Description

In cells undergoing apoptosis, oxidative stress, necrosis, and other cellular processes, the mitochondrial membrane potential ($\Delta \psi_m$) can become depolarized. For example, in cells undergoing 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 inner mitochondrial membrane potential ($\Delta \psi_m$), which can be detected using $\Delta \psi_m$-sensitive dyes. These cationic, lipophilic dyes accumulate within the mitochondria of healthy cells, but not within mitochondria that have lost $\Delta \psi_m$ due to induction of apoptosis or treatment with a mitochondrial uncoupler.

BD Pharmingen™ MitoStatus TMRE (Tetramethylrhodamine ethyl ester) is a fluorescent dye that is readily sequestered by active mitochondria, allowing for flow cytometric or imaging analysis to assess for apoptosis or mitochondrial depolarization. Non-apoptotic or cells with polarized mitochondria will fluoresce red while apoptotic or cells with depolarized mitochondria will have diminished levels of red fluorescence. MitoStatus TMRE (Tetramethylrhodamine ethyl ester) has an excitation maximum of 549 nm, but is well-excited by both the blue (e.g. 488 nM) and yellow-green (e.g. 561 nM) lasers. It has an emission maximum of 574 nm.

Panel 1. Flow cytometric analysis of TMRE staining in Jurkat Cells. Jurkat cells were treated with 0.025% DMSO (solid line histograms), 5 μM camptothecin (Left Plot, dashed line histogram) for 4 hr or 50 μM FCCP (20 min, Right Plot, dashed line histogram), and then stained with 100 nM BD Pharmingen™ MitoStatus TMRE (15 min, Cat. No. 564696).

Panel 2. Two-color flow cytometric analysis of TMRE staining in Jurkat Cells. Jurkat cells were treated with 0.025% DMSO (Left Plot) or 5 μM camptothecin (Right Plot) for 4 hr, then stained with 100 nM MitoStatus TMRE (15 min). Cells were resuspended in Annexin V Binding Buffer (Cat. No. 556454) and stained with APC Annexin V (15 min, Cat. No. 556419). Co-staining shows two main populations: healthy cells that are MitoStatus TMRE-positive and Annexin V-negative, and apoptotic or dead cells that are MitoStatus TMRE-negative and APC Annexin V-positive. Compared to the DMSO-vehicle treated control, camptothecin treatment increased the MitoStatus TMRE-negative and APC Annexin V-positive population, indicating that more cells are undergoing apoptosis. Loss of mitochondrial membrane potential is an early apoptotic marker so a small population of cells in transition is MitoStatus TMRE-negative and APC Annexin V-negative. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System.

Panel 3. Immunofluorescent imaging of TMRE in HeLa Cells. HeLa cells were treated with 0.02% DMSO (Left Image) or 1 μM staurosporine (Right Image) for 3 hr. Cells were stained (30 min) with 200 nM MitoStatus TMRE and 5 μg/mL Hoechst. Staining media was removed and replaced with DPBS. Compared to the vehicle-treated control, staurosporine-treated cells show a decrease in mitochondrial staining with TMRE, as well as pyknotic nuclei characteristic of apoptotic cells. Cells were imaged on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ software.

MitoStatus TMRE has been tested on mouse (data not shown).
Application Notes

Application

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<tr>
<td>Bioimaging</td>
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Recommended Assay Procedure:

**Dye Preparation**

Bring BD Pharmingen™ MitoStatus TMRE dye powder and fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg, Sigma D2650) to room temperature. Reconstitute TMRE dye powder in DMSO at a stock concentration of 0.2-1 mM. For example, 1 mg of BD Pharmingen™ MitoStatus TMRE can be dissolved in 1.94 mL of DMSO to yield a 1 mM stock solution (MW = 514.96 g/mol).

**Dye Storage**

Upon arrival, store the dye powder desiccated and protected from light at ≤ -20°C until use. The dye powder is stable for at least 12 months if stored as indicated. After reconstitution with DMSO, store the stock solution at ≤ -20°C in small aliquots. The stock solution is stable for at least 6 months if stored as indicated.

**Flow Cytometry Requirements**

Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence. Flow Cytometers (eg, BD FACSCanto™ II, BD LSRSortex™, BD™ LSR II, or BD Accuri™ C6) equipped with a blue (eg, 488 nm) or yellow-green (eg, 561 nm) laser can be used. TMRE dye fluorescence can be detected with filters commonly used for phycoerythrin (PE) (eg, 575/26 or 582/15 nm).

Fluorescence compensation is best achieved using the cell samples of interest. When designing multicolor fluorescent staining panels, please be aware of high fluorescence spillover into the following fluorochromes’ detectors: BD Horizon™ PE-CF594, BV605, BV650, or PerCP-Cy™5.5. We recommend titrating the dye and using the lowest concentration that provides adequate resolution of polarized and depolarized cell populations to reduce fluorescence spillover.

**Procedure**

**BD Pharmingen™ MitoStatus TMRE labeling of suspended cells for flow cytometric analysis**

1. Count cells to determine cell density. Adjust cell density to 1 × 10^6 cells/mL or less in fresh, pre-warmed cell culture media.
2. Stain cells in fresh, pre-warmed cell culture media or desired stain buffer with 20-200 nM BD Pharmingen™ MitoStatus TMRE in a polypropylene container by adding the stock solution directly to the cells at the desired concentration.
   a. *Note:* BD Pharmingen™ MitoStatus TMRE may also be added directly to the culture instead of staining in fresh media.
   b. *Note:* TMRE is known to stick to polystyrene. Samples should be stained in polypropylene containers.
   c. *Note:* To aid in flow cytometric gating, we recommend using control cells treated with vehicle alone and/or cells treated with a mitochondrial uncoupler, such as FCCP [Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, eg, Sigma Cat. No. C2920].
3. Incubate samples for 15-30 minutes at 37°C protected from light.
4. Wash cells twice with BD Pharmingen™ Stain Buffer (FBS).
5. Decant the supernatant and gently mix to disrupt the cell pellet.
6. Resuspend the cells in Stain Buffer (FBS).
7. Analyze cells by flow cytometry. Alternatively, counterstain cells with compatible fluorescent dyes or antibodies as desired and then analyze.

**BD Pharmingen™ MitoStatus TMRE labeling of adherent cells for flow cytometric analysis**

1. Adherent cells should be stained *in situ* at ≤70% confluence. Stain cells in fresh, pre-warmed cell culture media or desired stain buffer with 20-200 nM BD Pharmingen™ MitoStatus TMRE by adding the stock solution directly to the cells at the desired concentration.
   a. *Note:* BD Pharmingen™ MitoStatus TMRE may also be added directly to the culture instead of staining in fresh media.
   b. *Note:* To aid in flow cytometric gating, we recommend using control cells treated with vehicle alone and/or cells treated with a mitochondrial uncoupler, such as FCCP.
2. Incubate samples for 15-30 minutes at 37°C protected from light.
3. Wash cells twice with BD Pharmingen™ Stain Buffer (FBS).
4. Remove cells from the growth medium. We recommend using BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527).
5. Wash cells twice with BD Pharmingen™ Stain Buffer (FBS) or the equivalent.
6. Decant the supernatant and gently mix to disrupt the cell pellet.
7. Resuspend the cells in Stain Buffer (FBS).
8. Analyze cells by flow cytometry. Alternatively, counterstain cells with compatible fluorescent dyes or antibodies as desired and then analyze.
Notes:
1. This dye is not compatible with cellular fixation.
2. Each user should determine the optimal concentrations of reagents, cells, and conditions for the assay of interest. We recommend titrating the reagent in early experiments to obtain optimal results.
3. Cells may be stained in bulk prior to staining with fluorescent antibodies.

**BD Pharmingen™ MitoStatus TMRE labeling of cells for fluorescent imaging**
1. Stain cells in fresh, pre-warmed media with 20-200 nM BD Pharmingen™ MitoStatus TMRE.
2. Incubate samples for 15–30 minutes at 37°C protected from light.
3. Remove the staining solution and replace with pre-warmed DPBS.
4. Analyze cells by fluorescence imaging. Alternatively, counterstain cells with compatible fluorescent dyes or antibodies as desired and then analyze.

**Suggested Companion Products**

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**Product Notices**
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
4. Cy is a trademark of GE Healthcare.
5. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.

**References**


