MitoFlamma® Deep Red (live)

Catalog number : RMS1102

Component	Storage	Amount
MitoFlamma® Deep Red (live)	Freeze (-20 ℃), Protect from light	1 vial containing 100X lyophilized solid.

OVERVIEW

MitoFlamma® Deep Red is a mitochondria-selective deep red fluorescent dye that allows to observe mitochondrial morphology in living cells. It preferentially accumulates inside mitochondria regardless of mitochondrial membrane potential. MitoFlamma® Deep Red enables researchers to observe mitochondrial activity, localization and abundance, as well as monitoring the effect of drugs or other external stimuli on the mitochondrial function. MitoFlamma® Deep Red labeled samples might be used in various biological studies such as immunocytochemistry, *in situ* hybridization, microplate-based analysis, etc.

PARAMETERS

Instruments:	Fluorescence microscope
Excitation:	Cy5, APC filter set
Emission:	Cy5, APC filter set
Recommended plate:	Black well/Clear bottom
Instrument:	Flow cytometer
Excitation:	630 nm laser
Emission:	660 nm filter
Instrument specification(s):	Cy5, APC channel

PREPARATION OF STOCK SOLUTION

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

MitoFlamma® Deep Red stock solution (100X):
Dissolve a MitoFlamma® Deep Red in in molecular biology grade dimethylsulfoxide (DMSO) to make 100X stock solution.
* Add 100 μL of DMSO to MitoFlamma® Deep Red.

PREPARATION OF WORKING SOLUTION

MitoFlamma® Deep Red working solution 1X:
1 μL of 100X stock solution into 1 mL growth medium or buffer.
The working concentration can be in the range of 0.5X–2X.

MATERIALS REQUIRED BUT NOT PROVIDED

- DMSO
- PBS buffer or suitable growth medium for live cell imaging
- Aldehyde based fixatives such as paraformaldehyde for cell fixation (optional)
- Aldehyde based detergents such as Trition® X-100
- Micropipette
- Fluorescence microscope or Flow cytometry
- 37 ℃ incubator

EXPERIMENTAL PROTOCOLS

Staing adherent cells

1. Prepare MitoFlamma® Deep Red 100X stock in DMSO solution.
2. Dilute to stock solution in growth medium or Buffer.
3. Remove growth medium from cells.
4. Add MitoFlamma® Deep Red 1X working solution.
5. Incubate at 37 °C for 30 minutes. (or longer)
6. Replace the loading solution with fresh medium or PBS and observe cells using a fluorescence microscope.

Note: This protocols has been optimized for Hela cell line and it may need to be optimized with the particular cell types.

Staining suspension cells

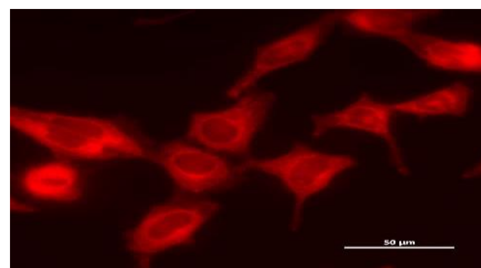
1. Pellet cells and aspirate the supernatant.
2. Resuspend pellet in MitoFlamma® Deep Red 1X working solution.
3. Incubate at 37 °C for 30 minutes. (or longer)
4. Centrifuge the cells, remove supernatant and resuspend cells in PBS.
5. Cells may be analyzed by flow cytometry (Cy5, APC channel) or fluorescence microscopy (Cy5, APC filter set).

Fixation

1. Fix the cells for 15 min in 4% parnformaldehyde at 37 ℃.
2. Aspirate the fixative and rinse the cell twice with PBS.

Note: Live cells stained with MitoFlamma® Deep Red can be fixed but fluorescence is not well retained. Subsequent permeabilization steps may also affect staining.

HELA CELL STAINING with MitoFlamma® Deep red



Hela cells were stained with MitoFlamma® Deep Red.

TECHNICAL SUPPORT

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