Lumiprobe
Life science solutions

Staining Mitochondria in Live Cells with LumiTracker® Mito Probes

LumiTracker® Mito probes are cationic, cell-permeant stains with mildly thiol-reactive chloromethyl moiety used for labeling live mitochondria. LumiTracker® Mito probes passively diffuse across the plasma membrane and selectively accumulate in active mitochondria based on their membrane potential. The LumiTracker® Mito probes differ in spectral characteristics and fixability, summarized in the next Table.

Probe	Catalog Number	MW	Ex (nm)*	Em (nm)*	Oxidation State	Kept after Fixation
Rosamine-based						
LumiTracker® Mito Orange CMTMRos	2252-x	427.38	555	578	Oxidized	Yes
LumiTracker® Mito Orange CM-H2TMRos	4367-x	392.93	555	578	Reduced	Yes
LumiTracker® Mito Red CMXRos	2251-x	531.53	581	600	Oxidized	Yes
Carbocyanine-based						
LumiTracker® Mito Green FM	3527-x	671.88	491	513	NA	No
LumiTracker® Mito Red FM	3170-x	724.00	590	643	NA	No

LumiTracker® Mito probes are primarily intended for use in live cells; however, rosamine-based probes are also kept after fixation and permeabilization during subsequent immunocytochemistry or *in situ* hybridization processing. Although some mitochondrial labeling can be accomplished in fixed cells with LumiTracker® Mito probes, the signal-to-noise ratios are typically not optimal. If labeling mitochondria in fixed cells is necessary, it is advisable to use anti-OxPhos antibodies.

Before you start

- The reduced rosamine LumiTracker® Mito probes are sensitive to oxidation, especially in solution, and must be stored under argon or nitrogen at lower -20 °C and protected from light. We recommend using reduced rosamine solutions immediately after their preparation.
- The commonly used dye concentration to stain cells is 25–500 nM. The working dilution depends on the cell type and density and should be defined experimentally.
- The reduced rosamine LumiTracker® Mito probes are generally loaded to cells at three- to five-fold higher concentrations than other LumiTracker® Mito probes.
- The optimal cell density and staining duration may vary by cell type. The staining protocol should be optimized in preliminary experiments for best results.

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Stock Solution Preparation

1. Before opening, each vial should be warm to room temperature.
2. Dissolve the lyophilized LumiTracker® Mito probe in high-quality, anhydrous dimethylsulfoxide to obtain a 1 mM stock solution.
3. Mix well until the dye has fully dissolved.
4. Store the stock solution in small aliquots at -20 °C or -80 °C away from light. Avoid repeated freeze-thaw cycles.

Staining Solution Preparation

- Dilute 1 mM LumiTracker® Mito stock solution to the final working concentration in the appropriate buffer or culture medium.

  Important! The reduced forms of the LumiTracker® Mito are susceptible to potential oxidases in serum. We do not recommend using complete media with these probes.

- The commonly used dye concentration to stain cells is 25–500 nM. The working dilution depends on the cell type, density, and application. If stained cells should be fixed and permeabilized, use a working concentration of 100–500 nM.
- For the LumiTracker® Mito Green FM probe, use a concentration of 20–200 nM. At high concentrations, this probe tends to stain other cellular structures.

  Important! Keep the dye concentration as low as possible to reduce potential artifacts and mitochondrial toxicity from overloading.

Cell Staining

Staining Adherent Cells

1. Grow cells on a sterile coverslip. Adherent cells can be stained directly on the coverslip.
2. Remove the media from the dish and add pre-warmed to 37 °C staining solution.
3. Incubate cells in the dark for 15–45 minutes under growth conditions appropriate for the particular cell type.
4. Replace the staining solution with fresh pre-warmed media or buffer.
5. Image cells using a fluorescence microscope or fluorescence microplate reader.

Staining Suspension Sells

1. Obtain a single-cell suspension.
2. Centrifuge cells and aspirate the supernatant.
3. Resuspend the cells gently in a pre-warmed to 37 °C staining solution.
4. Incubate cells in the dark for 15–45 minutes under growth conditions appropriate for the particular cell type.

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5. Centrifuge cells and aspirate the supernatant.
6. Resuspend cells in fresh pre-warmed medium or buffer.
7. Analyze cells using a flow cytometer, fluorescence microscope, or fluorescence microplate reader.
8. If immobilized cells on coverslips are needed, use poly-L-lysine-coated slides or coverslips before mounting.

Fixation and Permeabilization Cells after Staining

These steps are optional and may be needed for subsequent immunocytochemistry or in situ hybridization processing. Use the protocol described below for rosamine-based probes only. LumiTracker® Mito Green FM and LumiTracker® Mito Red FM are not retained well after fixation.

1. Wash the cells in fresh, pre-warmed buffer or growth medium after staining.
2. Carefully aspirate the buffer or medium.
3. Fix the cells for 15 minutes with fresh 2–4% formaldehyde prepared on buffer or growth medium.
4. Rinse the cells several times with buffer.
5. When permeabilization is needed, incubate fixed cells for 10 minutes in PBS containing 0.2% Triton X-100 and then wash in PBS. Alternatively, the cells may be permeabilized by incubating in ice-cold acetone for 5 minutes and then washed in PBS. This acetone-permeabilization step may also be used to improve the signal-to-background ratio.

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