

## Technical Data Sheet

## MitoStatus Red

## Product Information

Material Number: 564697

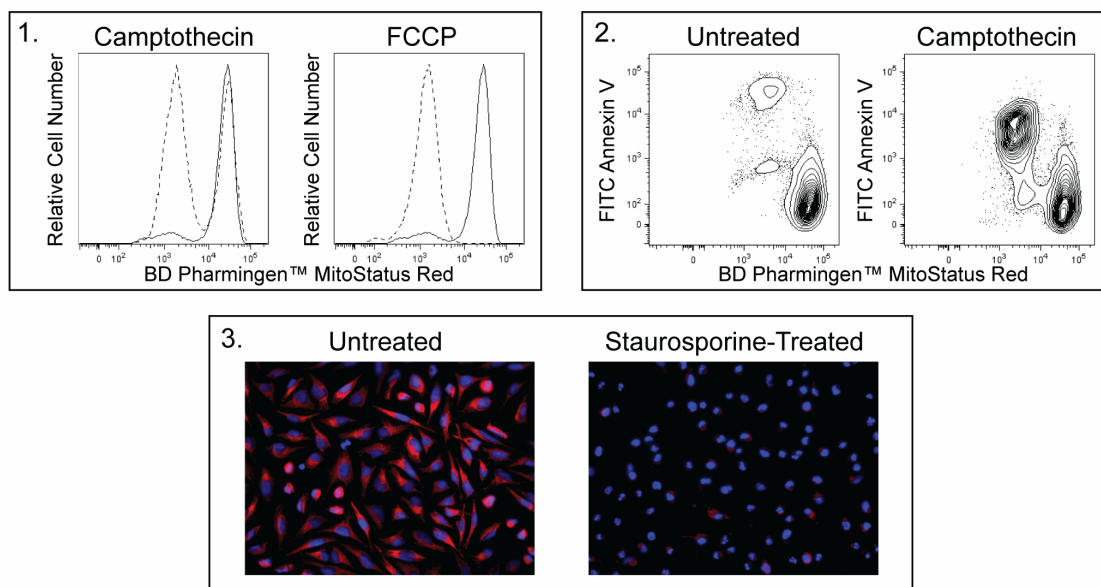
Size: 100 µg

## 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 Red 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. Apoptotic or cells with depolarized mitochondria will have diminished levels of fluorescence. MitoStatus Red has an excitation maximum of 622 nm and an emission maximum of 648 nm.

BD Pharmingen™ MitoStatus Red (Cat. No. 564697) is provided as 2 vials at 50 µg each.

**Analysis of Cells Stained with BD Pharmingen™ MitoStatus Red**

**Panel 1. Flow cytometric analysis of MitoStatus Red in Jurkat Cells.** Jurkat cells were treated with 0.025% DMSO (4 h, solid line histograms), 5 µM camptothecin (4 h, Left Plot, dashed line histogram) or 50 µM FCCP (20 min, Right Plot, dashed line histogram), and then stained with 100 nM BD Pharmingen™ MitoStatus Red (15 min, Cat. No. 564697).

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

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

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564697 Rev. 4



## Application Notes

### Application

Bioimaging	Tested During Development
Immunofluorescence	Tested During Development
Flow cytometry	Tested During Development

### Recommended Assay Procedure:

#### Preparation

Bring one 50 µg vial of BD Pharmingen™ MitoStatus Red dye powder and fresh cell culture-grade Dimethyl Sulfoxide (DMSO; eg, Sigma D2650) to room temperature. To reconstitute BD Pharmingen™ MitoStatus Red dye powder in DMSO at a stock concentration of 0.2 mM, add 460 µL of DMSO to one vial of dye powder.

#### Storage

Upon arrival, store the dye powder desiccated and protected from light at  $\leq -20^{\circ}\text{C}$  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  $\leq -20^{\circ}\text{C}$  in small aliquots. The stock solution is stable for at least 6 months if stored as indicated.

#### 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 LSRFortessa™, BD™ LSR II, or BD Accuri™ C6) equipped with a red laser (eg, 633 nm) can be used. This dye can be read out of filters commonly used for APC or Alexa Fluor® 647 (e.g., 660/20 or 670/30).

Fluorescence compensation is best achieved using a sample of the cells of interest. When designing multicolor fluorescent staining panels, please be aware of high spillover into the following fluorochromes' detectors: Alexa Fluor® 700, APC-H7, PerCP-Cy™5.5, BV711, or BV786. We recommend titrating the dye and using the lowest possible concentration that provides adequate resolution of polarized and depolarized populations for the cell type of interest to reduce spillover.

### Procedure

#### BD Pharmingen™ MitoStatus Red labeling of suspended cells for flow cytometric analysis

- Count cells to determine cell density. Adjust cell density to  $1 \times 10^6$  cells/ml or less in fresh, pre-warmed cell culture media.
- Stain cells in fresh, pre-warmed cell culture media or desired stain buffer with 20-200 nM BD Pharmingen™ MitoStatus Red in a polypropylene container by adding the stock solution directly to the cells at the desired concentration.
  - Note:* BD Pharmingen™ MitoStatus Red may also be added directly to the culture instead of staining in fresh media. Staining may also be performed in pre-warmed BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or pre-warmed Dulbecco's PBS. DPBS may provide increased resolution for some cell types, but can also result in increased background staining.
  - Note:* To aid in flow cytometric gating, we recommend using control cells treated with vehicle alone and/or a mitochondrial uncoupler, such as FCCP [Carbonyl cyanide 4 (trifluoromethoxy)phenylhydrazone, eg, Sigma Cat. No. C2920].
- Incubate samples for 15-30 minutes at  $37^{\circ}\text{C}$  protected from light.
- Wash cells twice with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656).
- Decant the supernatant and gently mix to disrupt the cell pellet.
- Resuspend the cells in Stain Buffer (FBS).
- Analyze cells by flow cytometry. Alternatively, counterstain cells with compatible fluorescent dyes or antibodies as desired and then analyze.

#### BD Pharmingen™ MitoStatus Red labeling of adherent cells for flow cytometry

- Adherent cells should be stained *in situ* at 70% confluence or less. Stain cells in fresh, pre-warmed cell culture media or desired stain buffer with 20-200 nM BD Pharmingen™ MitoStatus Red by adding the stock solution directly to the cells at the desired concentration.
  - Note:* BD Pharmingen™ MitoStatus Red may also be added directly to the culture instead of staining in fresh media. Staining may also be performed in pre-warmed BD Pharmingen™ Stain Buffer (FBS) or pre-warmed DPBS. DPBS may provide increased resolution for some cell types, but can also result in increased background staining.
  - Note:* To aid in flow cytometric gating, we recommend using control cells treated with vehicle alone and/or a mitochondrial uncoupler, such as FCCP.
- Incubate samples for 15-30 minutes at  $37^{\circ}\text{C}$  protected from light.
- Wash cells twice with BD Pharmingen™ Stain Buffer (FBS).
- Remove cells from the growth medium. We recommend using BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527).
- Wash cells twice with BD Pharmingen™ Stain Buffer (FBS).
- Decant the supernatant and gently mix to disrupt the cell pellet.
- Resuspend the cells in Stain Buffer (FBS) or equivalent.
- 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 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 Red labeling of cells for fluorescent imaging

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

#### Warnings and Precautions

**Danger:** BD Pharmingen™ MitoStatus Red is hazardous.

#### Hazard Statements:

Causes severe burns and eye damage.

#### Precautionary Statements:

Wear protective gloves/protective clothing/eye protection/face protection.

IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].

IF INHALED: Remove person to fresh air and keep comfortable for breathing.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

#### Suggested Companion Products

Catalog Number	Name	Size	Clone
561908	Hoechst 33342 Solution	1 mg	(none)
561527	Accutase™ Cell Detachment Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
556419	FITC Annexin V	200 Tests	(none)
556420	FITC Annexin V	100 Tests	(none)
560931	FITC Annexin V	25 Tests	(none)
556463	Propidium Iodide Staining Solution	2 mL	(none)
564061	Calcein AM	1 mg	
564060	Calcein Blue AM	1 mg	

#### Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. Cy is a trademark of GE Healthcare.
6. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
7. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

#### References

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