1. Abstract

Understanding in vitro cell viability and cytotoxicity in any number of model systems remains a challenging task. With recent advances in stem cell biology, it is now possible to study human embryonic stem (hES) cells and their progeny (i.e. cardiomyocytes, hepatocytes, and neurons). Currently, one of the major technical problems is growing undifferentiated hES cells as a monolayer at low numbers in a multi-well format. Combining Promega’s suite of biological assays and instrumentation with Stemina’s hES cell expertise, we can facilitate novel research to characterize the cellular and functional biology of hES cells.

Here, we report hES cell applications of Promega’s broad portfolio of homogeneous, cell-based assays designed to measure viability, apoptosis, cytotoxicity, and oxidative stress. CellTiter-Glo® and Caspase-Glo® 3/7 Assays measure cell viability and apoptosis, respectively, using an easy-to-read luminescent endpoint. Promega’s MultiTox-Flor provides an alternative approach to simultaneously measure fluorescence of both cell viability and cytotoxicity. Additionally, GSH-Glo™ can be used to measure drug responses facilitating oxidative stress, which may lead to programmed cell death.

The data presented highlights the cell-based utility of CellTiter-Glo®, Caspase-Glo®, MultiTox-Flor, MultiTox Cytotoxicity, and GSH-Glo™ assay results when hES cells were analyzed in a 96-well format.

2. Promega’s Tools and Expertise in Cell Viability, Cytotoxicity, and Apoptosis

2.1 CellTiter-Glo® Cell Viability Assay

The CellTiter-Glo® Luminiscent Cell Viability Assay measures the amount of ATP present in cells, which is directly correlated to the number of viable cells in culture.

2.2 Caspase-Glo® 3/7 Apoptosis Assay

Caspase-Glo® 3/7 Assay measures caspase-3 and caspase-7 activities through the detection of a luciferase product that results from a caspase cleavage reaction.

2.3 MultiTox-Flor Multiplex Cytotoxicity Assay

The MultiTox-Flor Multiplex Cytotoxicity Assay measures live and dead cells simultaneously with a single reagent addition and fluorescent readout.

2.4 GSH-Glo™ Glutathione Assay

The GSH-Glo™ Assay is a luminescent measure of cellular changes in glutathione (GSH) as a result of drug induced oxidative stress, potentially leading to apoptosis.

3. Growing hES Cells: Now in a 96-well Format

WA09 human embryonic stem (hES) cells were used for our studies. Routine hES cell maintenance was performed by growing hES cells on Matrigel (BD Biosciences) and in mTeSR1 medium (Stem Cell Technologies) in a 6-well culture. For assay studies, hES cells were seeded onto 96-well plates. To do so, hES cells were trypsinized for five minutes, washed, and resuspended in mTeSR1 medium. Cells were counted from an aliquot of the cell suspension in order to dilute the cell stock suspension for seeding into 96-well plates. Cell densities ranged from 0 to 50,000 cells per well of a 96-well plate.

5. hES cells (20k) were plated into wells of a 96-well plate and treated with 0-100 µg/mL BSO and incubated at 37°C, 5% CO₂ for 6 hours prior to performing the GSH-Glo™ Assay.

6. GSH-Glo™ Assay performance with different concentrations of BSO in the assay. The luminescent signal is linear with the number of cells per well, and the relationship is described by the following equation:

\[
\text{Luminescence (RLU)} = 1000000 + 200000 \times \text{hES Cells/Well}
\]

7. Summary

- hES cells can be cultured in a 96-well format at low cell number while maintaining proper morphology.
- CellTiter-Glo® and Caspase-Glo® 3/7 assays are sensitive, reliable, and reproducible over a broad range of hES cell numbers.
- hES cells demonstrate a low EC₅₀ value indicating a high sensitivity of hES cells to toxicant. (~10-fold higher than J urkat cells)
- MultiTox-Flor is an easy homogeneous assay to simultaneously measure hES cells viability and cytotoxicity.
- GSH-Glo™ is a fast, sensitive, stable luminescent detection system to measure glutathione levels as an indicator of oxidative stress tress in hES cells.
- Additional cell-based assays are on the way.

www.promega.com