

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

shown that submicromolar concentrations of these MitoTracker probes can be used to stain the mitochondria of NIH 3T3 fibroblasts, as well as of five fish cell lines (FHM, fathead minnow; BB, brown bullhead; BF-2, bluegill fry; EPC, epithelioma papprini cayprinid; and CCo, channel catfish ovary). The orange and red MitoTracker dyes are retained after permeabilization, and cells stained with these probes can be fixed, permeabilized and then double-labeled with an antibody directed against a mitochondrial protein or other cellular marker. Our MitoTracker reagents should also eliminate some of the hazards of working with pathogenic cells because, once the mitochondria are stained, the pathogenic cells can be treated with fixatives before the sample is analyzed.

MitoTracker Green FM (M-7514) stains mitochondria with a bright green, fluorescein-like fluorescence. MitoTracker Green FM 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 living cells immediately following addition of the stain without a wash step. As a companion to MitoTracker Green FM, we have introduced MitoFluor[™] Green (M-7502). MitoFluor Green has a structure similar to MitoTracker Green FM, but lacks its reactive chloromethyl moieties and is not as well retained following aldehyde fixation. Unlike the MitoTracker Orange CMTMRos and MitoTracker Red CMXRos derivatives, both MitoTracker Green FM and MitoFluor Green appear to preferentially accumulate in mitochondria regardless of mitochondrial membrane potential, making them important tools for determining mitochondrial mass. Using these probes, we have selectively stained mitochondria in both live and fixed cells. Furthermore, MitoTracker Green FM and MitoFluor Green are substantially more photostable than the widely

Introduction

Molecular Probes' MitoTracker[®] probes are a series of novel mitochondrion-selective dyes that are well retained during cell fixation. Conventional fluorescent stains for mitochondria — such as tetramethylrosamine and rhodamine 123¹ — are readily sequestered by the actively respiring organelles; however, they are subsequently washed out of cells once the mitochondria's membrane potential is lost. This characteristic severely limits their use in experiments in which cells must be treated with aldehyde fixatives or other agents that affect the energetic state of the mitochondria.

The cell-permeant MitoTracker probes contain a thiol-reactive chloromethyl moiety. Once the MitoTracker probe accumulates in the mitochondria, it can react with accessible thiol groups on peptides and proteins to form an aldehyde-fixable conjugate (Figure 1). Molecular Probes' scientists have

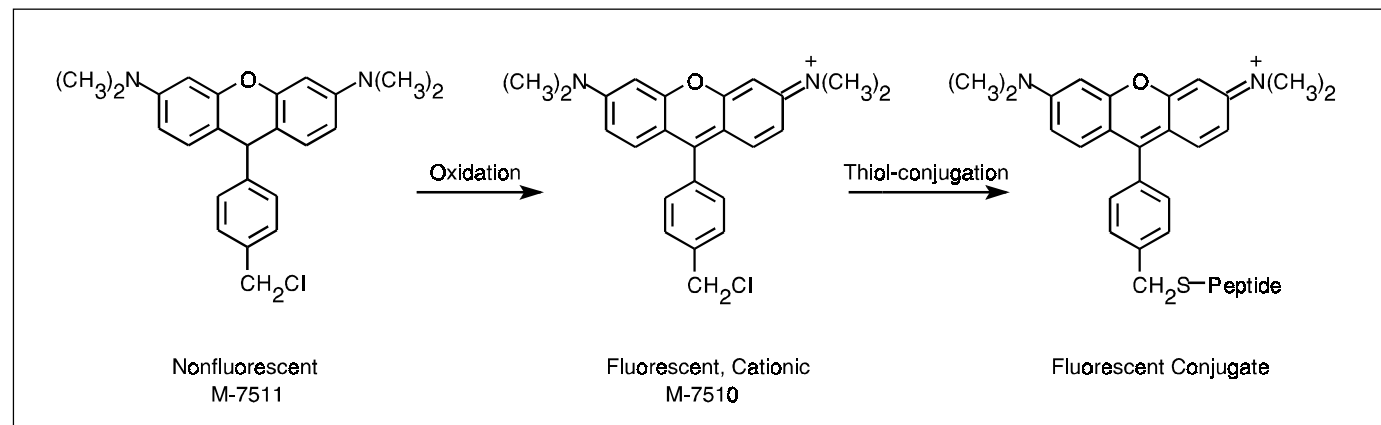


Figure 1. The intracellular reactions of our new fixable mitochondrion-selective dye, MitoTracker Orange CM-H₂TMRos. When this cell-permeant probe enters an actively respiring cell, it is oxidized to MitoTracker Orange CMTMRos and sequestered in the mitochondria, where it reacts with thiols on proteins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

used fluorescent dye rhodamine 123 and produce a brighter, more mitochondrion-selective signal at lower concentrations. Because their emission maxima are blue-shifted approximately 10 nm relative to the emission maximum of rhodamine 123, MitoTracker Green FM and MitoFluor Green produce a fluorescent staining pattern that should be better resolved from that of red fluorescent probes in double-labeling experiments.

In addition, we offer MitoTracker derivatives of both orange-fluorescent tetramethylrosamine (M-7510) and its nonfluorescent reduced form, dihydrotetramethylrosamine (M-7511), a probe that is oxidized to a fluorescent product within the mitochondria.² We have also prepared the chloromethyl derivatives of both X-rosamine (M-7512) and its reduced form (M-7513), which label mitochondria with a longer-wavelength fluorescence emission that is well resolved from fluorescein's emission. These four MitoTracker probes accumulate in active mitochondria, are well retained after cell fixation and, unlike the MitoTracker Green FM, are well retained after permeabilization.

Storage and Handling

The MitoTracker reagents are provided in specially packaged sets of 20 separate vials, each containing 50 µg lyophilized solid for reconstitution as required. The MitoFluor Green reagent is provided in a unit size of 1 mg. Upon receipt, the lyophilized solid 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 the vial, allow product to warm to room temperature. 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. Solutions of MitoTracker Green FM (M-7514), MitoFluor Green (M-7502) and the MitoTracker Orange CMTMRos and Mito-Tracker Red CMXRos derivatives (M-7510, M-7512) may be stored frozen at -20°C and protected from light. The reduced dihydro derivatives (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 same day that they are prepared.

Protocol

Cell Preparation and Staining

1.1 The concentration of probe for optimal staining will vary depending on the application. Here we suggest some initial conditions to use as guidelines. They may require modifications based on the particular cell type and the permeability of the cells or tissues to the probe, among other factors. In general, we have found that the reduced rosamine probes (M-7511, M-7513) should be loaded at three- to fivefold higher concentrations than the oxidized rosamines (M-7510, M-7512), MitoTracker Green FM (M-7514) and Mito-Fluor Green (M-7502). Dilute the 1 mM MitoTracker or MitoFluor stock

solution to the final working concentration in growth medium (with or without serum) or the buffer of choice. For live cell staining, we recommend working concentrations of 25–500 nM. For staining cells that are to be fixed and permeabilized, 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 reagents, we suggest using a slightly lower concentration (20–200 nM). At higher concentrations, these probes tend to stain other cellular structures.

1.2 For 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) probe-containing (25–500 nM) medium. 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 *Fluorescence Microscopy*). 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*. Please note that the MitoTracker Green FM and MitoFluor Green probes are retained after fixation, although the fluorescence intensity is decreased. Neither of these probes are well retained after permeabilization. MitoFluor Green, which lacks the mildly thiol-reactive chloromethyl groups, is designed primarily for live cell staining.

1.3 For suspension cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) probe-containing (25–500 nM) medium. Incubate the cells for 15–45 minutes under growth conditions appropriate for the particular cell type. Repellet 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. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization*. Please note that the MitoTracker Green FM and MitoFluor Green probes are retained after fixation, although the fluorescence intensity is decreased. Neither of these probes are well retained after permeabilization. MitoFluor Green, which lacks the mildly thiol-reactive chloromethyl groups, is designed primarily for live cell staining.

Alternatively, suspension cells may be attached to coverslips that have been treated with Cell-Tak® (Collaborative Biomedical Products; Bedford, MA); in this case, see step 1.2.

Fixation and Permeabilization

2.1 Before fixation, the cells must be washed at 37°C with Hanks' balanced salt solution (HBSS), phosphate-buffered saline (PBS) or any standard saline solution that does not

Table 1. Spectral characteristics of MitoTracker and MitoFluor probes.

Product Name	Catalog #	Ex (nm) ^{1,2}	Em (nm) ^{2,3}	Filter Set ⁴
MitoFluor Green	M-7502 ⁵	490	516	O-5715, O-5716
MitoTracker Green FM	M-7514 ⁵	490	516	O-5715, O-5716
MitoTracker Orange CMTMRos	M-7510 ⁶	554	576	O-5723, O-5728
MitoTracker Orange CM-H ₂ TMRos	M-7511 ⁶	554	576	O-5723, O-5728
MitoTracker Red CMXRos	M-7512 ⁶	579	599	O-5731, O-5733
MitoTracker Red CM-H ₂ XRos	M-7513 ⁶	579	599	O5731, O-5733

1. Fluorescence excitation maximum. **2.** Determined in methanol; values may vary somewhat in cellular environments. **3.** Fluorescence emission maximum. **4.** These Omega Optical bandpass filter sets are available from Molecular Probes. For further information on these and other filter sets, consult our *Handbook of Fluorescent Probes and Research Chemicals*, visit our Web site (www.probes.com) or contact our Technical Assistance Department. **5.** Nonfluorescent in aqueous solution. **6.** Nonfluorescent until oxidized.

contain serum. This step is especially important if the cells are attached to a Cell-Tak-coated coverslip or other amine-containing surface.

2.2 Standard fixation protocols using formaldehyde fixatives effectively crosslink the amines of the protein- or peptide-dye conjugate. Typically, we fix the cells for 15 minutes at 37°C using freshly prepared 3.7% paraformaldehyde in HBSS.

2.3 After fixation, the cells should be rinsed several times in PBS.

2.4 When the cells are going to be subsequently labeled with an antibody, a permeabilization step is often required to en-

hance the antigen's accessibility. Cells can be permeabilized by incubating in ice-cold acetone for 10 minutes. Following permeabilization, the cells should be rinsed 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

Molecular Probes offers high-quality Omega® Optical filter sets for fluorescence microscopy that are optimized to match the spectral properties of our dyes. Spectral characteristics for the MitoTracker and MitoFluor probes and their corresponding optical filter sets are summarized in Table 1.

References

1. Meth Cell Biol 29, 103 (1989);
2. Biochem Biophys Res Comm 175, 387 (1991).

Product List

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

Cat #	Product Name	Unit Size
M-7502	MitoFluor™ Green	1 mg
M-7514	MitoTracker® Green FM *special packaging*	20x50 µg
M-7511	MitoTracker® Orange CM-H ₂ TMRos *special packaging*	20x50 µg
M-7510	MitoTracker® Orange CMTMRos *special packaging*	20x50 µg
M-7513	MitoTracker® Red CM-H ₂ XRos *special packaging*	20x50 µg
M-7512	MitoTracker® Red CMXRos *special packaging*	20x50 µg

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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Molecular Probes, Inc.

PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

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Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV

PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5233419
eurotech@probes.nl

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