MitoBright LT Green
MitoBright LT Red
MitoBright LT Deep Red

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/MT10.pdf

General Information

Mitochondria are the principal cellular organelle for oxidative phosphorylation and the production of ATP and mitochondrial dysfunction is relevant to cancer, cell senescence and neurodegenerative diseases such as Alzheimer's and Parkinson's.

Methods for monitoring mitochondrial morphology, dynamics, and number are usually based on small fluorescent molecules or plasmid transfection techniques. The use of plasmids requires the target protein to be stably expressed, while small fluorescent molecules are widely used because they can simply be added to cells. Among commercially available small fluorescent molecules, those containing the chloromethyl moiety are commonly used. However, these dyes have some limitations, including short-term retention in cells, decreased fluorescence intensity in serum, and high background.

Dojindo's MitoBright LT dyes overcome these limitations. MitoBright LT dyes are designed to exhibit mitochondrial retention for long-term visualization. In addition, the MitoBright LT dyes show stronger fluorescence signals compared with other commercially available dyes that contain the chloromethyl moiety. The MitoBright LT dyes offer three different color options (Green, Red and Deep Red), and are provided as a ready-to-use DMSO solution. A working solution can easily be prepared in a single dilution step with growth medium or HBSS.

Contents

MT10 MitoBright LT Green
MT11 MitoBright LT Red
MT12 MitoBright LT Deep Red

Staining number possible by Unit Size (35 mm dish)

	Unit Size		
Code	20 µL	400 µL	400 µL x3
MT10	10	200	600
MT11			
MT12			

Storage Condition

MT10 Store at -20°C, protected from light and moisture.
MT11 Store at -20°C, protected from light and moisture.
MT12 Store at -20°C, protected from light and moisture.

Required Equipment and Materials

- Growth medium or HBSS
- Micropipettes

Preparation of Solutions

Preparation of MitoBright LT working solution

Dilute the 0.1 mmol/L MitoBright LT solution with growth medium to prepare a 0.1 µmol/L MitoBright LT working solution.

*Please use the MitoBright LT working solution within the day.

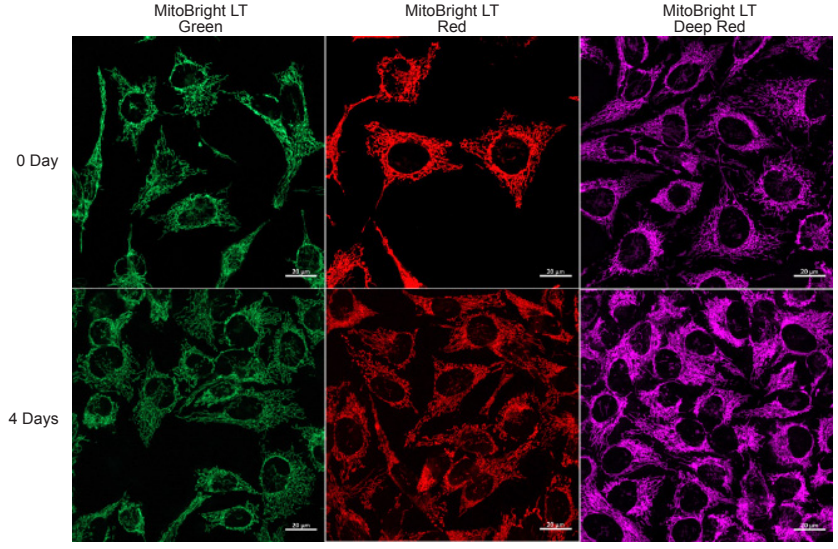
General protocol

1. Seed cells in a dish and culture them overnight in a 37°C incubator equilibrated with 95% air and 5% CO_2.
2. Discard the supernatant and wash the cells once with growth medium.
3. Add the 0.1 µmol/L MitoBright LT working solution to the cells and incubate them in a 37°C incubator equilibrated with 95% air and 5% CO_2 for 15 minutes.
4. Discard the supernatant and wash the cells twice with growth medium.
5. Add growth medium or HBSS to the cells, then observe the cells under a fluorescence microscope.

Usage Examples

Detection of fluorescent mitochondria in HeLa cells over time.

1. HeLa cells in MEM (containing 10% fetal bovine serum, 1% penicillin-streptomycin) were seeded on a µ-slide 8 well plate (ibidi) and cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO_2.
2. After the supernatant was removed, MitoBright LT working solution (0.1 µmol/L, 200 µL) was added and the cells cultured in a 37°C incubator equilibrated with 95% air and 5% CO_2 for 30 minutes.
3. The cells were then washed twice with 200 µL MEM.
4. MEM (without phenol red) was then added, and the cells were observed over time under a fluorescence microscope.



MitoBright LT Green
Excitation: 488 nm
Emission: 500–560 nm

MitoBright LT Red
Excitation: 561 nm
Emission: 560–620 nm

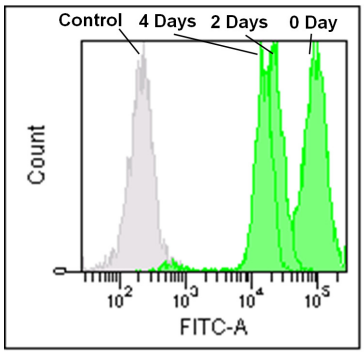
MitoBright LT Deep Red
Excitation: 640 nm
Emission: 650–700 nm

Figure 1 Images of mitochondrial fluorescence using MitoBright LT dyes in cells cultured for 4 days.

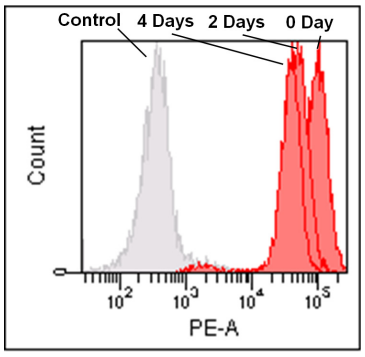
MT10 : MitoBright LT Green
MT11 : MitoBright LT Red
MT12 : MitoBright LT Deep Red
Revised on Aug. 27, 2019

Flow cytometry analysis of mitochondria in Jurkat cells over time.

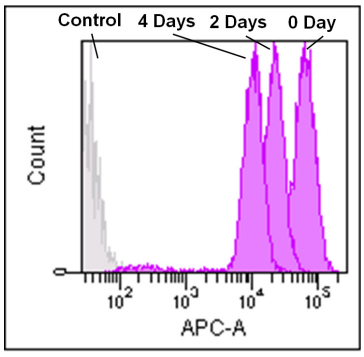
1. Jurkat cells in RPMI (containing 10% fetal bovine serum, 1% penicillin-streptomycin) were seeded in a dish and were cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO_2.
2. After the supernatant was removed, MitoBright LT working solution (0.1 μmol/L, 5 mL) was added, and the cells were cultured in a 37°C incubator equilibrated with 95% air and 5% CO_2 for 30 minutes.
3. The cells were then washed twice with 5 mL RPMI.
4. RPMI was then added and the cells were analyzed over time by flow cytometry.



MitoBright LT Green
Excitation: 488 nm
Emission: 515 nm–545 nm



MitoBright LT Red
Excitation: 488 nm
Emission: 564–604 nm



MitoBright LT Deep Red
Excitation: 633 nm
Emission: 650–670 nm

Figure 2 Flow cytometry analysis of mitochondria over time using MitoBright LT dyes.

Excitation and emission spectra of MitoBright LT dyes

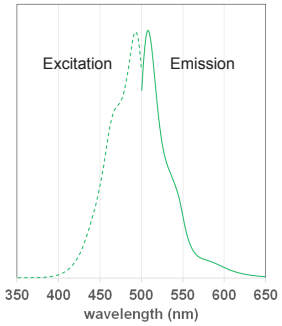


Figure 3 Excitation and emission spectra of MitoBright LT Green

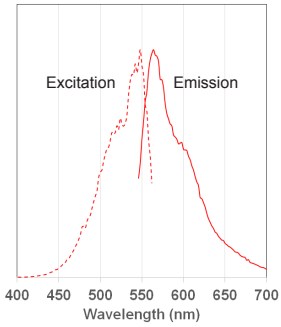


Figure 4 Excitation and emission spectra of MitoBright LT Red

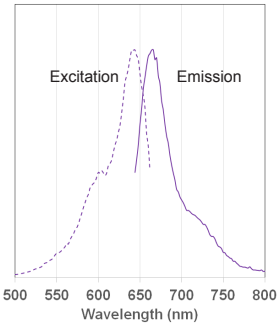
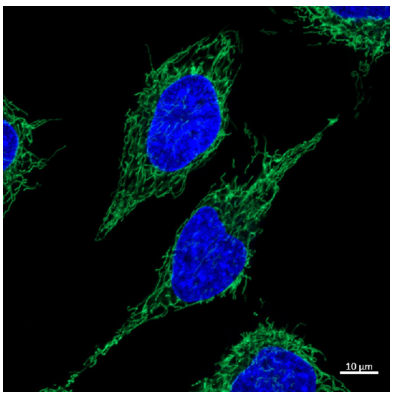
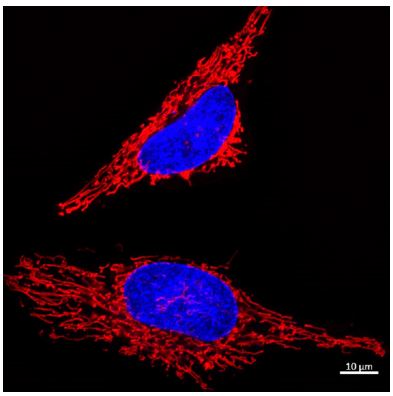


Figure 5 Excitation and emission spectra of MitoBright LT Deep Red

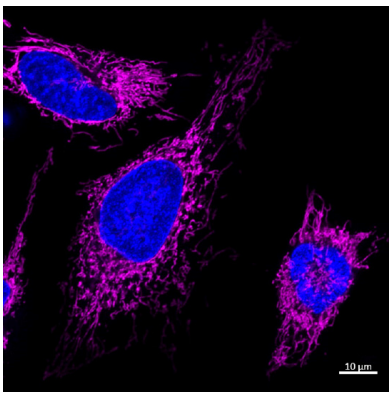
Fluorescence imaging of mitochondria using MitoBright LT dyes



Concentration of dye: 0.1 μmol/L
Cell line: HeLa cells
Excitation: 488 nm
Emission: 500–560 nm
Nuclear stain: Hoechst 33342



Concentration of dye: 0.1 μmol/L
Cell line: HeLa cells
Excitation: 561 nm
Emission: 560–620 nm
Nuclear stain: Hoechst 33342



Concentration of dye: 0.1 μmol/L
Cell line: HeLa cells
Excitation: 640 nm
Emission: 650–700 nm
Nuclear stain: Hoechst 33342

Figure 6 Images of mitochondrial fluorescence using LT MitoBright dyes.

If you need more information, please contact Dojindo technical service.

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