MitoBright IM Red for Immunostaining *Technical Manual*

General Information

Cells have intracellular organelles that function to produce energy, synthesize and degrade proteins. Typical organelles include the nucleus, mitochondria, endoplasmic reticulum, and lysosomes. Although research on the functions of these organelles has been actively conducted for some time, recent developments in imaging technology, such as super-resolution imaging, have revealed that not only the functions of each organelle but also the coordination among organelles is important. One such example is the mitochondria-associated endoplasmic reticulum membrane, which is the interface between mitochondria and the endoplasmic reticulum (ER). Visualization of mitochondria is necessary to observe the interaction between mitochondria and other organelles. Fluorescent protein labeling, immunostaining, and mitochondria-selective small molecule fluorochromes are commonly used for this purpose, although these methods have problems. The fluorescent protein labeling method requires gene transfer, and multiple staining may not be able to perform in the immunostain method depending on the animal species of the primary antibody. In addition, commercially available mitochondria-selective small molecule fluorescent dyes do not have good retention properties and are difficult to use in immunostaining.

To overcome the problems above, Dojindo has developed a new mitochondria-selective small-molecule fluorescent dye, MitoBright IM Red, which covalently binds to proteins and other biomolecules. MitoBright IM Red, therefore, is able to simultaneously perform the staining mitochondria and the immunostaining.

Contents

	1 Tube	3 Tubes
MitoBright IM Red for Immunostaining	20 µl x 1	20 µl x 3

Storage Condition

Store at -20°C

Required Equipment and Materials

- Growth medium or HBSS (Hanks balanced salt solution)
- Micropipettes

Preparation of Solutions

Preparation of MitoBright IM Red working solution

Dilute the 1 mmol/l MitoBright IM Red solution with growth medium (serum-free) to prepare a 1 µmol/l MitoBright IM Red working solution.

Note: The MitoBright IM Red working solution cannot be stored and must be freshly prepared each day and used within the day.

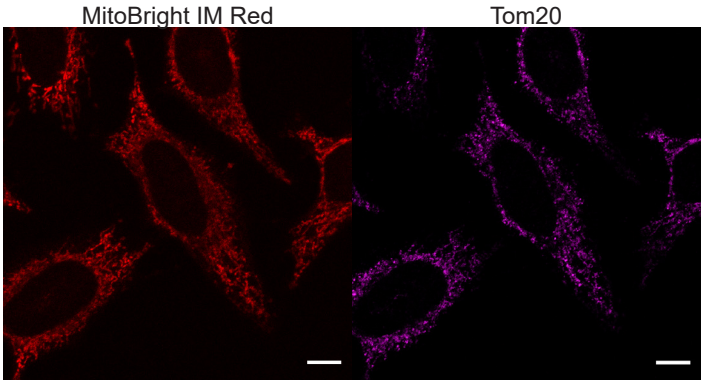
General protocol

1. Seed cells on a dish. Culture the cells at 37°C overnight in a 5% CO_2 incubator.
2. Discard the supernatant and wash the cells once with growth medium.
3. Add the 1 µmol/l MitoBright IM Red working solution to the dish and incubate at 37°C for 30 minutes in a 5% CO_2 incubator.
4. Discard the supernatant, and wash the cells once with growth medium.
5. Perform immunofluorescence staining.
6. Observe the cells under a fluorescence microscope.

Usage Examples

Multiple staining of MitoBright IM Red with Tom20 as a mitochondrial marker in HeLa cells.

1. HeLa cells (2.4×10^4 cells/well, 200 µl) in MEM (10% FBS) were seeded in a µ-slide 8 well plate and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, the cells were washed once with 200 µl of MEM.
3. MitoBright IM Red working solution (1 µmol/l, 200 µl) in MEM was added, and the cells were incubated at 37°C for 30 minutes in a 5% CO_2 incubator.
4. After removing the supernatant, the cells were washed twice with 200 µl of MEM.
5. Paraformaldehyde (4% v/v) in PBS (200 µl) was added, and the cells were incubated at room temperature for 15 minutes.
6. After removing the supernatant, the cells were washed three times with 200 µl of PBS.
7. Triton X-100 (0.1% v/v) in PBS (200 µl) was added, and the cells were incubated at room temperature for 10 minutes.
8. After removing the supernatant, 10% (v/v) Blocking One-P in PBS (200 µl) was added, and the cells were incubated at room temperature for 1 hour.
9. After removing the supernatant, anti-Tom20 antibody (1 µg/ml, 200 µl) was added, and the cells were incubated at room temperature for 1 hour.
10. After removing the supernatant, the cells were washed three times with 200 µl of PBS.
11. Alexa647 labeled anti-mouse IgG (2 µg/ml, 200 µl) was added, and the cells were incubated at room temperature for 1 hour.
12. After removing the supernatant, the cells were washed twice with 200 µl of PBS.
13. The cells were observed under a confocal laser scanning microscope (LSM 800, Zeiss).



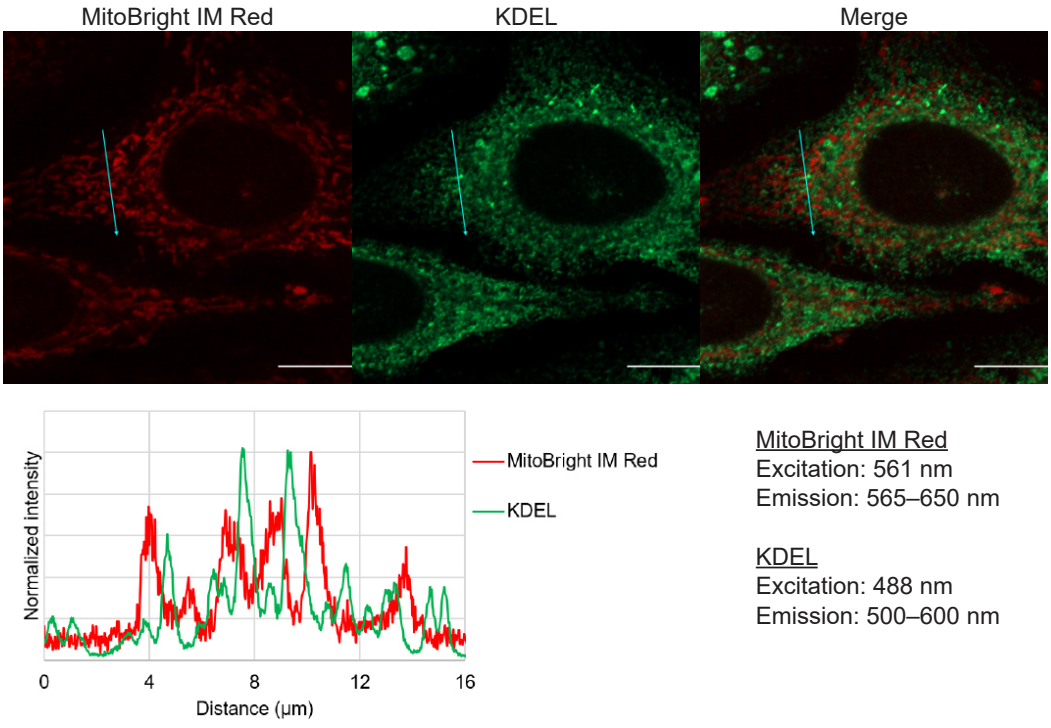
MitoBright IM Red
Excitation: 561 nm
Emission: 565–650 nm

Tom20
Excitation: 640 nm
Emission: 640–700 nm

Figure 1 Fluorescence images using MitoBright IM Red and Tom20.

Multiple staining of MitoBright IM Red with KDEL as an ER marker in HeLa cells.

1. HeLa cells (2.4 × 10^4 cells/well, 200 µl) in MEM (10% FBS) were seeded in a µ-slide 8 well plate and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, the cells were washed once with 200 µl of MEM.
3. MitoBright IM Red working solution (1 µmol/l, 200 µl) in MEM was added, and the cells were incubated at 37°C for 30 minutes in a 5% CO_2 incubator.
4. After removing the supernatant, the cells were washed twice with 200 µl of MEM.
5. Paraformaldehyde (4% v/v) in PBS (200 µl) was added, and the cells were incubated at room temperature for 15 minutes.
6. After removing the supernatant, the cells were washed three times with 200 µl of PBS.
7. Triton X-100 (0.1% v/v) in PBS (200 µl) was added, and the cells were incubated at room temperature for 10 minutes.
8. After removing the supernatant, 10% (v/v) Blocking One-P in PBS (200 µl) was added, and the cells were incubated at room temperature for 1 hour.
9. After removing the supernatant, anti-KDEL antibody (1 µg/ml, 200 µl) was added, and the cells were incubated at 4°C for overnight.
10. After removing the supernatant, the cells were washed three times with 200 µl of PBS.
11. Alexa488 labeled anti-mouse IgG solution (2 µg/ml, 200 µl) was added, and the cells were incubated at room temperature for 1 hour.
12. After removing the supernatant, the cells were washed twice with 200 µl of PBS.
13. The cells were observed under a confocal laser scanning microscope (LSM 800, Zeiss).



MitoBright IM Red
Excitation: 561 nm
Emission: 565–650 nm

KDEL
Excitation: 488 nm
Emission: 500–600 nm

Figure 2 Multiple staining images and line profiles of MitoBright IM Red and KDEL.

Excitation and emission spectra of MitoBright IM Red

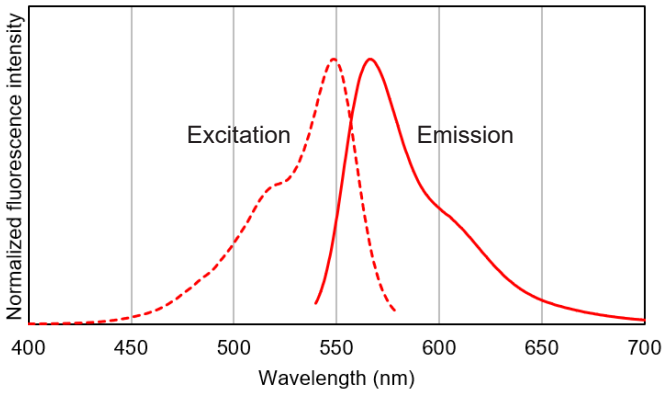


Figure 3 Excitation and emission spectra of MitoBright IM Red.

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