

Image-iT™ LIVE Mitochondrial and Nuclear Labeling Kit (I34154)

Quick Facts

Storage upon receipt:

- $\leq -20^{\circ}\text{C}$
- Protect from light
- Desiccate
- Avoid freeze-thaw cycles

Abs/Em:

- 579/599 nm (MitoTracker® Red CMXRos dye)
- 350/461 nm (Hoechst 33342 dye)

Number of assays: 500

Storage and Handling

Upon receipt, the kit should be stored upright, desiccated, and protected from light at $\leq -20^{\circ}\text{C}$. Vials should be allowed to warm to room temperature before opening. Avoid freeze-thaw cycles. When stored properly, components should be stable for at least 6 months.

Caution: Handle stock solutions containing DMSO with care, as DMSO is readily absorbed through the skin. It is important that vials be tightly closed and stored with desiccant.

Spectral Characteristics

MitoTracker Red CMXRos dye has excitation/emission maxima of approximately 579/599 nm; Hoechst 33342 dye has excitation/emission maxima of approximately 350/461 nm. Cells labeled with MitoTracker Red CMXRos and Hoechst 33342 dyes can be imaged using standard filter sets.

Materials Recommended but Not Provided

Hank's balanced salt solution (HBSS, available from Gibco (14025-092)).

Introduction

The Image-iT™ LIVE Mitochondrial and Nuclear Labeling Kit provides two stains — red-fluorescent MitoTracker® Red CMXRos dye and blue-fluorescent Hoechst 33342 dye — for highly selective mitochondrial and nuclear staining, respectively, in live green-fluorescent protein (GFP)-transfected cells. Using the protocol provided, the dyes can be combined into one staining solution to save labeling time and wash steps while still providing optimal staining.

Cell-permeant MitoTracker Red CMXRos dye provides highly selective mitochondrial staining with minimal background. Hoechst 33342 dye, a cell-permeant nucleic acid stain that is selective for DNA and spectrally similar to DAPI, is UV excitable and emits blue fluorescence when bound to DNA. These dyes should not interfere with GFP fluorescence, and both dyes are retained after formaldehyde fixation and permeabilization.

Materials

Kit Contents

- MitoTracker CMXRos dye (MW = 532, Component A), 50 μg
- Hoechst 33342 dye (Component B), 3 vials, each containing 400 μL at 1.0 mM in water
- Dimethylsulfoxide (DMSO) (Component C), 500 μL

Experimental Protocol

Reagent Preparation

Prepare a 200 μM MitoTracker Red CMXRos dye stock solution. Dissolve the entire contents (50 μg) of the vial of MitoTracker Red CMXRos dye (Component A) in 470 μL of DMSO (Component C) to yield a stock solution of 200 μM . When stored properly, the 200 μM MitoTracker Red CMXRos dye stock solution should be stable for at least 6 months; avoid freeze-thaw cycles.

Labeling Live Eukaryotic Cells

This is a general procedure for labeling live, cultured cells that are adhering to coverslips. The protocol was optimized for HeLa cells transfected with GFP. MitoTracker Red CMXRos dye and Hoechst 33342 dye are combined into one solution for single-step staining, but the two dyes can be used in separate labeling steps if desired, with a buffer wash between steps. Recommended times and concentrations may vary in different model systems and may require optimization.

1.1 Prepare labeling solution. Dilute the 200 μM MitoTracker Red CMXRos dye stock solution (prepared above) and the 1.0 mM Hoechst 33342 dye (Component B) into HBSS or appropriate cell medium with serum. A recommended concentration

for MitoTracker Red CMXRos dye is 10–50 nM; a recommended concentration for Hoechst 33342 dye is 1.0 µg/mL. Both dyes may be combined in a single staining solution.

1.2 Label cells. Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 15 minutes at 37°C.

1.3 Wash cells. When labeling is complete, remove the labeling solution and wash cells twice in cell medium. Unless cells will be

fixed, samples are ready to mount in warm HBSS or suitable buffer for imaging.

1.4 (Optional) Fix cells. Labeled cells can be fixed with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and staining with any additional counterstains.

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

Cat #	Product Name	Unit Size
I34154	Image-iT™ LIVE Mitochondrial and Nuclear Labeling Kit *counterstains for GFP-expressing cells*	1 kit

Contact Information

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