Multiple Methods for Detecting Apoptosis on the BD Accuri™ C6 Flow Cytometer

Technical Bulletin

Contents

Introduction

Apoptosis (programmed cell death) is an important biological process for both development and normal tissue homeostasis. Dysregulation of apoptotic pathways can lead to disease.

Methods for detecting apoptosis include Western blot, immunofluorescence, enzymatic assays, and flow cytometry. Flow cytometry is especially powerful because researchers can gain quantitative data on both apoptotic and dead cells within whole populations and cell subsets.

The BD Accuri C6 personal flow cytometer is well suited to the study of apoptosis. With the ability to detect four fluorochromes in addition to forward and side scatter, the instrument can perform most flow cytometric apoptosis assays, including Annexin V, caspase activation, PARP cleavage, mitochondrial change, and DNA fragmentation. Powerful yet easy to use, the BD Accuri C6 allows both new and experienced flow cytometry users to perform these established assays.

This technical bulletin presents examples of several popular BD Biosciences apoptosis kits (Table 1) run on the BD Accuri C6. It discusses the background of the assays and includes suggestions to optimize results.

Table 1. Selected methods for detecting apoptosis using flow cytometry.

Apoptosis Indicator	Assay	Examples	Cat. No.
Plasma membrane alterations (Phosphatidylserine exposure)	Annexin V binding assays • Single conjugates • Annexin V kits	Annexin V FITC Apoptosis Detection Kit	556570
		Annexin V PE Apoptosis Detection Kit	559763
Mitochondrial changes	JC-1 assays	BD™ MitoScreen (JC-1) Kit	551302
Caspase activation	Caspase activity assay kits and reagents	Caspase-3 Active Form PE Apoptosis Kit	550914
DNA fragmentation	TUNEL assays	APO-BrdU Apoptosis Detection Kit	556405
		APO-Direct Apoptosis Detection Kit	556381

Annexin V

Changes in the plasma membrane are one of the first detectable characteristics of the apoptotic process. In normal cells, phosphatidylserine (PS) molecules are confined to the inner leaflet of the plasma membrane (Figure 1). During apoptosis, these molecules externalize and can be bound to labeled Annexin V (FITC or PE) in the presence of calcium. Dead cells can be excluded with membrane-impermeant dyes such as propidium iodide (PI) or 7-AAD.

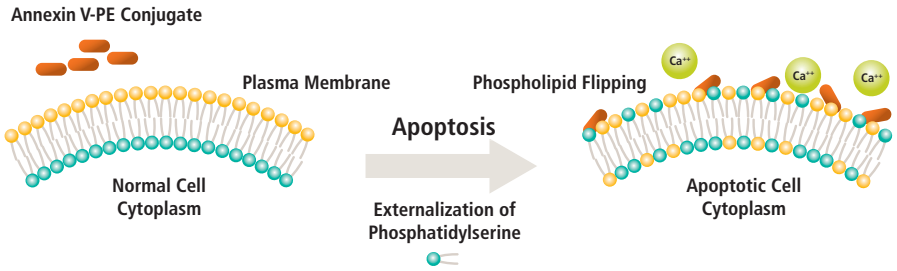

Figure 1. Changes in the plasma membrane are an early sign of apoptosis.

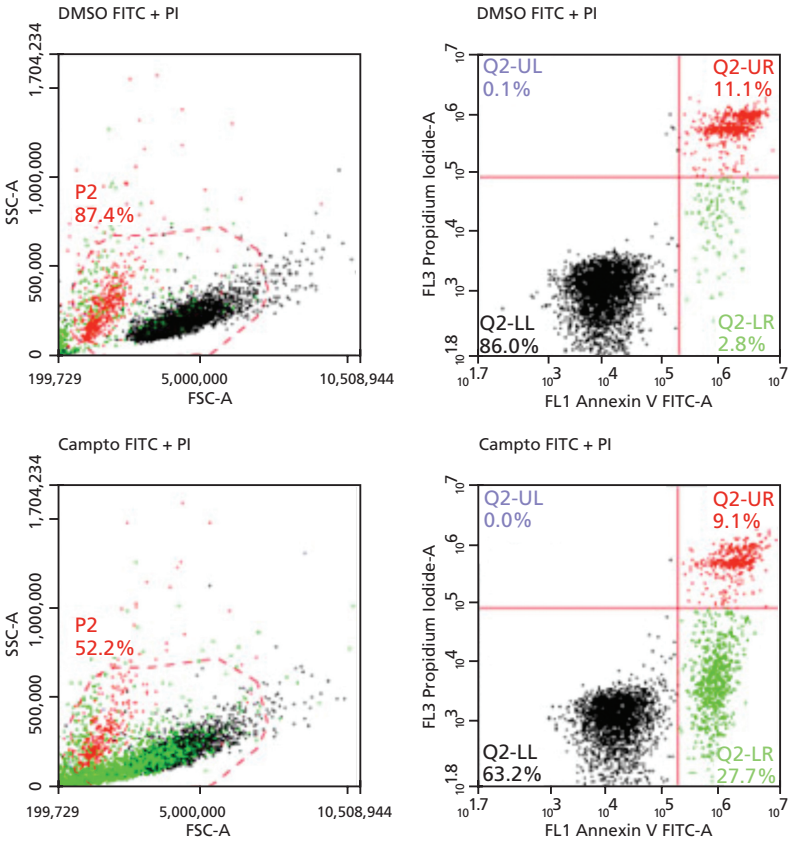

Figure 2. Flow cytometric analysis of FITC Annexin V staining.

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for 4 hours to induce apoptosis. Cells were stained with FITC Annexin V and PI according to the BD Pharmingen™ Annexin V FITC Apoptosis Detection Kit staining protocol (Cat. No. 556570). ***Results:*** *Camptothecin treatment (lower plots) resulted in an increase in early apoptotic cells (PI^-Annexin V^+, shown in green) compared to the DMSO control (upper plots). Dead cell (PI^+, red) and live cell (PI^-Annexin V^-, black) populations were easily distinguished. Data was acquired on a BD Accuri C6 flow cytometer using BD Accuri C6 software.*

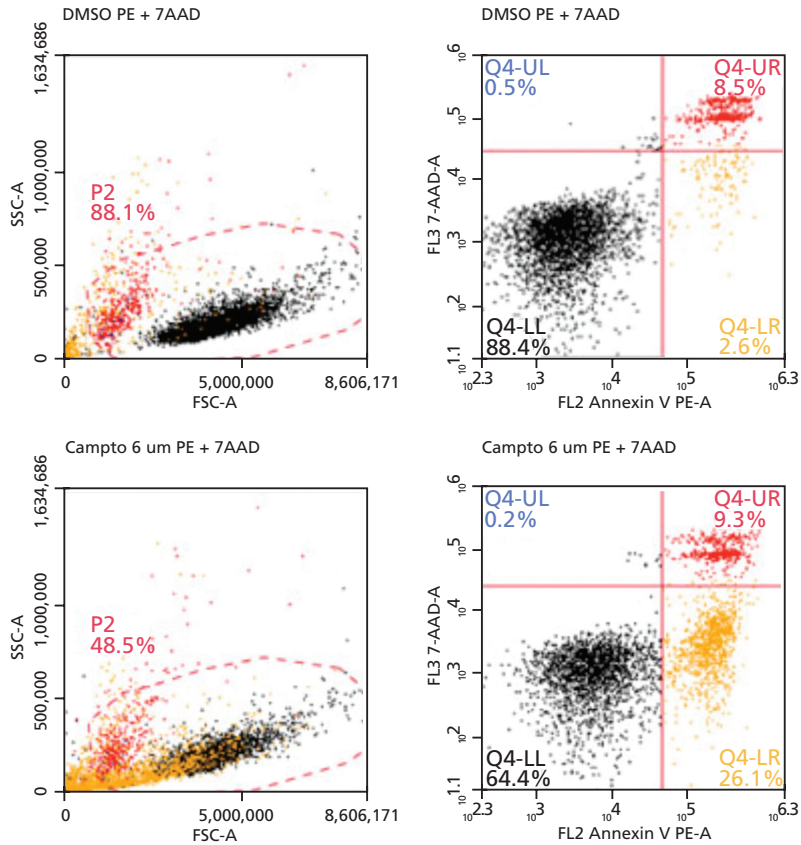

Figure 3. Flow cytometric analysis of PE Annexin V staining.

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for 4 hours to induce apoptosis. Cells were stained with PE Annexin V and 7-AAD according to the BD Pharmingen Annexin V PE Apoptosis Detection Kit staining protocol (Cat. No. 559763). ***Results:*** *Camptothecin treatment (lower plots) resulted in an increase in early apoptotic cells ($7\text{-}AAD^-$Annexin V^+, shown in orange), compared to the DMSO control (upper plots). Dead cell ($7\text{-}AAD^+$, red) and live cell ($7\text{-}AAD^-$Annexin V^-, black) populations were easily distinguished. Data was acquired on a BD Accuri C6 flow cytometer using BD Accuri C6 software.*

JC-1

Changes in mitochondrial membrane potential ($\Delta\Psi_m$) are another early marker for apoptosis. Lypophilic cationic fluorochromes such as JC-1 penetrate cells. In healthy cells, JC-1 accumulates in the mitochondria and forms aggregates. In apoptotic cells, however, JC-1 does not accumulate in the mitochondria and remains in the cytoplasm as monomers.

Because monomers and aggregates of JC-1 have different emission spectra, changes in $\Delta\Psi_m$ can be determined by comparing the ratio of fluorescence between the FL1 and FL2 channels.

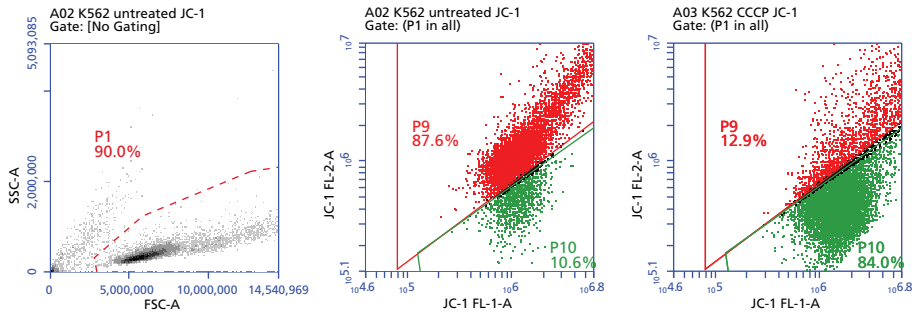

Figure 4. Flow cytometric analysis of BD MitoScreen staining.

K562 cells (human chronic myelogenous leukemia; ATCC CCL-243) were treated with 100 µM of CCCP (in DMSO) for 5 minutes at 37°C to induce apoptosis. The cells were stained with JC-1 (1:2,500 dilution in assay buffer) for 15 minutes at 37°C, according to the BD MitoScreen protocol (Cat. No. 551302). The cells were washed with assay buffer as described in the kit insert and collected on the BD Accuri C6 for 30 seconds on fast speed. ***Results:*** *CCCP treatment resulted in a shift in mitochondrial membrane potential (red to green). Data was acquired on a BD Accuri C6 flow cytometer using BD Accuri C6 software.*

Caspase-3

Changes in $\Delta\Psi_m$ as a result of apoptotic triggers make the mitochondrial membrane more permeable and release soluble proteins such as cytochrome c and procaspases. Procaspases are activated by protein cleavage and, in turn, cleave other proteins. This leads to a loss of cellular function and structure.

Several caspases are important for apoptosis, including caspase-3, -8, and -9. Methods of detecting caspase cleavage include fluorogenic substrates as well as antibodies specific to the cleaved (activated) forms of caspases.

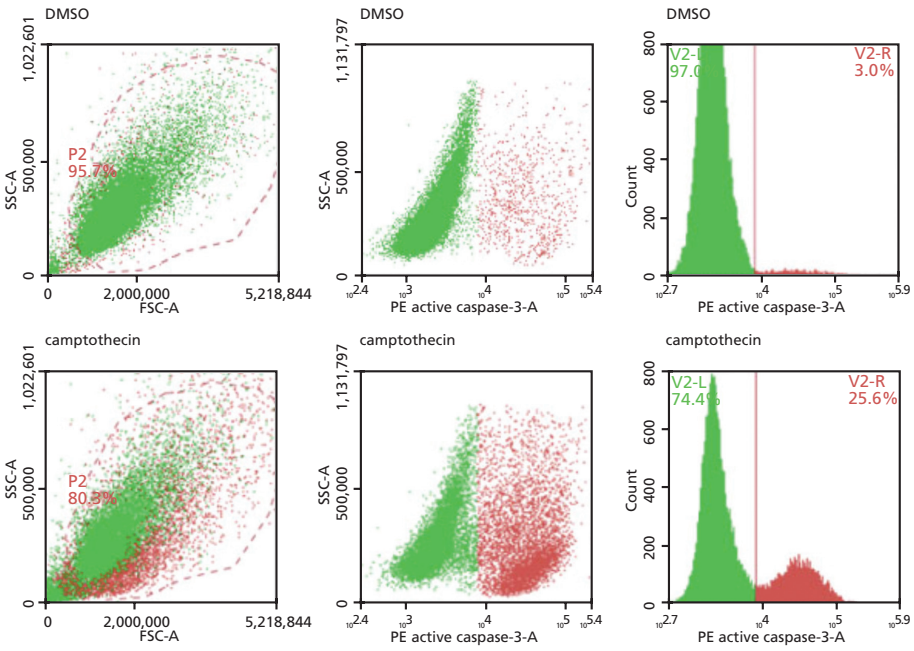

Figure 5. Flow cytometric analysis of active Caspase-3 staining.

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for 4 hours to induce apoptosis. Cells were permeabilized, fixed, and stained according to the BD Pharmingen Caspase-3 Assay Kit staining protocol (Cat. No. 550914). ***Results:*** *Camptothecin treatment (lower plots) resulted in an increase in active Caspase-3 expression (red) compared to the DMSO control (upper plots), which was primarily negative (green). Data was acquired on a BD Accuri C6 flow cytometer using BD Accuri C6 software.*

APO-BrdU and APO-Direct

DNA fragmentation is one of the final stages of apoptosis. BD Biosciences provides two kits to detect DNA fragmentation using flow cytometry.

The BD™ APO-BrdU Kit uses a reaction catalyzed by terminal deoxynucleotidyl transferase (TdT) to detect fragmentation. The method is often called end labeling or TUNEL (TdT dUTP nick end labeling). In this assay, TdT catalyzes a template-independent addition of bromolated deoxyuridine triphosphates (Br-dUTP) to the 3'-hydroxyl (OH) termini of double- and single-stranded DNA. After the Br-dUTP is incorporated, the cells are stained with labeled anti-BrdU, and the DNA terminal sites are identified using flow cytometry.

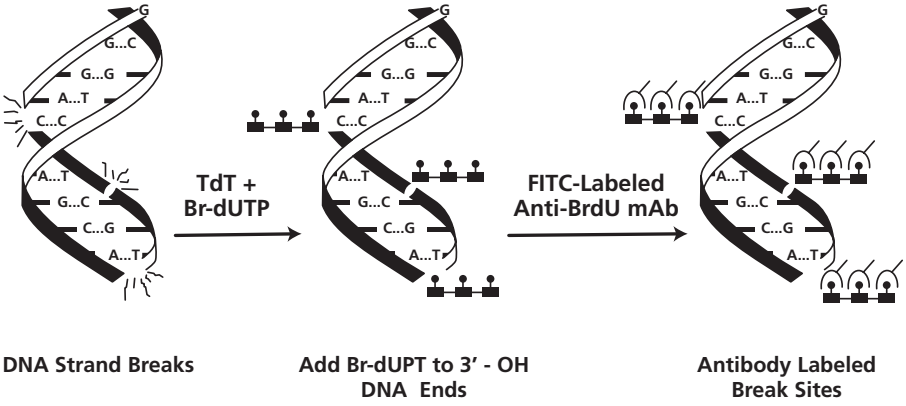

Figure 6. Schematic representation of APO-BrdU labeling.

The enzyme TdT catalyzes a template-independent addition of Br-dUTP to the 3'-hydroxyl ends of double- and single-stranded DNA. After Br-dUTP incorporation, DNA break sites are identified by a FITC-labeled anti-BrdU monoclonal antibody.

The BD™ APO-Direct Kit uses the same catalyst (TdT) in a single-step method that labels DNA breaks with a dUTP antibody.

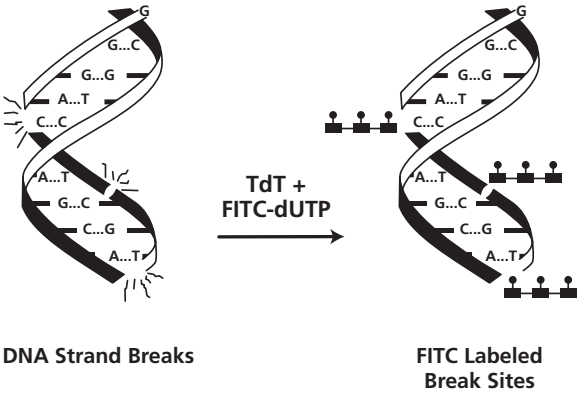

Figure 7. Schematic representation of APO-Direct labeling.

The enzyme TdT catalyzes a template-independent addition of FITC-labeled deoxyuridine triphosphates (FITC-dUTP) to the 3'-hydroxyl ends of double- and single-stranded DNA. When the FITC-labeled dUTPs are incorporated, the DNA break sites can be identified.

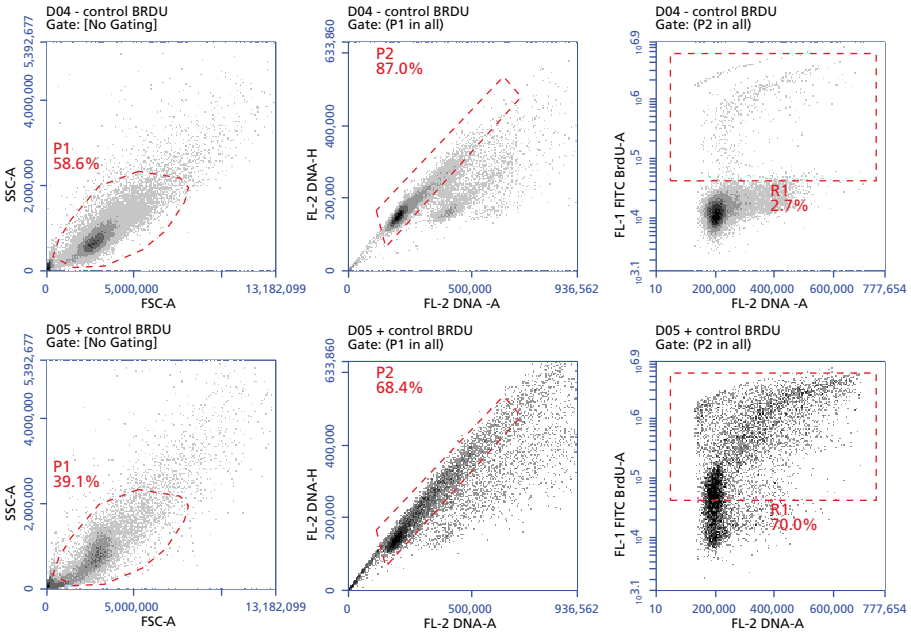

Figure 8. Flow cytometric analysis of APO-BrdU staining.

Positive control cells (human lymphoma cell line stimulated to undergo apoptosis, bottom row) and negative control cells (untreated lymphoma cell line, top row), both included in the BD APO-BrdU Kit (Cat. No. 556405), were stained according to the kit insert. Samples were collected for 30 seconds on fast speed on a BD Accuri C6 flow cytometer, and data was acquired using BD Accuri C6 software. Clumped cells were excluded by gating on the DNA Area vs Height plot (middle plots). ***Results:*** *The positive control cells showed a significant increase in apoptotic cells (R1) compared to the negative control.*

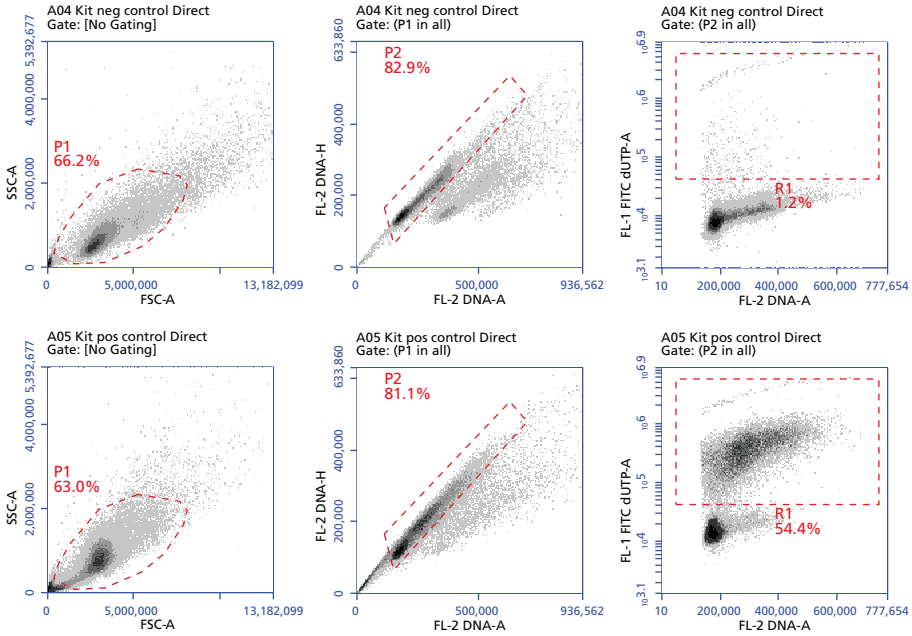

Figure 9. Flow cytometric analysis of APO-Direct staining.

Positive control cells (human lymphoma cell line stimulated to undergo apoptosis, bottom row) and negative control cells (untreated lymphoma cell line, bottom row), both included in the BD APO-Direct Kit (Cat. No. 556381), were stained according to the kit insert. Samples were collected for 30 seconds on fast speed on a BD Accuri C6 flow cytometer, and data was acquired using BD Accuri C6 software. Clumped cells were excluded by gating on the DNA Area vs Height plot (middle plots). ***Results:*** *The positive control cells showed a significant increase in apoptotic cells (R1) compared to the negative control.*

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