# PRODUCT INFORMATION



# MitoLite™ Green FM

Item No. 25162

Ex./Em. Max: 491/513 nm Supplied as: A solid -20°C Storage: Stability: ≥4 years

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

#### Description

MitoLite™ Green FM is a cell-permeable mitochondrial dye that passively diffuses across the plasma membrane and accumulates in mitochondria. MitoLite™ Green FM displays excitation/emission maxima of 491/513 nm, respectively, and can be used for live and dead cell applications, however, it is not well-retained after aldehyde or alcohol fixation.

# **Assay Protocol**

## 1. Prepare mitochondria-staining solution

- a. Warm the MitoLite™ Green FM solution to room temperature.
- b. Dilute 20 µl of 500X MitoLite™ Green FM into 10 ml of Hanks balanced salt solution with 20 mM HEPES (HHBS) or buffer of your choice.

Note 1: 20 µl of 500X MitoLite™ Green FM yields a volume of mitochondria-staining solution sufficient for one 96-well plate. Aliquot and store unused MitoLite™ Green FM stock solution at less than -15°C. MitoLite™ Green FM is light sensitive. Light exposure and repeated freeze-thaw cycles should be avoided.

Note 2: The optimal working concentration is application specific. Staining conditions may be modified according to cell type and/or permeability.

# 2. Prepare and stain the cells

### a. Adherent cells:

- Grow cells in a 96-well black wall/clear bottom plate (100 μl/well) or on coverslips inside a petri dish filled with appropriate culture medium.
- When cells reach the desired confluency, add an equal volume of the mitochondriastaining solution prepared in step 1b.
- iii. Incubate cells at 37°C, 5% CO<sub>2</sub> for 30 minutes to 2 hours.
- iv. Wash cells twice with pre-warmed (37°C) HHBS or buffer of your choice, then fill wells with buffer or growth medium.
- Observe cells using fluorescence technique of choice.

### b. Suspension cells\*:

- Centrifuge the cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
- Resuspend cell pellets in pre-warmed (37°C) growth medium and add an equal volume of ii. mitochondria-staining solution prepared in step 1b to cells.
- Incubate cells at 37°C, 5% CO<sub>2</sub> for 30 minutes to 2 hours.
- Wash cells twice with pre-warmed (37°C) HHBS or buffer of your choice, then fill wells with buffer or growth medium.
- Observe cells using fluorescence technique of choice.

\*Note 3: Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak $^{ exttt{B}}$ (BD Biosciences) and stained as adherent cells.

Note 4: If cells are not sufficiently stained it is recommended to increase either the labeling concentration or the incubation time to increase cellular dye accumulation.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFEI Y DAIA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

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