Vybrant® Lipid Raft Labeling Kits

V-34403 Vybrant® Alexa Fluor® 488 Lipid Raft Labeling Kit
V-34404 Vybrant® Alexa Fluor® 555 Lipid Raft Labeling Kit
V-34405 Vybrant® Alexa Fluor® 594 Lipid Raft Labeling Kit

Quick Facts

Storage upon receipt:

- ≤–20°C
- Desiccate
- Protect from light

Abs/Em: See Table 1

Table 1. Molecular Probes' Vybrant Lipid Raft Labeling Kits.

Catalog #	Cholera toxin subunit B conjugate	Abs/Em *
V-34403	Alexa Fluor 488	495/519
V-34404	Alexa Fluor 555	555/565
V-34405	Alexa Fluor 594	590/617

* Approximate absorption and fluorescence emission maxima, in nm, of the provided fluorescent cholera toxin subunit B conjugate.

Introduction

Molecular Probes' Vybrant® Lipid Raft Labeling Kits are designed to provide convenient, reliable and extremely bright fluorescent labeling of lipid rafts in live cells. Lipid rafts are detergent-insoluble, sphingolipid- and cholesterol-rich membrane microdomains that form lateral assemblies in the plasma membrane.[1-7] Lipid rafts also sequester glycophosphatidylinositol (GPI)-linked proteins and other signaling proteins and receptors, which may be regulated by their selective interactions with these membrane microdomains.[8-13] Recent research has demonstrated that lipid rafts play a role in a variety of cellular processes — including the compartmentalization of cell-signaling events,[14-21] the regulation of apoptosis [22-24] and the intracellular trafficking of certain membrane proteins and lipids [25-27] — as well as in the infectious cycles of several viruses and bacterial pathogens.[28-33] Examining the formation and regulation of lipid rafts is a critical step in understanding these aspects of eukaryotic cell function.

Our Vybrant Lipid Raft Labeling Kits provide the key reagents for fluorescently labeling lipid rafts *in vivo* with our bright and extremely photostable Alexa Fluor® dyes. Live cells are first labeled with the green-fluorescent Alexa Fluor 488, the orange-fluorescent Alexa Fluor 555 or the red-fluorescent Alexa Fluor 594 conjugate of cholera toxin subunit B (CT-B) (Table 1). This CT-B conjugate binds to the pentasaccharide chain of plasma membrane ganglioside G_{M1}, which selectively partitions into lipid rafts.[12,34,35] All of Molecular Probes' CT-B conjugates are prepared from recombinant CT-B and are completely free of the toxic subunit A, thus eliminating any concern for toxicity or ADP-ribosylating activity. An antibody that specifically recognizes CT-B is then used to crosslink the CT-B–labeled lipid rafts into distinct patches on the plasma membrane, which are easily visualized by fluorescence microscopy.[36,37]

Because they are compatible with various multilabeling schemes, the Vybrant Lipid Raft Labeling Kits can also serve as important tools for identifying physiologically significant membrane proteins that associate with lipid rafts. Cells can be labeled with other live-cell probes during the lipid raft labeling protocol or immediately following the antibody crosslinking step, depending on the specific labeling requirements of the other probes. Alternatively, once the lipid rafts have been labeled and crosslinked, the cells can be fixed for long-term storage or fixed and permeabilized for subsequent labeling with antibodies or other probes that are impermeant to live cells.

Materials

Contents

- Cholera toxin subunit B (recombinant) labeled with the Alexa Fluor 488, Alexa Fluor 555 or Alexa Fluor 594 dye (Component A), one vial containing 100 µg
- Anti–cholera toxin subunit B antibody (anti–CT-B), rabbit serum (Component B), one vial containing 500 µL
- 10X Phosphate-buffered saline (10X PBS) (Component C), pH 7.2, 100 mL

Each kit provides sufficient reagents to label 50 live-cell samples in a 2 mL assay.

Storage

Upon receipt, store the kit at ≤–20°C, desiccated and protected from light. When stored properly, this kit should be stable for at least 6 months from the date of purchase. To avoid multiple freeze-thaw cycles, the anti–CT-B rabbit serum (Component B) and the CT-B stock solution (prepared from Component A in step 1.2, below) may be divided into single-use aliquots before storage at ≤–20°C. For convenience, the 10X PBS (Component C) can be stored thawed at 2–6°C for 6 months.

Experimental Protocol

The following protocol describes the steps for labeling and crosslinking one 2 mL sample of 1×10^6 live cells in suspension. Alternatively, the same reagent volumes can be used for labeling and crosslinking adherent cells grown to subconfluent or confluent density on an 18 mm × 18 mm coverslip. This protocol can be scaled up when labeling more than one sample, or scaled down when incubation volumes are less than 2 mL.

Reagent Preparation

1.1 Prepare 1X phosphate-buffered saline (PBS). Add 2 mL of 10X PBS (Component C) to 18 mL of deionized water and chill before use. The resulting 20 mL of 1X PBS is sufficient for labeling one live-cell sample. Any remaining 1X buffer can be stored at ≤6°C for up to 1 week.

1.2 Prepare a stock solution of the fluorescent cholera toxin subunit B (CT-B) conjugate. After the Alexa Fluor dye–labeled CT-B (Component A) has warmed to room temperature, add 100 µL of the 1X PBS (prepared in step 1.1) to the vial and gently dissolve the solid. This 1 mg/mL stock solution can be stored at 2–6°C for up to 1 week. For longer storage, divide the solution into single-use aliquots and freeze at ≤–20°C for up to 6 months.

1.3 Prepare a working solution of the fluorescent CT-B conjugate. Add 2 µL of the 1 mg/mL CT-B conjugate stock solution (prepared in step 1.2) to a final volume of 2 mL chilled complete growth medium. This 1 µg/mL working solution is sufficient for one labeling using a 2 mL incubation volume. The working solution should be freshly prepared before each use.

1.4 Prepare a 200-fold dilution of the anti–CT-B antibody. Add 10 µL of the anti–CT-B rabbit serum (Component B) to a final volume of 2 mL chilled complete growth medium. This 200-fold dilution of the anti–CT-B rabbit serum is sufficient for one labeling using a 2 mL incubation volume and should be freshly prepared before each use. The undiluted rabbit serum can be stored for several weeks at 2–6°C. For longer storage, divide the undiluted rabbit serum into single-use aliquots and freeze at ≤–20°C for up to 6 months.

Labeling Protocol

2.1 Label the cells with the fluorescent CT-B conjugate. Centrifuge the cells and gently resuspend the cell pellet in chilled, complete growth medium. Centrifuge the cells again and gently resuspend the cell pellet in 2 mL of the fluorescent CT-B conjugate working solution (prepared in step 1.3). Incubate the cells for 10 minutes at 4°C. After this incubation, gently wash the cells several times with chilled 1X PBS.

2.2 Crosslink the CT-B–labeled lipid rafts with the anti–CT-B antibody. Centrifuge the cells and gently resuspend the cell pellet in 2 mL of the chilled anti–CT-B antibody working solution (the 200-fold dilution prepared in step 1.4). Incubate the cells for 15 minutes at 4°C. After this incubation, gently wash the cells several times with chilled 1X PBS.

2.3 Mount and visualize the labeled cells. (If the cells require aldehyde fixation for long-term storage or fixation and detergent permeabilization for subsequent labeling steps, then skip this step and continue to step 2.4.) Mount the cells in chilled 1X PBS and visualize by fluorescence microscopy using an appropriate filter set.

2.4 Fix the cells (optional). Fix the cells in chilled 1X PBS containing 4% formaldehyde for 15 minutes at 4°C, and then wash the cells several times with 1X PBS. Continue to step 2.6 if the cells do not require detergent permeabilization.

2.5 Permeabilize the cells (optional). Permeabilize the fixed cells with 1X PBS containing 0.1% Triton® X-100 for 10 minutes at room temperature. After permeabilization, wash the cells once in 1X PBS.

2.6 Mount and visualize the fixed cells. Mount the fixed (or fixed and detergent permeabilized) cells with an appropriate mounting medium (e.g., the medium provided in Molecular Probes' ProLong® Antifade Kit, P-7481), and then visualize by fluorescence microscopy using an appropriate filter set.

References

1. J Cell Biol 162, 365 (2003); **2.** J Lipid Res 44, 655 (2003); **3.** Eur J Biochem 269, 737 (2002); **4.** Science 290, 1721 (2000); **5.** Mol Membr Biol 16, 145 (1999); **6.** Trends Cell Biol 9, 87 (1999); **7.** Annu Rev Cell Dev Biol 14, 111 (1998); **8.** Proc Natl Acad Sci USA 100, 5813 (2003); **9.** J Immunol 170, 1329 (2003); **10.** J Membr Biol 189, 35 (2002); **11.** Proc Natl Acad Sci USA 98, 9098 (2001); **12.** J Cell Biol 147, 447 (1999); **13.** Mol Biol Cell 10, 3187 (1999); **14.** Biochim Biophys Acta 1610, 247 (2003); **15.** Annu Rev Immunol 21, 457 (2003); **16.** Mol Immunol 38, 1247 (2002); **17.** Nat Rev Immunol 2, 96 (2002); **18.** Biol Res 35, 127 (2002); **19.** Nat Rev Mol Cell Biol 1, 31 (2000); **20.** J Exp Med 190, 1549 (1999); **21.** J Cell Biol 143, 637 (1998); **22.** Immunity 18, 655 (2003); **23.** J Biol Chem 277, 39541 (2002); **24.** Biochem Biophys Res Commun 297, 876 (2002); **25.** Biol Chem 383, 1475 (2002); **26.** J Cell Biol 153, 529 (2001); **27.** J Cell Sci 114, 3957 (2001); **28.** J Virol 77, 9542 (2003); **29.** Exp Cell Res 287, 67 (2003); **30.** Traffic 3, 705 (2002); **31.** J Clin Virol 22, 217 (2001); **32.** Curr Biol 10, R823 (2000); **33.** J Virol 74, 3264 (2000); **34.** Biochemistry 35, 16069 (1996); **35.** Mol Microbiol 13, 745 (1994); **36.** J Cell Biol 141, 929 (1998); **37.** J Biol Chem 269, 30745 (1994).

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