APPLICATION NOTE

Cytotoxicity assessment using automated cell imaging and live/dead assays

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Benefits

- **Utilize an efficient no-wash homogenous assay protocol to measure cell viability**
- **Quantify live or dead cells accurately**
- **Generate statistically relevant results quickly with preconfigured analysis modules**

Introduction

Live/dead assays are utilized in a wide variety of research applications including investigation of cytotoxic effects of various compounds, treatments, or changes in gene expression. Automated cellular imaging and analysis provides an optimal method to assess cell viability and cell death. In this application note, we describe the use of the ImageXpress® Pico Automated Cell Imaging System and CellReporterXpress Automated Image Acquisition and Analysis Software to image cells treated with EarlyTox™ Live/Dead Assay Kit.

The EarlyTox Live/Dead Assay Kit contains markers for mammalian live and dead cells. Viable cells are stained with an intense green fluorescence in the cytosol by calcein AM. Non-fluorescent Calcein AM permeates the intact cell membrane where the acetoxymethyl (AM) group is cleaved by intracellular esterases, yielding the fluorescent calcein molecule. The dead cell marker, Ethidium homodimer-III (EthD-III), is non-fluorescent and non-permeable to an intact plasma membrane. When cell membrane integrity is compromised in association with cell death, EthD-III enters the cell and binds to nucleic acids, resulting in a bright red fluorescence in dead cells. Cytotoxic events that affect cell membrane integrity can be accurately assessed using this method. The assay kit enables characterization of a full concentration-response profile of test compounds. The no-wash, homogeneous assay eliminates washing steps that can wash away dead and dying cells. Fluorescent signals from calcein and EthD-III can be detected and utilized to produce high quality images and analysis using the ImageXpress Pico system and CellReporterXpress software.

Materials

- EarlyTox Live/Dead Assay Kit
  - Explorer Kit (2-plate size, Molecular Devices P/N R8340)
  - Bulk Kit (10-plate size, Molecular Devices P/N R8341)
- HeLa cells (ATCC P/N CCL-2)
- HeLa media
  - Minimum Essential Medium complete media supplemented with glutamine and serum
- Staurosporine (Sigma P/N S5921)
- Mitomycin C (Sigma P/N M4287)
- 384-well black, clear-bottom microplates (Corning Falcon P/N 62406-490)
- ImageXpress Pico Automated Cell Imaging System and CellReporterXpress software

Methods

HeLa cells, plated at 5,000 cells/well into a black, 384-well clear-bottom microplate, were grown overnight in a 37°C, 5% CO_2 incubator. The cells were treated for 24 hours with staurosporine (general protein kinase inhibitor and potential anti-cancer therapeutic) or mitomycin C (potent DNA crosslinker and chemotherapeutic) in quadruplicates with a 1:3 serial dilution starting at highest concentrations of 10 µM staurosporine and 300 µM mitomycin C.

After the compound treatment, the cells were stained with the Live/Dead assay kit reagents in combination with Hoechst 33342 nuclear dye (Thermo Fisher). Half of the volume in each well was removed and replaced with a 2x stain solution of Calcein AM and EthD-III. The final concentrations of stains were 2 µM Calcein AM and 3 µM EthD-III. The plates were then incubated at 37°C, 5% CO_2 for 30 minutes prior to the addition of Hoechst (6 µM final concentration). The cells were incubated at 37°C, 5% CO_2 for an additional 15 minutes. Immediately after the final incubation, the plates were imaged on the ImageXpress Pico system using a 10X Plan Fluor objective and the FITC, Texas Red, and DAPI channels for imaging Calcein AM, EthD-III, and Hoechst dyes, respectively. At this magnification, one field-of-view can capture up to 4000–4500 cells in a single image, yielding statistically relevant results.

Image analysis using Cell Scoring module

Images were analyzed using the Cell Scoring analysis module in CellReporterXpress software. The module identifies and differentiates live or dead cells. The Hoechst staining was used to identify total cells, and then cells were scored positive or negative for the specific stains, Calcein AM or EthD-III. Figure 1 shows images of positive and negative controls treated with and without staurosporine and the associated analysis masks indicating positive and negative cells. Separate analyses were performed to define the numbers and percentages of live (Calcein AM-positive) or dead (EthD-III-positive cells) cells.

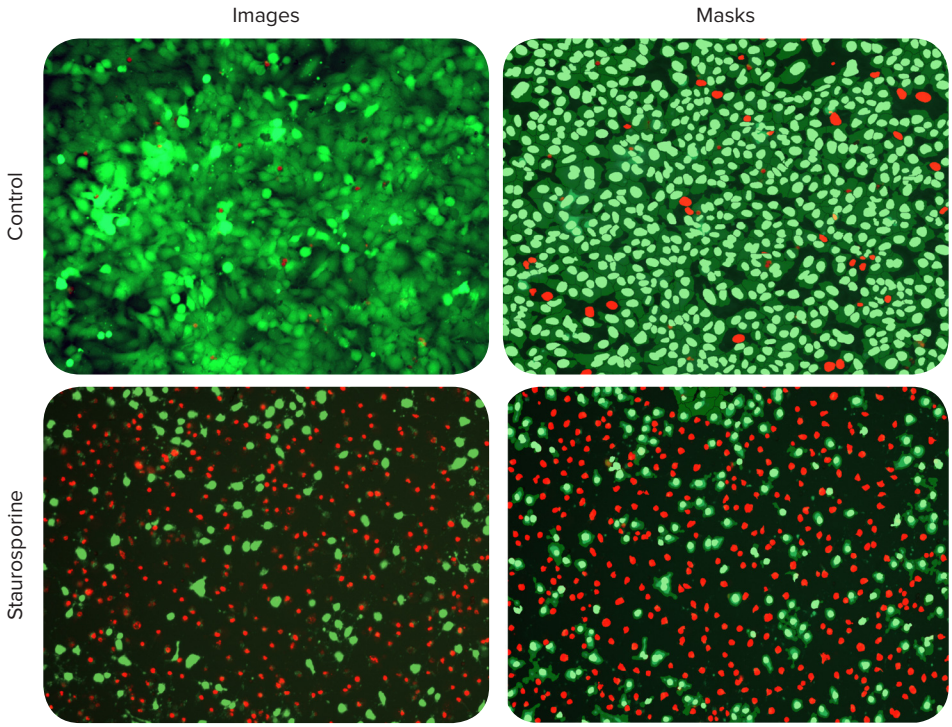

Figure 1. Representative images of negative control cells and cells treated with 0.1 µM of staurosporine. Left: 10x images of Hoechst nuclei stained (blue), Calcein AM-stained (green) and EthD-III-stained (red) HeLa cells. **Right:** Analysis masks show nuclei of live cells in green and nuclei of dead cells in red.

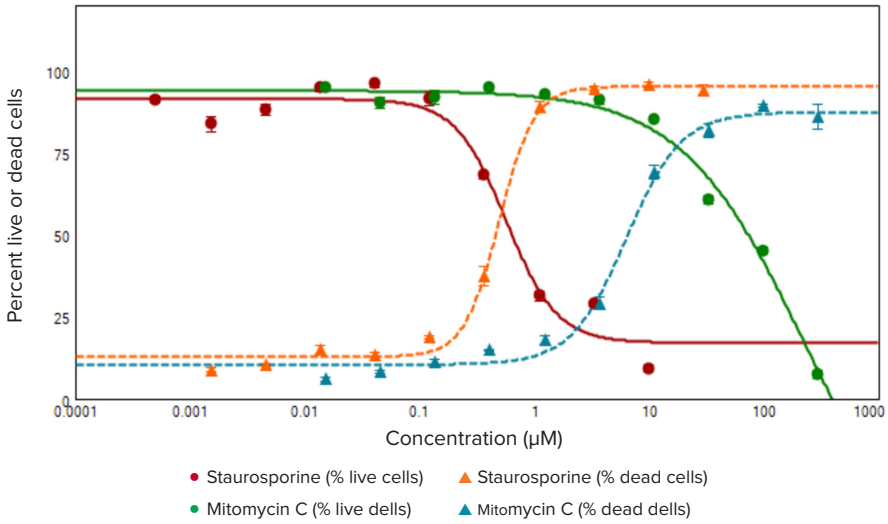

Figure 2. Concentration dependencies of the percentages of live and dead cells for HeLa cells treated with different concentrations of staurosporine or mitomycin C. Averages and standard deviations were derived from quadruplicates. The EC_{50} values produced by these curves are as follows: 0.569 µM staurosporine and 223 µM mitomycin C for % live cells, and 0.492 µM staurosporine and 6.305 µM mitomycin C for % dead cells.

EC_{50} toxicity calculation from dose-response curves

Live and dead cells were imaged, and quantitative cell scoring analysis was performed based on cells staining positive for either Calcein AM (green fluorescence) or EthD-III (red fluorescence) (Figure 1). Treatments of HeLa cells with staurosporine and mitomycin C both showed concentration-dependent increases in percentage of dead cells and decreases in percentage of live cells. Dose response curves displayed in Figure 2 plot the percentage of live cells versus compound concentration with EC_{50} values of 0.569 µM for staurosporine and 223 µM for mitomycin C. The percentage of dead cell curve produced EC_{50} values of 0.492 µM for staurosporine and 6.305 µM for mitomycin C.

Conclusion

The EarlyTox Live/Dead Assay Kit, in conjunction with the ImageXpress Pico system and CellReporterXpress software, enabled an accurate measurement of live and dead cells with an easy and efficient workflow. The automated imaging and quantitative analysis allows for the testing of cytotoxic compounds and is suited for assessment of cell viability for numerous biological assays.

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The ImageXpress Pico system features optics by Leica Microsystems.

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