Quantitation of Apoptosis, Necrosis and Cell Death Using High Content Screening

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Abstract

Apoptosis is involved in almost every physiologic and pathologic process in the body. Knowledge of the molecular mechanism of apoptosis has revealed new approaches for identifying small-molecule drugs that may effectively treat disorders including cancer, autoimmunity, stroke and osteoporosis. In addition, it is important to determine whether lead compounds are cytotoxic to normal cells in order to eliminate them early in the drug discovery process. We have developed a Live Dead application module and a Cell Health application module for high content screening on the Discovery-1™ High Content Screening System suitable for analysis of cell-based assays of apoptosis and necrosis.

A variety of commercially-available fluorescent probes have been developed to discriminate cells undergoing apoptosis from cells undergoing necrosis. Using Discovery-1, the different dyes can be visualized by their own unique fluorescence emission.

Both the Live Dead and Cell Health application modules use ABC (Adaptive Background Correction™) technology to segment cells from the background and handle uneven staining of samples. The functionality of this application was tested using multiple probes for apoptosis (both early and late) and necrosis. Samples were all acquired using Discovery-1 and analyzed using the new software modules.

Introduction

Cell based assays for cell health and cell death have become critical for drug discovery and development. Fluorescent probes for apoptosis and necrosis vary in selectivity, sensitivity and ease of use. Unfortunately, many of these probes do not provide good signal to noise or allow for differentiation of subpopulations of cells when run on a fluorescence plate reader. High content screening solves these problems by providing quantitative measurements on a cell by cell basis.

The Discovery-1 High Content Screening System was used to rapidly acquire and analyze fluorescent images of cells in multiple well plates. Cells were labeled with multiple fluorescent probes for apoptosis and cell death. The Live Dead and Cell Health application modules were used for automatic quantitative analysis of the images. The Live Dead module provides simple quantitative classification of cells using two fluorescent probes. The Cell Health module is designed for more comprehensive discrimination of living, apoptotic and dead via apoptosis or necrosis cells by using three fluorescent markers – one labeling all cells, one labeling apoptotic cells and one labeling dead cells. Cell-by-cell classification parameters and images are shown.

Materials and Method

Adherent Chinese Hamster Ovary (CHO-K1) cells were incubated with varying concentrations of staurosporine or DMSO for 6-12 hours prior to staining to induce apoptosis and necrosis and treated with the following probes prior to image acquisition and analysis on the Discovery-1 unit.

Live Dead

Figure 1 H33342 and PI. After treatment cells were stained with H33342 and PI diluted in PBS for 30-60 minutes @RT before image acquisition.

Cell Health

Figure 2a and 3a Molecular Probe's Vybrant #7 (H33342, PI, Yo-Pro-1). Probes in PBS were added to the media and incubated for 30 minutes at 4°C before image acquisition.

Figure 2b and 3b Molecular Probe's Vybrant #3 (H33342, PI, FITC-Annexin-V). After 45 minutes of incubation with Annexin-V and PI @RT, media was removed from the wells and replaced with 2% Paraformaldehyde containing H33342. Cells were incubated an additional 30 minutes at RT before image acquisition.

Figure 2c and 3c BIOMOL International's Mit-E-Ψ Mitochondrial Permeability Detection (H33342 + JC1) (courtesy of BIOMOL International) After 30 minutes of incubation with H33342 @ 37°C, media was replaced with JC-1 containing media (clarified by centrifugation) and incubated for 15 minutes at 37°C. Prior to image acquisition the media was replaced with warm 1X assay buffer.

Table 1: Fluorescent Probes Used

Fluorescent Probe	Optimum Wave-lengths (nm)	Compartment labeled	Cells labeled
Hoechst 33342 (H33342)	346/480	Nuclei	All nuclei however condensed chromatin (due to apoptosis or mitosis) labels more brightly.
YO-PRO-1	491/509	Nuclei	Apoptotic cells.
FITC Annexin V	496/578	Plasma Membrane	Apoptotic cells. Studies show that only ~30% of apoptotic actually label.
Propidium Iodide (PI)	535/617	Nuclei	Dead cells either through apoptosis or necrosis.
JC-1	498/525 or 585/595	Mitochondria	Labels green in apoptotic, red in non-apoptotic.

Figure 1: Cell Toxicity

Figure 1a.

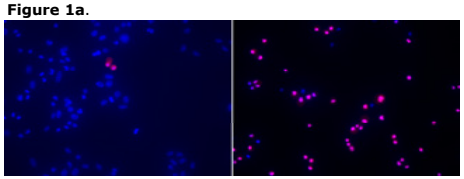
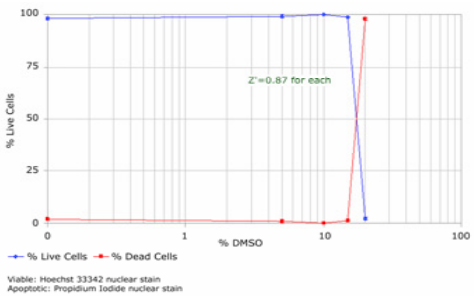


Figure 1b.



Figures 1a and 1b. Cells were imaged and analyzed using Discovery-1 and the Live Dead application module. Cells labeled with H33342 and PI. **Figure 1a**. Left: control, right: 20% DMSO. **Figure 1b**. Percentages of viable and non-viable cells were determined with increasing concentrations of DMSO.

Figure 2: Quantitation of Cell Health

Figure 2a. Vybrant #7 Cell Health assay.

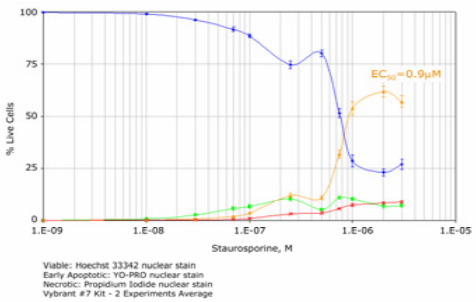


Figure 2b. Vybrant #3 Cell Health assay.

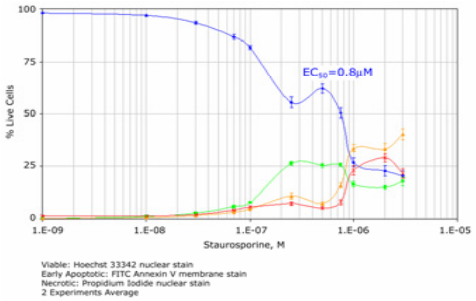
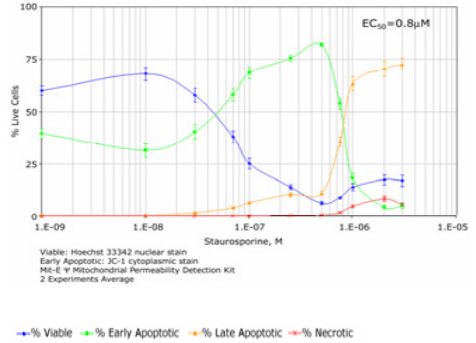


Figure 2c. Mit-E Ψ Cell Health assay.



Figures 2a, 2b, 2c. Cells were imaged and analyzed using Discovery-1 and the Cell Health application module. Percentages of viable, apoptotic and dead cells via apoptosis or necrosis were determined with increased concentrations of staurosporine.

Figure 3: Images of Cell Health Assay

Figure 3a. Vybrant #7 Cell Health assay images.

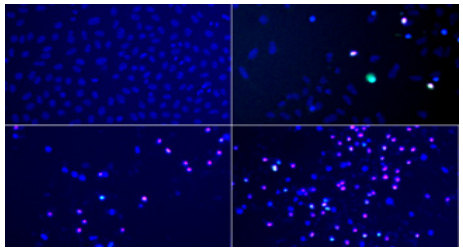


Figure 3b. Vybrant #3 Cell Health assay images.

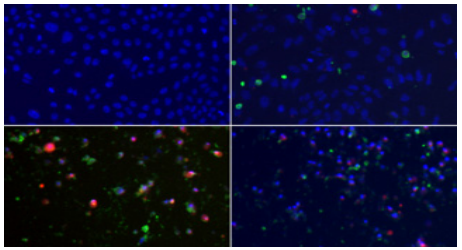
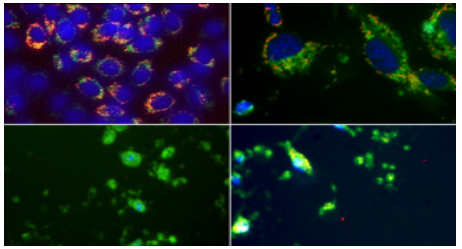


Figure 3c. Mit-E Ψ Cell Health assay images.



Figures 3a, 3b, 3c. Top left: control, top right: 0.1 µM staurosporine, bottom left: 1 µM staurosporine, bottom right: 3 µM staurosporine.

Conclusion

The Discovery-1 is an automated high content screening system that collects high quality fluorescent images of cells labeled with multiple fluorophores.

The Live Dead and Cell Health application modules can be used for quantitative analysis of cell viability. The modules may be used with extracellular, cytoplasmic or nuclear fluorescent probes to discriminate viable from non-viable cells and apoptotic from necrotic on a cell-by-cell basis.

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