Cell Navigator™ Mitochondrion Staining Kit *NIR Fluorescence*

Catalog number: 22670
Unit size: 500 Assays

Component	Storage	Amount
Component A: MitoLite™ NIR	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

Mitochondria are membrane-enclosed organelles found in most eukaryotic cells. Mitochondria generate most of the cellular supply of ATP. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process. Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, 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 in live cells with near infrared (NIR) fluorescence. The kit uses our proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The NIR fluorescent mitochondrial stain used in the kit is an ideal choice for imaging live cells and tissues where low background, and high signal-to-noise ratio is critical. The mitochondrial indicator, a hydrophobic compound, easily permeates intact live cells and trapped in mitochondria after it gets into cells. This fluorescent mitochondrial indicator is retained in mitochondria for a long time since it carries a cell-retaining group. This key feature significantly increases the staining efficiency. The kit can be readily adapted for many different types 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 and can be used for both proliferating and non-proliferating cells.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add Mitolite™ NIR working solution
3. Incubate at 37°C for 30 minutes to 2 hours
4. Analyze the cells under fluorescence microscope at Ex/Em = 660/693 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

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

Note 20 µL of 500X Mitolite™ NIR (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™ NIR working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Replace Mitolite™ NIR 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 = 660/693 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™ NIR working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Replace Mitolite™ NIR 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 = 660/693 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

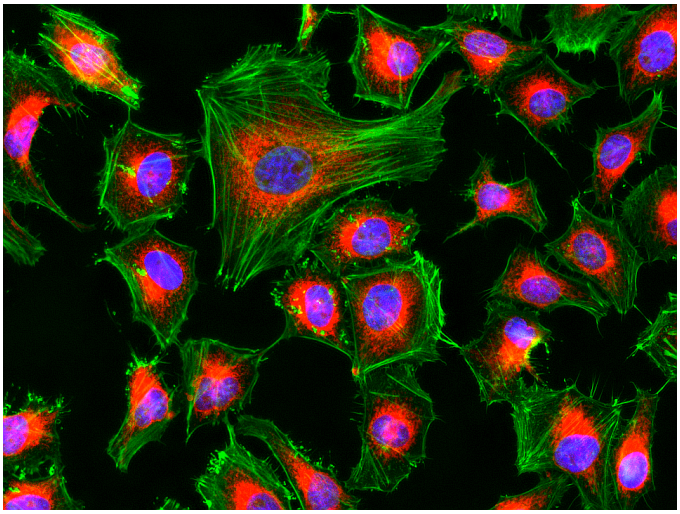

Figure 1.

Fluorescence images of HeLa cells stained with Cell Navigator™ Mitochondrion Staining Kit *NIR Fluorescence* using fluorescence microscope with a Cy5 filter set. Live cells were stained with mitochondria dye MitoLite™ NIR (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).