BD Pharmingen™ MitoStatus Reagents

Assessing Mitochondrial Membrane Potential on the BD Accuri™ C6 Flow Cytometer

Features

- Cationic, lipophilic dyes for assessment of mitochondrial membrane potential in a single fluorescence channel
- Detection of mitochondrial depolarization, a marker of apoptosis, autophagy, senescence, and other cellular processes
- Expanded choice and flexibility in multicolor panel design
- Tested for compatibility with the BD Accuri C6

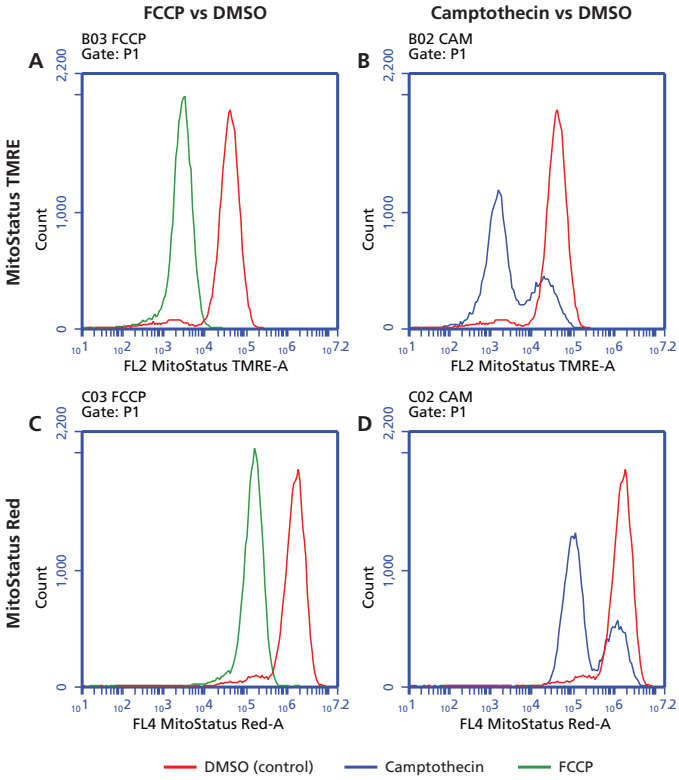

Figure 1. Detecting mitochondrial depolarization with MitoStatus reagents

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 0.025% DMSO vehicle control for 5 hours (red), 5 µM camptothecin for 5 hours to induce apoptosis (blue), or 50 µM FCCP mitochondrial uncoupler for 20 minutes (green). Cells were then stained with 100 nM of BD Pharmingen MitoStatus TMRE (upper plots) or MitoStatus Red (lower plots) for 15 minutes at 37°C in media, washed twice with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and acquired and analyzed on the BD Accuri C6 using BD Accuri™ C6 software. **Results: A, C.** Compared to the vehicle-treated controls, Jurkat cells treated with FCCP mitochondrial uncoupler show a decrease in fluorescence intensity due to depolarization of mitochondria. **B, D.** Camptothecin-treated cells show a mix of polarized and depolarized populations as they progress through apoptosis.

BD Pharmingen™ MitoStatus reagents can be used to assess a cell's inner mitochondrial membrane potential (Δψm), which is central to the study of apoptosis, autophagy, senescence, and other cellular processes. These cationic, lipophilic dyes accumulate within the mitochondria of healthy cells, but not within mitochondria that have lost Δψm due to induction of apoptosis or treatment with a mitochondrial uncoupler. Typically, the fluorescence intensity of healthy cells will be approximately 10-fold higher than cells whose mitochondrial membranes are depolarized.

On the BD Accuri™ C6 flow cytometer, BD Pharmingen™ MitoStatus TMRE dye is excited by the blue laser and BD Pharmingen™ MitoStatus Red dye by the red laser, allowing flexibility in multicolor panel design. Table 1 shows their fluorescence characteristics.

Characteristic	MitoStatus TMRE	MitoStatus Red
Excitation peak	549 nm	622 nm
Emission peak	574 nm	648 nm
Laser	488 nm (blue)	640 nm (red)
Detector	FL2	FL4
Equivalent fluorochromes*	PE	APC Alexa Fluor® 647

*Do not use these fluorochromes in the same tube with the corresponding MitoStatus dye.

Table 1. Fluorescence characteristics of MitoStatus dyes on the BD Accuri C6.

In cells undergoing apoptosis, oxidative stress, necrosis, and other cellular processes, the mitochondrial membrane can become depolarized. For example, in apoptosis, pro-apoptotic Bcl-2 family proteins cause mitochondrial outer membrane permeabilization (MOMP), resulting in the release of cytochrome c and the subsequent activation of caspase-9 and the apoptotic cascade. MOMP often correlates with the loss of Δψm, which can be detected using Δψm-sensitive dyes. Figure 1 shows that MitoStatus TMRE and MitoStatus Red clearly detect reduced Δψm in cells treated with FCCP (a mitochondrial uncoupler) or camptothecin (which induces apoptosis).

The MitoStatus dyes are similar to BD Pharmingen™ Annexin V dyes (Cat. Nos. 556570 and 559763), in that both can be used to detect apoptosis. While the MitoStatus dyes measure Δψm, Annexin V measures phosphatidylserine exposure. However, the MitoStatus dyes can be used in experiments where Annexin V is unsuitable, such as those that involve cells sensitive to calcium or certain adherent cells. Also, as Figure 2 shows, Δψm is often a slightly earlier sign of apoptosis than phosphatidylserine exposure. Finally, the MitoStatus dyes can detect not only apoptosis but also autophagy, senescence, and other processes that are associated with Δψm changes.

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In detecting Δψm status, the MitoStatus reagents are also similar to JC-1, used in the BD™ MitoScreen Kit (Cat. No. 551302). However, JC-1 is a ratiometric dye that requires two fluorescence channels, while the MitoStatus dyes require only one. JC-1 may still be preferable for cell types whose mitochondrial number can vary widely, since healthy cells with few mitochondria will bind little MitoStatus dye, and thus may appear falsely depolarized.

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.

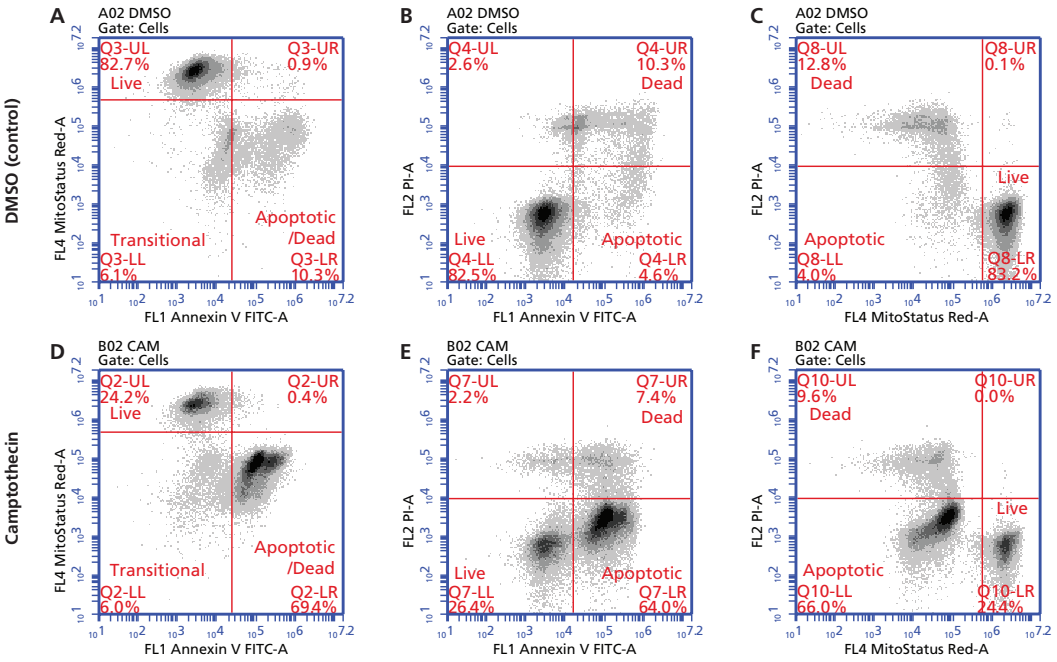

Figure 2. Detecting apoptosis with MitoStatus Red and Annexin V

Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 0.025% DMSO vehicle control (upper plots) or 5 µM camptothecin (lower plots) for 5 hours to induce apoptosis. Cells were then stained with 100 nM of BD Pharmingen MitoStatus Red for 15 minutes at 37°C in media, washed twice with BD Pharmingen Stain Buffer (FBS) (Cat. No. 554656), and stained with BD Pharmingen™ Annexin V FITC and propidium iodide (PI) according to the BD Pharmingen™ Annexin V FITC Apoptosis Detection Kit staining protocol (Cat. No. 556570). Cells were acquired and analyzed on the BD Accuri C6 using BD Accuri C6 software. **Results: A, D.** Co-staining with Annexin V FITC and MitoStatus Red revealed two major populations: live cells with polarized mitochondria and no phosphatidylserine exposure (UL), and apoptotic or dead cells with depolarized mitochondria and exposed phosphatidylserine (LR). A transitional population with depolarized mitochondria but no phosphatidylserine exposure (LL) reveals the loss of mitochondrial membrane potential to be a slightly earlier marker of apoptosis in these cells. **B, C, E, F.** Co-staining with either Annexin V FITC (B, E) or MitoStatus Red (C, F) and PI can detect live, apoptotic, and dead populations. Both co-stains reveal an increase in the number of apoptotic populations after camptothecin treatment, with similar percentages of live, apoptotic, and dead cells in the corresponding gates. (Quadrant gates are set based on the live cell population and adjusted along the PI axis to account for apoptotic cells being semi-permeable to PI.) Use of MitoStatus TMRE would yield similar results (data not shown).

Ordering Information

Description	Quantity	Cat.No.
BD Pharmingen™ MitoStatus TMRE	25 mg	**564696**
BD Pharmingen™ MitoStatus Red	100 µg	**564697**

Related Products

Description	Cat.No.
BD™ MitoScreen (JC-1) Kit	**551302**
BD Pharmingen™ Annexin V Apoptosis Detection Kit	**556570 (FITC)** **559763 (PE)**
BD Pharmingen™ Stain Buffer (FBS)	**554656**
BD Accuri™ C6 Flow Cytometer System	**653118**
BD CSampler™ Automated Sampling System	**653124**

Class1 Laser Product.

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