

“Sun Screening” with the Caspase-Glo™ Assay

Detecting UV Irradiation-Induced Apoptosis with the Caspase-Glo™ 3/7 Assay

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Abstract

Allergan is a global healthcare company devoted to neuromuscular, skin and eye care products and specialty pharmaceuticals. We are interested in compounds that inhibit the apoptotic effects of UV irradiation. Here we demonstrate an application of the Promega Caspase-Glo™ 3/7 Assay for high-throughput screening of anti-apoptotic compounds.

The Caspase-Glo™ Assay is quick and easy to use, making it well suited for high-throughput determination of caspase activity.

Introduction

The effects of UV irradiation on living cells is an area of growing interest, including UV-induced apoptosis. We are interested in high-throughput screening for compounds that protect against UV-induced apoptosis. UV irradiation induces cell death in a number of cell culture systems including CHO cells.

The apoptotic process can be visualized using Annexin-V/PI staining, Western Blot detection of caspase-3 activation as well as JC-1 dye monitoring of the change in mitochondrial membrane potential. However, these methods are not well suited to high-throughput assays. Combined with a fast, convenient and quantitative caspase-3/7 assay, the UV irradiation/CHO cell system can potentially provide a high-throughput means to evaluate the ability of compounds to interfere with apoptosis. Our goal was to assess the feasibility of the Promega Caspase-Glo™ 3/7 Assay^(a,b) as a fast and convenient method to quantitatively evaluate caspase-3/7 activation.

The Caspase-Glo™ 3/7 Assay

Activation of the caspase cascade is an integral event in the apoptotic pathway. Caspase-3 is one of the important “executioner” caspase enzymes, triggering the cleavage of numerous proteins that lead to the ordered breakdown of the cell. The detection of caspase-3 activation is frequently used as a positive marker for apoptosis. The Caspase-Glo™ 3/7 Assay provides a sensitive means of monitoring caspase activation in both cells and purified enzyme preparations. The assay uses a proluminescent substrate, DEVD-aminoluciferin containing the sequence recognized by caspase-3 and -7.

The assay provides the proluminescent caspase-3/7 substrate and a thermostable luciferase in a buffer system optimized for caspase-3/7 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. The liberated free aminoluciferin is consumed by luciferase, generating a “glow-type” luminescent signal (1). The luminescent signal produced is proportional to the amount of caspase activity present.

The Caspase-Glo™ 3/7 Assay achieves maximum sensitivity once the caspase and luciferase activities reach steady state, which occurs in approximately 1 hour. The precise time to reach maximum signal will depend on the assay system and culture conditions. The background luminescence in this assay is very low, resulting in excellent signal-to-background ratios and sensitivity not achieved by other caspase assay formats (1–3). The assay can detect as little as 0.1pg of active caspase-3 (4). Because of the sensitivity of this assay, fewer cells per well are required for accurate caspase activity measurements, and less recombinant enzyme is required if performing inhibitor screens.

The Caspase-Glo™ 3/7 Reagent produces luminescence that is directly proportional to caspase-3/7 activity. The luminescence is linear over four orders of magnitude of caspase concentration and over a broad range of cell densities (4). The luminescent signal is also stable with little decrease in signal intensity over two hours. This stability allows use of luminometers without reagent injectors, and luminescence measurements can be delayed where batch processing is desired.

Results

We used the Caspase-Glo™ 3/7 Assay to show that there is a time-dependent increase in the caspase-3/7 activity in the CHO cell culture after UV exposure. We subjected CHO cells to UV irradiation at increasing time points after a twenty-four-hour serum-free incubation (Figure 1). The results corroborate our previous observations using methods such as Annexin-V/PI staining, Western Blot detection of caspase-3 activation as well as JC-1 dye monitoring of the change in mitochondrial membrane potential. Because the CHO cell system we used required a transfer step after adding the Caspase-Glo™ Reagent, it was very important that the Caspase-Glo™ Reagent achieve complete lysis so the transfer would be uniform and consistent. This had been a problem with other assays; however, we did not experience inadequate cell lysis using the Caspase-Glo™ Reagent, and the assay provided the consistency needed for successful screening.

Caspase-Glo™ Assay... continued

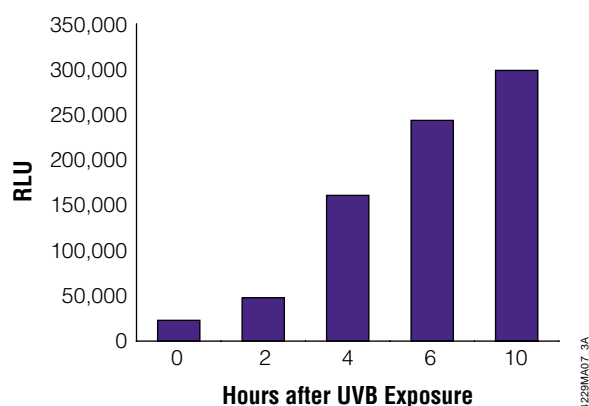


Figure 1. Specific caspase-3/7 activity in CHO cells post-UV treatment as determined by the Caspase-Glo™ 3/7 Assay. CHO cells were grown to 90% confluence in 5% FCS/F-12 media in 24-well plates. Media were then replaced with serum-free F-12 media. After twenty-four hours in the serum-free condition, groups of 4 wells were exposed to UV-B irradiation for a duration of 1 minute at time 0, or 2, 4, 6, or 10 hours later. At hour 10, each well was rinsed and filled with 0.3ml phosphate-buffered saline. The pan-caspase inhibitor, z-VAD-FMK (Calbiochem, San Diego, CA), was added at 10^{-6} M to two of the wells for each time point; these wells were used as nonspecific background controls. Caspase-Glo™ Reagent (0.5ml) was then added to each well. The plate was moderately shaken for 10 minutes on a plate shaker. A 200µl sample was transferred from each well to a 96-well, white-walled plate. This transfer step was necessary because our cells need to be grown in pretreated 24-well plates that are not white-walled and therefore are not suitable for the luminometer. Luminescence was measured using MicroLumatPlus LB96V (EG&G Berthold, Perkin Elmer Life Science) and expressed in Relative Light Units. Specific luminescence was calculated by subtracting the nonspecific background.

We previously had found that MAP kinase kinase inhibitor U0126 (10^{-6} M) inhibits UV-B-induced cell death in CHO cells using an LDH assay (CytoTox 96® Non-Radioactive Cytotoxicity Assay; Cat.# G1780). Using the Promega Caspase-Glo™ 3/7 Assay, we found that the activity of caspase-3/7 in UV-B-treated CHO cells is significantly lowered in the presence of 10^{-6} M U0126 (Figure 2). These results again corroborated with our observation that U0126 inhibits UV irradiation-induced apoptotic cell death in CHO cells, as visualized by methods such as Annexin-V/PI staining, Western Blot detection of Caspase-3 activation as well as JC-1 dye monitoring of the change in mitochondrial membrane potential.

Conclusions

Using UV-B treatment to induce apoptosis in CHO cell culture, we have found that caspase-3/7 activities as determined by the Caspase-Glo™ 3/7 Assay are consistent with our previous findings using other methodologies. Since the Caspase-Glo™ 3/7 Assay is extremely quick and easy to use, it is well suited to be a high-throughput assay for determining caspase-3/7 activities.

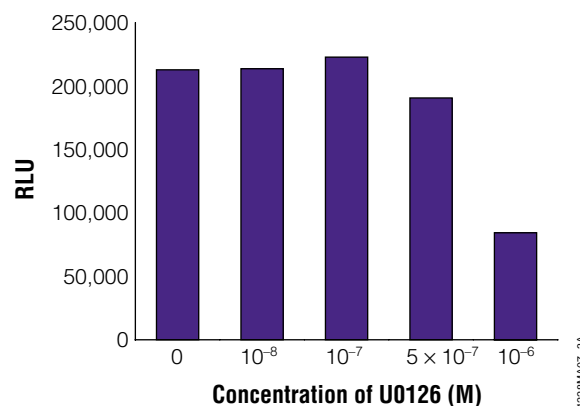


Figure 2. Protective effect of MAP kinase kinase inhibitor U0126 on UV-B-induced caspase-3/7 activation in CHO cells. The cells were treated as described for Figure 1 with the exception that the wells of serum-starved CHO cells were treated with 10^{-8} M, 10^{-7} M, 5×10^{-7} M and 10^{-6} M MAP kinase kinase inhibitor U0126 (Tocris, Ellisville, MO) 60 minutes prior to UV irradiation. The UV treatment lasted for 1 minute. Specific caspase-3/7 activity was then determined 6 hours later using the Caspase-Glo™ 3/7 Assay as described in Figure 1.

References

1. Karvinen, J. *et al.* (2002) *J. Biomol. Screen.* **7**, 233–231.
2. Preaudat, M. *et al.* (2002) *J. Biomol. Screen.* **7**, 267–274.
3. Gopala-Krishnan, S.M. *et al.* (2002) *J. Biomol. Screen.* **7**, 317–323.
4. O'Brien, M. *et al.* (2003) *Cell Notes* **6**, 13–15.

Protocol

- ◆ *Caspase-Glo™ 3/7 Assay Technical Bulletin* #TB323, Promega Corporation. (www.promega.com/tbs/tb323/tb323.html)

Ordering Information

Product	Size	Cat.#
Caspase-Glo™ 3/7 Assay ^(a,b)	2.5ml	G8090
	10ml	G8091
	100ml	G8092
CytoTox 96® Non-Radioactive Cytotoxicity Assay*	1,000 assays	G1780
Caspase Inhibitor Z-VAD-FMK	50µl	G7231
	125µl	G7232
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200–800 assays	G7890
	1,000–4,000 assays	G7891
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	1,000–4,000 assays	G7892
MEK Inhibitor U0126	5mg (5×1 mg)	V1121

* For Laboratory Use.

^(a) Patent Pending.

^(b) 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. If any product contains recombinant *Coleoptera* luciferase nucleic acid capable of producing light when expressed, a license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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