Cell Navigator™ Mitochondrion Staining Kit *Deep Red Fluorescence*

Catalog number: 22669
Unit size: 500 Assays

Component	Storage	Amount
Component A: MitoLite™ Deep Red FX660	Freeze (<-15 °C), Minimize light exposure	100 µL (500X DMSO stock solution)
Component B: Live Cell Staining Buffer	Freeze (<-15 °C), Minimize light exposure	50 mL

OVERVIEW

Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria and nuclei etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to label mitochondria of live cells in red fluorescence. The kit uses a proprietary 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. The labeling protocol is robust, requiring minimal hands-on time. It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add Mitolite™ Deep Red FX660 working solution
3. Incubate at 37°C for 30 minutes to 2 hours
4. Analyze the cells under fluorescence microscope at Ex/Em = 640/660 nm (Cy5 filter set)

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	Cy5 filter
Emission:	Cy5 filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

20 µL of 500X Mitolite™ Deep Red FX660 (Component A) into 10 mL of Live Cell Staining Buffer (Component B) to make Mitolite™ Deep Red FX660 working solution. Protect from light.

Note 20 µL of 500X Mitolite™ Deep Red FX660 (Component A) is enough for one 96-well plate. The optimal concentration of the fluorescent mitochondrial indicator varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

For adherent cells:

1. Grow cells either in a 96-well black wall/clear bottom plate (100 µL/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture medium.
2. When cells reach the desired confluence, add equal volume of Mitolite™ Deep Red FX660 working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Replace Mitolite™ Deep Red FX660 working solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).
5. Observe the cells using a fluorescence microscope with Cy5 filter set (Ex/Em = 640/660 nm).

   Note It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

For suspension cells:

1. Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
2. Resuspend the cell pellets gently in pre-warmed (37°C) growth medium, and add equal volume of Mitolite™ Deep Red FX660 working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Replace Mitolite™ Deep Red FX660 working solution with Hanks and 20 mM Hepes buffer (HH buffer) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration).
5. Observe the cells using a fluorescence microscope with Cy5 filter set (Ex/Em = 640/660 nm).

   Note It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained. Suspension cells may be attached to cover-slips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.

EXAMPLE DATA ANALYSIS AND FIGURES

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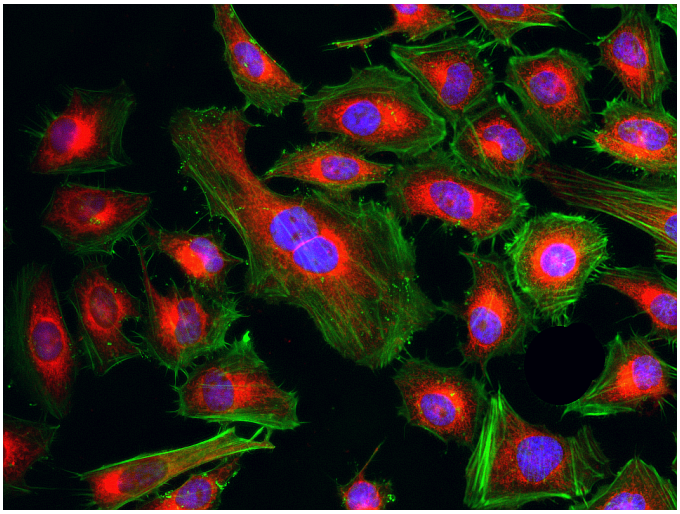

Figure 1.

Fluorescence images of HeLa cells stained with Cell Navigator™ Mitochondrion Staining Kit *Deep Red Fluorescence* using fluorescence microscope with a Cy5 filter set. Live cells were stained with mitochondria dye MitoLite™ Deep Red (Red). After fixation, the cells were labeled with F-actin dye iFluor™ 488-Phalloidin (Cat#23115, Green) and counterstained with Nuclear Blue™ DCS1 (Cat#17548, Blue).