Measuring Cell Health and Viability Sequentially by Same-Well Multiplexing Using the GloMax®-Multi Detection System

ABSTRACT Multiplexing, the execution of multiple assays sequentially, and in a single multiwell plate well, enables greater experimental understanding and data accuracy, as well as saving time and money. Here we highlight several applications of Promega multiplex-capable cell-based assays and the GloMax®-Multi Detection instrument.

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INTRODUCTION

As the cost of conducting biological research and obtaining more biologically relevant information increases, so too does the demand for cost-effective assays and reagents. Promega meets the challenge of rising research costs by providing cost-effective solutions. We have used our expertise in bioluminescence and cell-based assay design to develop multiplex-capable cell-based assays that save money, time and valuable samples, while providing more valuable information per well. We also offer versatile and sensitive detection equipment, such as the GloMax®-Multi Detection System (Cat.# E7031), which can be used to measure fluorescence and luminescence signals from our assays. We have shown that the GloMax®-Multi Detection System is optimal for detection of fluorescent and luminescent cell-based signals over a broad dynamic range (1,2).

Cell viability and cytotoxicity data can vary as a function of stimuli and growth conditions. Promega offers a variety of assays for collecting these data (Figure 1). Researchers rely on parameters such as viability, apoptosis, cytotoxicity (necrosis) and reporter activity as indicators of cell health in order to gain accurate biological information. By integrating or multiplexing assays for several of these parameters more information can be obtained from a single experiment.

Many Promega assays are offered in a homogeneous format, meaning that they can be performed in cell culture wells without first removing medium or washing cells. A homogeneous format allows researchers to multiplex assays, combining and measuring fluorescence and luminescence in a single well of a multiwell plate. For example, one might perform a fluorescent assay to measure cell viability followed by a luminescent caspase activity assay to measure apoptosis in the same plate well.

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To demonstrate the multiplex capabilities of Promega cell-based assays and the versatility and sensitivity of the GloMax®-Multi Detection System instrument, we multiplexed several fluorescent and

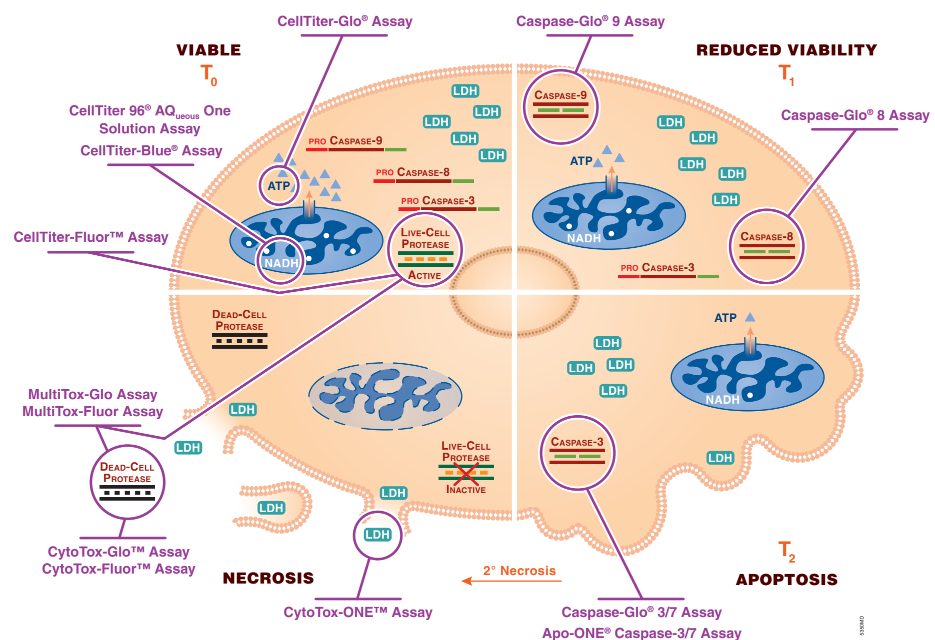

Figure 1. Promega offers a wide range of homogeneous assays for the study of cell metabolic health and cytotoxicity.

luminescent cell-based assays. These examples highlight the power of monitoring multiple cell parameters in a single sample.

FLUORESCENT/LUMINESCENT MULTIPLEX

Many Promega assays have been designed for simple multiplexing following a basic fluorescent assay-luminescent assay multiplexing protocol (Figure 2). In this protocol a fluorescent assay is performed first, using a concentrated assay reagent, followed by a luminescent assay. This protocol can be used for the assays highlighted in Table 1 but is not appropriate for all fluorescent and luminescent assays. Additional information on multiplexing options with other Promega assays has been published previously (3).

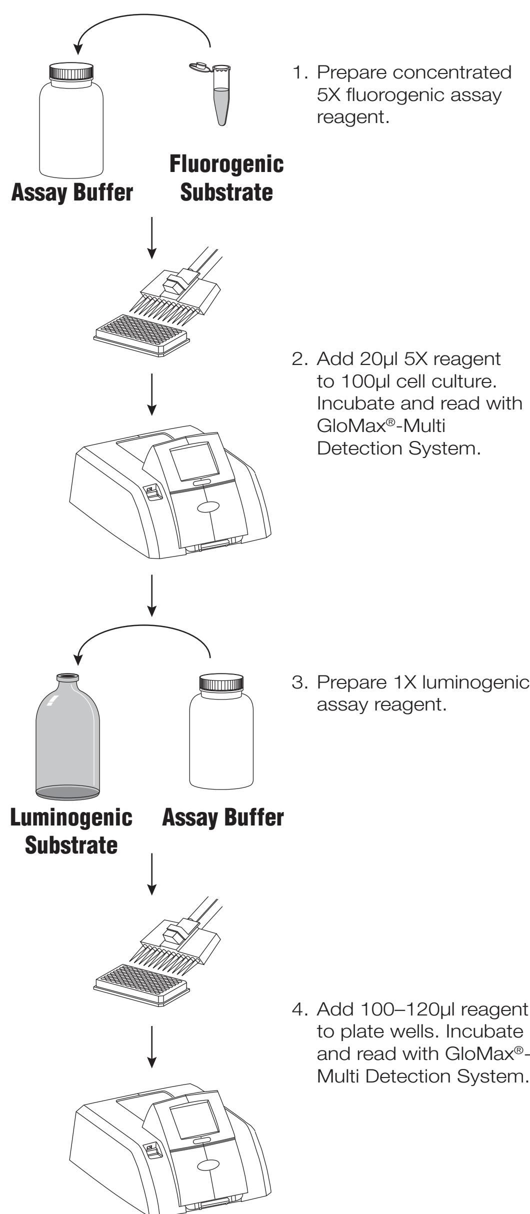

Figure 2. Fluorescent/luminescent multiplex protocol. Assays that are compatible with this simple multiplex protocol are outlined in Table 1.

MECHANISMS OF CELL DEATH

The MultiTox-Fluor Multiplex Cytotoxicity Assay[a] multiplexed with the Caspase-Glo® 3/7 Assay[b–d] provides a better understanding of the role of apoptosis in cytotoxicity (4). The MultiTox-Fluor Assay uses two fluorescent protease markers in a single assay, one marker for viable cells and the other for necrotic (dead) cells (5). In the MultiTox-Fluor Assay fluorescent live-cell signal from cleaved glycyl-phenylalanyl-amino-fluorocoumarin (GF-AFC) demonstrates an inverse correlation with the fluorescent dead-cell signal from cleaved bis-alanyl-alanyl-phenylalanyl-rhodamine 110 (bis-AAF-R110) as a function of increasingly toxic sodium butyrate concentrations (Figure 3). In the Caspase-Glo® 3/7 Assay, caspase-3/7 activity increases with increasing sodium butyrate concentrations as detected by cleavage of Z-DEVD-aminoluciferin. This correlates with the fluorescent R110 signal, indicating apoptosis to be a causative mechanism of cell cytotoxicity.

NORMALIZE WITH REPORTER ASSAYS

Use of the CellTiter-Fluor™ Cell Viability Assay[a] multiplexed with the ONE-Glo™ Luciferase Reporter Assay System[a,d,f] provides an understanding of gene expression in the context of cell viability. At specific concentrations, ionomycin and PMA work cooperatively

Table 1. Multiplexing of Promega Cell Viability, Apoptosis, Necrosis and Reporter Assays.

First Assay	Secondary Multiplex	Examples
Viability: CellTiter-Fluor™ Fluorescent GF-AFC (400 nm/505 nm)	**Viability**: CellTiter-Glo® Luminescent	Figure 2
	Apoptosis: Caspase-Glo® 3/7, 8 or 9 Luminescent	TB371, Figure 2
	Cytotoxicity: CytoTox-Glo™ Luminescent	TB358, Figure 2
	Reporter: ONE-Glo™, Bright-Glo™, Steady-Glo® Luminescent	Figure 2
Cytotoxicity: CytoTox-Fluor™ Fluorescent bis-AAF-R110 (485 nm/520 nm)	**Viability:** CellTiter-Glo® Luminescent	Figure 2
	Apoptosis: Caspase-Glo® 3/7, 8 or 9 Luminescent	Figure 2
	Reporter: Steady-Glo®, Bright-Glo™, ONE-Glo™ Luminescent	Figure 2
Viability and Cytotoxicity: MultiTox-Fluor Fluorescent bis-AAF-R110 (485 nm/520 nm) and GF-AFC (400 nm/505 nm)	**Viability:** CellTiter-Glo® Luminescent	Figure 2
	Apoptosis: Caspase-Glo® 3/7 Luminescent	Figure 2
	Reporter: Steady-Glo®, Bright-Glo™, ONE-Glo™ Luminescent	Figure 2

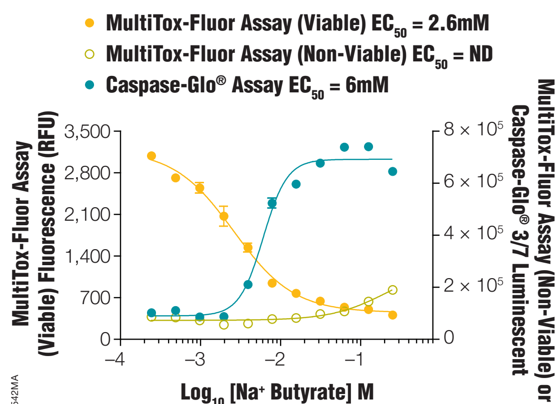

Figure 3. Multiplexing the MultiTox-Fluor Multiplex Cytotoxicity and Caspase-Glo® 3/7 Assays. K562 cells (5,000 cells/well, 100 µl/well) were treated with serial dilutions of the histone deacetylase (HDAC) inhibitor sodium butyrate in RPMI 1640/10% FBS. After 48 hours, the MultiTox-Fluor Reagent was prepared at 5X concentration and added to wells in 20 µl volumes. Fluorescence was measured on the GloMax®-Multi Detection System using the AFC (405 nm_{Ex}/495–505 nm_{Em}) and Blue (490 nm_{Ex}/515–570 nm_{Em}) optical kits. Then, Caspase-Glo® 3/7 Reagent was prepared and added to the wells (100 µl/well), followed by luminescence measurement using the instrument's luminescence module.

to stimulate NFAT-dependent gene expression. However, higher concentrations of ionomycin result in cytotoxicity. The luminescent signal (indicating cell transcription/translation) first increases in the presence of ionomycin and PMA (induction of *luc2P* gene expression), then decreases in response to an increasingly toxic concentration of ionomycin (Figure 4). Fluorescent (live-cell) signal from cleaved GF-AFC also decreases as a function of increasingly toxic ionomycin concentrations. These data demonstrate concordance between a decrease in cell viability and a decrease in genetic reporter (luciferase) activity as the direct result of cell cytotoxicity. If only reporter activity is measured, the impact of compound treatment on cell health may be overlooked.

CONCORDANCE OF VIABILITY ASSAYS

The CellTiter-Fluor™ Cell Viability Assay multiplexed with the CellTiter-Glo® Luminescent Cell Viability Assay[b,d,g] measures two different markers for cell viability. The CellTiter-Fluor™ Assay is based on a conserved, constitutive protease activity within live cells that serves as a biomarker of cell viability (4), while the CellTiter-Glo® Assay uses the metabolic marker ATP as an indicator of cell health (6). The fluorescent live-cell signal from cleaved GF-AFC correlates with the luminescent (cell health) signal from cell-generated ATP as a function of increasingly toxic concentrations of the inhibitor 17-AAG (Figure 5). These data illustrate the concordance of cell viability with metabolic cell health.

Sequential viability (fluorescence) and cytotoxicity (luminescence) measurements using the MultiTox-Glo Multiplex Cytotoxicity Assay provide a complete picture of cell health in a single kit.

MULTIPLEXED VIABILITY AND TOXICITY ASSAYS

Sequential viability (fluorescence) and cytotoxicity (luminescence) measurements using the MultiTox-Glo Multiplex Cytotoxicity Assay[a,b,d] provide a complete picture of cell health in a single kit. Fluorescent (live-cell) signal from cleaved GF-AFC demonstrates an inverse correlation with the luminescent (dead cell) signal from cleaved alanyl-alanyl-phenylalanyl-aminoluciferin (AAF-Glo™) as a function of increasingly toxic ionomycin concentrations (Figure 6). These data illustrate ratiometric concordance between cell viability and cytotoxicity measures within a single, "all-in-one" multiplex assay.

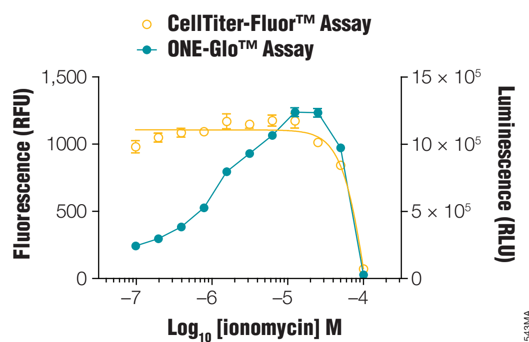

Figure 4. Multiplexing the CellTiter-Fluor™ Cell Viability and ONE-Glo™ Luciferase Assays. GloResponse™ NFAT-RE-*luc2P* HEK293 cells (10,000 cells/well, 100 µl/well) were treated with serial dilutions of ionomycin in DMEM/10% FBS (containing hygromycin B and PMA, which is a known inducer of NFAT transcriptional activity). After 6 hours at 37 °C, CellTiter-Fluor™ Reagent was prepared at 5X concentration and added to the wells in 20 µl volumes. After a 30-minute incubation at 37 °C, the fluorescence was measured on the GloMax®-Multi Detection System using the AFC (405 nm_{Ex}/495–505 nm_{Em}) optical kit. ONE-Glo™ Reagent (100 µl/well) was prepared and added to the wells, followed by luminescence measurement using the instrument's luminescence module.

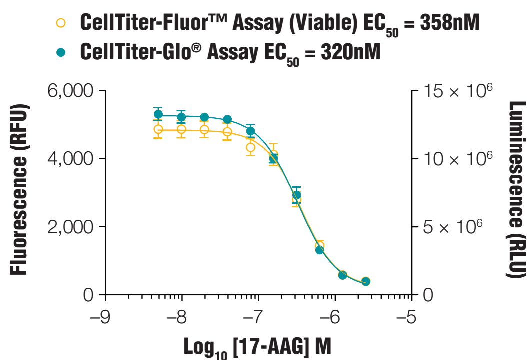

Figure 5. Multiplexing the CellTiter-Fluor™ Cell Viability and CellTiter-Glo® Luminescent Cell Viability Assays. K562 cells (5,000 cells/well, 100 µl/well) were treated with serial dilutions of the heat shock protein 90 (Hsp90) inhibitor 17-AAG in RPMI1640/10% FBS. After 72 hours, the CellTiter-Fluor™ Reagent was prepared at 5X concentration and added to wells in 20 µl volumes. Fluorescence was measured on the GloMax®-Multi Detection System using the AFC (405 nm_{Ex}/495–505 nm_{Em}) optical kit. Then, CellTiter-Glo® Reagent (100 µl/well) was prepared and added to the wells, followed by luminescence measurement using the instrument's luminescence module.

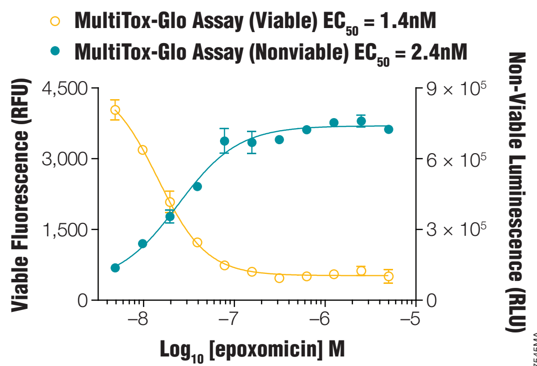

Figure 6. Measuring fluorescent and luminescent signals from the MultiTox-Glo Multiplex Cytotoxicity Assay. DU145 cells (5,000 cells/well, 100 µl/well) were treated with serial dilutions of the proteasome inhibitor epoxomicin in DMEM/10% FBS. After 48 hours, the MultiTox-Glo Reagents were prepared and added, as directed in the *MultiTox-Glo Multiplex Cytotoxicity Assay Technical Bulletin* #TB358, Section IV.C. Fluorescence was measured on the GloMax®-Multi Detection System using the AFC (405 nm_{Ex}/495–505 nm_{Em}) optical kit, followed by luminescence measurement using the instrument's luminescence module.

CONCLUSION

Multiplexing with Promega cell-based assays improves data quality and saves time, money, reagents and valuable samples, allowing researchers to generate multiple data sets from a single experiment. Multiplexing also minimizes environmental and user error resulting from experiment repetition. The GloMax®-Multi Detection System is an ideal platform for measuring fluorescence and luminescence signals, offering peak sensitivity and a broad dynamic range. Used together, Promega cell-based assays and the GloMax®-Multi Detection System enable the researcher to be more productive and efficient in the laboratory. More information about Promega cell-based assays and the GloMax®-Multi Detection System may be found at: **www.promega.com/applications/cellprolif/** and **www.promega.com/glomax/glomaxmulti.htm**

REFERENCES

1. Kopish, K. (2008) *Promega Notes* **98**, 3–5.
2. Zakowicz, H. *et al.* (2008) *Promega eNotes* online www.promega.com/enotes/applications/ap0085.htm.
3. Farfan, A. *et al.* (2004) *Cell Notes* **10**, 15–8.
4. Niles, A. *et al.* (2007) *Anal. Biochem.* **366**, 197–206.
5. Niles, A. *et al.* (2006) *Cell Notes* **15**, 11–5.
6. Hannah, R. *et al.* (2001) *Cell Notes* **2**, 11–3.

PROTOCOLS

- *MultiTox-Fluor Multiplex Cytotoxicity Assay Technical Bulletin* #TB348, Promega Corporation **www.promega.com/tbs/tb348/tb348.html**
- *MultiTox-Glo Multiplex Cytotoxicity Assay Technical Bulletin* #TB358, Promega Corporation **www.promega.com/tbs/tb358/tb358.html**
- *Caspase-Glo® 3/7 Assay Technical Bulletin* #TB323, Promega Corporation **www.promega.com/tbs/tb323/tb323.html**
- *CellTiter-Fluor™ Cell Viability Assay Technical Bulletin* #TB371, Promega Corporation **www.promega.com/tbs/tb371/tb371.html**
- *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288, Promega Corporation **www.promega.com/tbs/tb288/tb288.html**
- *ONE-Glo™ Luciferase Assay System Technical Manual*, #TM292, Promega Corporation **www.promega.com/tbs/tm292/tm292.html**
- *GloMax®-Multi Detection System Technical Manual* #TM297, Promega Corporation **www.promega.com/tbs/tm297/tm297.html**

ORDERING INFORMATION

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay*	10 ml	G8091
CellTiter-Fluor™ Cell Viability Assay*	10 ml	G6080
CellTiter-Glo® Luminescent Cell Viability Assay*	10 ml	G7570
CytoTox-Fluor™ Cytotoxicity Assay*	10 ml	G9260
CytoTox-Glo™ Cytotoxicity Assay*	10 ml	G9290
MultiTox-Fluor Multiplex Cytotoxicity Assay*	10 ml	G9200
MultiTox-Glo Multiplex Cytotoxicity Assay*	10 ml	G9270
ONE-Glo™ Luciferase Assay System*	10 ml	E6110
GloMax®-Multi Base Instrument	each	E7031
GloMax®-Multi Luminescence Module	each	E7041
GloMax®-Multi Fluorescence Module	each	E7051

Additional sizes are available.
*For Laboratory Use.

(a)Patent Pending.
(b)U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. Nos. 754312 and 785294 and other patents and patents pending.
(c)U.S. Pat. No. 7,148,030 and other patents pending.
(d)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.
(e)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents.
(f)Certain applications of this product may require licenses from others.
(g)U.S. Pat. No. 7,083,911, Australian Pat. No. 2002255553 and other patents pending.
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