Image-iT™ LIVE Intracellular Membrane and Nuclear Labeling Kit

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
CellTrace™ BODIPY® TR methyl ester (Component A, MW = 438)	500 µL	5.0 mM in DMSO	• ≤–20°C • Protect from light • Desiccate • Avoid freeze-thaw cycles • Store kit upright	When stored as directed, kit components should be stable for at least 6 months.
Hoechst 33342 dye (Component B, MW = 616)	3 vials, 400 µL each	1.0 mM in water		
Number of assays: 250				
Approximate fluorescence excitation and emission maxima: 598/625 nm, CellTrace™ BODIPY® TR methyl ester; 350/461 nm, Hoechst 33342 dye				

Introduction

The Image-iT™ LIVE Intracellular Membrane and Nuclear Labeling Kit provides two stains—red-fluorescent CellTrace™ BODIPY® TR methyl ester and blue-fluorescent Hoechst 33342 dye—for highly selective staining of the intracellular membranes and nuclei of live green-fluorescent protein (GFP)–transfected cells and tissues. The kit can also be used to stain fixed cells or tissues. 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 CellTrace™ BODIPY® TR methyl ester is retained after fixation with formaldehyde and permeabilization with Triton X-100. 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. Hoechst 33342 should not interfere with GFP fluorescence and is retained after fixation and permeabilization.

Spectral Characteristics

CellTrace™ BODIPY® TR methyl ester has excitation/emission maxima of approximately 598/625 nm, and Hoechst 33342 dye has excitation/emission maxima of approximately 350/461 nm. Samples stained with CellTrace™ BODIPY® TR methyl ester and Hoechst 33342 can be viewed using standard filter sets.

Before You Begin

Vials should be allowed to warm to room temperature before opening.

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.

Materials Required but Not Provided

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

Experimental Protocol

Labeling Live Eukaryotic Cells

This is a general procedure for labeling live, cultured cells that are adhering to coverslips. The protocol was optimized using HBSS for HeLa cells transfected with GFP. CellTrace™ BODIPY® TR methyl ester and Hoechst 33342 dye are combined into one solution for single-step staining, but the two dyes can be used in separate staining 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 5.0 mM CellTrace™ BODIPY® TR methyl ester (Component A) and the 1.0 mM Hoechst 33342 dye (Component B) into HBSS or cell-culture medium. A recommended concentration for CellTrace™ BODIPY® TR methyl ester is 1–10 µM; a recommended concentration for Hoechst 33342 stain is 2.0 µg/mL (3.3 µM). 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 10 minutes at 37˚C.

1.3 Wash and rest cells. When labeling is complete, remove the labeling solution, wash twice in cell-culture medium, and rest cells for 5 minutes in cell-culture medium at 37˚C. Unless the cells will be fixed, samples are ready for imaging.

1.4 (Optional) Fix cells. Labeled cells can be fixed with 4% formaldehyde for 15 minutes at 37˚C, followed by three 5-minute washes in PBS and staining with any additional counterstains. Cells may also be permeabilized (e.g., using 0.2% Triton X-100) as necessary.

Labeling Fixed Eukaryotic Cells

This protocol was optimized for formadehyde-fixed cells. CellTrace™ BODIPY® TR methyl ester 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.

2.1 Fix cells. Fix cells with 4% formaldehyde for 15 minutes at 37˚C.

2.2 Wash cells. Wash cells three times in HBSS. Do not permeabilize the cells.

2.3 Prepare labeling solution. Dilute the 5.0 mM CellTrace™ BODIPY® TR methyl ester (Component A) and the 1.0 µM Hoechst 33342 dye (Component B) into HBSS or cell-culture medium. A recommended concentration for CellTrace™ BODIPY® TR methyl ester is 1–10 µM; a recommended concentration for Hoechst 33342 stain is 2.0 µg/mL. Both dyes may be combined in a single staining solution.

2.4 Label cells. Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 10 minutes at room temperature.

2.5 Wash cells. When labeling is complete, remove the labeling solution and wash cells twice in HBSS or suitable buffer.

2.6 Prepare cells for viewing. Stain the cells with additional counterstains as desired and mount in HBSS or an aqueous antifade mounting medium such as ProLong® antifade reagent (available in the ProLong® Antifade Kit, P7481) or ProLong® Gold antifade reagent (P36930).

Labeling Live Embryos

This is a general procedure for labeling live embryos. The protocol was optimized for zebrafish embryos transfected with GFP. CellTrace™ BODIPY® TR methyl ester and Hoechst 33342 dye are combined into one solution for single-step staining, but the two dyes can be used in separate staining steps if desired, with a buffer wash between steps. Recommended times and concentrations may vary in different model systems and may require optimization.

3.1 Prepare labeling solution. Dilute the 5.0 mM CellTrace™ BODIPY® TR methyl ester (Component A) and the 1.0 mM Hoechst 33342 dye (Component B) into embryo-rearing medium. A recommended concentration for CellTrace™ BODIPY® TR methyl ester is 100 µM, plus 2% DMSO; a recommended concentration for Hoechst 33342 stain is 1.0 µg/mL. Both dyes may be combined in a single staining solution.

3.2 Label embryo(s). Label embryo(s) with labeling solution for 1 hour.

3.3 Wash embryo(s). When labeling is complete, remove the labeling solution and wash embryo(s) three times with unlabeled saline solution. Unless the embryo(s) will be fixed, it is now ready for imaging.

3.4 (Optional) Fix embryo(s). Labeled embryo(s) can be fixed with 4% formaldehyde, followed by three 5-minute washes in PBS and staining with any additional counterstains. Embryo(s) may also be permeabilized with Triton X-100 as necessary.

Labeling Fixed Embryos

This protocol was optimized for formadehyde-fixed embryos. CellTrace™ BODIPY® TR methyl ester 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.

4.1 Fix embryo(s). Fix embryo(s) with 4% formaldehyde, followed by washes in PBS and any additional counterstains. Embryo(s) may also be permeabilized (e.g., using Triton X-100) as necessary.

4.2 Wash embryo(s). Wash embryo(s) three times in buffer for 10 minutes each.

4.3 Prepare labeling solution. Dilute the 5.0 mM CellTrace™ BODIPY® TR methyl ester (Component A) and the 1.0 mM Hoechst 33342 dye (Component B) into embryo-rearing medium. A recommended concentration for CellTrace™ BODIPY® TR methyl ester is 100 µM, plus 2% DMSO; a recommended concentration for Hoechst 33342 stain is 1.0 µg/mL. Both dyes may be combined in a single staining solution.

4.4 Label embryo(s). Label embryo(s) with labeling solution for 1 hour.

4.5 Wash embryo(s). When labeling is complete, remove the labeling solution and wash embryo(s) three times with unlabeled saline solutions.

4.6 Prepare embryo(s) for viewing. Stain the embryo(s) with additional counterstains as desired and mount in HBSS or an aqueous antifade mounting medium such as ProLong® antifade reagent (available in the ProLong® Antifade Kit, P7481) or ProLong® Gold antifade reagent (P36930).

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

Cat #	Product Name	Unit Size
I34407	Image-iT™ LIVE Intracellular Membrane and Nuclear Labeling Kit *counterstains for GFP-expressing cells*	1 kit

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