

## CELLTITER-GLO™ LUMINESCENT CELL VIABILITY ASSAY: PRIMARY NEURONS AND HUMAN NEUROBLASTOMA SH-SY5Y CELLS

Georgyi V. Los, Ph.D., Chad Zimprich, B.S., and Randy Hoffman, B.S., Promega Corporation

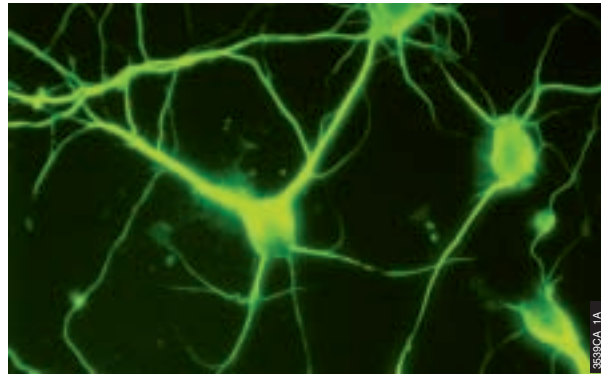
*This article demonstrates the use of the CellTiter-Glo™ Assay (Cat.# G7570, G7571, G7572, and G7573) for determining viability and testing for cytotoxicity in primary neuronal cell cultures.*

### Introduction

Primary neuronal cultures are experimental models that closely reflect the function of neurons in living organisms. Although primary neuronal cultures are thought to be the best experimental models for neurotoxicity testing and drug development, standard protocols for the generation and differentiation of these cell cultures are labor-intensive and yield relatively small quantities of differentiated neurons, limiting their usefulness in high-throughput screening. One way to overcome these limitations is to use screening technologies that allow quantitative measurements with small numbers of cells.

The CellTiter-Glo™ Luminescent Cell Viability Assay<sup>(a)</sup> is one such technology for working with small numbers of cells. This assay is based on the luciferase/luciferin reaction that, in the presence of Mg<sup>2+</sup> and ATP, produces oxyluciferin and releases energy in the form of luminescence (1). Since the luciferase reaction requires ATP, the luminescence produced is proportional to the amount of ATP present, an indicator of cellular metabolic activity (2).

In this article we present experimental data using the CellTiter-Glo™ Assay for quantitative analysis of viability of rat embryonic (E18) primary cerebrocortical, hippocampal and striatal (+SVZ) neurons, as well as human neuroblastoma SH-SY5Y cells. In some experiments, neurotoxicity was modeled by pretreatment of the cells with glutamate or MPP<sup>+</sup> ion. Glutamate is a major excitatory amino acid neurotransmitter in mammalian brain that plays an important role in synaptic plasticity, learning, and memory (3). At elevated extracellular concentrations, glutamate may act as a powerful neurotoxin capable of inducing severe excitotoxic damage to targeted neurons (4), a mechanism implicated in ischemia, hypoxia, status epilepticus, and several neurodegenerative diseases (5,6). MPP<sup>+</sup> (1-methyl-1,4-phenylpyridinium ion) is the main metabolite of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). MPP<sup>+</sup> can induce Parkinson's disease (PD)-like clinical symptoms and neuropathological destruction and has



**Figure 1. Fluorescent microscopy of rat primary hippocampal neurons.** Cells were fixed with 3.7% formaldehyde, immunostained with mouse monoclonal Anti-βIII-Tubulin Antibody (Cat.# G7121) at 1.0µg/ml followed by incubation with an AlexaFluor™-488-conjugated goat-anti mouse IgG (Molecular Probes).

been used to model PD. MPP<sup>+</sup> can also induce apoptosis in cerebellar granule cells, neuronally differentiated PC12 cells, mesencephalic-striatal co-cultures, and SH-SY5Y human neuroblastoma cells (7,8).

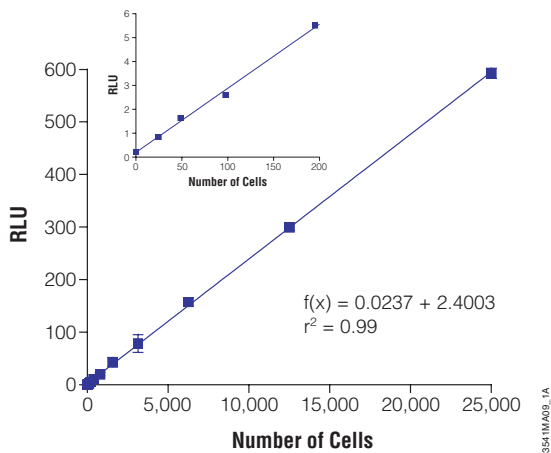
### Cell Culture Methods

Rat cerebrocortical, hippocampal and striatal [including subventricular zone (SVZ)] primary neurons were isolated as described (9,10). Briefly, embryonic day 18 (E18) rat brains were dissected to obtain cortex, hippocampus, and SVZ striatal tissues. The tissues were incubated in Hibernate™ E medium (Gibco) under previously described conditions (11) until the time of isolation. The cells were dissociated and plated on poly-D-lysine-coated glass or plasticware and cultured in serum-free Neurobasal™ medium (Gibco) with B27 supplement. The media was changed every 3 days.

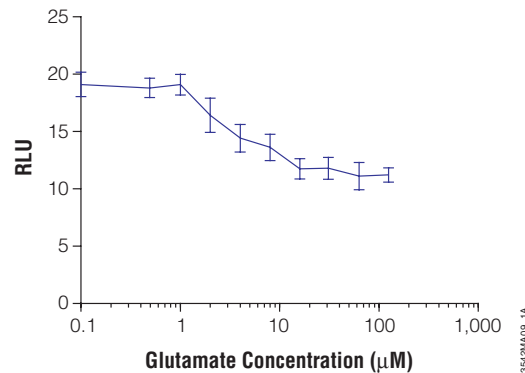
Human neuroblastoma SH-SY5Y cells obtained from ATCC (CRL-2266) were cultured in a 1:1 mixture of Ham's F12 nutrients and essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100IU/ml penicillin and 100µg/ml streptomycin as described (12). All cells were maintained in humidified 5% CO<sub>2</sub> atmosphere at 37°C.

### Cell Viability

Viability of the cells was verified by Trypan Blue exclusion assay (before plating) and by propidium iodide/fluorescein diacetate staining (for attached cells). Attached cells were rinsed twice with PBS (0.5ml/cm<sup>2</sup> of cell growing surface), and 0.5ml of PBS containing 15mg/ml of fluorescein diacetate (Sigma) and 4.6µg/ml of propidium iodide (Sigma) was added to the cells. After one minute, multiple images (8 images/cm<sup>2</sup> of cell



**Figure 2. The number of rat primary hippocampal neurons correlates with luminescent output.** Serial dilutions of rat (E18) primary hippocampal neurons in Neurobasal™/B27 medium were made in poly-D-lysine coated clear-bottom 96 well plates (100µl/well). An equal volume of CellTiter-Glo™ Reagent was added and mixed. The luminescence was recorded at 10 minutes using a DYNEX MLX® microtiter plate luminometer. Values represent the mean ± S.D. of 8 replicates of each cell number ( $r^2 = 0.99$  for 0–25,000 cells/well).



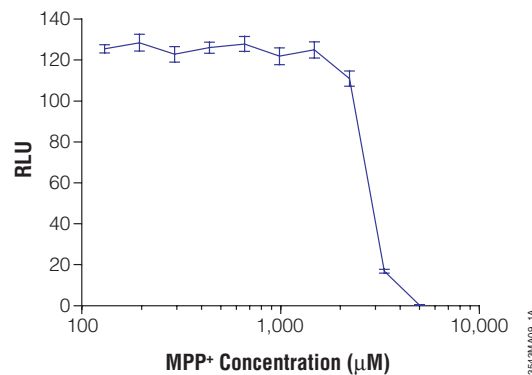
**Figure 3. Effect of glutamate on the viability of rat hippocampal neurons measured with the CellTiter-Glo™ Assay.** Rat (E18) primary hippocampal neurons (1,000 cells/well in 100µl of Neurobasal™/B27 medium) were plated in poly-D-lysine-coated clear-bottom 96 well plates. Cells were allowed to attach for 8 days. Medium was removed and cells were incubated with Mg<sup>2+</sup>-free Dulbecco's PBS or Mg<sup>2+</sup>-free Dulbecco's PBS with varying concentrations of L-glutamate for 2 hours at 37°C. Cells were returned to the original medium, and viability was assayed 24 hours later using the CellTiter-Glo™ Assay. Luminescence was recorded as in Figure 2 ( $n = 8$ ,  $p < 0.005$  for control vs.  $10^{-3}$ M glutamate).

growing surface) were taken with a Spot digital camera (Diagnostic Instruments, Inc.) on Axiovert® S100 inverted epifluorescent microscope (Nikon). Numbers of live (green) and dead (red) cells were calculated using Image-Pro® Plus (Media Cybernetics) software.

### Results and Summary

Figure 2 demonstrates the linearity ( $r^2 = 0.99$ ) of the Cell Titer-Glo™ Assay for rat E18 primary hippocampal neurons. Similar results were obtained with rat E18 primary cerebrocortical and striatal (+SVZ) neurons, human neuroblastoma SH-SY5Y cells and human neuronal progenitors. Figures 3 and 4 illustrate neurotoxicity studies using the CellTiter-Glo™ Assay.

These data demonstrate that the CellTiter-Glo™ Luminescent Cell Viability Assay can be used for quantitative analysis of viability of rat primary cerebrocortical, hippocampal and striatal (+SVZ) neurons as well as human neuroblastoma SH-SY5Y cells. The sensitivity of this assay allows more efficient use of primary neuronal cultures in life science research and in high-throughput screening for drug development and cytotoxicity testing.



**Figure 4. Effect of MPP+ on the viability of human neuroblastoma SH-SY5Y cells.** SH-SY5Y (2,000 cells/well in 100µl of F12/MEM + 10% FBS) were plated in clear-bottom 96 well plates. Cells were allowed to attach and grow for 24 hours. Then medium was replaced by F12/MEM without FBS, with or without varying concentrations of MPP+, and incubated for 24 hours. Viability was assayed using the CellTiter-Glo™ Assay, and the luminescence was measured as in Figure 2 ( $n = 8$ ;  $p < 0.005$  for control vs.  $10^{-3}$ M MPP+).

**References**

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**Ordering Information**

Product	Size	Cat. #
CellTiter-Glo™ Luminescent Cell Viability Assay <sup>(a)</sup>	10 × 100ml	G7573
	100ml	G7572
	10 × 10ml	G7571
	10ml	G7570

<sup>(a)</sup>Patent Pending.

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**PROMEGA SCIENTISTS TO PRESENT TALKS AT AMERICAN SOCIETY FOR CELL BIOLOGY**

**A Fast and Sensitive “Single Addition” Luminescent Cell Viability Assay**

Talk presented by Richard Moravec, M.S.  
 Research and Development, Promega Corporation  
 Sunday, December 9, 2001, 11:00am

We have developed a fast and sensitive, single-addition luminescent cell viability assay, the CellTiter-Glo™ Luminescent Cell Viability Assay<sup>(a)</sup>, which is based on the detection of ATP. The assay is performed by adding a single reagent directly to cells in culture. After mixing and incubating 10 minutes at ambient temperature, luminescence is measured with a luminometer or CCD imaging device. The luminescent signal is directly proportional to the number of viable cells, consistent with previously published reports using ATP as an indicator of cell viability. The luminescent signal produced has an extended half-life typically greater than 5 hours. The assay sensitivity is sufficient to detect 4 cells/well in a 384 well format and has a linear range of 3–4 logs, depending on cell type and plate format. We will present data demonstrating the use of the CellTiter-Glo™ Assay for cytotoxicity testing in a 384 well format and its compatibility with a variety of cell lines, media and solvents used to deliver drugs.

**The Apo-ONE™ Homogeneous Caspase-3/7 Assay: The “Number ONE” Solution for Apoptosis Detection**

Talk presented by Michael Curtin, B.S.  
 Cell Signaling and Neuroscience, Promega Corporation  
 Sunday December 9, 2001, 12:00pm

Promega Corporation has developed a one-step, highly sensitive, simple and robust assay to measure caspase-3 and caspase-7 activity. The Apo-ONE™ Homogeneous Caspase-3/7 Assay<sup>(b)</sup> uses a rhodamine 110-based substrate in a specially formulated lysis buffer. This buffer, which is added directly to cells in culture, allows the user to quickly and accurately measure the activities of caspase-3 and caspase-7. We will present data comparing the Apo-ONE™ Assay to other currently existing technologies. Particular regard will be given to the assay’s use with primary, suspension and adherent cell lines in both apoptotic induction and inhibition screens as well as in potency testing. Furthermore, the inherent flexibility of the assay will be demonstrated from data collected on the Biomek® (Beckman) 2000 automated module. These demonstrations will show how quickly and routinely the assay can be used as an early and accurate indicator of apoptotic processes.

<sup>(a)</sup>Patent Pending.

<sup>(b)</sup>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.

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