TECHNICAL NOTE

Apoptosis detection using EarlyTox Caspase-3/7-D NucView 488 Assay Kit on ImageXpress Micro systems

Introduction

Apoptosis is an important mechanism signaling programmed death of cells in normal processes such as embryonic development, as well as in diseases including cancer and neurodegenerative conditions.

The EarlyTox™ Caspase-3/7 NucView 488 Assay Kits enable detection of apoptosis within intact cell populations through use of the NucView 488 Caspase-3 substrate. This substrate consists of a fluorogenic DNA dye coupled to the caspase-3/7 DEVD recognition sequence. Initially non-fluorescent, it permeates the cell membrane. 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. The cells may be imaged alive without wash steps which may remove dead or apoptotic cells but the dye is also retained after fixing.

This flexible assay can be performed using an ImageXpress® Micro High Content Imaging System and MetaXpress® Analysis Software for calculating the incidence of apoptotic cells in a well.

Materials

- EarlyTox Caspase-3/7-D NucView 488 Assay Kit (Explorer Kit Cat. No. R8350 or Bulk Kit Cat. No. R8351, DMSO formulation)
- CHO M1WT3 cells
- Camptothecin
- 384-well black, clear-bottom microplates (Corning Falcon)
- DRAQ5 nuclear stain
- ImageXpress Micro High-Content Imaging System

Assay method

CHO cells were plated at 3,500 cells per well in 50 µL per well in a 384-well tissue culture treated microplate. They were allowed to attach and grow overnight in a incubator at 37°C, 5% CO_2. Then, wells were treated for 24 hours with a 1:2 dilution series of camptothecin (anti-cancer drug) from 100 µM down to 0.1 µM to induce apoptosis.

A 10 µM 2X working solution of NucView 488 substrate was prepared in warm culture medium. 25 µL was carefully aspirated from each well and replaced with 25 µL of 2X substrate solution for a final concentration of 5 µM. Cells were incubated at 37°C for 1 hour after which a 6X solution of DRAQ5 nuclear stain was added to each well in 10 µL increments for a final concentration of 2 µM. After 30 minutes incubation, plates were read on the ImageXpress Micro system using the FITC and Cy5 filter sets and a 10X Plan Apo objective (Figure 1). At this magnification, one field-of-view typically captures 1,100-1,400 nuclei so that one image yields statistically relevant results.

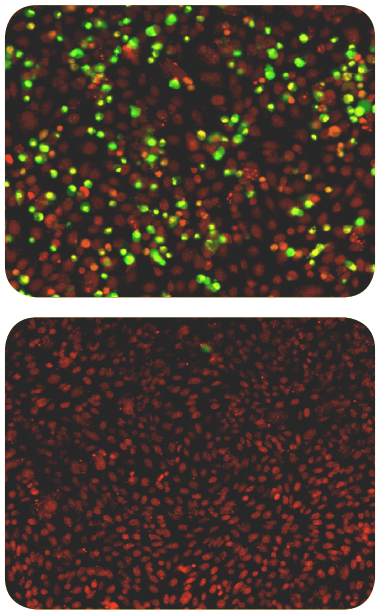

Figure 1. Image overlays of NucView Caspase 3/7 stained cells (green) and DRAQ5 nuclear stain (red). (Top) 50 µM camptothecin treated cells exhibit high incidence of apoptotic nuclei. **(Bottom)** Untreated controls show few nuclei with apoptotic staining.

Data analysis

Cells imaged on the ImageXpress Micro Imaging System were analyzed in MetaXpress software using the Cell Scoring module. With intuitive user input, the module identifies DRAQ5 stained nuclei for a total cell count and classifies Caspase 3/7 positive nuclei as apoptotic. Multiple parameters such as nuclear size, intensity, and percent apoptotic may be assessed. Wells with a cell count below a specific number can be omitted from analysis if gross toxicity in response to compound exposure interferes with the evaluation of apoptosis.

Results

Graphing the percent apoptotic cells vs. camptothecin concentration and applying a 4-parameter curve fit resulted in a concentration response curve with EC_{50} value of 5.7 µM (Figure 2). The EarlyTox Caspase-3/7 NucView 488 Assay Kit, used together with the ImageXpress Micro system and MetaXpress software, offers scientists a fast and robust image-based quantitation of apoptosis.

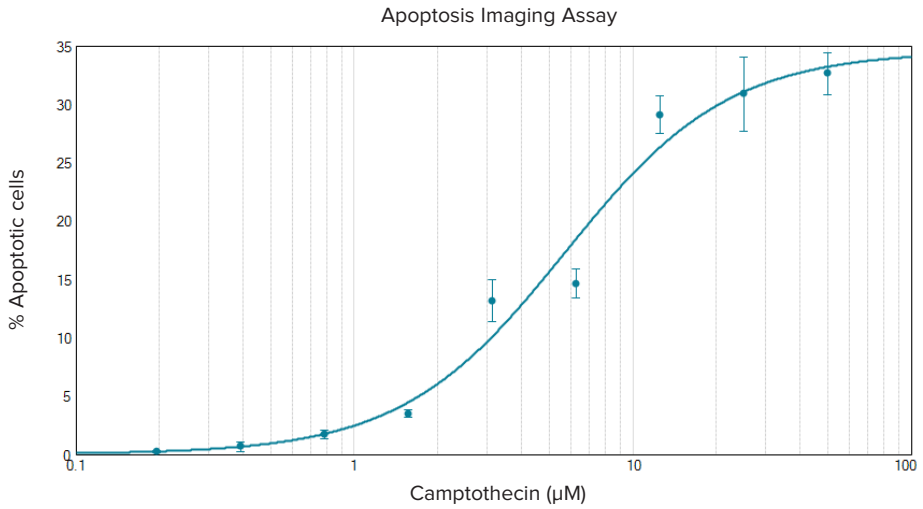

Figure 2. Dose-response curve for camptothecin-treated CHOm1 cells. Apoptotic cells (green fluorescent) and all cell nuclei (red fluorescent) were identified in the images and counted using MetaXpress software. A percentage of apoptotic cells is plotted. EC_{50} = 5.7 µM.

Compatible with this Molecular Devices system

ImageXpress Micro High-Content Imaging System

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