ab176747 CytoPainter Mitochondrial Staining Kit – NIR Fluorescence

Instructions for Use

For staining Mitochondria in live cells with our proprietary NIR probe.

This product is for research use only and is not intended for diagnostic use.

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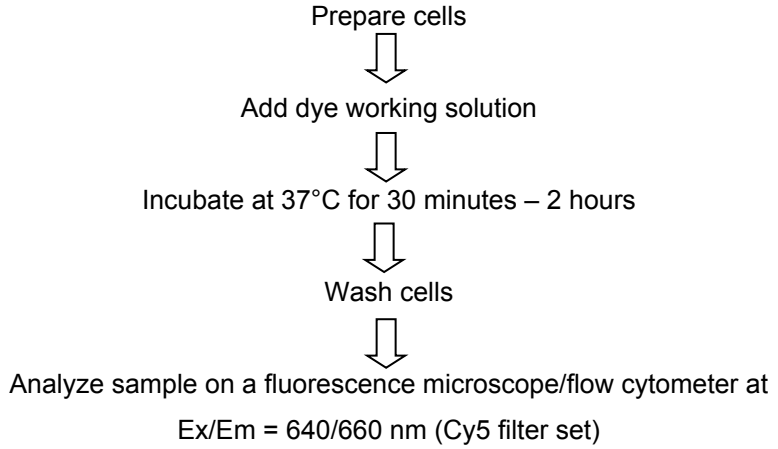
1. Introduction

CytoPainter Mitochondrial Staining Kit – NIR Fluorescence (ab176747) is designed to label mitochondria in live cells with red fluorescence. The kit uses our proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. The red fluorescent mitochondrial stain used in the kit has Ex/Em = 640/660 nm (Cy5 filter-compatible). 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.

Abcam's CytoPainter Mitochondrial Staining - NIR Fluorescence 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.

Mitochondria are membrane-enclosed organelles found in most eukaryotic cells. Mitochondria are sometimes described as "cellular power plants" because they 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. Although most of a cellular DNA is contained in the cell nucleus, the mitochondrion has its own independent genome.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
MitoNIR Indicator (500X DMSO solution)	1x 100 µL
Live Cell Staining Buffer	1x 50 mL

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light.

Thaw all the kit components to room temperature before staring the experiment.

5. Materials Required, Not Supplied

- HHBS Buffer (Hanks and 20 mM HEPES buffer) pH=7
- Microcentrifuge
- Pipettes and pipette tips
- Coverslips, petri dishes or well plates to grow cells

6. Assay Protocol

1. Reagent Preparation:

a) Warm MitoNIR Indicator to room temperature.

b) For a 1 x 96-well plate assay, prepare dye working solution by diluting 20 µL MitoNIR Indicator in 10 mL of Live Cell Staining Buffer.

NOTE: The optional concentration of the fluorescent lysosome indicator may vary depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability if the cells or tissues to the probe.

2. Sample Staining and Analysis:

2.1 Adherent cells:

a) Grow cells either in a 96-well back wall/clear bottom plate (100 µL/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture media.

b) Optional: If needed for your experiment, treat cells with test compounds for a desired period.

c) When cells reach the desired confluence, add equal volume of the dye-working solution (Step 1b).

d) Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes – 2 hours.

e) Wash cells twice with pre-warmed HBSS buffer at 37°C.

f) Fill the cell wells with HBSS or growth medium.

g) Observe cells using a fluorescence microscope fitted with a Cy5 filter set (Ex/Em = 640 – 660 nm).

2.2 Suspension cells:

a) Grow cells to desired number/cell density.

b) Optional: If needed for your experiment, treat cells with test compounds for a desired period.

c) Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.

d) Resuspend the cells gently in pre-warmed (37°C growth medium.

e) Add equal volume of the dye-working solution (Step 1b).

f) Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes – 2 hours.

g) Wash cells twice with pre-warmed HBSS buffer at 37°C.

h) Fill the cell wells with HBSS or growth medium.

i) Observe cells using a fluorescence microscope fitted with a Cy5 filter set (Ex/Em = 640 – 660 nm).

Note 1: 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.

Note 2: Suspension cells may be attached to cover-slips that have been treated and stained as adherent cells (see Step 2.1).

7. Data Analysis

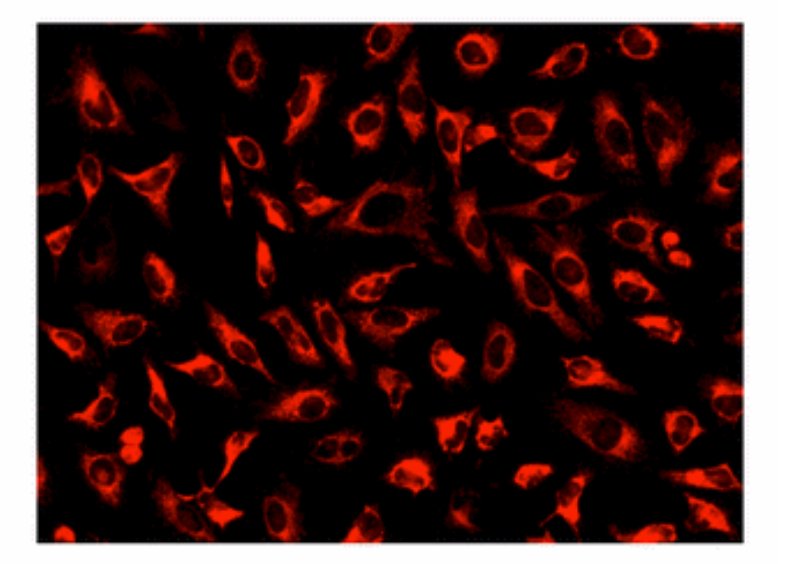


Figure 1. Image of HeLa cells stained with Abcam's CytoPainter Mitochondrial Staining Kit – NIR Fluorescence (ab176747) in a Costar black 96-well plate.

8. Troubleshooting

Problem	Reason	Solution
Mitochondria not sufficiently stained.	Too low dye concentration or incubation time insufficient	Increase concentration or incubation time
	Cells observed at incorrect wavelength	Ensure you are using appropriate filter settings
Cells do not appear healthy	Cells require serum to remain healthy	Add serum to stain and wash solutions. Try range 2 – 10% serum.
Nuclear counterstain is too bright	Different microscopes, cameras and filters may make some signals appear very bright	Reduce concentration of nuclear counterstain or shorten exposure time.

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