Characterizing Cellular Responses in 2-D and 3-D Culture Conditions with EarlyTox Cell Viability Assays

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OVERVIEW

Apoptosis is a highly regulated cellular program that causes cell death in normal processes such as embryonic development, as well as certain disease conditions. Components of apoptotic signaling pathways are also targets for drug discovery. To distinguish apoptosis from other mechanisms of cell death, one needs tools that to identify the signaling pathways involved. These should function in a wide variety of cell types and modes of growth, i.e., two-dimensional monolayer cell cultures and the more biologically relevant three-dimensional spheroids formed by many cell types under specific culture conditions. We present the results of cellular analysis performed on a number of cell lines relevant to drug discovery, using a new suite of EarlyTox™ cell viability assay kits optimized for use on SpectraMax® fluorescence microplate readers and ImageXpress® Micro imaging systems.

EARLYTOX CELL VIABILITY ASSAYS

EarlyTox Cell Viability Kits are a family of reagents for assessing cellular conditions, e.g., viability and apoptosis. Optimized primarily as homogeneous assays for fluorescence microplate readers, some of the assay kits can be used with cellular imaging systems.

Each assay follows the generalized workflow depicted below. Cells are seeded in microplates and treated experimentally. Reagents are then added and the plate is incubated. The results are detected using a fluorescence microplate reader or (for some assays) an imaging system.

Following are examples of how the different EarlyTox cell viability assays were used to assess viability and apoptosis in HeLa and other cells.

**EarlyTox Live/Dead Assay**

This assay uses separate markers for live and dead cells. Calcein AM is a green-fluorescent live-cell marker. Ethidium homodimer-III (EthD-III) is a red-fluorescent dye that stains the nuclei of dead cells. The kit is suitable for plate readers and imaging systems.

**EarlyTox Caspase-3/7 NuView™ 488 Assay Kit**

This assay enables detection of apoptosis in intact cell populations through the use of NuView 488 Caspase-3/7 substrate, which consists of a fluorogenic DNA dye coupled to the caspase-3/7 DEVD recognition sequence. Initially non-fluorescent, it permeates the cell membrane, and if the cell is apoptotic, the substrate is cleaved by caspase-3/7, releasing a dye that enters the nucleus and binds to DNA, resulting in bright green fluorescence. This magent is non-toxic to cells and can be used for kinetic studies of apoptosis on plate readers or imaging systems. A Masking Reagent provided in the kit reduces background to enable more accurate EC₅₀ determination on plate readers.

**EarlyTox Caspase-3/7 R110 Assay Kit**

This kit provides a single-step, homogenous assay that is specifically optimized for microplate readers. The fluorogenic substrate (Ac-DEVD-O-Me-R110) contains two DEVD consensus target sequences and is completely hydrolyzed in cell lysate by the enzymes in two successive steps. Hydrolysis of both DEVD peptides releases the green fluorescent dye rhodamine 110 (R110), resulting in a substantial fluorescent increase.

**EarlyTox Caspase-3/7 Glutathione Assay Kit**

This assay uses monoclonal antibody (MCA) to detect cellular GSH levels, which decline in the early stages of apoptosis. This blue fluorescent assay works in a homogeneous format on live cells and is suitable for both plate readers and imaging systems.

**HEPG2 SPHEROID ASSAY**

HEPG2 cells were seeded in a 96-well spheroid plate (Corning cat. 44520) at 1500 cells per well and allowed to grow and form spheroids for 48 hours, reaching an approximate diameter of 500 µm. They were treated with compounds for an additional 4 days. Spheroids were assayed for cell viability using the EarlyTox Live/Dead kit and imaged on the ImageXpress Micro Confocal High Content Imaging System. Whole spheroids were acquired in a single field of view using confocal-optics and a 10X objective.

**HEPG2 R110 & GLUTATHIONE ASSAYS**

**MCF-7, U937, JURKAT CELL VIABILITY**

Below are several examples of how the EarlyTox Live/Dead Assay was used to determine compound EC₅₀ values, as well as assess the effects of cytokine treatment on cell viability, in a variety of different cell lines. Both adherent (MCF-7) and suspension (Jurkat, U937) cells were tested.

**SUMMARY**

The EarlyTox Cell Viability suite of assay kits:
- Enables homogeneous work flows for faster data generation
- Works in a wide variety of cell types and culture conditions, including adherent monolayers, suspension cultures, and 3-D spheroids
- Are all optimized for use on SpectraMax fluorescence plate readers, with most also compatible with imaging systems (Live/Dead, NuView 488, and Glutathione)
- Includes pre-configured protocols with built-in analysis and curve plotting in SoftMax Pro Software