APPLICATION NOTE

Measuring cell viability and cytotoxicity with the EarlyTox Cell Integrity Kit

Introduction

The EarlyTox™ Cell Integrity Kit is an optimized set of reagents that simplifies the identification of live and dead cells. It can be used to measure the effects of different treatments on cell viability and to evaluate toxic effects mediated through a variety of mechanisms, including apoptosis and necrosis. The kit is designed to work with many cell types, both adherent and non-adherent. The protocol's simple workflow and reagent performance make it amenable to high-throughput screening.

The assay uses two DNA-binding dyes, a cell-permeant Live Red Dye and a cell-impermeant Dead Green Dye. Live Red Dye enters the nuclei of all cells, regardless of membrane integrity, and produces a bright red fluorescent signal. Dead Green Dye only enters dead cells, whose membranes are compromised, producing a bright green fluorescent signal (Figure 1). Live and dead cells can easily be identified on the basis of the fluorescent signal(s) detected by the imaging system.

The SpectraMax® MiniMax™ 300 Imaging Cytometer and SoftMax® Pro Software offer a complete imaging and data analysis package that complements the EarlyTox Cell Integrity Kit. Using the two-color fluorescence imaging capability of the instrument and the cellular analysis features of the software, cell viability can be assessed in less than five minutes for a 96-well plate.

EarlyTox Cell Integrity assay setup

HeLa cells were seeded at 5000 cells per well in a 384-well black-wall, clear-bottom microplate. Cells were allowed to attach and grow for 24 hours, and then cytotoxic compounds were added to the wells. After 24 hours of compound treatment, the EarlyTox Cell Integrity Kit was used to assess cell viability per the product insert instructions. The assay can be run in a homogeneous format, eliminating media removal or wash steps that could cause cell loss and inaccurate results. An optional masking reagent may be used to reduce background from media or test compounds.

Benefits

- **Higher signal allows shorter exposure times for fast results**
- **Designed to work with many cell types**
- **Streamlined image acquisition and analysis on a single system**

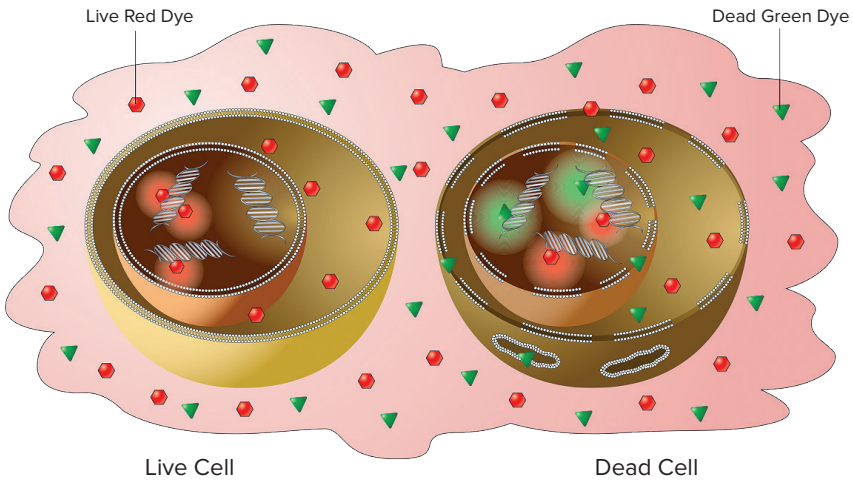


Figure 1. EarlyTox Cell Integrity Kit assay principle. Live and dead cells are distinguishable due to the properties of the two dyes used in the assay. Live Red Dye enters all cells and stains the nuclei red, while Dead Green Dye can only enter cells with compromised membranes, staining their nuclei green. Cells with only red fluorescence are detected and identified as live, while those exhibiting both red and green fluorescence are identified as dead.

Results

Cells were imaged with the SpectraMax MiniMax 300 Imaging Cytometer, using green (541 nm emission) and red (713 nm emission) channels (Figure 2A). All cell nuclei in the images were automatically identified by setting size and threshold for object identification in the red channel. Cells were counted as live or dead using the Classification feature in the software, which can distinguish between nuclei that label red only vs. nuclei labeled red and green (Figure 2B). A detailed set of output parameters provided by the software includes percentage of live cells for each well. IC_{50} curves were plotted using the 4-parameter curve fit in SoftMax Pro Software (Figure 3).

Conclusion

Used together with the SpectraMax MiniMax Imaging Cytometer, the EarlyTox Cell Integrity Kit offers a convenient way to determine live and dead cell populations by fluorescence imaging. Cytotoxicity mediated through a variety of cellular mechanisms can easily be monitored and quantified. SoftMax Pro Software distinguishes between live and dead cell staining patterns and plots the results automatically, saving time on analysis.

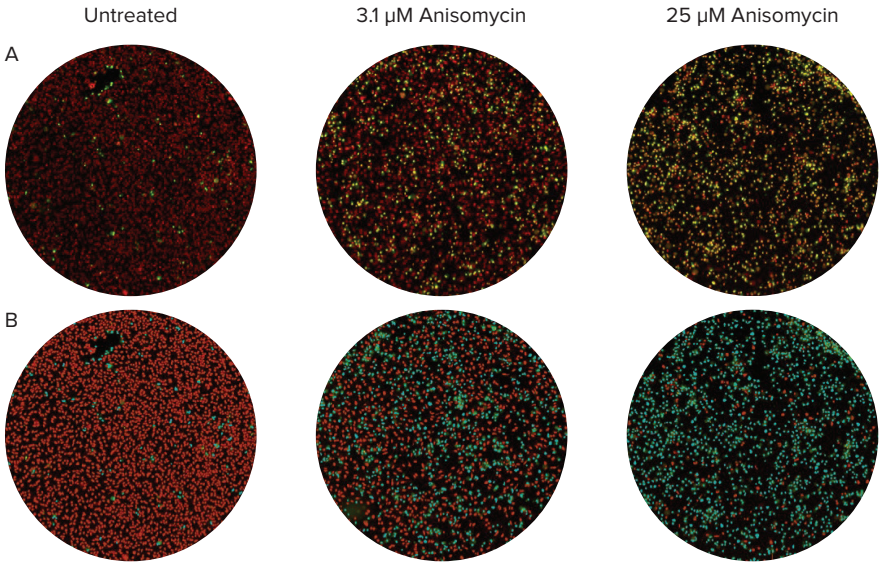


Figure 2. Analysis of untreated and compound-treated HeLa cells. A. Untreated cells (left panel) are mostly alive, with red fluorescent nuclei. At an intermediate concentration of compound (center panel), there is a mixture of live and dead cells. At high compound concentration (right panel), most cells are dead, with nuclei labeled both red and green. Images were acquired on the SpectraMax MiniMax 300 Imaging Cytometer. **B.** Cells were identified as live (red masks) or dead (blue masks) using the Classification feature in SoftMax Pro Software.

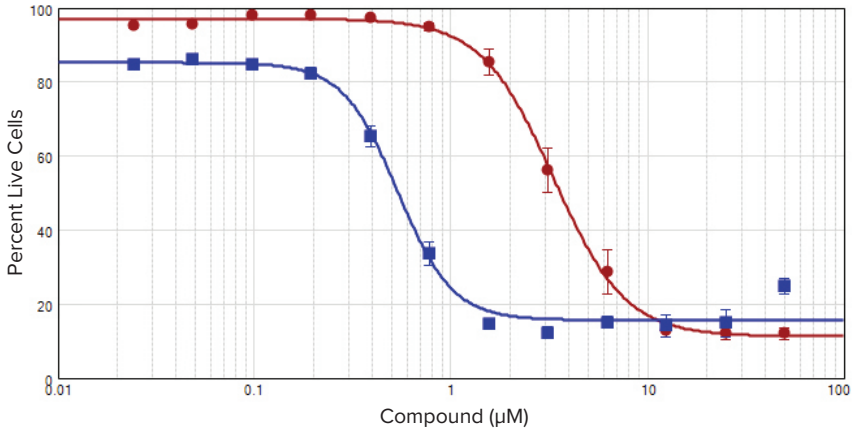
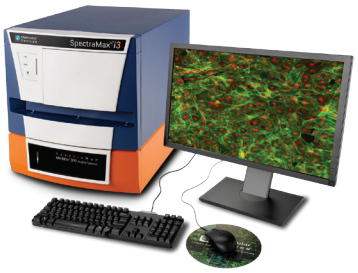


Figure 3. IC_{50} curves for cytotoxic compounds. HeLa cells were treated with anisomycin (red circles) or staurosporine (blue squares). Results were plotted using the 4-parameter curve fit in SoftMax Pro Software. IC_{50} for anisomycin was 3.3 µM, and IC_{50} for staurosporine was 0.53 µM; both values were consistent with published values.

Ordering information		
Reagent	**Description**	**Part number**
EarlyTox Cell Integrity Kit	(2) Reagent vials (one red, one green)* *Sufficient for (2) 96- or 384-well plates	R8213
EarlyTox Cell Integrity Kit	(2) Reagent vials (one red, one green)* *Sufficient for (10) 96- or 384-well plates	R8214
EarlyTox Live Green Dye	(1) Reagent vial* *Sufficient for (2) 96- or 384-well plates	R8215
EarlyTox Dead Green Dye	(1) Reagent vial* *Sufficient for (2) 96- or 384-well plates	R8216
EarlyTox Live Red Dye	(1) Reagent vial* *Sufficient for (2) 96- or 384-well plates	R8217

Compatible with these Molecular Devices systems



SpectraMax® MiniMax™ 300
Imaging Cytometer

ImageXpress® Micro XLS System

ImageXpress® Ultra System

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