

Dave Smith¹, Joby Jenkins¹, Brad Larson, B.A.², Tracy Worzella, M.S.²
¹TTP LabTech Limited, Cambridge, UK, ²Promega Corporation, Madison, WI

OVERVIEW

Here we demonstrate the ability to miniaturize the CellTiter-Glo® and Caspase-Glo® 3/7 assays for high-throughput cell-based screening in low-volume 384- and 1536-well formats. The TTP LabTech mosquito™ liquid handling system was used for delivery of cells and reagent. The linear range, %CV, and Z'-factor data obtained, demonstrate how these scalable assays and instrumentation offer ideal tools for HTS users studying drug effects on cells during the drug discovery process.

INTRODUCTION

There is growing interest in using whole cell assays for screening. By using a high-density well format, the quantities of expensive compounds can be substantially reduced, having a major impact on the cost of a screening campaign. Successful miniaturization of cell-based assays in low-volume, high-density plate formats requires a robust chemistry, as well as accurate liquid handling capabilities. The CellTiter-Glo® Luminescent Cell Viability Assay is a homogeneous "add-and-read" method for measuring ATP levels in metabolically active cells. The homogeneous Caspase-Glo® 3/7 Luminescent Assay measures the release of caspases 3 and 7, markers of impending cell death. We have developed automated CellTiter-Glo® and Caspase-Glo® 3/7 methods in low-volume 384 & 1536-well plate formats using TTP LabTech's mosquito™ liquid handling system. The mosquito™ liquid handling system offers flexible, accurate and disposable tip-based positive displacement pipetting at volumes down to 50nL. This system gives researchers the capability of dispensing viable cells into low-volume high density well formats. Mosquito™ can also be further integrated for hands-free automation.

We demonstrate the ability of the TTP LabTech mosquito™ liquid handling system to dispense viable cells, and reagent in low-volume 384 and 1536-well format. CVs from plates tested compare favorably to cells dispensed manually in 96-well format, as well as to cells robotically dispensed in 384-well format. Z'-Factor scores show that these are excellent assays, even in miniaturized format.

METHODS

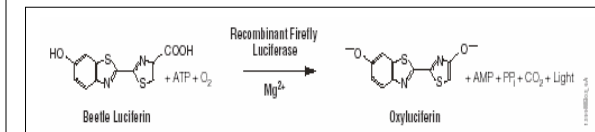


Figure 1. The luciferase reaction. For the CellTiter-Glo® and Caspase-Glo® 3/7 assays, mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg²⁺, ATP and molecular oxygen. Light signal is proportional to the number of viable cells in the CellTiter-Glo® Assay.

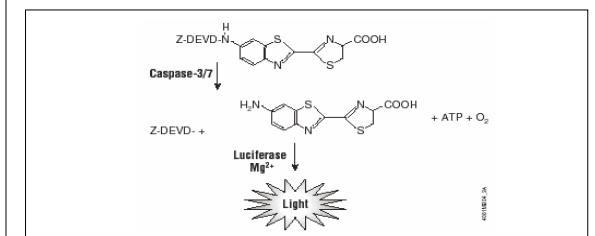


Figure 2. Cleavage of the pro-luminescent substrate containing the DEVD sequence in the Caspase-Glo® 3/7 Assay. Following cleavage by caspase-3 and -7, a substrate for luciferase (aminoluciferin) is released, resulting in the luciferase reaction and the production of light. Light signal correlates with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

A.

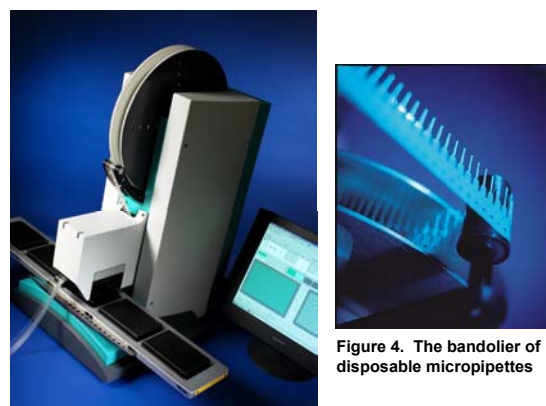


Figure 3. The mosquito™ liquid handling system

Figure 4. The bandolier of disposable micropipettes

B.

- high accuracy and precision at low volumes (from 1.2µL to 50nL)
- zero cross-contamination with no wash cycles through use of disposable pipettes
- ability to handle a wide range of fluid properties
- small footprint for benchtop use
- automation and ease of use
- disposable bandolier of miniature syringe-like micropipettes. Each bandolier containing 36,000 micropipettes at 4.5mm pitch, or 26,000 at 9mm pitch

TTP LabTech's mosquito™ hardware configuration. A) The TTP LabTech mosquito™ liquid handling system and disposable micropipette bandolier. B) Features of the mosquito™ that were useful for this application. Additional technical details can be obtained from TTP LabTech.

RESULTS

Chemistry	Assay Format	Assay Volume	Cell Conc.	% CV
CellTiter-Glo®	LV384	2.4µl	1000	2.93%
	1536	2.4µl	1000	5.09%
Caspase-Glo® 3/7	LV384	2.4µl	1000	4.70%
	1536	2.4µl	1000	4.35%

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 2.4µl.

For the CellTiter-Glo® Assay, %CV data was obtained by dispensing media and Jurkat cells at various volumes across a 384 or 1536-well plate, adding CellTiter-Glo® Reagent, then recording light units.

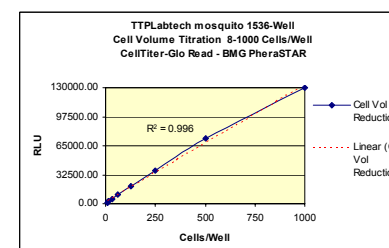
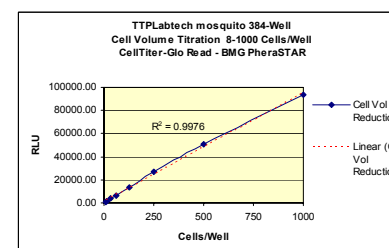
For the Caspase-Glo® 3/7 Assay, the cells were induced with anti-FAS antibody, dispensed across a 384 or 1536-well plate, followed by Caspase-Glo® 3/7 Reagent addition.

The %CV values listed here were calculated from the maximum cell number used in each titration series.

A.

Chemistry	Assay Format	Assay Volume	Tested (Cells/Well)	Cell Strain	Linearity R ² Value	Detection (Cells/Well)
CellTiter-Glo®	LV384	2.4µl	8-1000	Jurkat	0.9976	8
	1536	2.4µl	8-1000	Jurkat	0.996	8

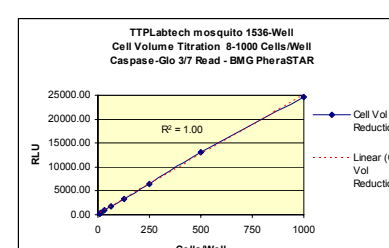
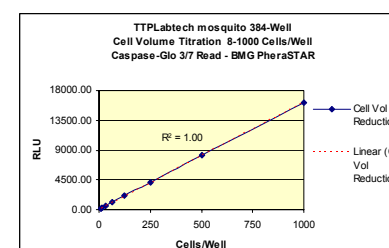
B.



C.

Chemistry	Assay Format	Assay Volume	Tested (Cells/Well)	Cell Strain	Linearity R ² Value	Detection (Cells/Well)
Caspase-Glo® 3/7	LV384	2.4µl	8-1000	Jurkat	1	16
	1536	2.4µl	8-1000	Jurkat	1	16

D.



Linearity and limit of detection for LV384- and 1536-Well formats for CellTiter-Glo® and Caspase-Glo® 3/7 Assays.

A & B: For the CellTiter-Glo® Assay, volumes of media and Jurkat cells were dispensed to create concentrations ranging from 1000 to 0 cells/well, in a total volume of 1.2µl. CellTiter-Glo® Reagent was then added to the plate.

C & D: For the Caspase-Glo® 3/7 Assay, Jurkat cells were treated with anti-FAS antibody for five hours. Media and cells were then dispensed using the same pattern as described above. Caspase-Glo® Reagent was then added to the plate.

Regardless of the volume tested, the R² 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 50 cells per well.

Chemistry	Well Format	Assay Volume	Z'-Factor Score
CellTiter-Glo®	LV384	2µl	0.76
		1µl	0.73
	1536	2µl	0.76
		1µl	0.68
Caspase-Glo® 3/7	LV384	2µl	0.74
		1µl	0.63
	1536	2µl	0.73
		1µl	0.63

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 assay¹. Z'-factor scores between 0.5 and 1 indicate an excellent screening assay. All volumes and well formats 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 10% Triton®-X 100 for one hour, 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 five hours, with the remaining half receiving no treatment. The Caspase-Glo® reagent was then added and light units were recorded.

CONCLUSIONS

1. The "add-mix-measure" format of the CellTiter-Glo® and Caspase-Glo® 3/7 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 TTP LabTech mosquito™ liquid handling system is a flexible and easy to use positive displacement nanoliter dispensing system for cell dispensing and performing low volume screening assays.
4. The programming software is straightforward, and easy to follow. Adjustments to steps within a protocol are simple making the creation of new or modified protocols a streamlined process.
5. The combination of Promega's cell-based assays, and the TTP LabTech mosquito™ liquid handling system offer a simple and reliable solution for those looking to automate and 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)

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