

MitoLite™ Green FM

Catalog number: 22695
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 22695)
MitoLite™ Green FM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

MitoLite™ Green FM is a green fluorescent mitochondrial stain designed for live-cell labeling of mitochondria. Unlike many mitochondrial dyes that rely strongly on mitochondrial membrane potential for accumulation, MitoLite™ Green FM shows mitochondrial localization with reduced dependence on membrane potential, making it useful for live-cell mitochondrial imaging workflows. MitoLite™ Green FM uses the same dye molecule as Thermo Fisher's MitoTracker Green FM and provides a comparable green-fluorescent mitochondrial staining option. It is suitable for live-cell fluorescence microscopy using common green fluorescence channels. The staining may also be retained after fixation under tested conditions.

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 Note

Bring the dye at room temperature before starting the experiment.

KEY PARAMETERS
Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

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).

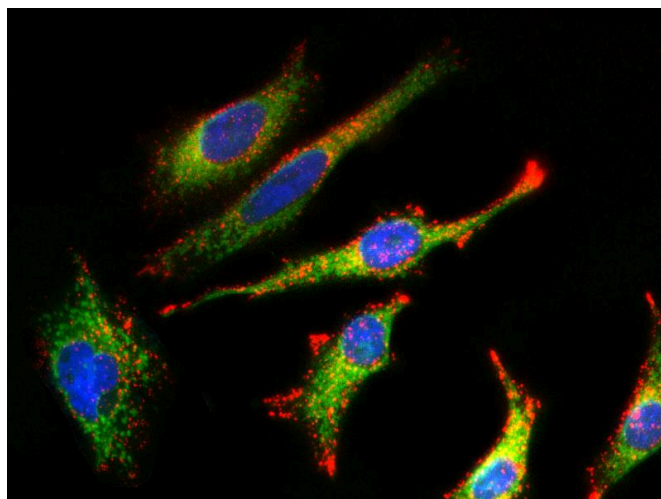
EXAMPLE DATA ANALYSIS AND FIGURES


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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