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## OVERVIEW

We have demonstrated the scalability of the CellTiter-Glo® and Caspase-Glo™ 3/7 assays for high-throughput cell-based screening in low-volume 384- and 1536-well formats. The Deerac Fluidics Equator™ NS 808 Eight Tip Low Volume Dispenser was used for non-contact delivery of sample and reagent. The multifunctional, PMT-based BMG LABTECH PHERAstar reader was used for signal detection. Excellent Z'-factor, %CV, and linear range data was obtained. Together, these scalable assays and instrumentation offer ideal tools for HTS users studying drug effects on cells during the drug discovery process.

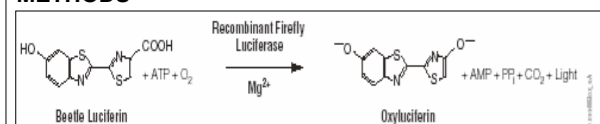
## INTRODUCTION

Using cells in the drug discovery process enables the screening of drug effects within the context of the cellular biological environment, allowing toxic compounds to be detected earlier and prevented from further advancement down the development pathway. As high-throughput screening evolves, the use of higher density, lower volume cell-based assays will become commonplace in many screening facilities. As a result, simple solutions will be needed for performing these assays in miniaturized formats such as low-volume 384 (LV384) and 1536. Such formats will require robust assays with simple "add-mix-measure" formats, but also deliver consistent results in single microliter volumes.

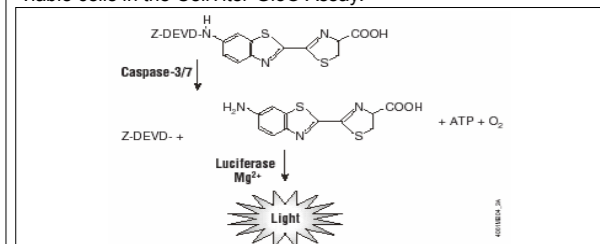
Here we demonstrate the capability of Promega's cell-based assays to meet the demands of automated high-throughput users. Assays were performed using the CellTiter-Glo® Luminescent Cell Viability Assay, a cell viability assay based on the quantitation of ATP in metabolically active cells, and the Caspase-Glo™ 3/7 Assay, a caspase assay that measures the activity of caspase-3 and -7. These assays were performed in LV384- and 1536-well formats. Cells and reagents were dispensed using the Deerac Fluidics Equator™ NS 808 Eight Tip Low Volume Dispenser, in volumes ranging from 20µl to 500nl. The BMG LABTECH PHERAstar, a PMT-based microplate reader, was used to measure luminescence in these high density plate formats.

We generated Z'-factor, %CV, linearity and limit of detection data to demonstrate the precision and sensitivity of these cell-based assays for automated high-throughput applications. These robust assays, combined with a high-precision pipetting system and sensitive detection instrumentation, enable the user to miniaturize their cell-based screening assays.

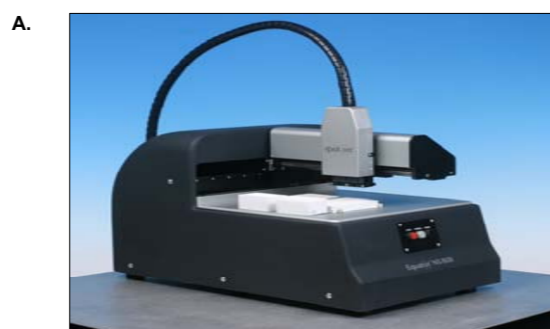
## METHODS



**Figure 1. The luciferase reaction.** For the CellTiter-Glo® and Caspase-Glo™ 3/7 assays, mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg<sup>2+</sup>, ATP and molecular oxygen. Light signal is proportional to the number of viable cells in the CellTiter-Glo® Assay.

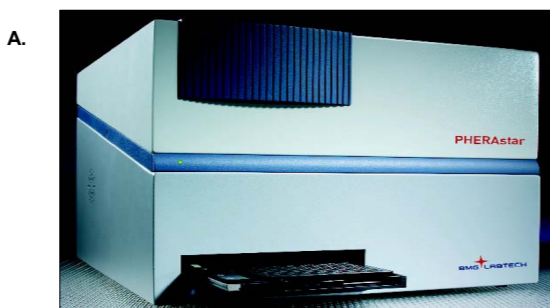


**Figure 2. Cleavage of the proluminescent substrate containing the DEVD sequence in the Caspase-Glo™ 3/7 Assay.** Following cleavage by caspase-3 and -7, a substrate for luciferase (aminoluciferin) is released, resulting in the luciferase reaction and the production of light. Light signal correlates with caspase -3 and caspase -7 activity.



- B.**
- spot-on™ proprietary dispensing technology
  - Fixed-tip non-contact reagent dispensing from 50nl to 20µl
  - Two-plate deck positions for 96, 384, or 1536-well plates
  - Speed: 200nl dispense into 1536-well plate in 15 seconds
  - On-the-fly and stepped dispensing capabilities
  - CVs less than 10%
  - Passive, active, or backwash station
  - Precision xyz positioning
  - Small footprint
  - User-friendly programming software

**Figure 3. Deerac Fluidics' hardware configuration.** A) The Deerac Fluidics Equator™ NS 808 Eight Tip Low Volume Dispenser. B) Features of the Equator™ system that were useful for this application. Additional technical details can be obtained from Deerac Fluidics.



- B.**
- Multifunctional – FI, FP, TRF, luminescence, absorbance
  - Sequential dual excitation
  - Simultaneous dual emission measurements
  - Automated Z-focus focal height adjustment
  - Reading capabilities for 6- to 1536-well plates
  - Uses two matched pairs of photomultiplier tubes for detection
  - User-friendly software for creating test set-ups and changing detection modes
  - Small footprint

**Figure 4. BMG LABTECH reader configuration.** A) The BMG LABTECH PHERAstar. B) Features of the PHERAstar that were useful for this application. Additional technical details can be obtained from BMG LABTECH.

## RESULTS

Chemistry	Assay Format	Assay Volume	Z'-Factor Score
CellTiter-Glo® Assay	LV384	20µl	0.85
		10µl	0.77
		5µl	0.68
		8µl	0.84
		5µl	0.85
	1536	8µl	0.82
		5µl	0.85
		2µl	0.82
		1µl	0.70
		1µl	0.62
Caspase-Glo™ 3/7 Assay	LV384	20µl	0.83
		10µl	0.78
		5µl	0.76
		8µl	0.77
		5µl	0.79
	1536	8µl	0.71
		5µl	0.79
		2µl	0.71
		1µl	0.62
		1µl	0.62

**Table 1. Z'-factor values in low-volume 384 and 1536-well formats for the CellTiter-Glo® and Caspase-Glo™ 3/7 Assays.** Z'-factor is a statistical calculation used to assess the robustness and precision of an assay<sup>1</sup>. Z'-factor scores between 0.5 and 1 indicate an excellent screening assay. All volumes tested for the CellTiter-Glo® and Caspase-Glo™ 3/7 Assays had Z'-factor scores greater than 0.5, indicating that they were all excellent assays.

The CellTiter-Glo® Z'-factor assays were performed by plating Jurkat cells and treating one-half of the plate with 10µM staurosporine for four hours, with the remaining half receiving no treatment. The CellTiter-Glo® Reagent was then added and light units were recorded.

The Caspase-Glo™ Z'-factor assays were performed by treating one-half of a plate of Jurkat cells with anti-FAS antibody for four hours, with the remaining half receiving no treatment. The Caspase-Glo™ reagent was then added and light units were recorded.

Chemistry	Assay Format	Assay Volume	Cell Number (per well)	%C.V.
CellTiter-Glo™ Assay	LV384	20µl	5,000	2.23
		10µl	2,500	3.21
		5µl	1,250	2.71
		2µl	500	2.53
		1µl	250	3.90
	1536	8µl	2,000	2.42
		5µl	1,250	2.73
		2µl	500	3.90
		1µl	250	3.26
		0.5µl	125	5.12
Caspase-Glo™ 3/7 Assay	LV384	20µl	5,000	4.98
		10µl	2,500	2.82
		5µl	1,250	8.95
		2µl	500	9.72
		1µl	250	6.12
	1536	8µl	2,000	5.27
		5µl	1,250	5.07
		2µl	500	7.78
		1µl	250	6.12
		0.5µl	125	8.53

**Table 2. Percent CV values for the CellTiter-Glo® and Caspase-Glo™ 3/7 Assays.** %CVs for all assays performed in LV384- and 1536-well formats were less than 10% in volumes as low as 500nl.

For the CellTiter-Glo® Assay, %CV data was obtained by plating a serial dilution of Jurkat or d293 cells, adding CellTiter-Glo® Reagent, then recording light units. For the Caspase-Glo™ 3/7 Assay, the cells were plated, induced with anti-FAS antibody or Triton®-X 100, followed by Caspase-Glo™ 3/7 Reagent addition. The %CV values listed here were calculated from the maximum cell number used in each titration series.

Assay Format	Assay Volume	Chemistry	Cell Range Tested (Cells/Well)	Cell Strain	Linearity (R <sup>2</sup> value)	Limit of Detection (Cells/Well)	
LV384	20µl	CellTiter-Glo® Assay	0-5,000	Jurkat	0.9987	10	
		Caspase-Glo™ 3/7 Assay	0-10,000	Jurkat	0.9944	156	
		CellTiter-Glo® Assay	0-2,500	Jurkat	0.9994	1	
		Caspase-Glo™ 3/7 Assay	0-5,000	Jurkat	0.9997	78	
		CellTiter-Glo® Assay	0-2,500	Jurkat	0.997	1	
	1536	8µl	Caspase-Glo™ 3/7 Assay	0-2,500	Jurkat	0.9975	78
			CellTiter-Glo® Assay	0-1,000	Jurkat	0.9951	1
			Caspase-Glo™ 3/7 Assay	63-1,000	Jurkat	0.9973	125
			CellTiter-Glo® Assay	0-4,000	Jurkat	0.9974	1
			Caspase-Glo™ 3/7 Assay	0-2,000	d293	0.9956	125
LV384	5µl	Caspase-Glo™ 3/7 Assay	0-4,000	Jurkat	0.9999	250	
		CellTiter-Glo® Assay	0-1,000	d293	0.9979	63	
		CellTiter-Glo® Assay	0-2,500	Jurkat	0.996	1	
		Caspase-Glo™ 3/7 Assay	0-1,250	d293	0.9954	78	
		Caspase-Glo™ 3/7 Assay	0-2,500	Jurkat	0.9996	156	
	1536	2µl	CellTiter-Glo® Assay	0-625	d293	0.9986	39
			CellTiter-Glo® Assay	0-1,000	Jurkat	0.993	1
			Caspase-Glo™ 3/7 Assay	0-500	d293	0.9942	31
			Caspase-Glo™ 3/7 Assay	0-1,000	Jurkat	0.9998	63
			CellTiter-Glo® Assay	1-250	d293	0.9934	16
LV384	1µl	CellTiter-Glo® Assay	0-500	Jurkat	0.9893	1	
		Caspase-Glo™ 3/7 Assay	0-250	d293	0.9903	16	
		Caspase-Glo™ 3/7 Assay	0-500	Jurkat	0.9991	31	
		CellTiter-Glo® Assay	4-125	d293	0.998	31	
		CellTiter-Glo® Assay	0-250	Jurkat	0.9901	1	
	1536	0.5µl	CellTiter-Glo® Assay	0-250	Jurkat	0.9981	31
			Caspase-Glo™ 3/7 Assay	0-250	Jurkat	0.9981	31

**Table 3. Linearity and limit of detection for LV384- and 1536-Well formats for CellTiter-Glo® and Caspase-Glo™ 3/7 Assays.** For the CellTiter-Glo® Assay, a serial dilution of Jurkat cells or d293 cells was plated, followed by addition of CellTiter-Glo® Reagent. For the Caspase-Glo™ 3/7 Assay, cells were serially diluted, plated, and apoptosis was induced in Jurkat cells as described in the text for Table 1. d293 cells were treated in 10% Triton® X-100 for one-hour before adding Caspase-Glo™ Reagent.

Regardless of the volume tested, the R<sup>2</sup> values were all greater than 0.99, indicating excellent linearity. The limit of detection for a majority of the assay volumes tested was less than 100 cells per well.

## CONCLUSIONS

1. The "add-mix-measure" format of the CellTiter-Glo® and Caspase-Glo™ Assays makes these chemistries highly amenable to automation and miniaturization.
2. Promega's CellTiter-Glo® and Caspase-Glo™ Assays have been successfully miniaturized as demonstrated by excellent Z'-factor, percent CV, linearity and limit of detection data.
3. The Deerac Fluidics Equator™ platform is a flexible and easy to use non-contact nanoliter dispensing system for cell dispensing and performing low volume screening assays.
4. The BMG LABTECH PHERAstar plate reader has sensitive reading capabilities for high density plates that are commonly used for HTS applications, all at a cost that is less than CCD-based detection.
5. The combination of Promega's cell-based assays, Deerac Fluidics' Equator™, and BMG LABTECH's PHERAstar plate reader offers a solution for the high-throughput automation user looking to miniaturize their cell-based screening assays.

## Reference

1. Zhang, J. et al. (1999) J. Biomol. Screening 4, p. 67-73.

## Protocols

Caspase-Glo™ 3/7 Assay Technical Bulletin #TB323  
[www.promega.com/tbs/tb323/tb323.html](http://www.promega.com/tbs/tb323/tb323.html)

CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin #TB288  
[www.promega.com/tbs/tb288/tb288.html](http://www.promega.com/tbs/tb288/tb288.html)