MitoTracker® Mitochondrion-Selective Probes

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Table 1. Contents and storage information.

Material	Amount	Storage	Stability
MitoTracker® probes	20 vials each containing 50 µg lyophilized solid	• ≤–20°C * • Desiccate • Protect from light • Avoid freeze-thaw cycles	When in solid form stored as directed, product is stable for at least 6 months.
Reduced rosamine MitoTracker® probes (M7511, M7513)	20 vials each containing 50 µg lyophilized solid	• ≤–20°C * • Store under argon or nitrogen • Desiccate • Protect from light • Avoid freeze-thaw cycles	

* Before opening a vial, allow the product to warm to room temperature.

Approximate fluorescence excitation and emission maxima, in nm: See Table 2.

Introduction

MitoTracker® Probes

The cell-permeant MitoTracker® probes contain a mildly thiol-reactive chloromethyl moiety for labeling mitochondria (Figure 1). Additionally, some MitoTracker® probes such as MitoTracker® Orange CMTMRos, MitoTracker® Orange CM-H_2TMRos, MitoTracker® Red CMXRos, MitoTracker® Red CM-H_2XRos, and MitoTracker® Deep Red are retained in the mitochondria after fixation. MitoTracker® Green FM and MitoTracker® Red FM are good live cell options.

To label mitochondria, cells are simply incubated with MitoTracker® probes, which passively diffuse across the plasma membrane and accumulate in active mitochondria. Once their mitochondria are labeled, the cells can be treated with an aldehyde-based fixative for samples that need fixation to allow further processing of the sample. Some of the MitoTracker® probes are also retained after permeabilization with some detergents during subsequent processing steps (e.g., immunocytochemistry or *in situ* hybridization). 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.

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, use the MitoTracker® probes—a series of patented

mitochondrion-selective stains that are concentrated by active mitochondria and retained during cell fixation.[1]

The MitoTracker® probes differ in spectral characteristics and fixability (Figure 2, Table 2). MitoTracker® probes are provided in specially packaged sets of 20 vials, each containing 50 µg for reconstitution as required.

Rosamine- and carbocyanine-based MitoTracker® dyes: We offer rosamine-based MitoTracker® dyes which include MitoTracker® Orange CMTMRos (Cat. no. M7510), a derivative of tetramethylrosamine, and MitoTracker® Red CMXRos (Cat. no. M7512), a derivative of X-rosamine. Reduced MitoTracker® dyes, MitoTracker® Orange CM-H_2TMRos (Cat. no. M7511) and MitoTracker® Red CM-H_2XRos (Cat. no. M7513), which are derivatives of dihydrotetramethylrosamine and dihydro-X-rosamine, respectively, are also available. These reduced probes do not fluoresce until they enter live cells, where they are oxidized to the corresponding fluorescent mitochondrion-selective probe and then sequestered in the mitochondria.

The carbocyanine-based MitoTracker® dyes include MitoTracker® Red FM (Cat. no. M22425), MitoTracker® Green FM dye (Cat. no. M7514), and MitoTracker® Deep Red FM (Cat. no. M22426).

MitoTracker® Red CMXRos, MitoTracker® Red FM, and MitoTracker® Deep Red FM are all well suited for multicolor labeling experiments because their red fluorescence is well resolved from the green fluorescence of other probes.

Some of the MitoTracker® probes are well retained after fixation and permeabilization (Table 2). While some mitochondrial labeling may be achieved in fixed cells using MitoTracker® dyes, the signal-to-noise ratios are not generally favorable and these dyes are recommended for use in live cells only. If mitochondrial labeling of fixed cells is desired, using anti-OxPhos antibodies available from Invitrogen (for example, Cat. no. A21355) is a good option.

Before You Begin

Materials Required but Not Provided

- DMSO
- Suitable buffer or growth medium for live cell imaging
- Aldehyde based fixatives such as formaldehyde for cell fixation
- Aldehyde based detergents such as Triton® X-100

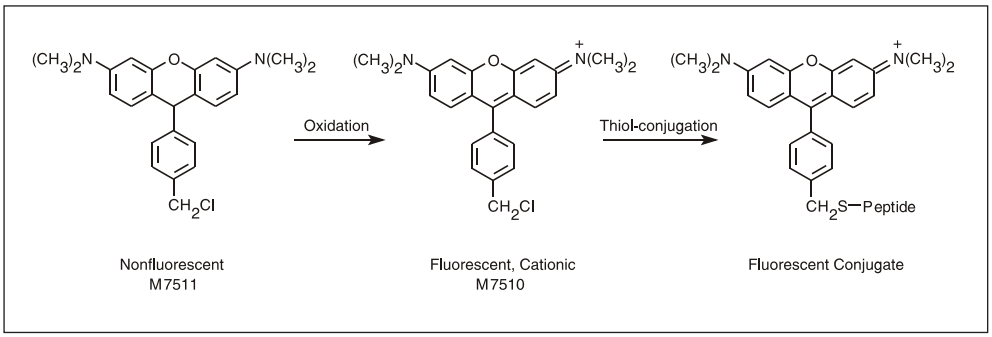

Figure 1. The intracellular reactions of our fixable mitochondrion-selective dye, MitoTracker® Orange CM-H_2TMRos. When this cell-permeant probe enters an actively respiring cell, it is oxidized to MitoTracker® Orange CMTMRos and sequestered in the mitochondria, where it reacts with thiols on proteins and peptides to form an aldehyde-fixable conjugate.

Table 2. MitoTracker® Mitochondrion-Selective Probes

	Product	Catalog Number	Ex (nm) *	Em (nm) *	Oxidation State	Retained after Fixation
	Rosamine-based MitoTracker® Probes					
Buy Now	MitoTracker® Orange CMTMRos	M7510	554	576	Oxidized	Yes
Buy Now	MitoTracker® Orange CM-H_2TMRos	M7511 ‡	554	576	Reduced	Yes
Buy Now	MitoTracker® Red CMXRos	M7512	579	599	Oxidized	Yes
Buy Now	MitoTracker® Red CM-H_2XRos	M7513 ‡	579	599	Reduced	Yes
	Carbocyanine-based MitoTracker® Probes					
Buy Now	MitoTracker® Green FM	M7514 †	490	516	NA	No
Buy Now	MitoTracker® Red FM	M22425	581	644	NA	No
Buy Now	MitoTracker® Deep Red FM	M22426	644	665	NA	Yes

* Fluorescence excitation (Ex) and emission (Em) maxima determined in methanol; values may vary somewhat in cellular environments.
†Nonfluorescent in aqueous solution. ‡Nonfluorescent until oxidized. NA = Not applicable.

Preparing Stock Solutions

Before opening a vial, allow the product to warm to room temperature.

To prepare a stock solution, dissolve the lyophilized MitoTracker® 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 (M7511, M7513) 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 immediately after they are prepared. Store all other solutions of the MitoTracker® dyes frozen at ≤–20°C and protected from light.

Experimental Protocols

Cell Preparation and Staining

The concentration of probe for optimal staining varies by application. The initial conditions suggested here are guidelines that may 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, the reduced rosamine MitoTracker® probes (M7511, M7513) are loaded at three- to five-fold higher concentrations than other MitoTracker® probes.

1.1 **Preparing staining solutions.** Dilute 1 mM MitoTracker® stock solution (see *Preparing Stock Solutions* for preparation) to the final working concentration in appropriate buffer or growth medium. Because the reduced forms of the MitoTracker® probes are susceptible to potential oxidases in serum, we do not recommend using complete media with these dyes.

General Guidelines: Use working concentrations of 25–500 nM. For staining cells that are to be fixed and permeabilized (see *Fixation and Permeabilization after Staining*), use a working concentration of 100–500 nM. To reduce potential artifacts and mitochondrial toxicity from overloading, keep the concentration of dye as low as possible. For the MitoTracker® Green FM probes, use 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 confluency, remove the media from the dish and add prewarmed (37°C) staining solution containing MitoTracker® probe (prepared in step 1.1). While incubation times vary depending on the model system and probe used, incubation for 15–45 minutes under growth conditions appropriate for the particular cell type is generally sufficient but may need to be optimized. After staining is complete

replace the staining solution with fresh prewarmed media or buffer and observe cells using a fluorescence microscope or fluorescence microplate reader. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization after Staining*.

1.3 Staining suspension cells. Centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) staining solution containing the MitoTracker® probe (prepared in step 1.1). While incubation times vary depending on the model system and probe used, incubation for 15–45 minutes under growth conditions appropriate for the particular cell type is generally sufficient but may need to be optimized. After staining is complete, re-pellet the cells by centrifugation and resuspend cells in fresh prewarmed medium or buffer. Cells maybe analyzed by flow cytometry, microplate-based analysis, or fluorescence microscopy. If immobilized cells on coverslips are needed, use poly-D-lysine to coat the slides or coverslips before mounting. If the cells are to be fixed and permeabilized, continue to *Fixation and Permeabilization after Staining*.

Optional: Fixation and Permeabilization after Staining

After staining live cells with a MitoTracker® dye, it is often useful to fix and permeabilize the cells for subsequent manipulations. For example, fixation and permeabilization allows you 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 MitoTracker® Red FM are not retained well after fixation.

2.1 Washing the cells. After staining, wash the cells in fresh, pre-warmed buffer or growth medium.

2.2 Fixing the cells. Carefully remove the medium/buffer covering the cells, and replace it with freshly prepared, pre-warmed buffer or growth medium containing 2–4% formaldehyde. For MitoTracker® Red CMXRos, we have found that fixing with 3.7% formaldehyde in complete growth medium at 37°C for 15 minutes works well for endothelial cells.

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

2.4 Permeabilization (optional). When permeabilization is needed for subsequent steps such as immunocytochemistry, incubate fixed cells in buffer containing detergent such as Triton® X-100. Following permeabilization, rinse the cells in buffer and proceed with immunocytochemistry procedure. We found that incubating the endothelial cells for 10 minutes in PBS containing 0.2% Triton® X-100 works well.

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 in improving the signal-to-background ratio.

Fluorescence Microscopy

Spectral characteristics for the MitoTracker® probes are summarized in Table 2.

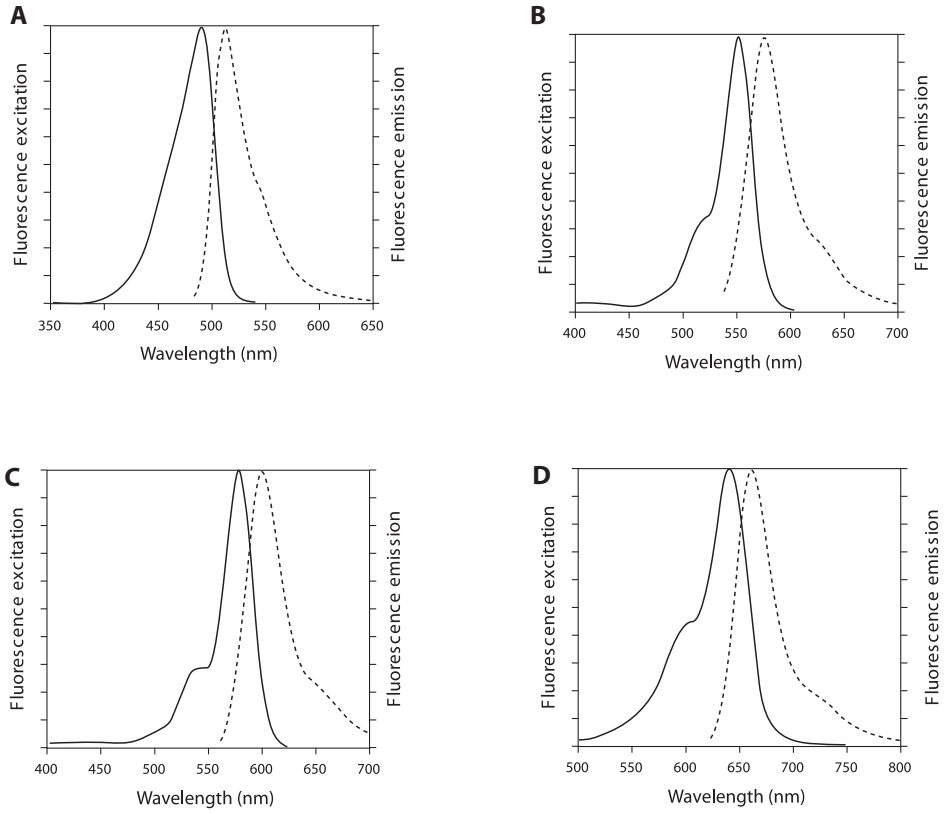

Figure 2. Fluorescence excitation and emission spectra for MitoTracker® Green FM (A), MitoTracker® Orange (B), MitoTracker® Red CMXRos (C), and MitoTracker® Deep Red (D). All spectra were determined in methanol.

References

1. J Histochem Cytochem 44, 1363 (1996); **2.** Cytometry 39, 203 (2000); **3.** Mol Cell Biochem 172, 171 (1997).

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

Cat. no.	Product Name	Unit Size
M22426	MitoTracker® Deep Red FM *special packaging*	20 × 50 µg
M7514	MitoTracker® Green FM *special packaging*	20 × 50 µg
M7511	MitoTracker® Orange CM-H_2TMRos *special packaging*	20 × 50 µg
M7510	MitoTracker® Orange CMTMRos *special packaging*	20 × 50 µg
M22425	MitoTracker® Red FM *special packaging*	20 × 50 µg
M7513	MitoTracker® Red CM-H_2XRos *special packaging*	20 × 50 µg
M7512	MitoTracker® Red CMXRos *special packaging*	20 × 50 µg
Related Products		
A21355	anti-OxPhos Complex V inhibitor protein, mouse IgG1, monoclonal antibody	100 µg
M36008	MitoSOX™ Red mitochondrial superoxide indicator *for live-cell imaging*	10 × 50 µg

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