Apoptosis, DNA Damage, and Cell Proliferation Kit

Features

- Useful for determining the effects of small molecules, radiation, and other environmental stressors on cell cycle, proliferation, apoptosis, and DNA damage
- Conserve precious sample with the measurement of multiple parameters in a single tube
- Tested on both human and mouse samples
- Compatible with most flow cytometers with two or more lasers such as the BD Accuri® C6 and the BD FACSCalibur™*

Table 1. Kit Contents.

Name	Clone	Format	Laser	Purpose
Anti-BrdU	3D4	PerCP-Cy™5.5	Blue	Detection of cell proliferation
Anti-H2AX (pS139)	N1-431	Alexa Fluor® 647	Red	Detection of DNA damage
Anti-Cleaved PARP (Asp214)	F21-852	PE	Blue	Detection of apoptosis
DAPI (optional)	—	—	UV	Determination of DNA content

This kit also contains BrdU, DNase, buffers, and a detailed protocol.

Many factors including stress, radiation, environmental exposure, and treatment with small molecules can lead to changes in cell cycle, apoptosis, DNA damage, and cell proliferation. The Apoptosis, DNA Damage, and Cell Proliferation (ADDCP) Kit contains key markers for the simultaneous determination of these important cellular states by multicolor flow cytometry saving time and samples, and improving experimental results.

Detection of Cell Proliferation by BrdU

Bromodeoxyuridine (BrdU) is an analog of the DNA precursor thymidine. When cells are incubated in the presence of BrdU, the molecule is incorporated into newly synthesized DNA and can be detected with antibodies against BrdU. Thousands of scientific papers have been published using BrdU incorporation to measure cell proliferation.[1]

Determination of DNA Damage Using Phosphorylated H2AX

H2AX is a member of the histone H2A protein family. When DNA strand breaks occur, H2AX is rapidly phosphorylated at serine 139 by ataxia telangiectasia mutated (ATM), ATM-Rad-3-related (ATR), DNA protein kinase (DNA-PK), and potentially other proteins. Hundreds to thousands of H2AX molecules are phosphorylated per double-stranded DNA break.

Phosphorylation of H2AX leads to the recruitment of DNA damage repair proteins at the site of DNA damage. It has been reported that detection of phosphorylated H2AX by flow cytometry is significantly more sensitive for the detection of DNA damage than the single-cell gel electrophoresis (comet) assay.[2]

Measurement of Apoptosis with Cleaved PARP

Poly (ADP-ribose) polymerase-1 is a 116 kDa enzyme involved in DNA repair and maintenance of genomic integrity. During the early phases of apoptosis (programmed cell death), caspase-3 is activated by cleavage. Caspase-3, in turn, cleaves PARP into 24- and 89-kDa fragments, thereby inactivating it.[3] Clone F21-852 specifically recognizes the 89-kDa cleaved fragment and does not recognize intact PARP. Detection of cleaved PARP by flow cytometry is routinely used for the study of apoptosis.

How the Kit Works

Cells are in vitro–labeled, or mice can be in vivo–labeled with BrdU. After labeling, cells can be stained with cell surface markers, if desired. Samples are then fixed, permeabilized, and treated with DNase. The DNase treatment helps to expose the BrdU epitopes. Following this treatment, cells are simultaneously stained with fluorochrome-labeled anti-BrdU, cleaved PARP, and H2AX. DAPI staining can be performed at this step to determine DNA content. Cells are resuspended in staining buffer and analyzed by flow cytomtery.

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

*Detection of DAPI requires excitation by a UV laser.

BD

Apoptosis, DNA Damage, and Cell Proliferation Kit

References

1. Li C. Specific cell cycle synchronization with butyrate and cell cycle analysis. *Methods Mol Biol*. 2011;761:125-136.
2. Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. *In Vivo*. 2008;22:305-309.
3. Krishnakumar R, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. *Mol Cell*. 2010;39:8-24.

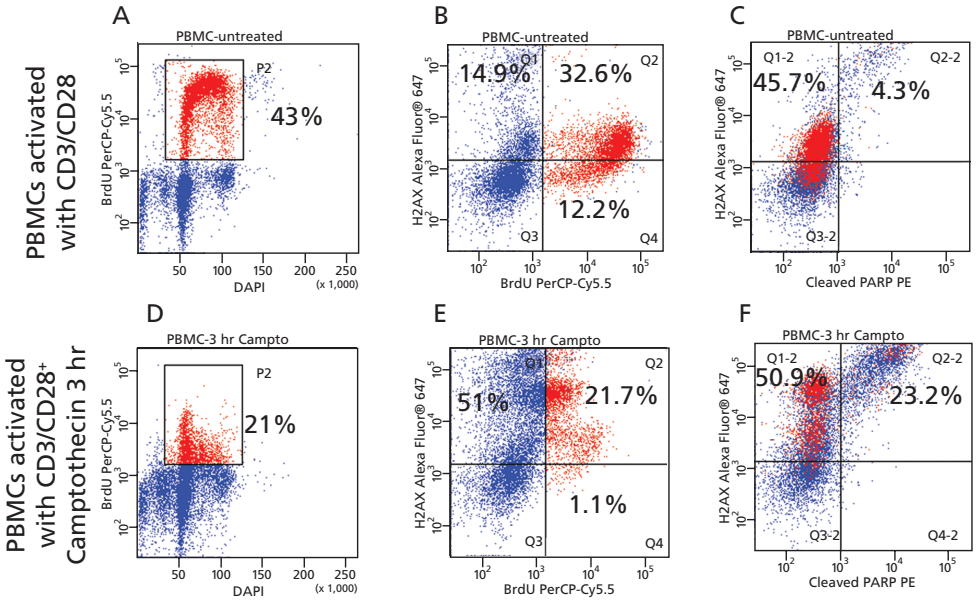

Figure 1. PBMCs were stimulated with Anti-CD3/CD28–coated Dynabeads® for 3 days, then harvested and washed, and then replated with either 5 mM of camptothecin or left untreated as controls. The treated cell group was cultured for 3 hours with camptothecin, then washed and replated for an additional 2 hours allowing cells to recover. The untreated group was cultured for 5 hours. Both groups (control and treated) were pulsed with 50 mM of BrdU during the final 1 hour of culture. Cells were harvested, washed with staining buffer, then fixed then analyzed using the Apoptosis, DNA Damage, and Cell Proliferaiton Kit (Cat. No. 562253).

Figures A–C are the untreated control group and figures D–F are the camptothecin-treated group. From the BrdU vs DAPI profile (figures A and D), it is apparent that camptothecin disrupted the cells' ability to incorporate BrdU or decreased cell proliferation. The untreated group shows a characteristic horseshoe pattern with 43% of the cells incorporating BrdU during the 1-hour pulse, compared with 21% of the cells incorporating BrdU in the treated group. The H2AX signal (figures B and E) also show differences. The untreated control has a low signal intensity of H2AX with approximately 47% of the cells staining positive. The majority of the H2AX cells are on the BrdU$^+$ (proliferating) population. The camptothecin-treated group shows both a higher signal intensity and a higher percentage of positive cells (72%), with the majority of the staining on the BrdU$^-$ population (non-proliferating). The cleaved PARP (apoptosis) signal is shown in figures C and F. Untreated cells show low levels of cleaved PARP (4.3%). The camptothecin-treated group has a much higher percentage of cleaved PARP positive cells (23.2%). The percentage of H2AX$^+$/PARP$^-$ cells is not that different when comparing the untreated cells (45.7%) and the camptothecin-treated cells (50.9%). The difference is in the signal intensity. Nearly all of the cells in this experiment that are positive for cleaved PARP are also positive for H2AX.

Ordering Information

Description	Cat.No.
Apoptosis, DNA Damage, and Cell Proliferation Kit (50 Tests)	**562253**

Contents

Monoclonal Antibodies
BrdU PerCP-Cy5.5
H2AX (pSer139) Alexa Fluor® 647
Cleaved PARP (Asp214) PE
Other Reagents
BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution
BD Perm/Wash™ Buffer
BD Cytofix/Cytoperm™ Plus Permeabilization Buffer
DAPI
BrdU
DNase

23-13576-00

BD Biosciences
2350 Qume Drive
San Jose, CA 95131
US Orders: 855.236.2772
Technical Service: 877.232.8995
answers@bd.com
bdbiosciences.com