

Automating Promega Cell-Based Assays in Multiwell Formats

By Tracy Worzella, B.S., and Brad Larson, B.A., Promega Corporation

Abstract

In this article, we demonstrate the use of liquid handling workstations for performing cell-based assays. The feasibility of scale-up and minimal manipulation required to perform these assays makes them candidates for implementation on a variety of automated workstations. This article specifically describes the adaptation of Promega cell-based assays to the Beckman Coulter Biomek® 2000 and FX workstations in both 96- and 384-well formats. We have automated the Caspase-Glo™ 3/7 Assay, Apo-ONE® Homogeneous Caspase-3/7 Assay, CytoTox-ONE™ Homogeneous Membrane Integrity Assay, CellTiter-Glo® Luminescent Cell Viability Assay, and CellTiter-Blue™ Cell Viability Assay.

Promega's simple and scalable "add-mix-measure" cell viability and apoptosis assay formats make these assays ideal for multiwell plates and high-throughput screening on any workstation.

Introduction

Cell-based assays have become a quick, lower-cost means to test hit or lead compounds for toxic effects before proceeding with drug development. The term "cell-based assay" describes any procedure that uses live cells for the evaluation of effects on toxicity, viability, proliferation or other specific cellular processes following the addition of a test compound. Of particular interest in drug discovery is the ability to analyze ADME/Tox (Absorption, Distribution, Metabolism, Elimination/ Toxicity) properties for compounds of interest. Cell-based assays provide a platform in which these properties and processes can be tested.

The process of performing a cell-based assay generally includes plating cells, equilibrating to culture conditions, adding a test compound, and measuring output from treatment. These assays can be made easier by automating the process. Depending on the needs and financial resources of the user, a single step or multiple steps of the assay process can be adapted to an automated workstation. These workstations, whether they are liquid handlers or fully-integrated systems with incubators, shakers and plate readers, allow increased throughput for sample processing. Automation also removes the requirement for hands-on assay performance, resulting in less error and higher reproducibility.

We have incorporated and validated several Promega cell-based assays on the Beckman Coulter Biomek® 2000 and FX automated workstations in both 96- and 384-well formats to meet the varying needs of the automation user. Table 1 highlights the assays that we have automated and validated on the Biomek® platforms. Using these cell-based assay reagents, we have automated the final step in the assay process, allowing the measurement of output following treatment. The simple and scalable "add-mix-measure" cell viability and apoptosis assay formats make these assays ideal for multiwell plates and high-throughput screening on any workstation.

Table 1. Promega Automated Cell-Based Assays. The assays listed are validated on the Biomek® 2000 and FX platforms in both 96- and 384-well formats.

Assay	Type	Assay Measurement	Output
Caspase-Glo™ 3/7 Assay	Apoptosis	Caspase-3/7 Activity	Luminescent
Apo-ONE® Homogeneous Caspase-3/7 Assay	Apoptosis	Caspase-3/7 Activity	Fluorescent
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	Membrane Integrity	LDH Release	Fluorescent
CellTiter-Glo® Luminescent Cell Viability Assay	Cell Viability	ATP	Luminescent
CellTiter-Blue™ Cell Viability Assay	Cell Viability	Metabolic Capacity/ Resazurin Reduction	Fluorescent

Automated Method Development

Automated, single-plate methods were developed for five Promega cell-based assays in 96- and 384-well format on the Biomek® 2000 and FX workstations. Each method is a computer program that the robot will follow in order to perform the assay. These instructions encompass all the liquid handling steps, physical manipulations of each assay plate across the deck and operation of any devices on the deck.

To perform a cell-based assay on an automated workstation, the deck of the workstation is manually set up by adding labware, reagents, tip boxes and the sample plate. Once the method is started, the robot will perform all steps without the need for manual intervention. Due to the simplicity of the assays being automated, and the methods written for each assay, the number of deck positions required to perform each assay

Automating Promega Cell-Based Assays... continued

is minimal. Typically, only 3 to 4 deck positions are required for the single-plate Biomek® 2000 and FX methods (Figure 1). While this number may vary depending on the method, each automated assay will fit onto any deck layout that a robotic platform may have. This will also make it possible to create multiplate methods that are able to fit onto the original deck layout without the need for stacking capabilities.

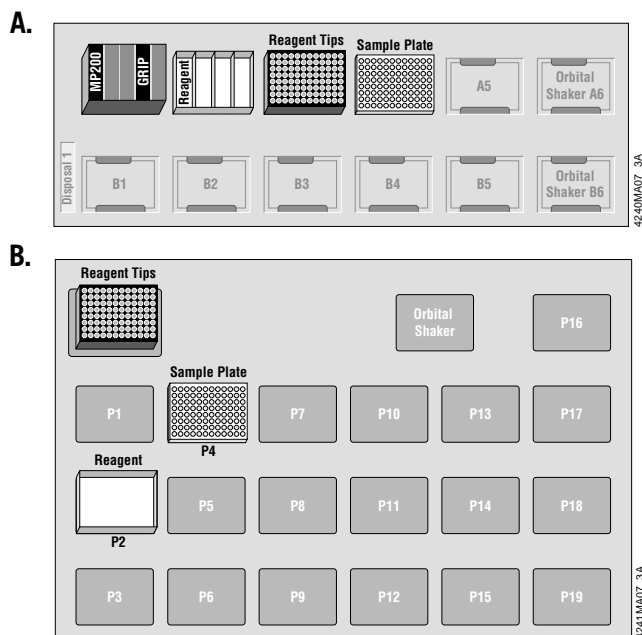


Figure 1. General deck configurations for cell-based assays on the Beckman Coulter Biomek® 2000 (Panel A) and Biomek® FX (Panel B). The deck configurations shown above are a general representation for cell-based assays on the Biomek® 2000 and FX. Additional labware, such as tip boxes, reagent reservoirs, or assay plates, will be required depending on the assay and the individual method being performed. An orbital shaker is required with each deck layout.

The automated cell-based assay methods we developed perform three steps on the robotic platform (Table 2). Additional steps, including assay plate incubations and recording data from fluorescent or luminescent assays, are performed offline (separate from the robotic platform). These steps can be automated, depending on the level of automation required on the robotic platform being used. This fact, along with the methods' simplicity, and minimal deck requirements, allows these assays to meet the needs of low-, medium-, or high-throughput research laboratories.

Validated methods to run these assays and other chemistries are available for download at www.promega.com/automethods/. In addition, the hardware, labware requirements and instructions are available as automated protocols and are available at www.promega.com/tbs/.

Table 2. General Method for Single-Plate Automated Cell-Based Assays.

1. Transfer volume of reagent prescribed in the Automated Protocol to the assay plate containing blank, control, cell culture, or purified enzyme suspension.
2. Transfer the assay plate to the orbital shaker on the robotic platform, and mix reagent and samples.
3. Transfer the assay plate back to its original position on the deck.

The assays include an incubation period, which varies with the assay. The CytoTox-ONE™ Assay includes an optional addition of Stop Solution at the conclusion of the method.

Validation and Results

Each single-plate method was validated according to the performance criteria for each assay. Validation of these methods provides confidence that the methods have been thoroughly tested and will provide consistent, reproducible results in any setting. Results indicate that these automated assays provide reproducible data that often equals or exceeds that achieved when performing the assays manually (data not shown). This also represents a time savings to researchers by allowing them to proceed directly to sample processing.

The criteria that we use for validation include the sensitivity of each assay, as well as linearity across a set range of cell or purified enzyme concentrations. Assay robustness and reproducibility are tested as well using Z'-factor determination (1).

Z'-factor is a statistical value used to determine an assay's robustness and reproducibility by comparing its dynamic range to the data variation. A Z'-factor value of 1.0 indicates a perfect assay. A Z'-factor value greater than 0.5 indicates excellent assay quality.

Table 3. Z'-Factor for Automated Cell-Based Assays Developed on the Biomek® 2000 and FX Platforms.

Automated Assay	Cell-Based Platform	Robotic Well Format	Assay Plate Z'-Factor
Apo-ONE®	Biomek® 2000	96	0.80
Homogeneous		384	0.58
Caspase-3/7 Assay	Biomek® FX	96	0.77
		384	0.76
CellTiter-Glo® Luminescent	Biomek® 2000	96	0.76
		384	0.61
Cell Viability Assay	Biomek® FX	96	0.80
		384	0.70
Caspase-Glo™ 3/7 Assay	Biomek® 2000	96	0.63
		384	0.62
	Biomek® FX	96	0.90
		384	0.84
CellTiter-Blue™	Biomek® 2000	96	0.88
Cell Viability Assay		384	0.70
	Biomek® FX	96	0.90
		384	0.77
CytoTox-ONE™	Biomek® 2000	96	0.56
Homogeneous		384	0.68
Membrane Integrity Assay	Biomek® FX	96	0.68
		384	0.61

The Z'-factor was determined for each automated assay. Analyses were completed in 96- and 384-well formats using the Biomek® 2000 and Biomek® FX platforms. The results from each analysis are shown in Table 3. All assays and formats had a Z'-factor greater than 0.5.

Summary

We have demonstrated the ability to automate Promega's cell-based assays in both 96- and 384-well formats. Z'-factors for each assay format were above 0.5, indicating that they were all excellent assays.

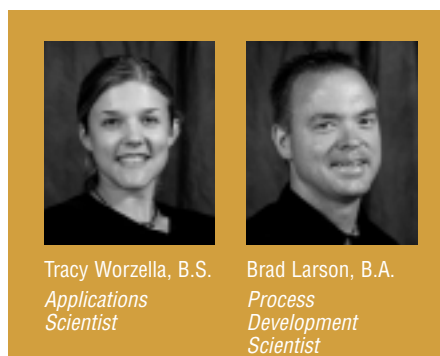
The minimal deck requirements of the automated methods for these cell-based assays make them flexible enough to meet the needs of low-, medium- or high-throughput laboratories.

Reference

- Zhang, J. *et al.* (1999) *J. Biomol. Screening* **4**, 67–73.

Protocols

- ◆ *Automated Apo-ONE® Homogeneous Caspase-3/7 Assay Protocol #EP012*, Promega Corporation.
(www.promega.com/tbs/ep012/ep012.html)
- ◆ *Automated CellTiter-Blue™ Cell Viability Assay Protocol #EP015*, Promega Corporation.
(www.promega.com/tbs/ep015/ep015.html)
- ◆ *Automated Caspase-Glo™ 3/7 Assay Protocol #EP017*, Promega Corporation.
(www.promega.com/tbs/ep017/ep017.html)



Ordering Info

Product	Size	Cat.#
Caspase-Glo™ 3/7 Assay ^(a,b)	2.5ml	G8090
	10ml	G8091
	100ml	*G8092
Apo-ONE® Homogeneous Caspase-3/7 Assay ^(c) (HTP)	1ml	G7792
	10ml	G7790
	100ml	*G7791
CytoTox-ONE™ Homogeneous Membrane Integrity Assay ^(a)	200–800	*G7890
	1,000–4,000	*G7891
CellTiter-Glo® Luminescent Cell Viability Assay ^(a,b)	10 × 100ml	*G7573
	100ml	*G7572
	10 × 10ml	*G7571
	10ml	G7570
CellTiter-Blue™ Cell Viability Assay	20ml	G8080
	100ml	*G8081
	10 × 100ml	*G8082

*Kit size appropriate for automation.

^(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.

^(c) 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.

Apo-ONE and *CellTiter-Glo* are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. *Caspase-Glo*, *CellTiter-Blue* and *CytoTox-ONE* are trademarks of Promega Corporation.

Biomek is a registered trademark of Beckman Coulter, Inc.