Revised: 20–June–2005

Image-iT™ LIVE Lysosomal and Nuclear Labeling Kit (I34202)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light
- Desiccate
- Avoid freeze-thaw cycles

Ex/Em:

- 577/590 nm (LysoTracker® Red DND-99 dye)
- 350/461 nm (Hoechst 33342 dye)

Number of assays: 500

Introduction

The Image-iT™ LIVE Lysosomal and Nuclear Labeling Kit provides two stains — red-fluorescent LysoTracker® Red DND-99 dye and blue-fluorescent Hoechst 33342 dye — for highly selective staining of lysosomes and the nucleus, respectively, in live green-fluorescent protein (GFP)–transfected cells. When used according to the sample protocol, cell-permeant LysoTracker Red DND-99 dye provides highly selective lysosomal staining with minimal background. A significant amount of specific staining is retained after formaldehyde fixation, although some cytoplasmic background staining may be seen. 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. This dye should not interfere with GFP fluorescence and is retained after fixation and permeabilization.

It is not recommended that the dyes be combined into one staining solution. The dyes should be used in separate labeling steps, with Hoechst 33342 staining first.

Materials

Kit Contents

- **LysoTracker Red DND-99 dye** (Component A), 50 µL at 1.0 mM in dimethylsulfoxide (DMSO)
- **Hoechst 33342 dye** (Component B), 3 vials, each containing 400 µL at 1.0 mM in water

Storage and Handling of Stock Solutions

Upon receipt, the kit should be stored upright, desiccated, and protected from light at ≤–20˚C. Avoid freeze-thaw cycles. Vials should be allowed to warm to room temperature before opening. When stored properly, components should be stable for at least 6 months.

Note: Handle stock solutions containing DMSO with care. DMSO (found in the LysoTracker Red DND-99 dye solution) is readily absorbed through the skin. Vials should be tightly closed and stored with desiccant.

Spectral Characteristics

LysoTracker Red DND-99 dye has excitation/emission maxima of approximately 577/590 nm; Hoechst 33342 dye has excitation/emission maxima of approximately 350/461 nm. Cells labeled with LysoTracker Red DND-99 dye and Hoechst 33342 dye can be imaged using standard filter sets.

Materials Recommended 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 has been optimized using HBSS for HeLa cells transfected with GFP. LysoTracker Red DND-99 and Hoechst 33342 dyes must be used in separate staining steps. Recommended times and concentrations may vary in different model systems and may require optimization.

1.1 Prepare labeling solutions. Dilute the 1.0 mM solution of Hoechst 33342 dye (Component B) into HBSS or the appropriate medium for the cell line being used; a recommended concentration for Hoechst 33342 dye is 2.0 µg/mL. Dilute the 1.0 mM of LysoTracker Red DND-99 dye into a separate volume of HBSS; a recommended concentration for LysoTracker Red DND-99 dye is 100 nM. Note: the two dyes cannot be combined in one staining solution.

1.2 Stain cells with Hoechst 33342 dye. Apply a sufficient amount of 2.0 µg/mL Hoechst 33342 labeling solution (prepared above) to cover cells adhering to coverslip(s). Incubate for 5 minutes at 37˚C.

1.3 Wash cells. When labeling is complete, remove the labeling solution and wash cells twice in room-temperature HBSS or suitable buffer.

1.4 Stain cells with LysoTracker Red. Apply a sufficient amount of 100 nM LysoTracker Red DND-99 labeling solution (prepared above) to cover cells adhering to coverslip(s). Incubate for 1 minute at room temperature. Longer label times may result in unwanted cytoplasmic background, perhaps due to an alkalinizing effect on the lysosomes.

1.5 Wash and view. Quickly remove labeling solution and gently wash the cells. Unless the cells will be fixed, samples are ready to be mounted in HBSS or suitable buffer for imaging.

1.6 (Optional) Fix cells. Wash the stained cells gently with HBSS or suitable buffer and fix with 4% formaldehyde for 15 minutes at 37˚C. Wash in HBSS or suitable buffer and add any additional counterstains. Fixation may cause some cytoplasmic background.

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

Cat #	Product Name	Unit Size
I34202	Image-iT™ LIVE Lysosomal and Nuclear Labeling Kit *counterstains for GFP-expressing cells*	1 kit

Contact Information

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.

Please visit our website — **probes.invitrogen.com** — for the most up-to-date 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