

MitoTracker[®] and MitoFluor[™] Mitochondrion-Selective Probes

Quick Facts

Storage upon receipt:

- -20°C
- Avoid freeze–thaw cycles
- Desiccate
- Protect from light

Ex/Em: See Table 1

Solvent for stock: DMSO

keeping the dye associated with the mitochondria after fixation (Figure 1). To label mitochondria, cells are simply incubated in submicromolar concentrations of a MitoTracker probe, which passively diffuses across the plasma membrane and accumulates in active mitochondria. Once their mitochondria are labeled, the cells can be treated with an aldehyde-based fixative to allow further processing of the sample. Because most of the MitoTracker probes are also retained after permeabilization with detergents or organic solvents, the sample continues to exhibit the fluorescent staining pattern characteristic of live cells during subsequent processing steps (e.g., immunocytochemistry, *in situ* hybridization or electron microscopy). In addition, MitoTracker probes eliminate some of the difficulties of working with pathogenic cells because, once the mitochondria are stained, the cells can be treated with fixatives before the sample is analyzed.

The MitoTracker probes differ in spectral characteristics and fixability (Table 1). MitoTracker probes are provided in specially packaged sets of 20 vials, each containing 50 µg for reconstitution as required.

Orange-, Red- and Deep Red-Fluorescent MitoTracker Dyes. We offer MitoTracker Orange CMTMRos (M-7510), a derivative of tetramethylrosamine, and MitoTracker Red CMXRos (M-7512), a derivative of X-rosamine, as well as our newest MitoTracker dyes, MitoTracker Red 580 (M-22425) and MitoTracker Deep Red 633 (M-22426). MitoTracker Red CMXRos, MitoTracker Red 580 and MitoTracker Deep Red 633 are all well suited for multicolor labeling experiments because their red fluorescence is well resolved from the green fluorescence of other probes. Reduced MitoTracker dyes, MitoTracker Orange CM-H₂TMRos (M-7511) and MitoTracker Red CM-H₂XRos (M-7513), which are derivatives of dihydrotetramethylrosamine and dihydro-X-rosamine, respectively, are also available. The reduced probes do not fluoresce until they enter an

Introduction

MitoTracker Dyes – Fixable Mitochondrion-Selective Probes

Although conventional fluorescent stains for mitochondria, such as tetramethylrosamine and rhodamine 123, are readily sequestered by functioning mitochondria, these stains are easily washed out of cells once the mitochondria experience a loss in membrane potential. This characteristic limits the use of such conventional stains in experiments that require cells to be treated with aldehyde fixatives or with other agents that affect the energetic state of the mitochondria. To overcome this limitation, Molecular Probes has developed the MitoTracker[®] probes — a series of patented mitochondrion-selective stains that are concentrated by active mitochondria and well retained during cell fixation.¹

The cell-permeant MitoTracker probes contain a mildly thiol-reactive chloromethyl moiety that appears to be responsible for

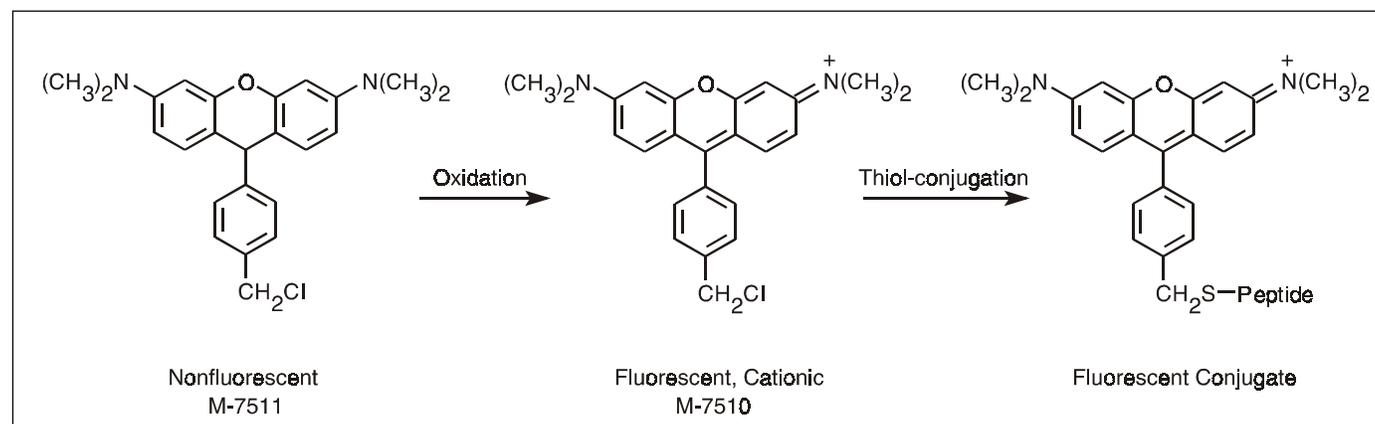


Figure 1. The intracellular reactions of our fixable mitochondrion-selective dye, MitoTracker Orange CM-H₂TMRos. When this cell-permeant probe enters an actively respiring cell, it is oxidized to MitoTracker Orange CMTMRos and sequestered in the mitochondria, where it reacts with thiols on proteins and peptides to form an aldehyde-fixable conjugate.

Table 1. Spectral characteristics of MitoTracker and MitoFluor probes.

Product	Catalog Number	Ex (nm) *	Em (nm) *	Fixable?
MitoTracker Green FM	M-7514 †	490	516	No
MitoTracker Orange CMTMRos	M-7510	554	576	Yes
MitoTracker Orange CM-H ₂ TMRos	M-7511 ‡	554	576	Yes
MitoTracker Red CMXRos	M-7512	579	599	Yes
MitoTracker Red CM-H ₂ XRos	M-7513 ‡	579	599	Yes
MitoTracker Red 580	M-22425	581	644	Yes
MitoTracker Deep Red 633	M-22426	644	665	Yes
MitoFluor Green	M-7502 †	490	516	No
MitoFluor Red 589	M-22424	588	622	No
MitoFluor Red 594	M-22422	598	630	No
MitoFluor Far Red 680	M-22423	680	>700	No

* Fluorescence excitation and emission maxima determined in methanol; values may vary somewhat in cellular environments. † Nonfluorescent in aqueous solution. ‡ Nonfluorescent until oxidized.

actively respiring cell, where they are oxidized to the corresponding fluorescent mitochondrion-selective probe and then sequestered in the mitochondria. All of these MitoTracker probes are well retained after fixation and permeabilization.

Green-Fluorescent MitoTracker Green FM. Mitochondria in cells stained with nanomolar concentrations of MitoTracker Green FM dye (M-7514) exhibit bright green, fluorescein-like fluorescence.² MitoTracker Green FM dye has the added advantage that it is essentially nonfluorescent in aqueous solutions, only becoming fluorescent once it accumulates in the lipid environment of mitochondria. Hence, background fluorescence is negligible, enabling researchers to clearly visualize mitochondria in live cells immediately following addition of the stain, and without a wash step. Furthermore, MitoTracker Green FM dye is substantially more photostable than the widely used fluorescent dye rhodamine 123 and produces a brighter, more mitochondrion-selective signal at lower concentrations. Because its emission maxima is blue-shifted approximately 10 nm relative to the emission maximum of rhodamine 123, MitoTracker Green FM dye produces a fluorescent staining pattern that should be better resolved from that of red-fluorescent probes in double-labeling experiments. Although MitoTracker Green FM dye can selectively stain mitochondria both in live cells and in cells that have been fixed, the dye is not well retained after cell permeabilization.

MitoFluor Dyes – Nonfixable Mitochondrion-Selective Probes

MitoFluor Red 594 and MitoFluor Far Red 680 Dyes. We offer two mitochondrial membrane potential-sensing dyes that have long-wavelength fluorescence emission: MitoFluor™ Red 594 dye (M-22422) and MitoFluor Far Red 680 dye (M-22423). MitoFluor Red 594 dye was designed for optimal excitation by the 594 spectral line of the He-Ne laser. This long-wavelength

probe provides a clear spectral window below 600 nm for the simultaneous detection of green-fluorescent labels, other site-selective probes and green-fluorescent protein chimeras. MitoFluor Far Red 680 dye, also known as rhodamine 800, is a mitochondrial potential sensor with absorption and fluorescence emission in the near-infrared spectral region. Accumulation of MitoFluor Far Red 680 dye by active mitochondria produces a slight red-shift in its absorption and fluorescence emission peaks that is accompanied by a marked decrease in fluorescence intensity.³ Although its fluorescence is not directly visible and must be captured using an infrared light-sensitive detector such as a CCD camera, MitoFluor Far Red 680 dye offers advantages when working with tissue, blood and other biological fluids prone to high absorbance or autofluorescence at shorter wavelengths.⁴ The MitoFluor Red 594 and MitoFluor Far Red 680 dyes can only be used to stain live cells and are not retained after fixation.

MitoFluor Red 589 Dye. MitoFluor Red 589 dye (M-22424) appears to accumulate in mitochondria regardless of mitochondrial membrane potential, making it a useful stain in both live and fixed cells. The probe has absorption and emission peaks at 588 nm and 622 nm, respectively, and can be viewed with common filter sets appropriate for the Texas Red dye. Like our other MitoFluor Red dyes, MitoFluor Red 589 dye provides a clear spectral window below 600 nm for double-labeling with green-fluorescent probes.

MitoFluor Green Dye. As a companion to MitoTracker Green FM dye, we offer MitoFluor Green dye^{2,5} (M-7502). MitoFluor Green dye has a structure similar to MitoTracker Green FM dye, but lacks its reactive chloromethyl moieties, is not as well retained following aldehyde fixation, and does not survive cell permeabilization. Like MitoTracker Green FM dye, it can selectively stain mitochondria both in live cells and in cells that have been fixed. MitoFluor Green dye is also substantially more photostable than rhodamine 123, producing a brighter, more mitochondrion-selective signal at lower concentrations. Because its emission maxima is blue-shifted relative to the emission maximum of rhodamine 123, MitoFluor Green dye produces a fluorescent staining pattern that should be better resolved from that of red-fluorescent probes in double-labeling experiments.

Storage and Handling

The MitoTracker probes are provided in specially packaged sets of 20 separate vials, each containing 50 µg of lyophilized solid for reconstitution as required. The MitoFluor Green, MitoFluor Red 589 and MitoFluor Red 594 probes are provided in unit sizes of 1 mg. The MitoFluor Far Red 680 probe is supplied in a unit size of 10 mg. Upon receipt, the lyophilized solids should be stored desiccated at -20°C until required for use. When stored as solids, these reagents are stable for at least six months. **AVOID REPEATED FREEZING AND THAWING.**

Before opening a vial, allow the product to warm to room temperature. To prepare a stock solution, dissolve the lyophilized product in high-quality, anhydrous dimethylsulfoxide (DMSO) to a final concentration of 1 mM; the molecular weight (MW) is indicated on the product label. The reduced rosamine MitoTracker probes (M-7511, M-7513) are quite sensitive to oxidation, especially in solution, and must be stored under argon or nitrogen, at -20°C and protected from light. It is preferable to use solutions of the dihydro derivatives on the day that they are prepared. All other solutions of the MitoTracker and MitoFluor dyes can be stored frozen at -20°C and protected from light.

Protocol

Cell Preparation and Staining

1.1 Preparing staining solutions. The concentration of probe for optimal staining will vary by application. The initial conditions suggested here are guidelines that may need to be modified based on the particular cell type or on other factors, such as the permeability of the cells or tissues to the probe. In general, we have found that the reduced rosamine MitoTracker probes (M-7511, M-7513) should be loaded at three- to fivefold higher concentrations than other MitoTracker and MitoFluor probes. Dilute the 1 mM MitoTracker or MitoFluor stock solution (see *Storage and Handling* for preparation) to the final working concentration in growth medium, e.g., Dulbecco's modified Eagle medium (D-MEM), with or without serum to match the medium that the cells were grown in. For live-cell staining, we recommend working concentrations of 25–500 nM. For staining cells that are to be fixed and permeabilized (see *Fixation and Permeabilization after Staining*), we suggest using a working concentration of 100–500 nM. To reduce potential artifacts from overloading, the concentration of dye should be kept as low as possible. For the MitoTracker Green FM and MitoFluor Green probes, we suggest using a slightly lower concentration (20–200 nM). At higher concentrations, these probes tend to stain other cellular structures.

1.2 Staining adherent cells. Grow cells on coverslips inside a Petri dish filled with the appropriate culture medium. When cells have reached the desired confluence, remove the medium from the dish and add the prewarmed (37°C) growth medium containing the MitoTracker or MitoFluor probe (prepared in step 1.1). Incubate the cells for 15–45 minutes under growth conditions appropriate for the particular cell type. Then replace the loading solution with fresh prewarmed medium and observe the cells using a fluorescence microscope fitted with the correct filter set (see Table 1). If the cells do not appear to be sufficiently stained, we recommend either increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the mitochondria once the cells have been transferred to fresh medium. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization after Staining*. The MitoFluor probes are not well retained after fixation and permeabilization.

1.3 Staining suspension cells. Centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) medium containing the MitoTracker or MitoFluor probe (prepared in step 1.1). Incubate the cells for 15–45 minutes under growth conditions that are appropriate for the particular cell type. Re-pellet the cells by centrifugation and resuspend in fresh prewarmed medium. Again, if the cells are not sufficiently stained, we recommend increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the mitochondria once the cells have been transferred to fresh medium. Alternatively, suspension cells may be attached to coverslips that have been treated with BD Cell-Tak™ Cell and Tissue Adhesive (BD Bioscience, Bedford, MA); in this case, see step 1.2. If the cells are to be fixed and permeabilized, continue

to *Fixation and Permeabilization after Staining*. The MitoFluor probes are not well retained after fixation and permeabilization.

1.4 Staining fixed cells. The MitoTracker Green FM, MitoFluor Green, MitoFluor Red 589, MitoTracker Red 580 and MitoTracker Deep Red 633 probes may be used to stain cells fixed in formaldehyde (see step 2.2, below). Following fixation, the cells should be rinsed in PBS. Incubate the fixed cells (on slides or in suspension) for 10–20 minutes in PBS containing 10–200 nM of probe. Note that the loading concentration is lower and staining time is shorter than in the procedure for staining live cells. After the incubation, wash the cells at least once with fresh PBS.

Fixation and Permeabilization after Staining with MitoTracker Dyes

After staining live cells with one of the MitoTracker dyes, it is often convenient to fix the cells in formaldehyde and to permeabilize them with Triton® X-100. For example, fixation and permeabilization makes it possible to probe for other intracellular structures by immunocytochemistry. Most of the MitoTracker dyes are well-retained following fixation and permeabilization using the protocol described below. However, MitoTracker Green FM and the MitoFluor dyes are not retained well. Note: Mitotracker Red 580 is not compatible with Triton X-100 detergent permeabilization.

2.1 Washing the cells. After staining, wash the cells in fresh, prewarmed growth medium. This step is especially important if the cells are attached to a BD Cell-Tak Adhesive-coated coverslip or another amine-containing surface.

2.2 Fixing the cells. Carefully remove the growth medium covering the cells, and replace it with freshly prepared, prewarmed growth medium containing 3.7% formaldehyde. Note, if the growth medium contains serum, then the formaldehyde solution should be prepared in growth medium containing serum. Incubate at 37°C for 15 minutes.

2.3 Rinsing the cells. After fixation, rinse the cells several times in PBS.

2.4 Permeabilization. When the cells are going to be subsequently labeled with an antibody, a permeabilization step is usually required to enhance the antigen's accessibility. Incubate the fixed cells in PBS containing 0.2% Triton X-100 at room temperature for 5 minutes. Following permeabilization, rinse the cells in PBS. Alternatively, the cells may be permeabilized by incubating in ice-cold acetone for 5 minutes, and then washed in PBS. Even when cells are not going to be labeled with an antibody, this acetone-permeabilization step may be useful because it appears to improve signal retention.

Fluorescence Microscopy

Spectral characteristics for the MitoTracker and MitoFluor probes are summarized in Table 1.

References

1. J Histochem Cytochem 44, 1363 (1996); 2. Cytometry 39, 203 (2000); 3. J Biochem (Tokyo) 121, 29 (1997); 4. Anal Biochem 279, 142 (2000); 5. Mol Cell Biochem 172, 171 (1997).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
M-22423	MitoFluor™ Far Red 680	10 mg
M-7502	MitoFluor™ Green	1 mg
M-22424	MitoFluor™ Red 589	1 mg
M-22422	MitoFluor™ Red 594	1 mg
M-22426	MitoTracker® Deep Red 633 *special packaging*	20 x 50 µg
M-7514	MitoTracker® Green FM *special packaging*	20 x 50 µg
M-7511	MitoTracker® Orange CM-H ₂ TMRos *special packaging*	20 x 50 µg
M-7510	MitoTracker® Orange CMTMRos *special packaging*	20 x 50 µg
M-22425	MitoTracker® Red 580 *special packaging*	20 x 50 µg
M-7513	MitoTracker® Red CM-H ₂ XRos *special packaging*	20 x 50 µg
M-7512	MitoTracker® Red CMXRos *special packaging*	20 x 50 µg

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