Cell Navigator® Mitochondrion Staining Kit *Orange Fluorescence with 405 nm Excitation*

Catalog number: 22673
Unit size: 500 Assays

Component	Storage	Amount (Cat No. 22673)
Component A: MitoViolet™ 550	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL, 500X DMSO stock solution)
Component B: Live Cell Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (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, 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 orange fluorescence of large Stokes Shift. The kit uses a proprietary dye that selectively accumulates in mitochondria probably via the mitochondrial membrane potential gradient. 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 mitochondrial stain used in the kit is quite photostable, making the fluorescence quite robust. 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 MitoViolet™ 550 working solution
3. Incubate at 37°C for 30 minutes to 2 hours
4. Wash and Replace with growth medium
5. Analyze the cells under fluorescence microscope at Ex/Em = 405/550 nm (Violet filter set)

Important Note

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

KEY PARAMETERS

Fluorescence microscope

Emission	Violet filter
Excitation	Violet filter
Recommended plate	Black wall/clear bottom

CELL PREPARATION

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

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X MitoViolet™ 550 (Component A) into 10 mL of Live Cell Staining Buffer (Component B) to make MitoViolet™ 550 working solution. Protect from light. **Note:** 20 µL of 500X MitoViolet™ 550 (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.

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 MitoViolet™ 550 working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Wash and replace MitoViolet™ 550 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 Violet filter set (Ex/Em = 405/550 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 MitoViolet™ 550 working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Wash and replace MitoViolet™ 550 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 Violet filter set (Ex/Em = 405/550 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

Placeholder for image details

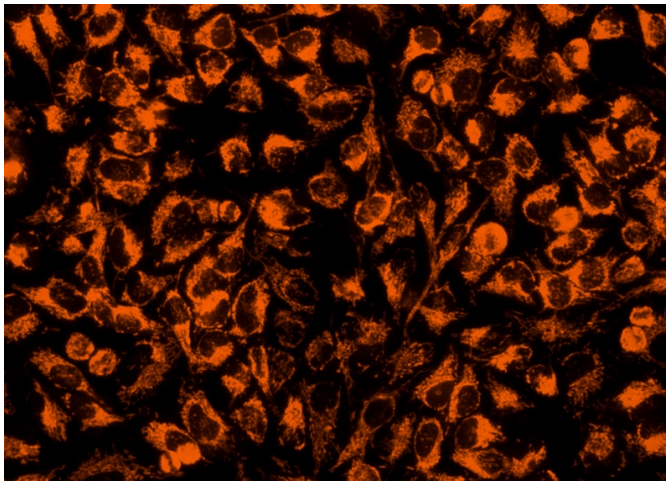

Figure 1. Image of HeLa cells stained with Cell Navigator® Mitochondrion Staining Kit in a Costar black wall/clear bottom 96-well plate.

DISCLAIMER

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