Q&A

technically speaking

Cell Viability Assays

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Abstract

Promega offers many tools for measuring apoptosis in cells, tissues and lysates. The Apo-ONE™ Homogeneous Caspase-3/7 Assay provides an accurate means of detecting apoptotic activity, while the CellTiter-Glo™ Luminescent Cell Viability Assay allows rapid, scalable determination of cell viability. This article examines these tools in the context of Promega's complete line of cell research products, including the CellTiter 96® Assays and CytoTox 96® Non-Radioactive Cytotoxicity Assay.

For more information on the Apo-ONE™ Homogeneous Caspase-3/7 Assay and the CellTiter-Glo™ Luminescent Cell Viability Assay, see the articles on pages 2 and 6 in this issue of *Promega Notes*.

Q What different types of cell death might cells undergo?

A number of mechanisms by which eukaryotic cells die have been described in the literature (1,2). Apoptosis, or "programmed cell death," may be triggered by several factors including genetic programming and receptor-mediated activation. These triggers result in the activation of specific molecular cascades involving a family of proteases known as caspases (cysteine-aspartic acid specific proteases; 2). Changes in cell structure and organization have been documented that include cell blebbing and chromosomal DNA fragmentation. The latter, a feature of the final stages of the apoptotic cascade, generates nucleosomal fragments in multiples of 200bp that can be visualized as a "ladder" on an agarose gel.

A second type of cell death, called necrosis, is an energy-independent process that is characterized by the break up of the cell membrane and subsequent ionic imbalances within the cell. A localized inflammatory response is observed in necrotic tissue in vivo. At the molecular level, activated ATPases and DNase play an important role in the incipient death of the cell. While chromosomal DNA fragmentation occurs in necrotic cells, this fragmentation produces a general smear, unlike the sized fragments observed in apoptosis.

Q What is the Apo-ONE™ Assay? How can I use it in my apoptosis detection experiments?

The Apo-ONE™ Homogeneous Caspase-3/7 Assay[a] (Cat.# G7790) is an easy-to-use system for the measurement of both caspase-3 and caspase-7 activities (3,4). The kit is comprised of two components: the Caspase Substrate Z-DEVD-R110 and the Apo-ONE™ Homogeneous Caspase-3/7 Buffer. These two components are mixed into a single, convenient, homogeneous caspase reagent that can be added directly to samples.

The substrate carries a rhodamine 110 group (excitation at 499nm; emission at 521nm) flanked by consensus peptides. Upon release from the substrate, the rhodamine 110 produces a fluorescent signal that is proportional to the caspase-3/7 cleavage activity. Protocols are provided in the Technical Bulletin (#TB295) for using the Apo-ONE™ Assay directly on cell cultures in a 96-well format or on purified caspase preparations. Volumes can be adjusted to suit other plate formats, provided that a 1:1 ratio of homogeneous reagent to sample is maintained.

Q What is the principle behind the CellTiter-Glo™ Luminescent Cell Viability Assay?

The CellTiter-Glo™ Luminescent Cell Viability Assay[b] (Cat.# G7570) is a convenient, fast and effective system for analyzing cell viability based on the amount of ATP produced by cells in culture. ATP levels are rapidly depleted in dead or dying cells, so the assay is specific for metabolically active cells (5,6). The assay components—the CellTiter-Glo™ Buffer and the CellTiter-Glo™ Substrate—are mixed into a single CellTiter-Glo™ Reagent that can be added directly to serum-supplemented cell cultures.

The chemistry of the CellTiter-Glo™ Assay is based on the firefly luciferase reaction in which beetle luciferin is converted to luciferin oxide in the presence of ATP, magnesium and oxygen. Data can be obtained in as little as ten minutes after the addition of the reagent to the culture. Assay sensitivity is higher than that of conventional colorimetric cell proliferation detection systems. We have also shown that, for the CellTiter-Glo™ Assay, linearity is maintained over a large range of cell numbers (e.g., for Jurkat cells, linearity is maintained from 0 to 50,000 cells per

well). The prepared CellTiter-Glo™ Reagent contains ATPase inhibitors that prevent the breakdown of ATP from viable cells after lysis.

Q Can I use the CellTiter-Glo™ Assay for processing large numbers of plates?

Yes. The two-component format of the CellTiter-Glo™ Assay lends itself well to processing of large numbers of plates. Moreover, the possibility of pipetting errors, a potential problem in many multiple-component cell proliferation assays, is significantly reduced. In addition, the luciferase reaction generates a “glow-type” luminescent signal that has a half-life of approximately 5 hours, thereby allowing analysis of a large number of plates without a concomitant loss of signal. These attributes make the assay ideally suited to automation on many robotic platforms.

Q I am considering both the CellTiter-Glo™ Assay and the CellTiter 96® AQ_{ueous} One Solution Assay. Which would be most suitable for my experiments?

The CellTiter-Glo™ Luminescent Cell Viability Assay allows data collection in as little as 10 minutes after the addition of the CellTiter-Glo™ Reagent to the cell cultures. This contrasts with the 1- to 4-hour incubation required for the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay[(c)] (Cat.# G5421). Such fast data collection is of primary importance in investigations that require processing of large numbers of plates. In addition, the increased sensitivity of the CellTiter-Glo™ Assay is a tremendous advantage when dealing with small numbers of cells. For example, 50 Jurkat cells generate a luminescent signal that is three standard deviations higher than background. However, only the CellTiter 96® AQ_{ueous} One Solution Assay provides the option of returning a plate to 37°C for further color development if required.

Q How do the CytoTox 96® and CellTiter 96® Assays compare to radiolabeling methods such as $[^3H]$-thymidine and ^{51}Cr?

Comparative studies performed using both the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Cat.# G4000) and $[^3H]$-thymidine assays have shown significant correlation between the formazan absorbance measurements of the CellTiter 96® Assays and the radioactivity measurements of $[^3H]$-thymidine incorporation assays. Likewise, experiments comparing the CytoTox 96® Non-Radioactive Cytotoxicity Assay and ^{51}Cr release have shown similar correlations.

Q What instrumentation is required for each of the cell proliferation and apoptosis assays?

As indicated above, the CellTiter-Glo™ Assay uses firefly luciferase to generate a luminescent signal based on the amount of ATP present in the samples analyzed. A luminometer is therefore required for measurement of luciferase activity and subsequent establishment of ATP levels. Injector-based luminometers are not recommended given the length of time for lysis. The Apo-ONE™ Homogeneous Caspase-3/7 Assay requires a fluorometer. The CellTiter 96® AQ_{ueous} One Solution Assay and the CytoTox 96® Non-Radioactive Cytotoxicity Assay require a spectrophotometer that can measure absorbance at wavelengths ranging from 490nm to 570nm.

References

1. Sperandio, S., de Belle, I. and Bredesen, D. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 14376–14381.
2. Villa, P., Kaufmann, S.H. and Earnshaw, W.C. (1997) *TIBS* **22**, 388–392.
3. Niles, A. and Humpal-Winter, J. (2001) *Cell Notes* **2**, 2–3.
4. *Apo-ONE™ Homogeneous Caspase-3/7 Assay Technical Bulletin*, #TB295, Promega Corporation.
5. Hannah, R. *et al.* (2001). *Cell Notes* **2**, 11–13
6. *CellTiter-Glo™ Luminescent Cell Viability Assay Technical Bulletin*, #TB288, Promega Corporation.

[(a)] 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.

[(b)] Patent Pending.

[(c)] 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.

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Technical Questions?
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