Flow cytometry analysis of dose response for apoptosis induction

Introduction

Dose response curves are a standard research technique used to evaluate therapeutic efficacy of cancer drugs. The use of flow cytometry enables accurate simultaneous evaluation of multiple analytes, in a statistically relevant sample size in a short time. This application note discusses the use of the Invitrogen™ Attune™ NxT Flow Cytometer with Autosampler to evaluate the effectiveness of several cancer drugs in inducing apoptosis in cancer cells, with dose response curves.

Jurkat cells, comprising human T lymphocyte cells used to study acute T cell leukemia, were grown and treated in 96-well plates with various concentrations of different cancer drugs as described below. These cells were evaluated for toxicity using an apoptosis reporter assay and analyzed on the Attune NxT Flow Cytometer. Cell toxicity was measured using the fluorogenic substrate Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent, which reacts with the enzymes caspase-3 and caspase-7, which are activated during apoptosis. The ability to add the CellEvent Caspase-3/7 Green Detection Reagent directly to the live cells in the medium without the need to wash, fix, or permeabilize the cells is ideal for this endpoint assay experiment. Flow cytometry is especially useful for this application, as it can quickly provide quantitative data on a statistically relevant number of cells at the single-cell level, across a large concentration range of samples, in a short time.

Materials

- Attune NxT Flow Cytometer (Cat No. A24858) with Autosampler (Cat. No. 4473928)
- Jurkat acute T cell leukemia cell line, clone E61 (ATCC™ TIB-152™ cells)
- 96-well U-bottom polystyrene tissue culture plates (Cat. No. 163320)
- Invitrogen™ CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit (Cat. No. C10427)
- Staurosporine (Cat. No. BP2541100)
- Apoptosis Inducer Set (EMD Millipore, Cat. No. APT800)
- Gibco™ Dulbecco's phosphate-buffered saline (DPBS), no calcium, no magnesium (Cat. No. 14190-144)

Methods

Jurkat cells were plated at 50,000 cells per well in a round-bottom, 96-well plate. Staurosporine, camptothecin, cycloheximide, or etoposide were added to individual wells to various final concentrations ranging from 100 µM to 2 nM (each in triplicate) in a final volume of 150 µL per well (Figure 1). Cells were incubated with the various drug concentrations at 37°C and in 5% CO_2 for 4 hours. After the 4-hour incubation, 10 µL of an 8 mM solution (16X stock solution) of the apoptosis indicator, CellEvent Caspase-3/7 Green Detection Reagent, was added to each well and incubated for an additional 30 minutes.

Data acquisition

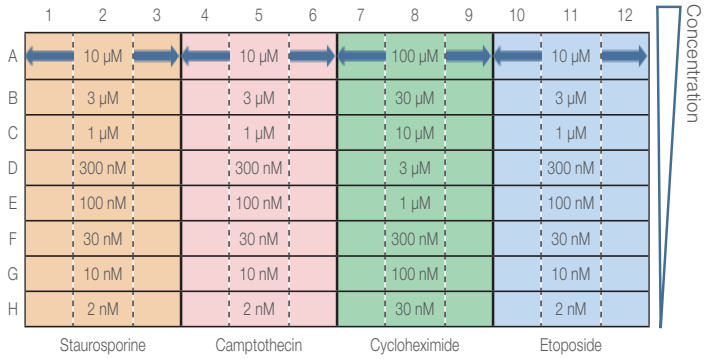

Figure 1. Plate setup showing triplicate treatments with four drugs at various concentrations.

Data on cellular apoptosis via caspase-3/7 activity were acquired on an Attune NxT Flow Cytometer (4-laser) with Autosampler with the Invitrogen™ Attune™ NxT Software included in the system. A total of 20,000 cells were acquired from each well at a speed of 200 µL/min. Each sample was mixed one time, with one rinse after each sample. Fluorescence from the CellEvent Caspase-3/7 Green Detection Reagent was detected in the BL1-H channel using 488 nm excitation and the 530/30 nm bandpass filter for detection. A forward scatter (FSC) threshold of 10,000 was used to eliminate debris. An FSC vs. side scatter (SSC) dot plot was created with a polygon gate around the main population of cells. This population was then displayed on a BL1-H fluorescence histogram with gates on the positive and negative peaks.

Data analysis

Data analysis was performed by placing two gates on the histogram for fluorescence of the CellEvent Caspase-3/7 Green Detection Reagent in the BL1-H channel—one around the negative peak and one around the positive peak. The percent total statistic was displayed as the gate label (Figure 2). The data below show that staurosporine is a potent initiator of apoptosis at high concentration (Figure 2A), while at low concentration minimal toxicity is observed (Figure 2B). Alternatively, data from the cells treated with etoposide (Figures 2C and 2D) show a low percentage of cell death at either concentration tested.

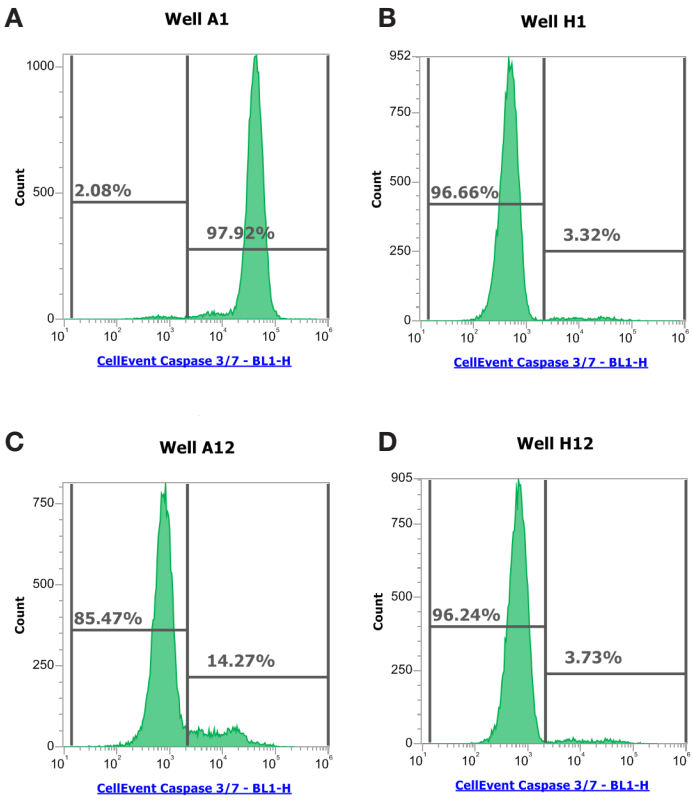

Figure 2. Representative histograms of the Jurkat T cell line treated with either a high or low dose of two different drugs. Cells were treated with **(A, B)** staurosporine or **(C, D)** etoposide as described in the "Methods" section. No gating was performed before cells were displayed on these histograms.

The Attune NxT Software allows for easy statistical analysis of the data, which can be easily exported to create dose response curves in a spreadsheet or graphics program. Statistics from the whole plate were exported as a single CSV file and analyzed using GraphPad Prism™ statistical software. The mean ± SD of the triplicates for each treatment condition are displayed in Figure 3.

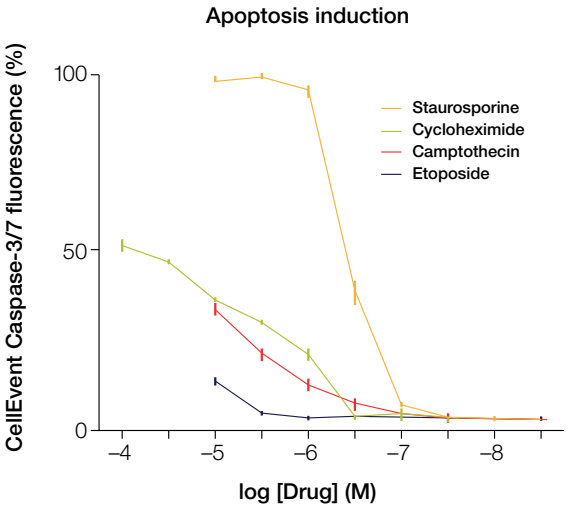

Figure 3. Data generated from the dose response curves from Jurkat cells treated with various concentrations of 4 different cancer drugs. Apoptosis was measured using CellEvent Caspase-3/7 Green Detection Reagent, which detects the cleavage and activation of the pro-apoptotic enzymes caspase-3 and -7. A range of 8 different concentrations of each drug was tested, with each condition tested in triplicate.

The "Heat Map" tab in Attune NxT Software was used to create a heat map displaying percentage of total cells in the CellEvent Caspase-3/7 Green–positive gate for a quick visual evaluation of the plate results (Figure 4).

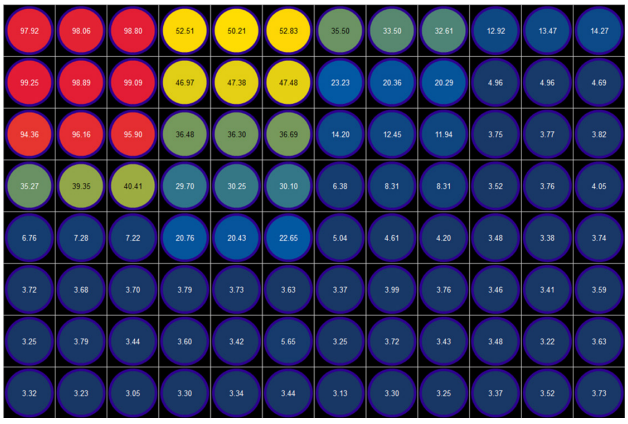

Figure 4. Heat map providing a quick visual evaluation of plate results. This view uses a range of colors along with a numerical value to indicate the percentage of cells in each well that were apoptotic, as indicated by a positive signal from CellEvent Caspase-3/7 Green Detection Reagent. The % positive is displayed in each well, and the color transitions from purple to red as the percentage increases.

Conclusion

This application note demonstrates an easy and effective way to perform the standard research technique of generating dose response curves to evaluate therapeutic efficacy of several cancer drugs, using the Attune NxT Flow Cytometer with Autosampler. Flow cytometry is an especially useful technique to evaluate drug effectiveness, as it quickly provides quantitative data at the single-cell level on a large sample size across a wide range of drug concentrations.

High-throughput flow cytometers with an automated plate acquisition device have grown in popularity, as the need to evaluate a higher number of samples has increased significantly. Conversely, use of flow cytometry has traditionally been limited in many high-throughput protocols due to a high propensity for clogging, depending on the sample type. With the exception of our Attune NxT Flow Cytometer, most flow cytometers cannot handle larger, clumpy cells, and end up with a system clog. This results in loss of samples, delay in timelines, and requiring hands-on monitoring of the instrument. The Attune NxT Flow Cytometer was developed with the goal of removing barriers to flow cytometry testing to enable new research; this includes bringing the power of flow cytometry to the high-throughput screening of cancer cells.

References

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