BD Apoptosis, DNA Damage and Cell Proliferation Kit and Template

Cell Status Assays on the BD Accuri™ C6 Flow Cytometer

Features

- Preconfigured kit, protocol, and software template
- Assess apoptosis, DNA damage, and cell proliferation on the BD Accuri C6
- Support studies involving cleaved PARP, H2AX, and BrdU
- Conserve precious sample by measuring multiple parameters in a single tube
- Enable quick and easy setup and analysis using the BD Accuri C6

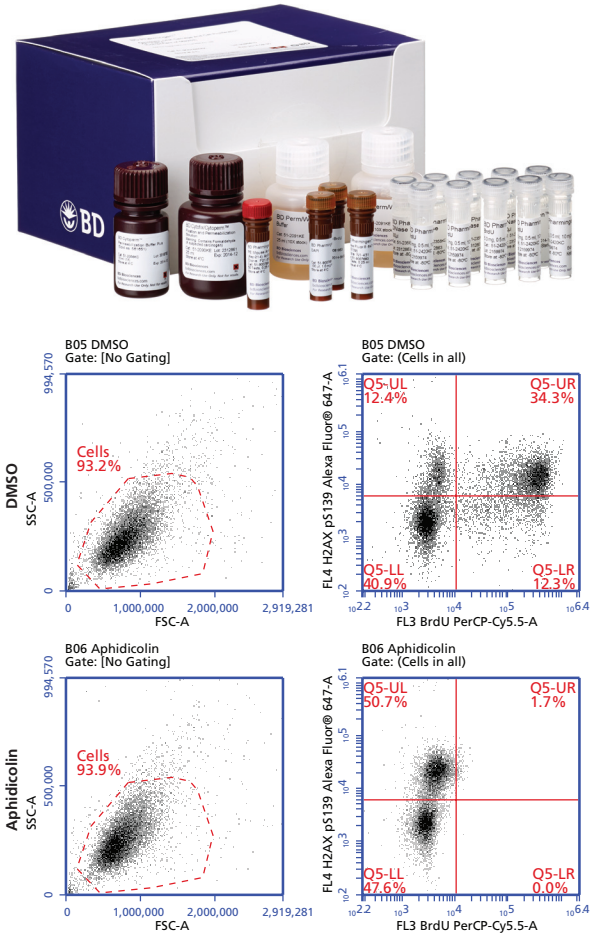

Figure 1. Analysis of cell proliferation on the BD Accuri C6

Jurkat cells (human T-cell leukemia: ATCC TIB-152) were treated with compound vehicle (DMSO) or the S-phase inhibitor aphidicolin (3 µg/mL) for 4 hours at 37°C. BrdU (10 µM) was added from the BD Pharmingen Apoptosis, DNA Damage and Cell Proliferation Kit (Cat. No. 562253) during the last hour of treatment. The cells were harvested and stained according to the kit instructions, acquired on a BD Accuri C6 using the kit template, and analyzed using BD Accuri C6 software. **Results:** A light scatter gate (Cells) was drawn around the cell population (left plots). With DMSO treatment alone (upper plots), 46.6% of the cells were proliferating ($BrdU^+$, UR and LR). After treatment with aphidicolin (lower plots), proliferating cells ($BrdU^+$, UR and LR) all but vanished, while the percentage and signal intensity of H2AX expression remained similar to the control.

Visit **bdbiosciences.com** for more information.

The **BD Pharmingen™ Apoptosis, DNA Damage and Cell Proliferation Kit** (Cat. No. 562253), protocol, and software template for the BD Accuri™ C6 flow cytometer simplify the simultaneous analysis of important cellular states in human samples. The kit, which supports studies using bromodeoxyuridine (BrdU), phosphorylated H2AX, and cleaved Poly (ADP-ribose) polymerase-1 (PARP), includes fluorescent antibodies, buffer systems, and other reagents needed for acquisition and analysis. A BD Accuri™ C6 software template matched to the kit includes a predefined workspace, markers, regions, gates, and parameter names for quick and easy setup and analysis.

Figures 1 and 2 show data on the BD Accuri C6 using the preconfigured kit and software template.

Many factors, including stress, radiation, environmental exposure, and treatment with small molecules, can lead to changes in apoptosis, DNA damage, cell proliferation, and other cellular events. The kit uses three antibodies to detect these events. It identifies proliferating cells with antibodies to BrdU, which has previously been incorporated into the cells' newly synthesized DNA. It identifies damaged DNA with antibodies to H2AX, which is rapidly phosphorylated at DNA strand breaks (and less rapidly at replicating DNA sites). Finally, the kit identifies apoptotic cells with antibodies to a PARP fragment, cleaved from PARP by caspase-3 in the early phases of apoptosis.

To use the kit, incubate the cells with BrdU (included) to incorporate it into proliferating cells. Then, stain the cells for cell surface markers, if desired. Fix and permeabilize the samples and treat them with DNase, which helps to expose the BrdU epitopes. Stain the cells simultaneously with fluorochrome-labeled anti-BrdU, cleaved PARP, and H2AX. Finally, resuspend the cells in staining buffer and analyze them on the BD Accuri C6 using the free software template.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer (Cat. No. 653118) 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.

BD Apoptosis, DNA Damage and Cell Proliferation Kit and Template

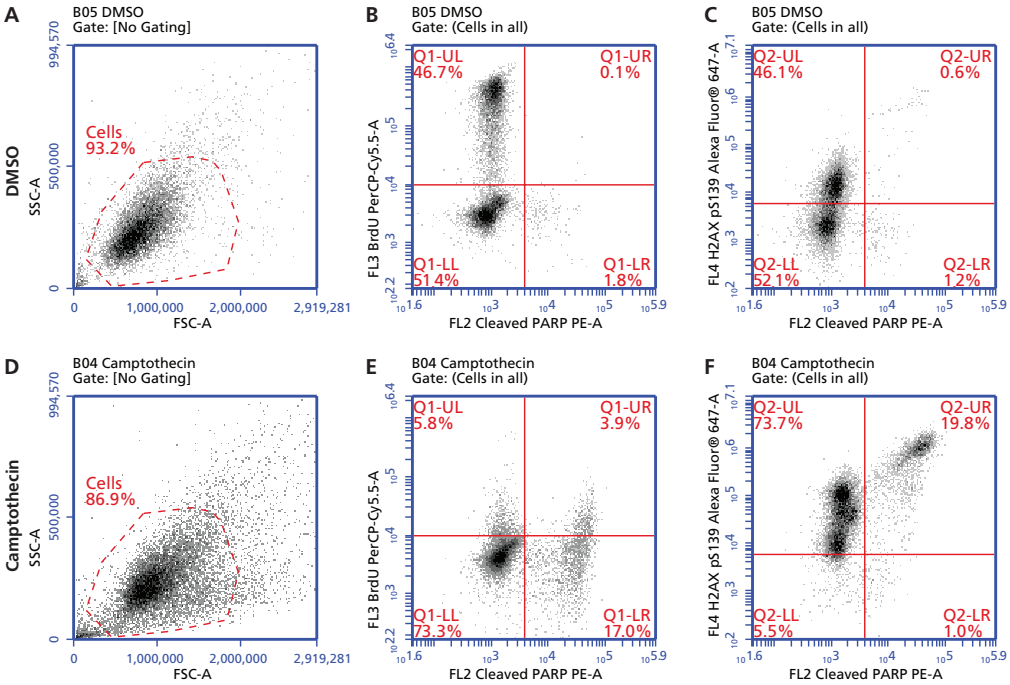

Figure 2. Analysis of apoptosis, DNA damage, and cell proliferation on the BD Accuri C6

Jurkat cells (human T-cell leukemia: ATCC TIB-152) were treated with compound vehicle (DMSO) or the topoisomerase I inhibitor camptothecin (6 µM) for 4 hours at 37°C to induce apoptosis. BrdU (10 µM) was added from the BD Pharmingen Apoptosis, DNA Damage and Cell Proliferation Kit (Cat. No. 562253) during the last hour of treatment. The cells were harvested and stained according to the kit instructions, acquired on a BD Accuri C6 using the kit template, and analyzed using BD Accuri C6 software. **Results:** With DMSO treatment alone (upper plots), approximately 46.7% of the cells were proliferating (BrdU$^+$, plot B) and very few were apoptotic (cleaved PARP$^+$, plots B and C). The DMSO control had a low signal intensity of H2AX expression with 46.1% of the non-apoptotic cells staining positive (plot C). After treatment with camptothecin (lower plots), apoptotic cells (cleaved PARP$^+$, plots E and F) increased and proliferating cells (BrdU$^+$, plot E) decreased, as expected. Among non-apoptotic cells, H2AX expression increased both in percentage (73.7%) and signal intensity (plot F), indicating DNA damage.

Ordering Information

All kits and their associated software templates are available at **bdbiosciences.com/go/templates**.

Description	Clone	Quantity	Number of Tests	Cat. No.
BD Pharmingen™ Apoptosis, DNA Damage and Cell Proliferation Kit, containing:				
BrdU PerCP-Cy™5.5	3D4	250 µL	50 tests	**562253**
H2AX (pSer139) Alexa Fluor® 647	N1-431	250 µL		
Cleaved PARP (Asp214) PE	F21-852	250 µL		
BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution		25 mL		
BD Perm/Wash™ Buffer		25 mL		
BD Cytofix/Cytoperm™ Plus Permeabilization Buffer		10 mL		
DAPI (not used with BD Accuri C6)		100 µL		
BrdU solution		0.5 mL		
DNAse		300 µL		

Related Products

Description	Cat. No.
BD Pharmingen™ Annexin V Apoptosis Detection Kit	**556570 (FITC) 559763 (PE)**
BD Pharmingen™ BD™ MitoScreen (JC-1) Kit	**551302**
BD Pharmingen™ Active Caspase-3 Apoptosis Kit	**550480 (FITC) 550914 (PE)**
BD Pharmingen™ BrdU Flow Kit	**559619 (FITC) 552598 (APC)**
BD Cycletest™ Plus DNA Reagent Kit	**340242**
BD Accuri™ C6 Flow Cytometer System	**653118**
BD CSampler™ Automated Sampling System	**653124**

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

23-16738-00

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