Automated ApoTox-Glo Assay to Simultaneously Assess Cell Viability, Cytotoxicity and Apoptosis

Sarah Shultz1, Andrew Niles1, Jason Greene2, Gaby Neumann2 and Peter Banks2
1 Promega Corporation, 2600 Woods Hollow Road, Madison, Wisconsin 53711 USA
2 BioTek Instruments, Inc., Highland Park, PO Box 998, Winooski, Vermont 05404 USA

Abstract

Cell-based assays are useful tools to investigate in vitro responses to a variety of target compounds and cell-signaling molecules. Paramount measures are often desirable, particularly in secondary screening, to generate more biologically relevant data. The evaluation of multiple parameters can become quite labor intensive and time consuming if conducted in parallel, so the use of simple multiplex assays executed with automation help to improve efficiency and reduce operator involvement.

Here, we introduce an automated ApoTox-Glo cell-based assay application for simultaneously measuring three parameters: cell-viability, cytotoxicity, and apoptosis. The method combines two fluorescent and luminescent assay chemistries offered by Promega (Caspeir-Glo®-3/7 and MultiTox-Fluor™-Assay) in the same assay well to extract information about viability, cytotoxicity and caspase activation events. These parameters are particularly useful to define the mechanisms associated with a cytotoxic profile. All dispensing steps are automated and performed using the BioTek Precision Microplate Pipetting System. Fluorescence and luminescence measurements are performed using the BioTek Synergy™2 Multi-Mode Microplate Reader.

Lab Automation Instrumentation

Figure 1. The BioTek Synergy™ 2 is a high-performance multi-mode microplate reader.

Figure 2. The BioTek Precision™ XS Automated Sample Processor is a precise liquid handling system used for dispensing steps and is easily programmed to implement the ApoTox-Glo assay. This compact robotic instrument combines a single-channel sample processing head, an eight-channel pipetting head, and an eight-channel bulk reagent dispenser to control all liquid handling steps of the assay.

Figure 3. The BioTek Synergy™ 2 Multi-Mode Microplate Reader provides detection capability for all three assays.

Fluorescent and Luminescent ApoTox-Ldo Assay

Apoptosis is a process by which cells are eliminated through programmed cell death. This process is important for maintaining normal tissue structure and function and is controlled by both environmental and intrinsic signals. Apoptosis is characterized by the execution of two key events: the activation of caspases and the exposure of phosphatidylserine on the outer surface of the plasma membrane. The activation of caspases and phosphatidylserine exposure are two sensitive markers of apoptosis.

The ApoTox-Glo assay is comprised of the Promega MultiTox-Fluor® Assay and Caspase-Glo®-3/7 Assay. The MultiTox-Fluor™ Assay is a non-radioactive assay that measures the viability of live and dead cells in a single well sample. For live cell assessment, live-cell protease activity is measured by the fluorogenic, cell-permeable substrate Gly-Pro-7-amino-4-methylcoumarin (Gly-Pro-AMC) (S3F-APC). For this live-cell protease activity marker labels only live cells because it is cleaved only in the presence of membrane integrity and leakage into the surrounding culture medium, and thus does not contribute to the dead cell measurement. For dead cell assessment, a second protease activity marker, the cell-impermeant peptide substrate bovine (Bz-Ala-Ala-Pro-Val)-tritiated-110 (Bo-AA-PV)-3 is used to measure the activity of a dead-cell protease from cells that have lost membrane integrity and leaked the biomarker into the surrounding culture medium. The Caspase-Glo®-3/7 Assay is a luminescent assay that measures caspase-3 and -7 activities in cultures of cells, which are indicative of apoptosis. The assay is based on a luminescent, cell-permeable substrate, which is converted to a luminescent signal when cleaved. This substrate is then added in an additional 25µL volume and luminescence measured on the BioTek Synergy™ 2 Multi-Mode Microplate Reader.

ApoTox-Glo Assay Protocol

1. Dispense cells in culture medium into assay plates.
2. Serially dilute and dispense compounds across the assay plate and incubate for the desired test exposure period (i.e., 24 hours).
3. Dispense MultiTox-Fluor® Reagent to all wells of the assay plate, shake briefly, incubate at 37°C for 30 minutes, and measure fluorescence (live-cell fluor 400Ex/505Em; dead-cell fluor 485Ex/520Em).
4. Dispense Caspase-Glo®-3/7 Reagent to all wells of the assay plate, shake briefly to lyse cells, incubate at room temperature for 30 minutes, and measure luminescence.
5. Repeat steps 3-4 at each treatment time point.

ApoTox-Glo assay results

Figure 4. ApoTox-Glo assay of Hep G2 cells after 24 hour treatment with bortezomib (A) or staurosporine (B). Proteolytic biomarkers for cell viability and cytotoxicity were measured using the MultiTox-Fluor™ Assay. Caspase-3/7 activity was then measured in the same well using the Caspase-Glo®-3/7 Assay. As expected, both staurosporine and bortezomib compound treatments for 24 hours resulted in a dose-dependent decrease in viability, an increase in cytotoxicity, and an increase in caspase-3/7 activity consistent with apoptosis.

Hep G2 cells were plated in 384-well format at a density of 5,000 cells per 10µL volume of DMEM plus 10% fetal bovine serum and allowed to equilibrate for two hours. Staurosporine and bortezomib were towed serially diluted and added to wells in 10µL volumes. Plates were incubated at 37°C in 5% CO2 for 24 hours. MultiTox-Fluor® Reagent was prepared in 10µL of each substrate in 5:1, Assay Buffer, and S2, was used per well. The plate was mixed and incubated for 30 minutes at 37°C. Fluorescence measurements of viability and cytotoxicity were then read at two wavelengths on the BioTek Synergy™ 2 Multi-Mode Microplate Reader. Caspase-Glo®-3/7 Assay was then read in an additional 25µL volume and luminescence measured on the BioTek Synergy™ 2 Multi-Mode Microplate Reader after 30 minutes at room temperature.

Summary

Together Promega cell-based assays can be used with the BioTek Precision Microplate Pipetting System to provide a simple automated Triplex application to evaluate the effects of compounds on cell viability, cytotoxicity, and apoptosis over time.

Implementing this automated ApoTox-Glo assay provides a small-scale, cost-effective cell-based screening method particularly well suited for in vitro toxicology applications in academic labs, growing biotechnology companies, and for secondary screening environments.

Figure 5. The BioTek Synergy™ 2 Multi-Mode Microplate Reader provides detection capability for all three assays.

The Precision Power™ PC Software that accompanies the instrument makes stepping through the assay protocol very straightforward and easy. Specifically, a 5-step ApoTox-Glo assay protocol was created (described below).

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Together Promega cell-based assays can be used with the BioTek Precision Microplate Pipetting System to provide a simple automated Triplex application to evaluate the effects of compounds on cell viability, cytotoxicity, and apoptosis over time.