ASSESSING CELL VIABILITY IN PRIMARY CULTURES USING A LUMINESCENT, ATP-BASED ASSAY: APPLICATIONS FOR THE CELLTITER-GLO® ASSAY

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Here we highlight previously unpublished data and several peer-reviewed publications in which primary cell cultures were studied using the CellTiter-Glo® Assay. The sensitivity of the CellTiter-Glo® Assay allows researchers to conserve sample when working with extremely precious primary cell cultures.

Introduction

Although immortalized cell lines have provided invaluable information about cell biology to researchers, primary cell culture offers a more relevant system for the study of cell function, disease states and patient therapy. However, working with primary cells in culture presents numerous challenges, including the requirement for unique cell supplements and growth conditions (1). Primary cell cultures are sensitive to apoptosis due to contact inhibition, serum concentration, and their three dimensional (3D) environment, and many conditions for optimal growth and proliferation of primary cells remain unknown (2).

To help researchers, screening systems that use small numbers of cells may be used to measure cell viability as a function of various growth factors and environments. Once growth conditions are optimized, these screening systems may be used further to determine the effect of compounds on cytotoxicity, aiding drug discovery and development.

ATP is a valid parameter of cell viability and overall metabolic health. The CellTiter-Glo® Luminescent Cell Viability Assay[a–c] is based on the luciferase/luciferin reaction, which in the presence of Mg^{2+} and ATP, produces oxyluciferin and energy in the form of light. By establishing ATP as the limiting reaction component, this assay accurately measures viability and cytotoxicity of primary neurons (3). Likewise, because neither luciferase nor luciferin is naturally found in mammalian cells, the assay is highly sensitive, detecting as few as four cells/well in a 384- or 1536-well format (4; Figure 1). These qualities make the CellTiter-Glo® Assay ideal for large-scale screening of primary cell cultures.

Primary Neuronal Cell Cultures

Unpublished Data

Amyloid Beta (Aβ) peptide is cytotoxic to neurons. Figure 1 depicts previously unpublished data in which neurons harvested from embryonic mouse cortex were exposed to Aβ. The Aβ-induced cytotoxicity was measured using the CellTiter-Glo® Assay.

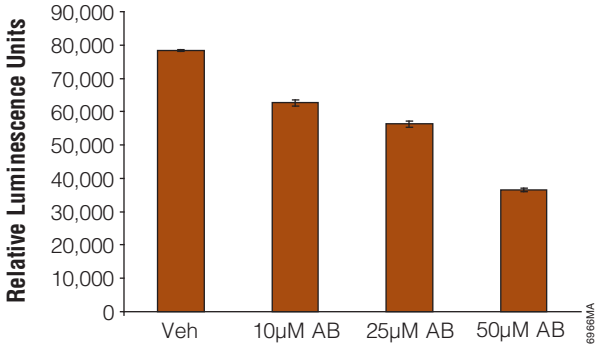

Figure 1. Primary neuronal cell cytotoxicity is a function of Aβ concentration. Neurons were harvested from embryonic mouse cortex and seeded into PDL/laminin-coated 96-well plates (Greiner flat bottom, opaque white) containing Neurobasal™ cell culture medium (Invitrogen) supplemented with 2% B-27 and 0.5 mM glutamine. After 7 days in culture, the medium was supplemented with different concentrations of Aβ-peptide. Cells were cultured an additional 48 hours before the medium was replaced with 100 µl PBS. Plates were maintained at room temperature for 45 minutes, after which 85 µl of CellTiter-Glo® Reagent was added to each well (modified procedure from Technical Bulletin #TB288, Promega Corporation). Plates were shaken on an orbital shaker for 2 minutes at 200 rpm and maintained at room temperature for an additional 10 minutes before being read on a plate reader set to luminescence mode. Data presented with the kind permission of M. Hendrickson and J. Beck, Mithridion Corporation.

Published Data

Zhao, Z. *et al.* (2006) A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. *BMC Neuroscience* **7**, 29.

Amyotrophic lateral sclerosis (ALS) is influenced by mitochondrial dysfunction brought on by a defective superoxide dismutase (SOD) gene. SOD is responsible for ATP production, which when suboptimal leads to neuronal cell death. To better understand this pathway, primary spinal cord cultures deficient in SOD1 were prepared from two-week-old mouse embryos. After ten days in culture, these neurons were treated with various ketones and assayed for improved cell viability via the CellTiter-Glo® Assay. The ketone bodies restored mitochondrial function, leading to higher ATP readings and improved cell viability.

Determining Viability of Primary Cells in Culture

Primary Immune Cells

Trushin, S.A. *et al.* (2007) Glycoprotein 120 binding to CXCR4 causes p38-dependent primary T cell death that is facilitated by, but does not require cell-associated CD4. *J. Immunol.* **178**, 4846–53.

Human immunodeficiency virus-1 (HIV-1) infection results in the depletion of CD4 T cells through as yet unknown mechanisms. Primary human CD4 T cells were isolated from the blood of healthy and HIV-positive volunteers and maintained in RPMI 1640 medium supplemented with 10% FBS. After 24–36 hours, the cells were incubated with gp120, anti-CD4, or anti-CXCR4 antibodies, all of which are known to induce cell death. Reduced cell viability following specified incubation periods was analyzed with ELISA and confirmed by a measured decrease in live cells using the CellTiter-Glo® Assay. CD4 T cell apoptosis, via a p38-dependent MAPK pathway, was induced when gp120 bound to either the CD4 or CXCR4 molecules.

Flaherty, D.M. *et al.* (2006) Human alveolar macrophages are deficient in PTEN. *J. Biol. Chem.* **281**, 5058–64.

Human alveolar macrophages play an important role in inflammation and immunity in the lung. The cells are resistant to apoptosis due in part to the pro-survival phosphatidyl-inositol (PI)2 3-kinase/Akt pathway. Akt itself is negatively regulated by PTEN. Primary cultures of alveolar macrophages were obtained by bronchoalveolar lavage, after which they were exposed to the PI 3-kinase inhibitor LY294002. The cells were also transfected with an adenoviral vector containing the PTEN transgene. Cell viability was measured using the CellTiter-Glo® Assay. Inhibition of either PI 3-kinase or activation of Akt resulted in apoptotic initiation, which was indicated earlier by the measured reduction of cellular ATP via the CellTiter-Glo® Assay.

Primary Tumor Cells

Chen, E.I. *et al.* (2007) Adaptation of energy metabolism in breast cancer brain metastases. *Cancer Res.* **67**, 1472–86.

Brain metastases are a serious complication of breast cancer, affecting nearly 20% of patients. To better understand metastatic pathways, circulating tumor cells were collected from stage IV breast cancer patients. The cells were injected into severe combined immunodeficient (SCID) mice and recovered from their brains or femurs 6 weeks later. Recovered cells were seeded in 96-well plates at a concentration of 2.5×10^4 cells per well, and the CellTiter-Glo® Assay was used to measure ATP. Data indicated that breast cancer cells that metastasize to the brain use metabolic pathways far different than those typically found in tumor cell microenvironments.

Stem Cells

Salinas, C.N. *et al.* (2007) Chondrogenic differentiation potential of human mesenchymal stem cells photo-encapsulated within poly(ethylene glycol)-arginine-glycine-aspartic acid-serine thiol-methacrylate mixed-mode networks. *Tissue Eng.* **13**, 1025–34.

Mesenchymal stem cells (MSCs) are an attractive target for cartilage repair due to their ease of isolation, multipotency, and proliferative potential. Poly(ethylene glycol) (PEG)-based polymers are often used to differentiate MSCs into chondrocytes; however, the matrix is insufficient for sustained cell viability. To study novel methods for cellular viability enhancement, adult MSCs were cultured in stem cell medium after being extracted from a single donor. Cells were encapsulated in PEG hydrogels containing the peptide sequence arginine-glycine-aspartic acid-serine (RGDS) and assessed for viability by the CellTiter-Glo® Assay. RGDS-containing samples maintained a higher level of viable cells compared with nonRGDS-containing samples, as well as a gene expression profile conducive to chondrogenesis.

Summary

These studies demonstrate that the CellTiter-Glo® Assay can be used to assess primary cell culture viability, including that of primordial/stem cells. The CellTiter-Glo® Assay provides a homogeneous assay, sensitive to four cells, allowing researchers to obtain reliable viability data while conserving precious primary and stem cell samples.

References

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2. Cossu, G. and Mavilio, F. (2000) *J. Clin. Invest.* **105**, 1669–74.
3. Los, G.V. *et al.* (2001) *Neural Notes* **20**, 5–7.
4. Moravec, R. *et al.* (2001) *Cell Notes.* **2**, 14–16.

Protocol

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

Ordering Information

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10 ml	G7570

For Laboratory Use. Available in additional sizes.

[a]U.S. Pat. Nos. 6,602,677 and 7,241,584, Australian Pat. No. 754312 and other patents and patents pending.

[b]U.S.Pat. No. 7,083,911 and other patents pending.

[c]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.

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