Introduction

The CellTiter-Glo™ Assay 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 reconstituting 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 achieved 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® AQueous One Solution Cell Proliferation Assay® (MTS-based assay). Although each assay measures a different indicator of metabolism, both assays give a similar ED50 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.

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


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,
CellTiter-Glo™ Luminescent Cell Viability Assay

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 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 actinomycin D (1µg/ml final) and incubated for 20 hours. Cytotoxicity was determined using either the CellTiter-Glo™ Assay or the CellTiter 96® AQueous One Solution Assay (n = 4) in the presence of actinomycin D (1µg/ml final) and incubated for 20 hours. Cytotoxicity was determined using either the CellTiter-Glo™ Assay or the CellTiter 96® AQueous One Solution Assay. 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.

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The ED50 values determined by both assays were approximately (8µg/ml).

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


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 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® AQueous One Solution Assay (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.
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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

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(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.

For Laboratory Use.

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