Automating dose-response cell cycle analysis with the BD CSampler™ Plus

Cell and cancer biology applications for the BD Accuri™ C6 Plus flow cytometer

Features

- Easy-to-use automated sampling from tube racks or plates for walkaway convenience
- Maintain both adherent and non-adherent cells in suspension over lengthy experiments
- Culture, prepare, run and analyze multiple samples in the same plate
- Ideal for dose-response studies involving many samples
- New BD Pharmingen™ EdU Click Proliferation Kits reduce preparation steps for cell cycle analysis

Figure 1. BD CSampler™ Plus automatic sampling accessory

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 cell cycle, cellular viability, apoptosis, mitochondrial membrane potential, DNA damage and/or proliferation. With the BD Accuri™ C6 Plus personal flow cytometer, you can perform dose-response studies right on your benchtop.

Dose-response studies can be time-consuming and labor-intensive, however, because of the sheer number of samples to process. The BD CSampler Plus automatic sampling accessory (Figure 1) can help. This option offers reliable, easy-to-use automation while adding minimal footprint to the BD Accuri C6 Plus. The BD CSampler Plus supports:

- 48-well plates
- 96-well plates
- 96-deep-well plates
- 24-tube rack (included) for standard 12 x 75-mm tubes

You can count on the BD CSampler Plus to keep your cells in suspension over the course of your dose-response experiment. Figure 2 shows that counts of Jurkat and HeLa cells remained consistent across runs of both a 24-tube rack and a 96-well plate. You can select up to three agitation cycles per well, as well as up to three wash cycles to minimize cross-well contamination.

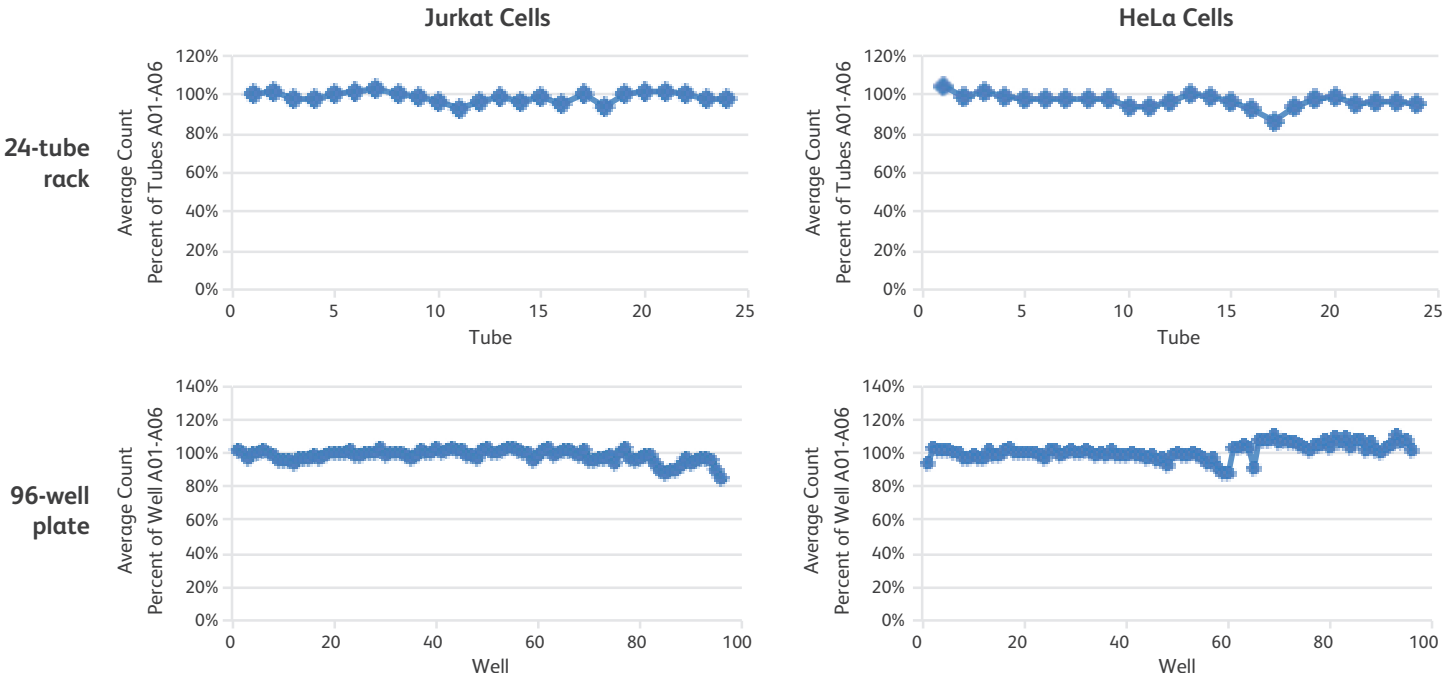

Figure 2. Consistent counting of Jurkat and HeLa cells using the BD CSampler Plus

Live Jurkat (left) or HeLa (right) cells were loaded into the BD CSampler Plus 24-tube rack (top) or 96-well round bottom plate (bottom) at 1 x 10^6 cells/mL in 500 µL (tubes) or 200 µL (well plate) total volume of BD Pharmingen™ Stain Buffer (FBS, Cat. No. 554656). Immediately after loading, tubes or plates were acquired on a BD Accuri C6 Plus with the BD CSampler Plus for 30 seconds per well at a high flow rate and agitation between each well, and analyzed using BD Accuri™ C6 Plus software. **Results:** Graphs show tube or well count as a percentage of the average of the first six tubes or eight wells, and data is averaged between two instruments. Sample-to-sample variation within one instrument was <20% in all conditions.

Figure 3 shows a typical dose-response experiment analyzing the effects of nocodazole administration on the cell cycle in two types of cancer cells. Nocodazole is an antineoplastic agent that interferes with the polymerization of microtubules, causing cells to arrest in the G_2 or M phase of the cell cycle. Cell cycle was assessed by staining with the DNA dye propidium iodide (PI) and the thymidine analog ethynyl-deoxyuridine (EdU), which is incorporated into newly synthesized DNA during the S phase. Results allow segmentation of cells in the G_0/G_1 phase, S phase and G_2/M phase. In this multiplexed assay, an antibody against phosphohistone H3 (pHH3) was added to further differentiate M-phase cells from cells in other phases.

The dot plots show cell cycle analyses for untreated MDA-MB-468 breast cancer cells (Figure 3A) as well as a representative sample of cells treated with 100 nM of nocodazole (Figure 3B). As expected, many treated cells were arrested in the M phase, showing more than a 10-fold increase over untreated cells. The plots also showed more than a three-fold increase in G_2 phase cells, with corresponding decreases in other cell cycle phases.

Figure 3C shows dose-response cell cycle curves for both breast cancer cell lines. The two cell lines responded similarly to nocodazole, although M-phase arrest was apparent in MDA-MB-231 cells at a lower dosage (30 nM) than MDA-MB-468 cells (100 nM). The reduction in M-phase cells at high dosages can be attributed to cells arresting earlier in G_2, thus never progressing to M, and to loss due to cell death.

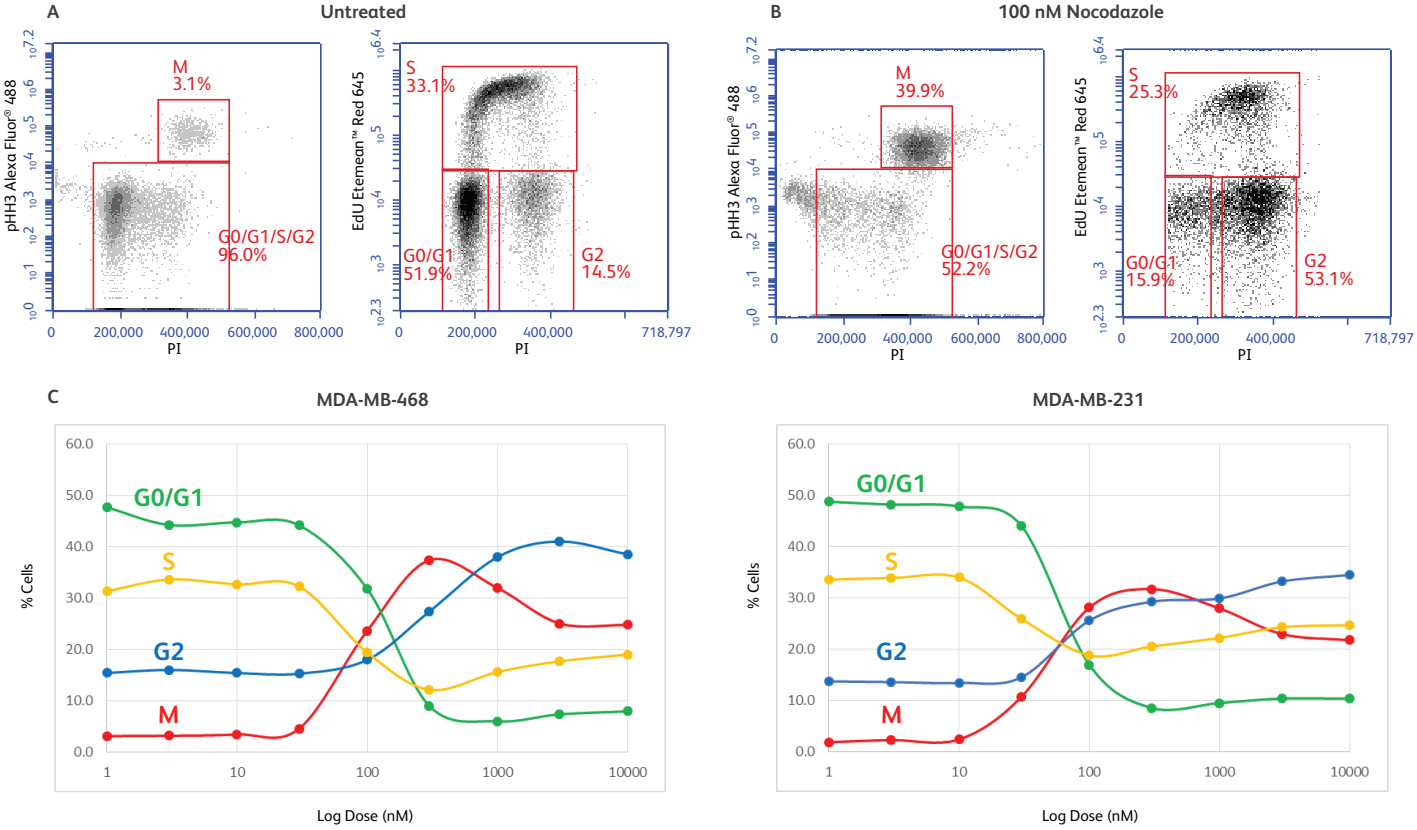

Figure 3. Dose-response effects of nocodazole on the cell cycle for two breast cancer cell lines

MDA-MB-468 and MDA-MB-231 breast cancer cells were treated with escalating doses of the microtubule depolymerizing agent nocodazole (Sigma; 1–10,000 nM) for 16 hours. Cells were then pulsed with 10 µM of EdU for 1 hour, harvested from culture, stained for EdU content using the BD Pharmingen 647 EdU Click Proliferation Kit (Cat. No. 565456), subsequently stained with BD Pharmingen Alexa Fluor® 488 Rat anti-Histone H3 (pS28, Cat. No. 558610), and then stained for DNA content with BD Pharmingen Propidium Iodide Staining Solution (Cat. No. 556463) and 0.1 mg/mL of RNase A. Cells were acquired in a 96-well plate using a BD Accuri C6 Plus and BD CSampler Plus, and analyzed using BD Accuri C6 Plus software. **Results: A.** Cell cycle analysis of untreated MDA-MB-468 cells. M-phase cells were identified as $pHH3^+$ (left plots). G_0/G_1, S and G_2-phase cells were then discriminated based on EdU and DNA content (right plots), where G_0/G_1-phase cells are EdU^-/2N, S-phase cells are EdU^+/2N-4N and G_2-phase cells are EdU^-/4N. **B.** A representative cell cycle analysis of MDA-MB-468 cells treated with 100 nM of nocodazole showed an increase of cells in the M and G_2 phases. **C.** Percentages of each cell cycle phase were plotted against nocodazole concentration to identify cell cycle changes with nocodazole treatment. As nocodazole dosage increased, MDA-MB-468 cells (left graph) began to arrest in the G_2 and M phases, with a concurrent reduction in cells in the G_0/G_1 and S phases. Arrest in the G_2 and M phases showed slightly different concentration kinetics, with cells arresting into the M phase at lower nocodazole concentrations than into the G_2 phase. The decrease in M-phase cells at high concentrations of nocodazole can be attributed to cells arresting earlier in G_2, thus never progressing to M, and to loss due to cell death. Similar kinetics were observed for MDA-MB-231 cells (right graph), although M-phase arrest was apparent at a lower concentration than in MDA-MB-468 cells (30 nM and 100 nM, respectively).

With the BD CSampler Plus, you can culture, prepare, run and analyze the samples in the same plate, without transferring them to individual tubes. Besides streamlining experimental workflow, automation offers walkaway convenience at runtime, allowing you to focus on science instead of experiment management.

The EdU kit used in this experiment also streamlined and accelerated its workflow. By employing click chemistry, the EdU kit can perform cell cycle analysis with fewer preparation steps than bromodeoxyuridine (BrdU), a widely used thymidine analog. For maximum panel design flexibility, BD offers EdU kits for both the blue and red lasers.

Easy to use, simple to maintain and affordable, the BD Accuri C6 Plus personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors and four fluorescence detectors. A compact and transportable design, fixed laser alignment, pre-optimized detector settings and automated instrument QC result in a system that is simple to use.

Ordering information	
Description	Cat. No.
BD Accuri™ C6 Plus Flow Cytometer System	660517
BD Accuri™ C6 Plus Workstation Computer and Software	661391
BD CSampler™ Plus Automated Sampling System (optional)	660519
BD Pharmingen™ 647 EdU Click Proliferation Kit	565456
BD Pharmingen™ 488 EdU Click Proliferation Kit	565455

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