

MitoLite™ Green FM

Catalog number: 22695

Unit size: 10x50 ug

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

OVERVIEW

MitoLite™ Green FM is the same molecule to the MitoTracker Green FM (M7514, ThermoFisher). It is green-fluorescent mitochondrial stain. Unlike other MitoLite probes, MitoLite™ Green FM appears to localize to mitochondria, much less depending on mitochondrial membrane potential. The dye stains live cells, but it is not well-retained after aldehyde fixation. It is one of few mitochondrial probes that can stain mitochondria in dead cells.

AT A GLANCE

Protocol Summary

1. Prepare 1 mM MitoLite™ Green FM stock solution
2. Prepare 20-200 nM MitoLite™ Green FM staining solution
3. Remove the growth media from the cells
4. Add MitoLite™ Green FM staining solution to cells
5. Incubate at 37°C for 30 minutes
6. Wash cells and replace with 1x Hanks and 20mM Hepes Buffer (HH buffer)
7. Observe cells using a fluorescence microscope with FITC filter set

Important Bring the dye at room temperature before starting the experiment.

KEY PARAMETERS

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

Instrument: Flow cytometer
 Excitation: 488 nm laser
 Emission: 530/30 nm filter
 Instrument specification(s): FITC channel

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™ Green FM stock solution:

Dissolve one vial MitoLite™ Green FM (50 ug) in 74 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™ Green FM staining solution:

Dilute 1 mM MitoLite™ Green FM stock solution to the final working concentration in HH buffer. The working concentration can be in the range of 20–200 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™ Green FM 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 FITC 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™ Green FM 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 flow cytometry (530/30 nm filter- FITC channel) or fluorescence microscopy (FITC filter set).

EXAMPLE DATA ANALYSIS AND FIGURES

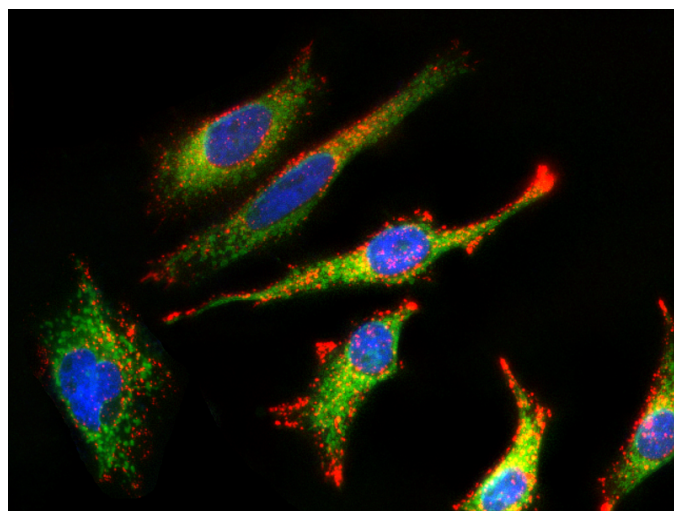


Figure 1.

Fluorescence images of HeLa cells stained with MitoLite™ Green FM using fluorescence microscope with a FITC filter set (Green). Live cells were co-stained with lysosome dye LysoBrite™ Red (Cat#22645, Red) and nuclei stain Nuclear Violet™ LCS1 (Cat#17543, Blue).

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