Dose–Response Studies Using Flow Cytometry
Cancer Biology Applications on the BD Accuri™ C6

Features

- Assess the effects of candidate compounds on cell viability
- Multiplex indicators of cellular status and function in the same assay
- Perform statistical analyses, and draw dose–response curves in a spreadsheet or graphics program

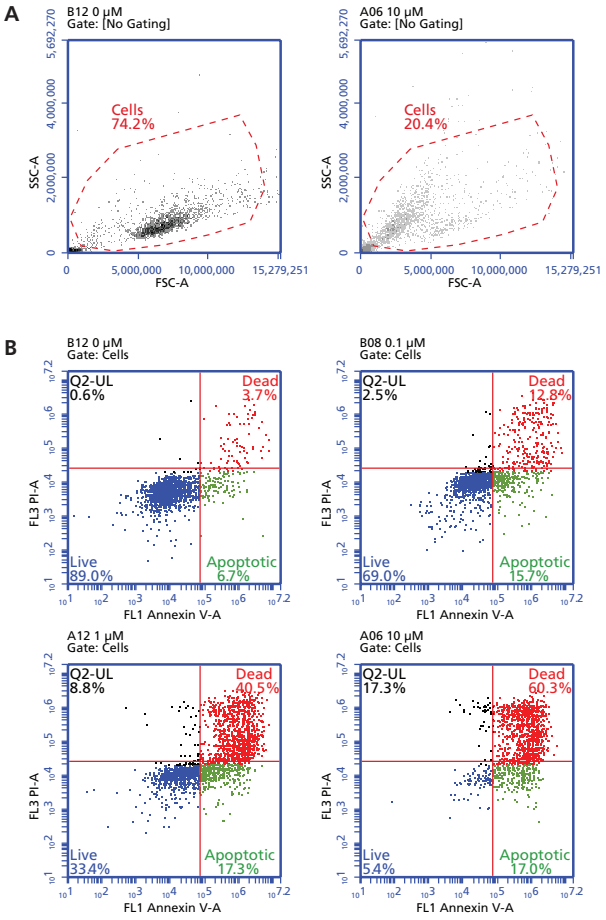

Figure 1. Dose–response analysis of live, apoptotic, and dead cells using flow cytometry

MDA-MB-231 cells (human epithelial breast adenocarcinoma; ATCC) were treated with varying doses of camptothecin (Sigma-Aldrich™); from 0.03 µM to 100 µM. Cells treated with vehicle only (DMSO) were used as a reference control. Cell culture supernatant was collected and pooled with cells disassociated with BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527) prior to staining with the BD Pharmingen™ Annexin V: FITC Apoptosis Detection Kit I (Cat. No. 556547). Data was acquired on a BD Accuri C6 flow cytometer and analyzed using BD Accuri C6 software. **Results: A**. Cells were initially gated based on light scatter properties. Upon treatment, dying cells shrink, as showed by reduced forward scatter (FSC) and increased side scatter (SSC). It is important to draw the cell gate to include both live and apoptotic/dead cells in the analysis. **B.** Cells were then analyzed for expression of Annexin V and PI to identify live (Annexin V^-PI^-, blue), apoptotic (Annexin V^+PI^-, green), and dead (Annexin V^+PI^+, red) cells after 48 hours of treatment. As shown in representative dot plots, live cells (blue) generally decreased at higher camptothecin doses, corresponding to an increase of apoptotic (green) and dead cells (red).

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One fundamental cancer research assay tests candidate compounds for their effects on tumor cells. Flow cytometry is especially well suited for dose–response studies because it can rapidly and accurately assess multiple markers simultaneously. In a single assay, you can assess a compound's effects on cellular viability, apoptosis, mitochondrial membrane potential, DNA damage, and/or proliferation.

Figure 1 shows an example dose–response experiment on the BD Accuri™ C6 personal flow cytometer using the MDA-MB-231 cancer cell line. Cells were treated for 48 hours with varying doses of camptothecin, which induces apoptosis by inhibiting the DNA enzyme topoisomerase I. Multiple parameters were assessed by flow cytometry. Specifically, apoptosis was measured using Annexin V, which detects externalization of phosphatidylserine molecules on the plasma membrane. Dead cells were identified using the DNA binding dye propidium iodide (PI), which enters cells with compromised cell membranes. Both reagents are included in the BD Pharmingen™ Annexin V: FITC Apoptosis Detection Kit I (Cat. No. 556547). Live cells were identified by excluding apoptotic and dead cells.

Results showed that higher doses of camptothecin exposure increased the percentages of both apoptotic and dead cells. Across the sequential plots in the figure, you can see the cells transition from live (Annexin V^-PI^-, blue) to apoptotic (Annexin V^+PI^-, green) to dead (Annexin V^+PI^+, red).

This experiment shows the kind of informative data you can generate using the BD Accuri C6 for dose–response studies. In contrast with conventional assays such as the tetrazolium dye MTT, which measures only viability, flow cytometry allows you to simultaneously assess multiple parameters and understand how candidate drug compounds might trigger different pathways of cell death.

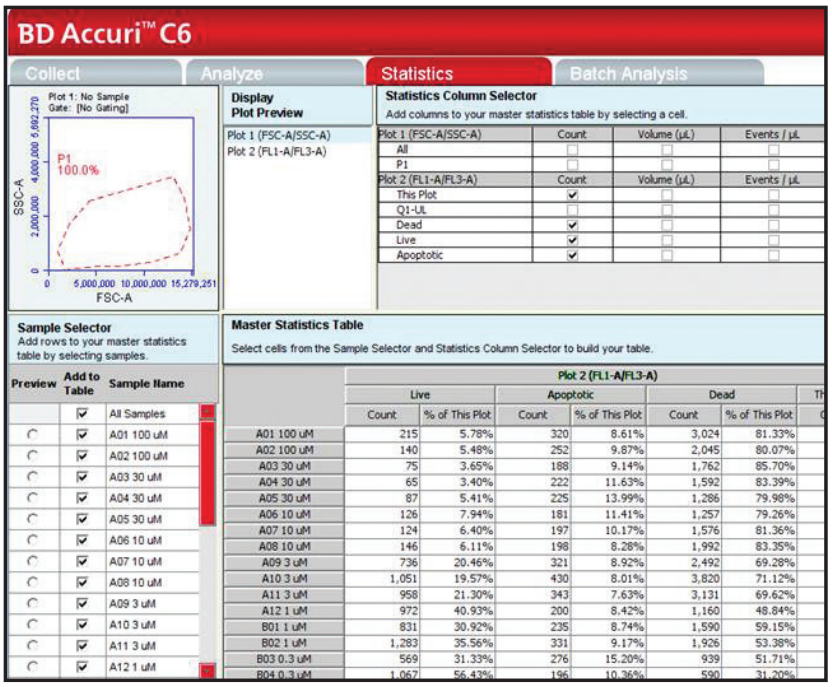
Figure 2 demonstrates how the data can be displayed and tabulated in BD Accuri™ C6 software. The software is designed to be intuitive and easy to learn and operate for both novice and experienced flow cytometry users.

Figure 3 shows a dose–response curve for this experiment. The software makes it easy to perform statistical analysis on the data, with which you can create dose–response curves in a spreadsheet or graphics program. In this experiment the IC_{50} value for camptothecin was 0.54 µM.

A personal flow cytometer in the lab provides many advantages for cell and cancer biology studies. When cells are ready for analysis or rare tumor samples arrive, it's crucial to have a flow cytometer at hand, ready to go.

BD

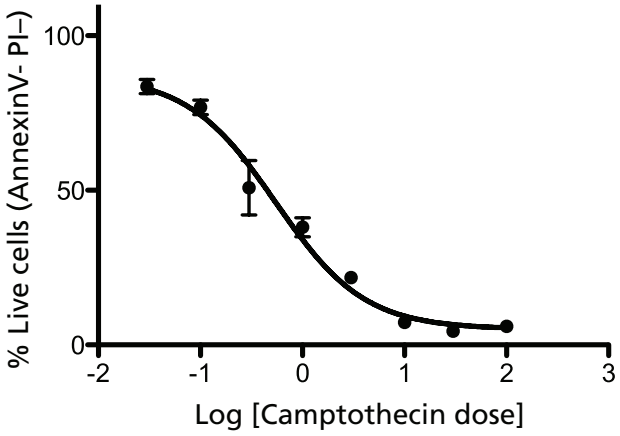
Dose–Response Studies Using Flow Cytometry



Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. A compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use. A nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory (Cat. No. 653124) offers automated sampling from 24-tube racks or multiwell plates.

Figure 2. Tabulated data on the Statistics tab

The Statistics tab of the BD Accuri C6 software allows for fast and simple statistical analysis. You can display and tabulate your data—in this case, the frequency and absolute count of live, apoptotic, and dead cells.



Camptothecin Concentrations
0.03 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM
30 µM
100 µM

Figure 3. Dose–response curve showing effects of camptothecin dosage on cell viability

The statistical data generated by the BD Accuri C6 software (Figure 2) was used to produce a dose-response curve in GraphPad Prism® Software (GraphPad™). The graph shows the frequency of live MDA-MB-231 cells after 48 hours of camptothecin treatment (as shown in Figure 1), plotted on the y axis as a percentage of live control cells treated with DMSO only. The logarithm of camptothecin dose was plotted on the x axis. **Results:** The relationship between camptothecin dose and cell viability, as measured by flow cytometry, resulted in a typical sigmoid curve.

Ordering Information

Description	Cat.No.
BD Accuri™ C6 Flow Cytometer System	**653118**
BD CSampler™ Automated Sampling System (optional)	**653124**
BD Pharmingen™ Annexin V: FITC Apoptosis Detection Kit I	**556547**
BD Pharmingen™ Annexin V: PE Apoptosis Detection Kit I	**559763**
BD™ Accutase™ Cell Detachment Solution	**561527**

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