CELLTITER-GLO™ LUMINESCENT CELL VIABILITY ASSAY FOR CYTOTOXICITY AND CELL PROLIFERATION STUDIES

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We briefly review the use of the CellTiter-Glo™ Luminescent Cell Viability Assay (Cat.# G7570, G7571, G7572, G7573) in three recently published, peer-reviewed articles. The CellTiter-Glo™ Assay was used to measure cytotoxicity in drug screening experiments and to investigate cell proliferation in prostate cancer cells.

Introduction

The CellTiter-Glo™ Luminescent Cell Viability Assay[a] is a highly sensitive method for assaying cell proliferation and cytotoxicity. The assay uses the luciferase reaction to measure ATP, a global indicator of cellular metabolism. While providing a powerful tool for the life science researcher, the "add, mix, measure" format of the assay also satisfies the need for a simple and rapid tool for high-throughput screening (Figure 1). The assay is performed by adding a single reagent directly to cells in culture and requires no extractions or washes.

The CellTiter-Glo™ Reagent is prepared by reconsituting the lyophilized CellTiter-Glo™ Substrate with the CellTiter-Glo™ Buffer. The final volume of CellTiter-Glo™ Reagent used depends on the final volume of cells and test compounds. The Reagent is added to culture wells, plates are agitated briefly, and luminescence is detected. Reproducible luminescence is acheived as long as a 1:1 ratio of Reagent to sample volume is maintained. Figure 2 shows that the CellTiter-Glo™ Assay produces results comparable to other cell viability assay formats. A TNFα cytotoxicity assay was performed on L929 cells, comparing the detection methods of the CellTiter-Glo™ Assay and CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay[b] (MTS-based assay). Although each assay measures a different indicator of metabolism, both assays give a similar ED_{50} value.

The three articles highlighted here use the CellTiter-Glo™ Assay to address a variety of biological questions ranging from fundamental questions of signaling in cancer to drug cytotoxicity.

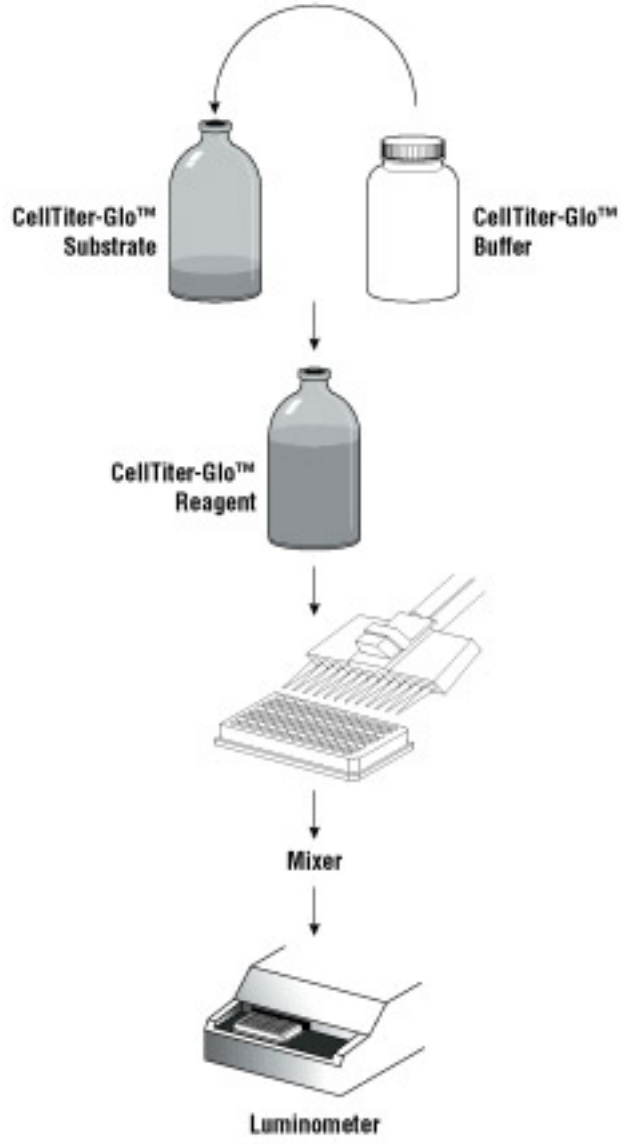

Figure 1. Schematic diagram of the CellTiter-Glo™ Luminescent Cell Viability Assay protocol.

Inhibition of ligand-mediated HER2 activation in androgen-independent prostate cancer

Mendoza, N. *et al. (2002) Can. Res.* **62**, 5485–88.

This paper investigated the role of HER2 tyrosine kinase activation in the development of androgen-independent (AI) prostate cancer. HER2 overexpression and activation is implicated in the development of androgen independence in prostate cancer, but studies have provided conflicting results about HER2 expression. These authors studied HER2 expression and activation in the AI prostate cancer cell line,

AI 22Rv1. Using Western analysis they determined that this cell line expresses HER2, EGFR and HER3. (HER2 is known to form active heterodimers with HER3 and EGFR).

They next investigated heregulin activation of HER2 in these cells using the CellTiter-Glo™ Luminescent Cell Viability Assay. Cells were seeded into black 96-well plates and allowed to adhere overnight. The next day the cells were serum starved for 24 hours and then treated with different concentrations of rhuMAb C24 (an inhibitor of HER2 activation) for 1 hour followed by treatment with heregulin or TNFα. Cells were incubated 4 days before measuring cell proliferation.

Cells treated with heregulin showed significant cell proliferation compared to control cells, while TNFα did not induce the same proliferation. The inhibitor antibody rhuMAb affected heregulin-induced proliferation in a dose-dependent manner.

Action of a novel anticancer agent, CHS 828, on mouse fibroblasts: Increased sensitivity of cells lacking poly (ADP-Ribose) polymerase-I

Lövborg, H. *et al. (2002) Can. Res.* **62**, 4206–11.

These authors investigated the role of PARP-1 in modulating cellular response to the anti-cancer drug CHS 828. The primary mechanism of CHS 828 toxicity is unknown, but some investigations have shown that it can induce an increase in extracellular acidification rate, suggesting that mitochondrial respiration may be affected by the drug. Immortalized embryonic fibroblasts (MEFs) were obtained from PARP-1$^{-/-}$ mice. The effect of CHS 828 on proliferation of these cells was assessed by two methods. One assessed the conversion of a tetrazolium salt to a colored formazan product, reflecting the reducing potential of the cell. The authors also used the CellTiter-Glo™ Luminescent Cell Viability Assay to assess cell viability. Both assays indicated that mouse fibroblasts lacking PARP-1 showed a decrease in cell proliferation when treated with CHS 828. The authors observed a dose-dependent induction of p53 protein by CHS 828 in normal, but not PARP-1$^{-/-}$ MEFs.

Comparative in vitro sensitivities of human immune cell lines, vaginal and cervical epithelial cell lines and primary cells to candidate microbicides Nonoxynol 9, C31G, and sodium dodecyl sulfate

Krebs, F.C. *et al.* (2002) *Antimicrobial Agents Chemother.* **46**, 2292–98.

Screening compounds for cytotoxicity to human cells in vitro is an important first step in identifying potential chemotherapeutic agents and microbicides. This study compares the sensitivity of primary cells to that of cell lines for in vitro cytotoxicity screening of potential vaginal microbicides. Specifically the authors investigated human vaginal epithelial

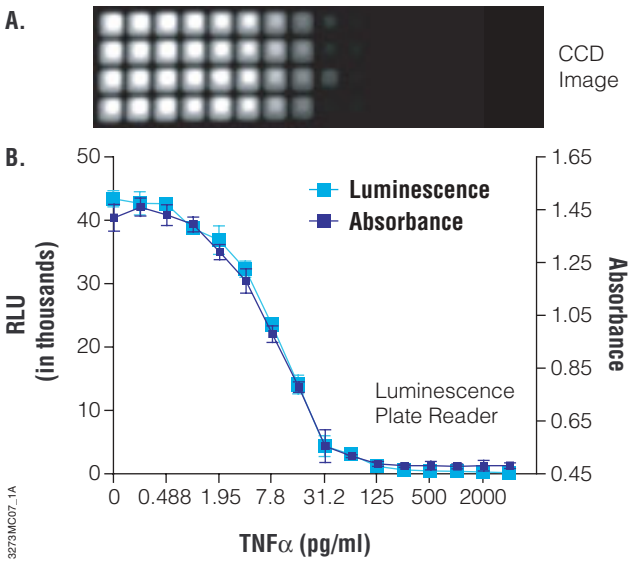

Figure 2. The CellTiter-Glo™ Assay and CellTiter 96® AQ$_{ueous}$ One Solution Assay give comparable results of TNFα cytotoxicity in L929 cells. Cells were plated (1,000 cells/well) and allowed to attach and grow for 24 hours. Varying concentrations of TNFα were added (n = 4) in the presence of actinomcyin D (1µg/ml final) and incubated for 20 hours. Cytotoxicity was determined using either the CellTiter-Glo™ Assay or the CellTiter 96® AQ$_{ueous}$ One Solution Assay (described in Moravec *et al. Cell Notes* **2**, 14–16). **Panel A**. Luminescence was visualized with an Alpha Innotech Multi-Image Light Cabinet CCD camera (30 second exposure at medium sensitivity). Each column of replicates corresponds to the mean data point on the graph as determined by the luminometer. **Panel B.** The ED$_{50}$ values determined by both assays were approximately equivalent (8pg/ml).

cells and cell lines and human immune cells and cell lines for sensitivity to three potential microbicides. Results using MTT-based cell viability assays suggested that as human vaginal keratinocytes transition from primary cells to immortalized cells, their in vitro cytotoxicity profile changes. To determine if the MTT-based assay affected the observed differences in sensitivity, the authors used the CellTiter 96® AQ$_{ueous}$ Non-Radioactive Cell Proliferation Assay[b] (MTS-based) and the CellTiter-Glo™ Assay (ATP-based) to measure cytotoxicity in primary cells and immortalized cell lines. All three assays gave comparable results. The authors concluded that in vitro cytotoxicity assays using established cell lines can be used to rank the relative cytotoxicity of candidate vaginal microbicides.

The assay provides a powerful tool for the life science researcher, and the "add, mix, measure" format of the assay satisfies the needs of high-throughput users.

Conclusion

These three studies demonstrate the utility of the CellTiter-Glo™ Luminescent Cell Viability Assay for in vitro cytotoxicity screening or cell proliferation studies. The assay is useful for answering fundamental research questions about the molecular events involved in cell proliferation, for investigating the mechanism of cytotoxicity of chemotherapeutic compounds, or for in vitro cytotoxicity screening of candidate microbicides or other therapeutic compounds. The simple protocol makes the CellTiter-Glo™ Assay amenable to high-throughput screening applications. ■

Ordering Information

Product	Size	Cat.#
CellTiter-Glo™ Luminescent Cell Viability Assay[a]	10ml	G7570
	10 × 10ml	G7571
	100ml	G7542
	10 × 100ml	G7573
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*[b]	200 Assays	G3582
	1,000 Assays	G3580
	5,000 Assays	G3581
CellTiter 96® Non-Radioactive Cell Viability Assay (MTT)	1,000 Assays	G4000
	5,000 Assays	G4100

*For Laboratory Use.

[a]Patent Pending.
[b]The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida and is licensed exclusively to Promega Corporation.

Protocol

CellTiter-Glo™ Luminescent Cell Viability Assay Technical Bulletin #TB288, Promega Corporation

www.promega.com/tbs/tb288/tb288.html