MitoLite™ Red CMXRos

Catalog number: 22698
Unit size: 10x50 ug

Component	Storage	Amount
MitoLite™ Red CMXRos	Freeze (<-15 °C), Minimize light exposure	10x50 ug

OVERVIEW

MitoLite™ Red CMXRos is chemically same to the MitoTracker™ Red CMXRos (ThermoFisher). MitoLite™ Red CMXRos is a cationic dye that selectively accumulates in mitochondria probably vial the mitochondrial membrane potential gradient. The mitochondrial indicator is a hydrophobic compound that easily permeates intact live cells, and trapped in mitochondria after it gets into cells. This fluorescent mitochondrial indicator is retained in mitochondria for long time since the indicator carries a cell-retaining group. This key feature significantly increases its staining efficiency. MitoLite™ Red CMXRos is well-retained after aldehyde fixation.

AT A GLANCE

Protocol Summary

1. Prepare 1 mM MitoLite™ Red CMXRos stock solution
2. Prepare 100-500 nM MitoLite™ Red CMXRos staining solution
3. Remove the growth media from the cells
4. Add MitoLite™ Red CMXRos staining solution to cells
5. Incubate at 37°C for 30 minutes
6. Wash cells and repalce with 1x Hanks and 20mM Hepes Buffer (HH buffer)
7. Observe cells using a fluorescence microscope with TRITC filter set

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	TRITC filter set
Emission:	TRITC filter set
Recommended plate:	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

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.

MitoLite™ Red CMXRos stock solution:
Dissolve one vial MitoLite™ Red CMXRos (50 ug) in 94 uL high-quality, anhydrous dimethylsulfoxide (DMSO) to make 1 mM stock solution. Note: Keep the stock solution frozen at ≤–15°C and protected from light.

PREPARATION OF WORKING SOLUTION

MitoLite™ Red CMXRos staining solution:
Dilute 1 mM MitoLite™ Red CMXRos stock solution to the final working concentration in HH buffer.

Note The working concentration can be in the range of 100–500 nM.

SAMPLE EXPERIMENTAL PROTOCOL

Staining adherent cells:

1. Grow cells to reach the desired confluency.
2. Remove the growth media from the cells.
3. Add MitoLite™ Red CMXRos staining solution to each well.
4. Incubate at 37°C for 30 minutes.
5. Wash cells and replace with 1x Hanks and 20mM Hepes Buffer (HH buffer).
6. Observe cells using a fluorescence microscope with TRITC filter set.

Note The staining protocols is good for HeLa cell line and it may need to be optimized with the particular cell types.

Staining suspension cells:

1. Centrifuge cells to a pellet and aspirate the supernatant.
2. Resuspend the cells gently in MitoLite™ Red CMXRos staining solution.
3. Incubate at 37°C for 30 minutes.
4. Centrifuge the cells, remove supernatant and resuspend cells in fresh HH buffer.
5. Cells may be analyzed by fluorescence microscopy (TRITC filter set).

EXAMPLE DATA ANALYSIS AND FIGURES

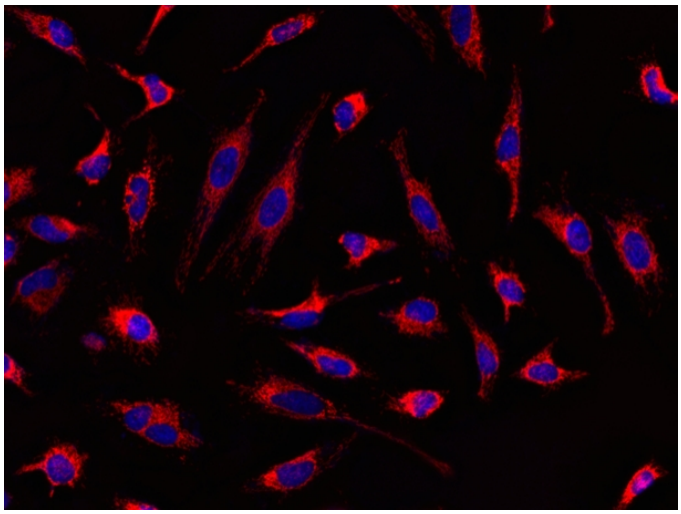

Figure 1. Fluorescence images of HeLa cells stained with MitoLite™ Red CMXRos. Hela cells were stained with 0.1uM MitoLite™ Red CMXRos in HH buffer at 37°C for 30mins, and then washed twice with HH bufer. Cells were imaged with fluorescence microscope using a TRITC filter set (Red). Nuclei were stain with Nuclear Violet™ LCS1 (Cat#17543) and viewed with DAPI filter set (Blue).

DISCLAIMER