MitoTracker™ Dyes for Flow Cytometry

Catalog Numbers M46750, M46751, M46752, M46753

Pub. No. MAN0026032 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

MitoTracker™ Dyes for Flow Cytometry are cell–permeant reagents that label mitochondria in live cells. To label mitochondria, cells are simply incubated with MitoTracker™ Dyes, which passively diffuse across the plasma membrane and accumulate in active mitochondria. They are provided at an optimized concentration with protocols for flow cytometry. This allows for more precise analysis with both conventional and spectral flow cytometry. A range of available colors are detected with standard flow cytometry filters.

Contents and storage

Each vial of MitoTracker™ Dyes provides sufficient material for 20 flow cytometry assays.

Catalog numbers that appear as links open the web pages for those products.

Product	Cat. No.	Amount	Storage[1]
MitoTracker™ Green FM for Flow Cytometry	M46750	5 vials (100 assays total)	≤-20°C protected from light and moisture
MitoTracker™ Red FM for Flow Cytometry	M46751		
MitoTracker™ Red CMXRos for Flow Cytometry	M46752		
MitoTracker™ Deep Red FM for Flow Cytometry	M46753		

[1] When stored as directed, kit components are stable for at least 6 months.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
PBS, pH 7.4	10010031
DMSO, Anhydrous	D12345

Spectral properties of MitoTracker™ Dyes

Table 1 MitoTracker™ Dyes - Selective Probes

Product	Excitation laser (nm)	Excitation (nm)	Emission (nm)
MitoTracker™ Green FM	488	490	516
MitoTracker™ Red FM	561	581	644
MitoTracker™ Red CMXRos	561	579	599
MitoTracker™ Deep Red FM	633	644	665

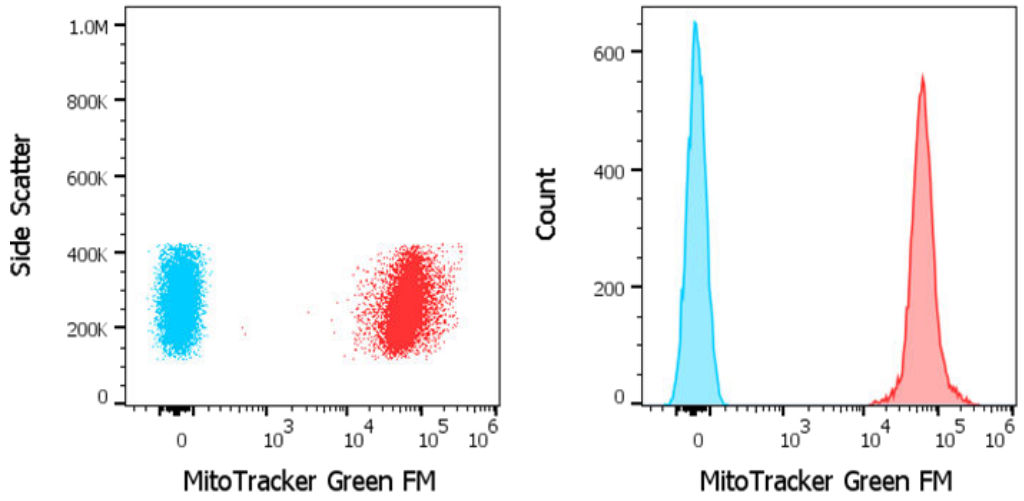

Figure 1 MitoTracker™ Green FM signal in Jurkat cells
Jurkat cells (human T-lymphocyte cell line) were stained with MitoTracker™ Green FM for Flow Cytometry for 15 minutes at 37°C. Cells were then washed and acquired on an Attune™ NxT Flow Cytometer using a 488-nm laser for excitation and 530/30-nm emission filter. Data shows overlay of stained (red) and unstained cells (blue).

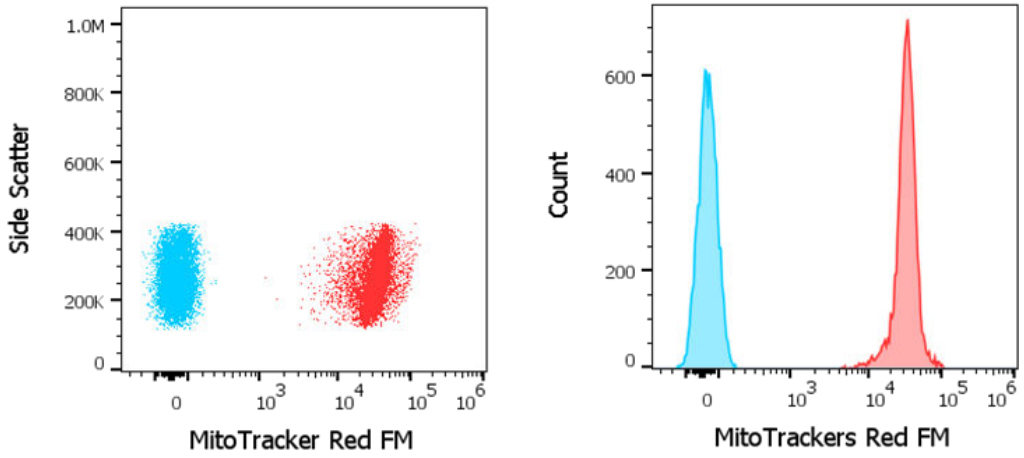

Figure 2 MitoTracker™ Red FM signal in Jurkat cells
Jurkat cells (human T-lymphocyte cell line) were stained with MitoTracker™ Red FM for Flow Cytometry for 15 minutes at 37°C. Cells were then washed and acquired on an Attune™ NxT Flow Cytometer using a 561-nm laser for excitation and 620/15-nm emission filter. Data shows overlay of stained (red) and unstained cells (blue).

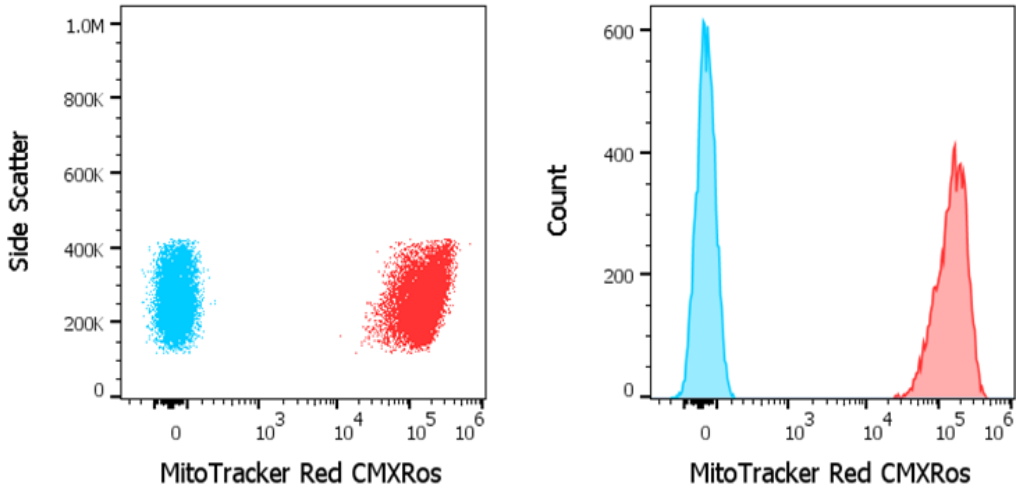

Figure 3 MitoTracker™ Red CMXRos signal in Jurkat cells
Jurkat cells (human T-lymphocyte cell line) were stained with MitoTracker™ Red CMXRos for Flow Cytometry for 15 minutes at 37°C. Cells were then washed and acquired on an Attune™ NxT Flow Cytometer using a 561-nm laser for excitation and 620/15-nm emission filter. Data shows overlay of stained (red) and unstained cells (blue).

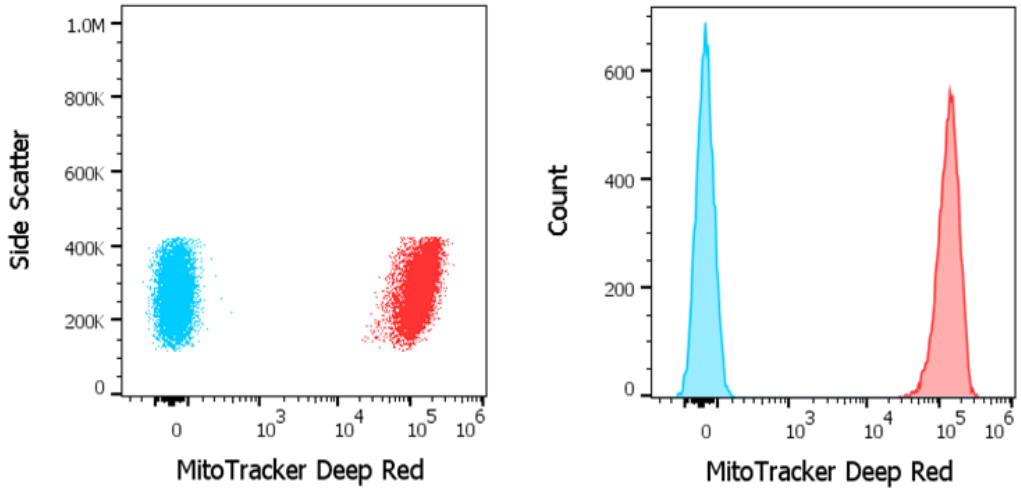

Figure 4 MitoTracker™ Deep Red FM signal in Jurkat cells
Jurkat cells (human T-lymphocyte cell line) were stained with MitoTracker™ Deep Red FM for Flow Cytometry for 15 minutes at 37°C. Cells were then washed and acquired on an Attune™ NxT Flow Cytometer using a 638-nm laser for excitation and 670/14-nm emission filter. Data shows overlay of stained (red) and unstained cells (blue).

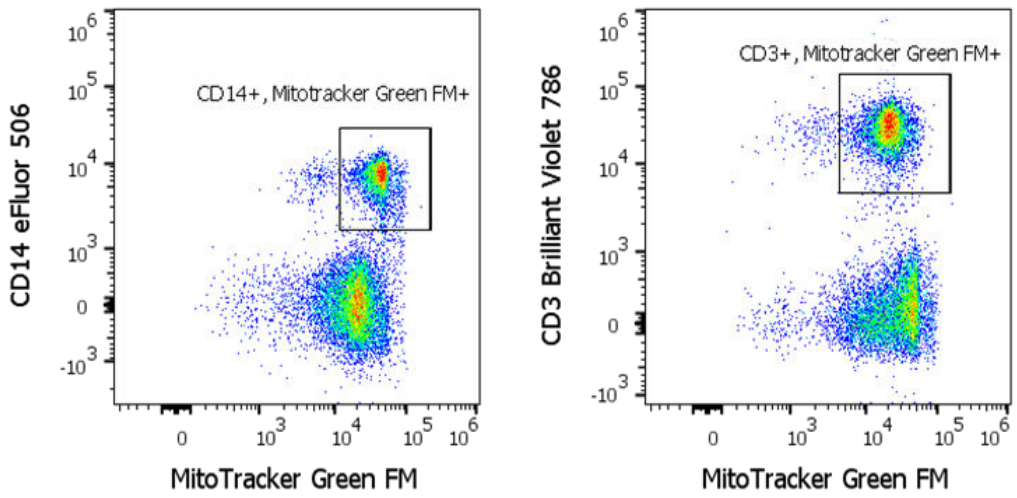

Figure 5 Multiplex of MitoTracker™ Green FM in CD3+ T cells and CD14+ monocytes
Peripheral blood mononuclear cells (PBMCs) were stained with MitoTracker™ Green FM for 15 minutes at 37°C, followed by a wash and additional stain with anti-CD3 Brilliant Violet 786 and anti-CD14 eFluor™ 506, then SYTOX™ Blue Dead Cell Stain. Cells were acquired on the Attune™ NxT Flow Cytometer, V6. Cells were gated based on forward- and side-scatter profiles, then singlet cells and live cells. CD3+ T cells and CD14+ monocytes/macrophages are shown with MitoTracker™ Green FM.

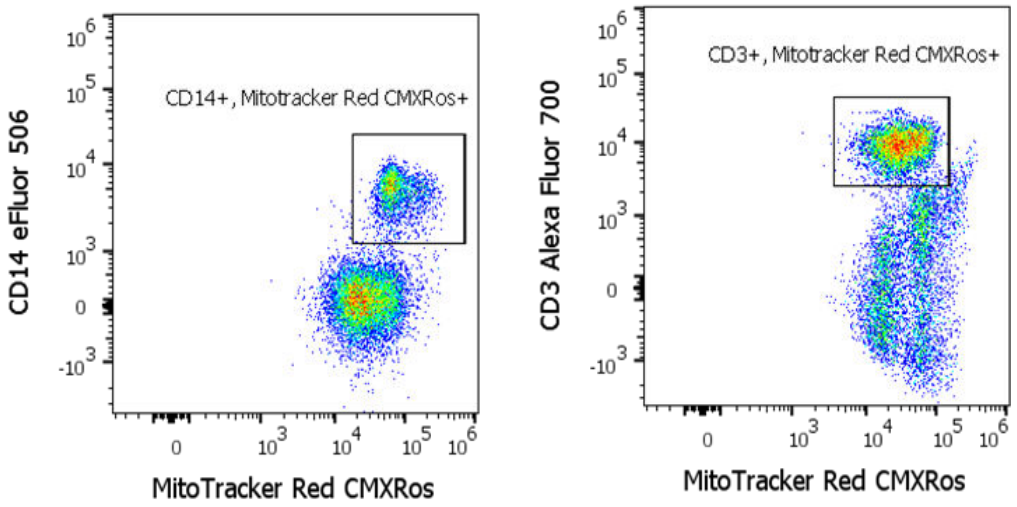

Figure 6 Multiplex of MitoTracker™ Red CMXRos in CD3+ T cells and CD14+ monocytes
Peripheral blood mononuclear cells (PBMCs) were stained with MitoTracker™ Red CMXRos for 15 minutes at 37°C, followed by a wash and additional stain with anti-CD3 Alexa Fluor™ 700 and anti-CD14 eFluor™ 506, then SYTOX™ Blue Dead Cell Stain. Cells were acquired on the Attune™ NxT Flow Cytometer, V6. Cells were gated based on forward- and side-scatter profiles, then singlet cells and live cells. CD3+ T cells and CD14+ monocytes/macrophages are shown with MitoTracker™ Red CMXRos.

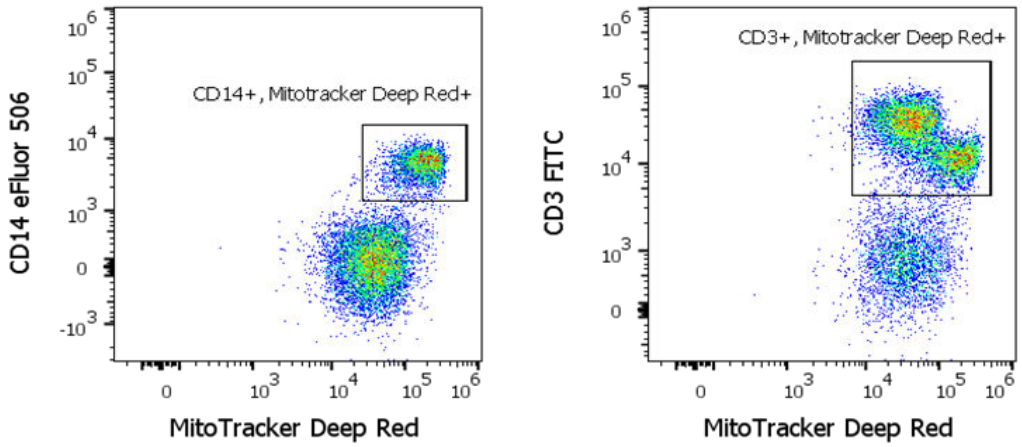

Figure 7 Multiplex of MitoTracker™ Deep Red FM in CD3+ T cells and CD14+ monocytes
Peripheral blood mononuclear cells (PBMCs) were stained with MitoTracker™ Deep Red FM for 15 minutes at 37°C, followed by a wash and additional stain with anti-CD3 FITC and anti-CD14 eFluor™ 506, then SYTOX™ Blue Dead Cell Stain. Cells were acquired on the Attune™ NxT Flow Cytometer, V6. Cells were gated based on forward- and side-scatter profiles, then singlet cells and live cells. CD3+ T cells and CD14+ monocytes/macrophages are shown with MitoTracker™ Deep Red FM.

Experimental Protocols

The following protocols have been used in cell types including peripheral blood cells, splenocytes, and many cell lines. Cells should be labeled in a solution, such as phosphate-buffered saline (PBS). Buffers containing proteins such as bovine serum albumin should not be used. If another staining reaction is to be performed on the same sample, determine the optimal sequence for the two procedures. The dyes are generally added as the last step in a staining protocol and not washed out before analysis.

Dye preparation

The reactive dye is supplied in individual vials, each with enough material for 20 samples. Once reconstituted, the DMSO dye solution can be stored frozen, protected from light and moisture.

1. Bring one vial of dye to room temperature.
2. Add 20 µL of DMSO to the vial of dye, then mix well. Visually confirm that all the dye is dissolved.

Cell staining (low-throughput method)

1. Pipette approximately 1 × 10^6 cells in suspension to each flow cytometry tube.
2. Label cells with desired reagents, such as antibodies and LIVE/DEAD™ Fixable Viability dyes (see **thermofisher.com/livedead**).
3. Wash with PBS, then discard supernatant.
4. Resuspend cells in 1 mL of PBS.
5. Add 1 µL of reconstituted dye, then mix well.
6. Incubate at preferred temperature (37°C is recommended) for 30–60 minutes, protected from light.
7. Analyze cells by flow cytometry using an appropriate excitation and emission channel.

Cell staining (medium-throughput method)

1. Pipette approximately 1 × 10^6 cells in suspension to each flow cytometry tube.
2. Label cells with desired reagents such as antibodies and LIVE/DEAD™ Fixable Viability dyes.
3. Prepare a 1X solution of reactive dye by adding 1 µL of reconstituted reactive dye per 1 mL PBS, then mix well. For example, for 10 tubes add 10 µL dye to 10 mL PBS.
4. Wash with PBS, then discard supernatant.
5. Resuspend each tube of cells in 1 mL of 1X dye solution.
6. Incubate at preferred temperature (37°C is recommended) for 30–60 minutes, protected from light.
7. Analyze cells by flow cytometry using an appropriate excitation and emission channel.

Cell staining (high-throughput method)

1. Pipette approximately 1 × 10^5 cells in suspension to each well of a 96-well plate.
2. Label cells with desired reagents such as antibodies and LIVE/DEAD™ Fixable Viability dyes.
3. Prepare a 1X solution of reactive dye by adding 12 µL of reconstituted reactive dye to 12 mL PBS, then mix well.
4. Wash cells in a 96-well plate with PBS, then discard supernatant.
5. Add 100 µL of 1X dye solution to cells in each well.
6. Incubate at preferred temperature (37°C is recommended) for 30–60 minutes, protected from light.
7. Analyze cells by flow cytometry using an appropriate excitation and emission channel.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	14 January 2021	New document created for MitoTracker™ Dyes.