MitoLite™ Dyes

Introduction

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.

AAT Bioquest's MitoLite™ reagents are a set of fluorogenic probes to label mitochondria of live cells. The proprietary mitochondrial dyes selectively accumulate in mitochondria probably via the mitochondrial membrane potential gradient. The mitochondrial indicators are hydrophobic compounds that easily permeate intact live cells and trapped in mitochondria after they get into cells. The fluorescent mitochondrial indicators are retained in mitochondria for a long time since they carry a cell-retaining group. This key feature significantly increases the staining efficiency, making them useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity.

Chemical and Physical Properties

Catalog Number	MitoLite™ Dyes	Unit	Molecular weight	Excitation	Emission	Fixable?
22674	MitoLite™ Blue FX490	500 tests	~ 600	350 nm	490 nm	Yes
22675	MitoLite™ Green EX488	500 tests	~ 400	498 nm	520 nm	NO
22676	MitoLite™ Orange FX570	500 tests	~ 500	545 nm	575 nm	Yes
22677	MitoLite™ Red FX600	500 tests	~ 800	575 nm	600 nm	Yes
22678	MitoLite™ Deep Red FX660	500 tests	~ 700	640 nm	659 nm	Yes
22679	MitoLite™ Orange 405	500 tests	~ 500	399 nm	550 nm	NO
22690	MitoLite™ NIR FX690	500 tests	~ 700	660 nm	692 nm	Yes

Storage and Handling Conditions

The The MitoLite™ stock solutions provided are 500X in DMSO. They should be stable for at least 6 months if store at <-15 ºC, and avoid light and freeze/thaw cycles.

Assay Protocol with MitoLite™ Dyes

Brief Summary

Prepare cells → Add dye working solution → Incubate at 37 °C for 30 minutes to 2 hours → Wash the cells → Analyze under fluorescence microscope

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare mitochondria-staining solution:

1.1 Warm MitoLite™ dyes to room temperature.

1.2 Prepare dye working solution by diluting 20 µL of 500 X Mitolite™ dyes to 10 mL of Hanks and 20 mm Hepes buffer or buffer (HBSS) of your choice.
Note 1: 20 µL of Mitolite™ dye is enough for one 96-well plate. Aliquot and store unused MitoLite™ dye stock solutions at < -15 °C. Protect it from light and avoid repeated freeze-thaw cycles.
Note 2: The optimal concentration of the fluorescent mitochondria indicators 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.

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2. Prepare and stain cells:

2.1 For adherent cells: a). 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. When cells reach the desired confluence, add equal volume of the dye-working solution (from Step 1.2). b). Incubate the cells in a 37 ºC, 5% CO_2 incubator for 30 minutes to 2 hours. c). Replace the dye-loading solution or wash (especially for cat#22679) the cells with pre-warmed (37 ºC) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Fill the cell wells with HBSS or growth medium. d). Observe the cells using a fluorescence microscope fitted with a desired filter set.
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.

2.2 For suspension cells: Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellets gently in pre-warmed (37 ºC) growth medium, and add equal volume of the dye-working solution (from Step 1.2). Incubate the cells in a 37 ºC, 5% CO_2 incubator for 30 minutes to 2 hours. Replace the dye-loading solution or wash (especially for cat#22679) the cells 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). Fill the cell wells with HBSS or growth medium. Observe the cells using a fluorescence microscope fitted with a desired filter set.

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 with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Step 2.1).

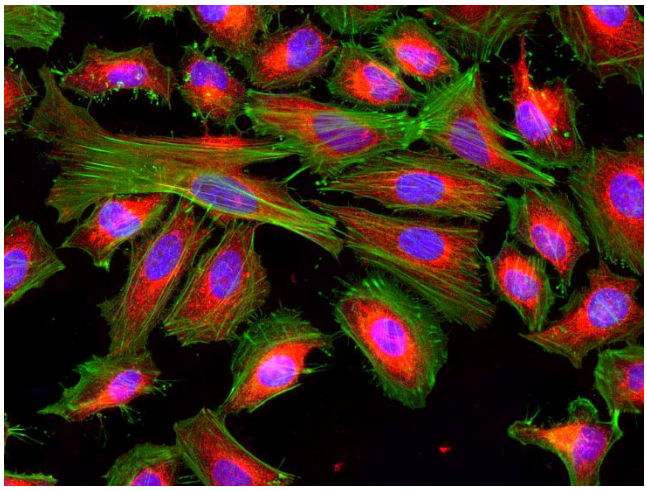

Figure 1. Fluorescence image of HeLa cells stained with mitochondria dye MitoLite™ Red FX600 (Cat#22677, Red) using fluorescence microscope with a Cy5 filter set. After fixation, the cells were labeled with F-actin dye iFluor™ 488-Phalloidin (Cat#23115, Green) and counterstained with Nuclear Blue™ DCS1 (Cat#17548, Blue).

References

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3. Martinez-Caballero S, Peixoto PM, Kinnally KW, Campo ML. (2007) A fluorescence assay for peptide translocation into mitochondria. Anal Biochem, 362, 76.
4. Swayne TC, Gay AC, Pon LA. (2007) Fluorescence imaging of mitochondria in yeast. Methods Mol Biol, 372, 433.
5. Duffy CF, MacCraith B, Diamond D, O'Kennedy R, Arriaga EA. (2006) Fast electrophoretic analysis of individual mitochondria using microchip capillary electrophoresis with laser induced fluorescence detection. Lab Chip, 6, 1007.