



MitoFlamma® Green (live)

Catalog number : RMS1101

Component	Storage	Amount
MitoFlamma® Green (live)	Freeze (-20 °C), Protect from light	1 vial containing 100 X lyophilized solid.

OVERVIEW

MitoFlamma® Green is a mitochondria-selective green fluorescent dye that allows to observe mitochondrial morphology in living cells. It preferentially accumulates inside mitochondria regardless of mitochondrial membrane potential. MitoFlamma® Green 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® Green labeled samples might be used in various biological studies such as immunocytochemistry, *in situ* hybridization, microplate-based analysis, etc.

PARAMETERS

Instruments: Fluorescence microscope
 Excitation: FITC filter set
 Emission: FITC filter set
 Recommended plate: Black well/Clear bottom

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

PREPARATION OF STOCK SOLUTION

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

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

PREPARATION OF WORKING SOLUTION

MitoFlamma® Green 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 Triton® X-100
- Micropipette
- Fluorescence microscope or Flow cytometry
- 37 °C incubator

EXPERIMENTAL PROTOCOLS

Staining adherent cells

1. Prepare MitoFlamma® Green 100X stock in DMSO solution.
2. Dilute to stock solution in growth medium or Buffer.

3. Remove growth medium from cells.
4. Add MitoFlamma® Green 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

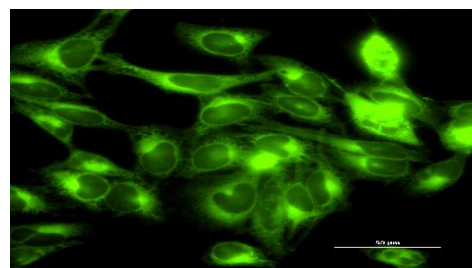
1. Pellet cells and aspirate the supernatant.
2. Resuspend pellet in MitoFlamma® Green 1X working solution.
3. Incubate at 37 °C for 30 minutes. (or longer)
4. Centrifuge the cells, remove supernatant and resuspend cells in PBS.
5. Cells may be analyzed by flow cytometry (FITC channel) or fluorescence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

Fixation

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

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

HELA CELL STAINING with MitoFlamma® Green



HeLa cells were stained with MitoFlamma® Green.

TECHNICAL SUPPORT

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