspase when screening for caspase inhibitors.

Faster Results: The maximum signal (and maximum sensitivity) of the assay is reached in as little as 30 minutes after reagent addition (Figure 3, Panel B), and unlike fluorescence assays, the signal does not depend on accumulation of cleaved product.

Advantages of the Caspase-Glo® 2 Assay (continued)

Broad Dynamic Range: The assay is linear over four logs of caspase-2 concentration and can detect caspase-2 activity at concentrations as low as 0.2mU/ml (0.6pM; Figures 2 and 4).

High-Quality Assay: The assay demonstrates an excellent Z´-factor value, a statistical value that compares the dynamic range of an assay to data variation. Z´-factor values greater than 0.5 indicate excellent assay quality (15). The Caspase-Glo® 2 Assay gave a Z´-factor value of 0.85 in 384-well plates using 0.05U/ml of caspase-2 (Figure 5).

Increased Accuracy: The assay provides accurate results for kinetic studies of inhibitors (Figure 6). Because of the sensitivity, inhibitor studies can be done using the luminescent substrate at concentrations significantly less than the K_m. In the case of competitive inhibitors, if the substrate is used at concentrations signficantly less than the K_m, the $IC_{50} = K_i$ (16).

Batch-Processing Capability: The coupled-enzyme, homogeneous format results in a continuous signal, providing excellent stability and allowing plates to be read over an extended period of time (Figure 3, Panel A). Luminometers with reagent injectors are not required.

Flexibility: Inhibitors can be added with the reagent to inhibit non-specific cleavage of the substrate (Figures 6 and 10).

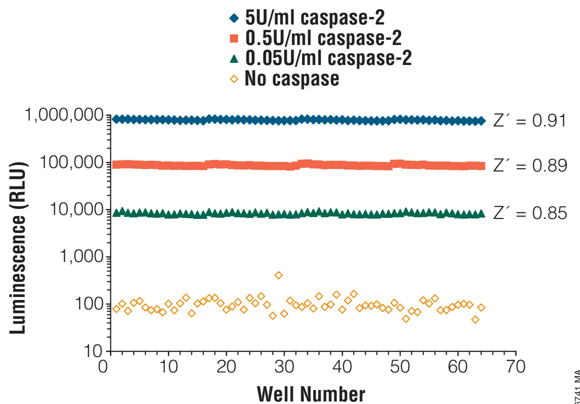


Figure 5. Z´-factor analysis. Z´-factor values (15) for the Caspase-Glo® 2 Assay were calculated using recombinant caspase-2 (5U, 0.5U, and 0.05U/ml) and a no-caspase blank. Caspase-2 was diluted in 10mM HEPES (pH 7.0), 2mM DTT, 0.1% Prionex® as a carrier. Assays were performed in a total volume of 20µl in a 384-well plate. Luminescence was recorded on a BMG PHERAstar luminometer at 30 minutes. Z´-factor values ranged from 0.85 to 0.91 for this assay. One unit of caspase-2 is defined as the amount of enzyme required to cleave 1pmol of substrate/minute at 30°C using 200 µM Ac-LEHD-pNA as substrate. In this case, 1 unit of caspase-2 is equivalent to 10ng protein.

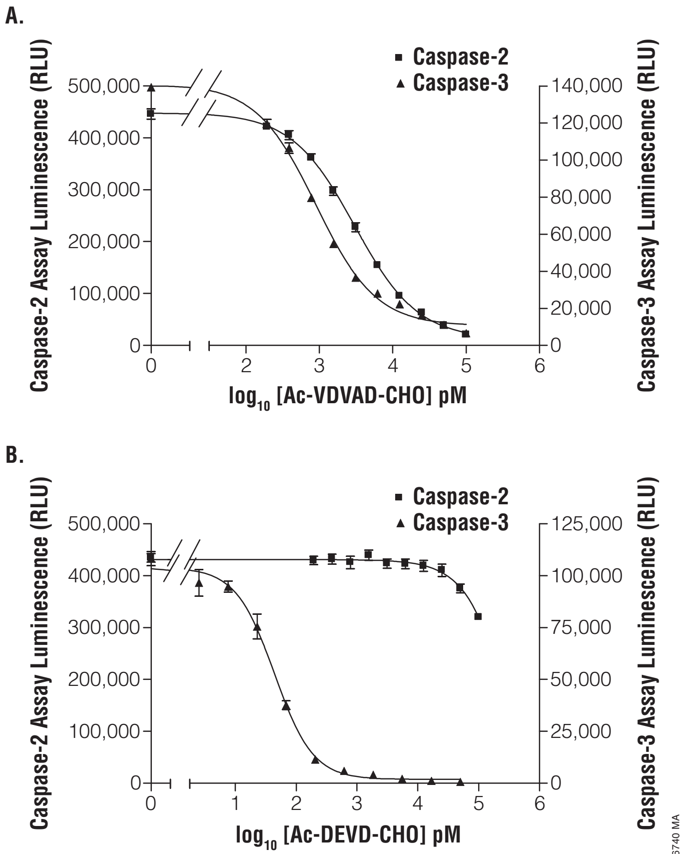


Figure 6. Determination of IC_{50} values for caspase-2 inhibitors. The inhibitor concentrations that result in 50% inhibition (IC_{50}) of caspase-2 activity were determined for the caspase inhibitors Ac-VDVAD-CHO and Ac-DEVD-CHO using the Caspase-Glo® 2 Assay. **Panel A.** The competitive inhibitor, Ac-VDVAD-CHO, was resuspended in DMSO, serially diluted, and combined with caspase-2 (0.5U/ml) or caspase-3 (5U/ml) in HEPES (pH 7.0), 2mM DTT and 0.1% Prionex® in 96-well plates. **Panel B.** The competitive inhibitor, Ac-DEVD-CHO, was prepared and incubated with caspase-2 or -3 as described above. The maximum DMSO concentration is <0.01% for both inhibitors. The Z-VDVAD-Glo™ Substrate was used at 20µM. Luminescence was recorded 40–60 minutes after reagent addition, and GraphPad Prism® software was used to calculate the IC_{50}. The IC_{50} for Ac-VDVAD-CHO with caspase-2 was 3.0nM and for caspase-3 was 0.9nM, comparable to the published K_i of 3.5nM for caspase-2 and 1.0nM for caspase-3 using fluorescent substrates (14,17). The Ac-VDVAD-CHO is equally effective on the two caspases, confirming that this inhibitor cannot be used to assign specificity (Panel A). The IC_{50} for Ac-DEVD-CHO for caspase-2 was >500nM and for caspase-3 was 0.04nM. The relative potency of the inhibitor for caspase-2 and for caspase-3 is the same as that published using fluorescent substrates (14,17). The Ac-DEVD-CHO inhibitor is highly selective for caspase-3 over caspase-2 and can be used to select for caspase-2 activity (Panel B).

2. Product Components and Storage Conditions

Product	Size	Cat.#
Caspase-Glo® 2 Assay	10ml	G0940

For Laboratory Use. Cat.# G0940 provides sufficient reagents for 100 assays at 100µl/assay or 200 assays at 50µl/assay in 96-well plates, or 400 assays at 25µl/assay in 384-well plates. Includes:

- 10ml Caspase-Glo® 2 Buffer
- 100µl Z-VDVAD-Glo™ Substrate
- 1 bottle Luciferin Detection Reagent

Product	Size	Cat.#
Caspase-Glo® 2 Assay	50ml	G0941

For Laboratory Use. Cat.# G0941 provides sufficient reagents for 500 assays at 100µl/assay or 1,000 assays at 50µl/assay in 96-well plates, or 2,000 assays at 25µl/assay in 384-well plates. Includes:

- 50ml Caspase-Glo® 2 Buffer
- 500µl Z-VDVAD-Glo™ Substrate
- 1 bottle Luciferin Detection Reagent

Storage Conditions: Store the Caspase-Glo® 2 Assay components at –20°C protected from light. The Caspase-Glo® 2 Buffer may be thawed and stored at 4°C for 4 months with no loss in signal. The Z-VDVAD-Glo™ Substrate can be refrozen and stored at –20°C for 1 year with minimal loss of signal. Caspase-Glo® 2 Reagent (combined Z-VDVAD-Glo™ Substrate, Caspase-Glo® 2 Buffer and Luciferin Detection Reagent) can be stored frozen at –20°C or at 4°C for 1 month with minimal loss of activity. The reagent can be thawed and frozen several times with minimal loss of signal.

3. Reagent Preparation and Storage

1. Thaw the Caspase-Glo® 2 Buffer, and equilibrate both the buffer and lyophilized Luciferin Detection Reagent to room temperature (22–25°C) before use.
2. Reconstitute the Luciferin Detection Reagent in the amber bottle by adding Caspase-Glo® 2 Buffer (10ml for Cat.# G0940, 50ml for Cat.# G0941). The Luciferin Detection Reagent should go into solution easily in less than one minute.
3. Thaw the Z-VDVAD-Glo™ Substrate, and mix well by vortexing briefly before use.

4. Prepare the Caspase-Glo® 2 Reagent by adding the Z-VDVAD-Glo™ Substrate to the resuspended Luciferin Detection Reagent. For Cat.# G0940, add 100µl of Z-VDVAD-Glo™ Substrate to the 10ml of Luciferin Detection Reagent. For Cat.# G0941, add 500µl of the Z-VDVAD-Glo™ Substrate to the 50ml of Luciferin Detection Reagent. Mix by swirling or inverting the contents to obtain a homogeneous solution. The Z-VDVAD-Glo™ Substrate will be at 40µM concentration in the Caspase-Glo® 2 Reagent. The apparent K_m for the substrate is 40µM.

5. Allow the Caspase-Glo® 2 Reagent to sit at room temperature for 30–60 minutes prior to use. This allows time for the removal of any contaminating free aminoluciferin, ensuring maximal sensitivity. Although free aminoluciferin is not detected by HPLC, it is present in trace amounts (Figure 8).

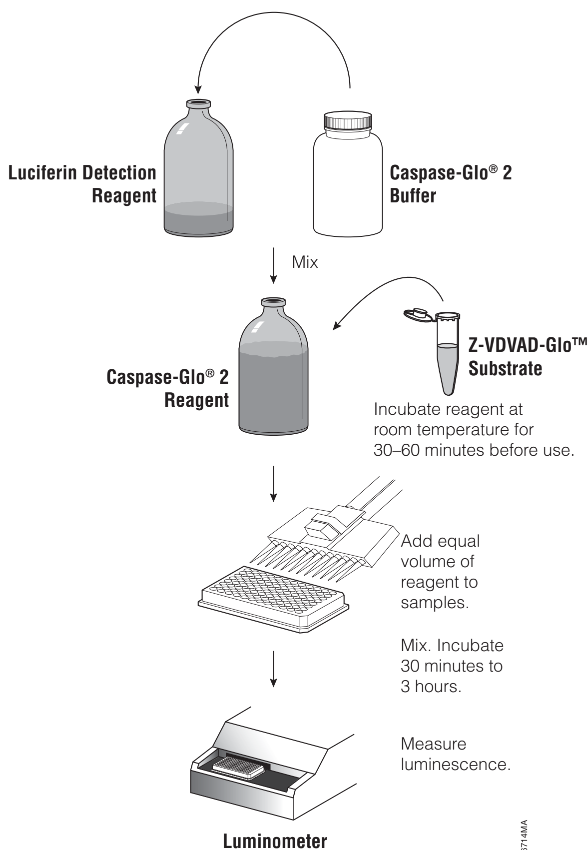


Figure 7. Flow diagram showing preparation and use of the Caspase-Glo® 2 Reagent.

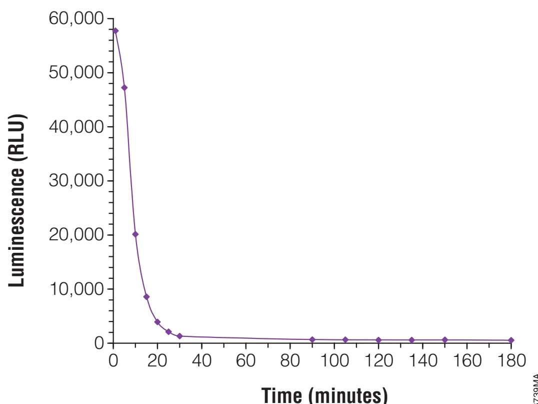


Figure 8. Time course for removal of free aminoluciferin from the Caspase-Glo® 2 Reagent. The Z-VDVAD-Glo™ Substrate was added to the bottle of reconstituted Luciferin Detection Reagent, and a time course of luminescence loss was recorded. Trace amounts of free aminoluciferin are present in the substrate and are removed by incubation with the reconstituted Luciferin Detection Reagent. To achieve maximal assay sensitivity with minimal background luminescence, the prepared Caspase-Glo® 2 Reagent should be incubated for at least 30 minutes at room temperature before use.

4. Assay for the Detection of Caspase-2 Activity

Directions are given for performing the Caspase-Glo® 2 Assay in a total volume of 100µl using 96-well plates and a luminometer. However, the assay can be adapted easily to different volumes provided that the 1:1 ratio of Caspase-Glo® 2 Reagent volume to sample volume is preserved (e.g., 25µl of sample + 25µl Caspase-Glo® 2 Reagent in a 384-well format).

Materials to be Supplied by the User

- white multiwell plates (black plates may be used, but RLU will be reduced)
- multichannel pipette or automated pipetting station for delivery of Caspase- Glo® 2 Reagent
- plate shaker or other device for mixing multiwell plates
- luminometer capable of reading multiwell plates
- caspase-2 enzyme (e.g., BIOMOL International, Cat.# SE-175,or Calbiochem, Cat.# 218813)
- optional: Prionex® Carrier (Centerchem, Inc. Norwalk, CT)

4.A. Assay Conditions

Prepare the following reactions to detect caspase-2 activity (or inhibition of activity) in purified enzyme preparations:

Blank: Caspase-Glo® 2 Reagent + vehicle control for test compound or inhibitor, if used (no test compound or inhibitor included)

Positive Control: Caspase-Glo® 2 Reagent + vehicle control + purified caspase-2 enzyme

Assay: Caspase-Glo® 2 Reagent + test compound + purified caspase-2 enzyme

The blank is used as a measure of any background luminescence associated with the test compound vehicle and Caspase-Glo® 2 Reagent and should be subtracted from experimental values. The positive control is used to determine the maximum luminescence obtainable with the purified enzyme system. Vehicle refers to the solvent used to dissolve the inhibitor or test compound.

Notes:

1. Prepare the Caspase-Glo® 2 Reagent as described in Section 3, and mix thoroughly before starting the assay. Allow the Reagent to sit at room temperature at least 30 minutes before use to remove any contaminating free aminoluciferin.
2. The final concentration of caspase-2 should be within the linear range of the assay (Figure 2).
3. The recommended caspase-2 dilution buffer is 10mM HEPES (pH 7.0), 2mM DTT and 0.1% Prionex® carrier (optional as a carrier if low enzyme concentrations are used).
4. Use identical enzyme concentrations for the assay and positive control reactions.
5. For gentle mixing you may use a plate shaker.
6. The maximal luminescent signal will be reached in ~30 minutes and will have a half-life of several hours (Figure 3).

4.B. Standard Assay (96-well, 100µl Final Reaction Volume)

1. Add 50µl of Caspase-Glo® 2 Reagent to each well of a white 96-well plate containing 50µl of blank, control or test sample.

   Note: If reusing tips, be careful not to touch pipette tips to the wells containing samples to avoid cross-contamination.

2. 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 convenience of reading time (Figure 3, Panel A).

   Note: Maximal signal is reached typically within ~30 minutes using purified caspase-2 (Figure 3, Panel B). At this time, sensitivity is optimal. Temperature fluctuations will affect the luminescent readings; if the room temperature fluctuates too much, a constant-temperature incubator may be desired.

3. Record luminescence with a plate-reading luminometer.

4.C. Considerations for Monitoring Caspase-2 Activity in Cells

The substrate sequence VDVAD is optimized for caspase-2 because of the preference of caspase-2 for a pentapeptide over a tetrapeptide, but the substrate is also cleaved by caspases-3 and -7 (14; Figure 9). The buffer contains a lysis reagent enabling use of the assay to measure caspase activities in cultured cells; the Z-VDVAD-aminoluciferin substrate will detect caspase-2, as well as caspases-3 and -7 in cultured cells. The caspase-2 inhibitor, Ac-VDVAD-CHO, cannot be used to assess specificity of the signal because the inhibitor also inhibits caspases-3 and -7 (14; Figure 6, Panel A). However, the caspase-3/7 inhibitor, Ac-DEVD-CHO, is preferentially selective for caspases-3 and -7 over caspase-2. The K_i of Ac-DEVD-CHO for caspase 3 is 0.2nM and for caspase-7 it is 0.3nM vs. a K_i of 1.7µM for caspase-2 (14,17; Figure 6, Panel B). This inhibitor can be used at a low concentration (between 20 and 60nM) to inhibit caspases-3 and -7 without affecting caspase-2 activity (Figure 6, Panel B). In addition, the proteasome also can cleave the Z-VDVAD-aminoluciferin substrate (data not shown). In order to select for caspase-2 activity, the selective caspase-3/7 inhibitor, Ac-DEVD-CHO, and the proteasome inhibitor, MG-132, can be combined with the Caspase-Glo® 2 Assay to inhibit nonspecific activities (Figure 10).

If the caspase-2 activation is temporally segregrated from the activation of caspases-3 and -7, then cross-reactivity is not a concern; however, if there is temporal overlap between activation of caspase-2 and the effector caspases, the effector caspases need to be inhibited in order to detect caspase-2. There are very few confirmed cell models for caspase-2 activation, and there are no caspase-2-specific inhibitors. In order to determine whether the caspase-3/7 inhibitor, Ac-DEVD-CHO could be used in combination with the Caspase-Glo® 2 Assay to counter-select for caspase-2 activity in cell culture, we performed a caspase-addition (spiking) experiment. Caspase-2, caspase-3, or caspase-2 plus -3 were added to cultured cells (Figure 10). The amounts of caspase added were adjusted to give comparable activity and to be comparable to levels of caspase-3 detected in apoptotic cells. The results demonstrated that caspase-3 activity can be selectively inhibited with the Ac-DEVD-CHO inhibitor, without affecting caspase-2 activity (Figure 10). If the amount of caspase-2 activity in apoptotic cells is significantly less than that for caspases-3 and -7 and is temporally coincident with caspase-3 or -7 activation, then a peptide-based activity assay is not suitable for distinguishing caspase-2 activity because of the activity of the effector caspase. The sensitivity of the bioluminescent caspase-2 assay, in combination with the Ac-DEVD-CHO and MG-132 inhibitors, provides the necessary tool to detect caspase-2 activity in cells.

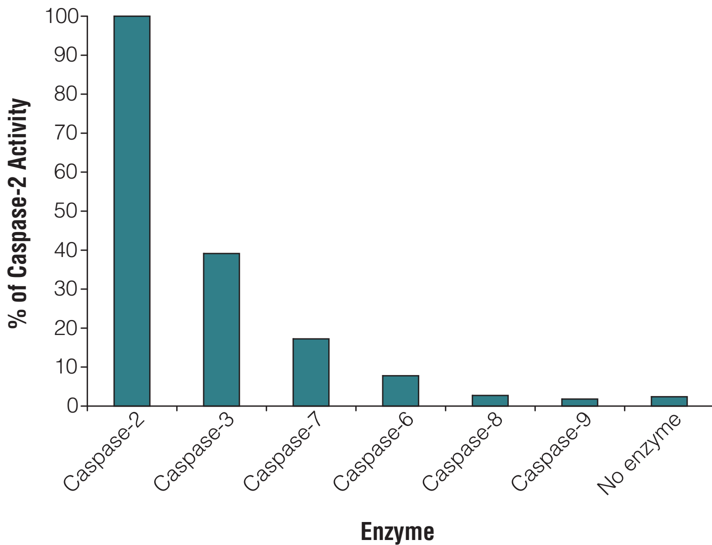


Figure 9. Cross-reactivity of the Caspase-Glo® 2 Assay with other caspases. The caspase enzymes (BIOMOL International) were tested at 0.5U/ml (for caspases-2, -6, -8, and -9) or 0.625U/ml (for caspases-3 and -7). The caspase enzymes were diluted in 10mM HEPES (pH 7.2), 2mM DTT, and 0.1% Prionex® as a carrier. Luminescence was recorded as relative light units (RLU) on a GloMax® 96 Microplate Luminometer (Cat.# E6501) 30 minutes after adding the Caspase-Glo® 2 Reagent. Results are presented as a percentage of the RLU obtained from the caspase-2 activity. Caspase-3, and to a lesser degree, caspase-7 exhibit significant cross-reactivity on the Z-VDVAD-aminoluciferin substrate.

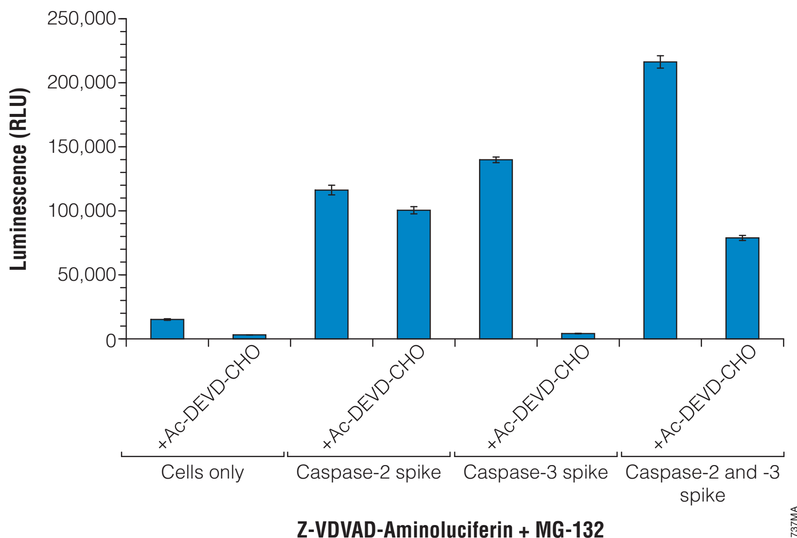


Figure 10. Inhibition of caspases-2 and -3 after addition into cultured cells. Caspase-2 (0.1U/ml) or caspase-3 (2U/ml) or both were added to 10,000 Jurkat cells/well cultured in RPMI-1640 with 10% fetal bovine serum in 100µl volume. The proteasome inhibitor MG-132 (60µM) was added to the Caspase-Glo® 2 Reagent. The reagent with or without added Ac-DEVD-CHO inhibitor (60nM) was added to the cultured cells along with the added caspases. Luminescence was read on a GloMax® 96 Microplate Luminometer (Cat.# E6501) after 90 minutes to allow the inhibitors to equilibrate. The cultured cells alone contained minimal Z-VDVADase activity that was inhibited by Ac-DEVD-CHO. The caspase-3 activity added into the cell culture was completely inhibited by the 60nM Ac-DEVD-CHO, whereas the caspase-2 activity was not affected. The difference in the signal from cells with added caspase-2 with or without inhibitor can be attributed to endogenous Z-VDVADase activity within the cells.

5. General Considerations

Sensitivity

The bioluminescent Caspase-Glo® 2 Assay is more sensitive than comparable fluorescent assays for several reasons. Fluorescence substrates generally depend on a shift in the excitation/emission wavelengths after cleavage by the protease; consequently, there is some overlap in the emission spectra of the substrate before and after cleavage, creating substantial inherent background. The luminescent substrate (Z-VDVAD-aminoluciferin) is not a substrate for luciferase until it is cleaved, greatly reducing inherent background. Furthermore, the homogeneous, coupled-enzyme format of the assay insures that any contaminating free aminoluciferin is consumed before beginning the assay (Figure 8). In a fluorescence assay, any contaminating free fluorophore remains and contributes to background. The only background in this bioluminescent assay is due to the spontaneous hydrolysis of the Z-VDVAD-aminoluciferin substrate. Thus the inherent background is low, resulting in large signal-to-noise ratios (Figure 4). The low background also allows a broad range of linearity for the assay (~4 logs of caspase-2 concentration; Figures 2 and 4). The assay sensitivity allows the researcher to use less enzyme if screening for caspase-2 inhibitors. We recommend using ≤1U/ml of caspase-2 per well (based on BIOMOL International human recombinant caspase-2 with a specific activity of 100U/µg); at higher concentrations the kinetics of the assay are compromised due to product inhibition of the luciferase reaction. The assay is not dependent on accumulation of cleaved product because the light output is a result of the luciferase consuming aminoluciferin as soon as it is produced. Maximum signal and sensitivity are achieved as soon as the caspase-2 and luciferase activities reach a steady state. Typically this occurs in ~30 minutes; therefore, the assay is extremely sensitive in a short time frame.

Temperature

The intensity and rate of decay of the luminescent signal from the Caspase-Glo® 2 Assay depends on the rate of the luciferase reaction. Environmental factors that affect the rate of the luciferase reaction change the intensity of light output and the stability of the luminescent signal. Temperature is one factor that affects the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates to a constant temperature prior to performing the assay. For batch-mode processing of multiple assay plates, take precautions to ensure complete temperature equilibration. Plates removed from a 37°C incubator and placed in tall stacks at room temperature will require longer to equilibrate to a consistent temperature than plates arranged in a single layer.

5. General Considerations (continued)

Chemicals

The chemical environment of the luciferase reaction will affect the enzymatic rate and thus luminescence intensity. Solvents used for various chemical compounds may interfere with the luciferase reaction, affecting the light output from the assay. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, has minimal effect on light output at final concentrations up to 5%.

Mixing

Mixing is not absolutely required after adding the Caspase-Glo® 2 Reagent, although it may aid in reproducibility between wells. A plate shaker may be used to gently mix plate contents.

6. References

1. Troy, C.M. and Shelanski, M.L. (2003) Caspase-2 redux. *Cell Death Differ.* **10**, 101–7.
2. Kumar, S. (2007) Caspase function in programmed cell death. *Cell Death Differ.* **14**, 32–43.
3. Tu, S. *et al*. (2006) In situ trapping of activated initiator caspases reveals a role for caspase-2 in heat shock-induced apoptosis. *Nat. Cell Biol.* **8**, 72–7.
4. Milleron, R.S. and Bratton, S.B. (2006) Heat shock induces apoptosis independently of any known initiator caspase-activating complex. *J. Biol. Chem.* **281**, 16991–7000.
5. Samraj, A.K. *et al*. (2007) Loss of caspase-9 reveals its essential role for caspase-2 activation and mitochondrial membrane depolarization. *Mol. Biol. Cell* **18**, 84–93.
6. Ho, P-K. and Hawkin, C.J. (2005) Mammalian initiator apoptotic caspases. *FEBS J.* **272**, 5436–53.
7. Troy, C.M. *et al*. (2001) Death in the balance: Alternative participation of the caspase-2 and -9 pathways in neuronal death induced by nerve growth factor deprivation. *J. Neurosci.* **21**, 5007–16.
8. Lassus, P., Opitz-Araya, X., and Lazebnik, Y. (2002) Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. *Science* **297**, 1352–4.
9. Schweizer, A., Briand, C., and Grutter, M.G. (2003) Crystal structure of caspase-2, apical initiator of the intrinsic apoptotic pathway. *J. Biol. Chem.* **278**, 42441–7.
10. Lin, C-F. *et al*. (2004) Sequential caspase-2 and caspase-8 activation upstream of mitochondria during ceramide- and etoposide-induced apoptosis. *J. Biol. Chem.* **279**, 40755–61.
11. Wagner, K.W., Engels, I.H., and Deveraux, Q.L. (2004) Caspase-2 can function upstream of bid cleavage in the TRAIL apoptosis pathway. *J. Biol. Chem.* **279**, 35047–52.
12. Robertson, J.D. *et al*. (2002) Caspase-2 acts upstream of mitochondria to promote cytochrome c release during etoposide-induced apoptosis. *J. Biol. Chem.* **277**, 29803–9.

13. Seth, R. *et al*. (2005) p53-dependent caspase-2 activation in mitochondrial release of apoptosis-inducing factor and its role in renal tubular epithelial cell injury. *J. Biol. Chem*. **280**, 31230–9.

14. Talanian, R.V. *et al*. (1997) Substrate specificities of caspase family proteases. *J. Biol. Chem*. **272**, 9677–82.

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

16. Brandt, R.B., Laux, J.E. and Yates, S.W. (1987) Calculation of inhibitor Ki and inhibitor type from the concentration of inhibitor for 50% inhibition for Michaelis-Menten enzymes. *Biochem. Med. Metab. Biol*. **37**, 344–9.

17. Garcia-Calvo, M. *et al*. (1998) Inhibition of human caspases by peptide-based and macromolecular inhibitors. *J. Biol. Chem*. **273**, 32608–13.

7. Related Products

Luminescent Caspase Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay	2.5ml	G8090
Caspase-Glo® 6 Assay	10ml	G0970
Caspase-Glo® 8 Assay	2.5ml	G8200
Caspase-Glo® 9 Assay	2.5ml	G8210

For Laboratory Use. Available in Additional Sizes.

Caspase Inhibitor

Product	Size	Cat.#
Caspase Inhibitor Ac-DEVD-CHO	100µl	G5961

Other Protease Assays

Product	Size	Cat.#
Proteasome-Glo™ Cell-Based Assay	10ml	G8660
Proteasome-Glo™ 3-Substrate System	10ml	G8531
Proteasome-Glo™ Chymotrypsin-Like Assay	10ml	G8621
Proteasome-Glo™ Tyrpsin-Like Assay	10ml	G8631
Proteasome-Glo™ Caspase-Like Assay	10ml	G8641
DPPIV-Glo™ Protease Assay	10ml	G8350
Calpain-Glo™ Protease Assay	10ml	G8501

For Laboratory Use. Available in Additional Sizes.

7. Related Products (continued)

Cell Viability and Cytotoxicity Assays

Product	Size	Cat.#
CellTiter-Fluor™ Cell Viability Assay*	10ml	G6080
CellTiter-Glo® Luminescent Cell Viability Assay (ATP)*	10ml	G7570
MultiTox-Fluor Multiplex Cytotoxicity Assay*	10ml	G9200
CytoTox-Fluor™ Cytotoxicity Assay*	10ml	G9260
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*	200 assays	G3582
CellTiter-Blue® Cell Viability Assay (resazurin)	20ml	G8080
CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH)	200–800 assays	G7890

*For Laboratory Use. Available in Additional Sizes.

[a]U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294, European Pat. No. 1131441 and other patents pending.

[b]U.S. Pat. Nos. 7,148,030 and 7,384,758 and other patents pending.

[c]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.

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