

# 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	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Protect from light</li> <li>• Desiccate</li> <li>• Avoid freeze-thaw cycles</li> <li>• Store kit upright</li> </ul>	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		
<b>Number of assays:</b> 250				
<b>Approximate fluorescence excitation and emission maxima:</b> 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  $\mu$ M; a recommended concentration for Hoechst 33342 stain is 2.0  $\mu$ g/mL (3.3  $\mu$ M). 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 formaldehyde-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 formaldehyde-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

**Contact Information**

**Molecular Probes, Inc.**

29851 Willow Creek Road  
Eugene, OR 97402  
Phone: (541) 465-8300  
Fax: (541) 335-0504

**Customer Service:**

6:00 am to 4:30 pm (Pacific Time)  
Phone: (541) 335-0338  
Fax: (541) 335-0305  
probesorder@invitrogen.com

**Toll-Free Ordering for USA:**

Order Phone: (800) 438-2209  
Order Fax: (800) 438-0228

**Technical Service:**

8:00 am to 4:00 pm (Pacific Time)  
Phone: (541) 335-0353  
Toll-Free (800) 438-2209  
Fax: (541) 335-0238  
probestech@invitrogen.com

**Invitrogen European Headquarters**

Invitrogen, Ltd.  
3 Fountain Drive  
Inchinnan Business Park  
Paisley PA4 9RF, UK  
Phone: +44 (0) 141 814 6100  
Fax: +44 (0) 141 814 6260  
Email: euroinfo@invitrogen.com  
Technical Services: eurotech@invitrogen.com

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 Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

**Limited Use Label License No. 223: Labeling and Detection Technology**

The manufacture, use, sale or import of this product may be subject to one or more patents or pending applications owned or licensed by Invitrogen Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity) in a manner consistent with the accompanying product literature. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the most restrictive terms apply. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents that are owned or controlled by Invitrogen Corporation and/or Molecular Probes, Inc. which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2007, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.