BioTracker™ NIR770 Cytoplasmic Membrane Dye

Live Cell Dye

Cat. # SCT114

pack size: 100µL

Store at Room Temp

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Lipophilic carbocyanine dyes are widely used for labeling neurons in tissues by retrograde labeling, and to label membranes in a wide variety of cell types. The dyes are weakly fluorescent in aqueous phase, but become highly fluorescent in lipid bilayers. Staining is highly stable with low toxicity and very little dye transfer in between cells, making the dyes suitable for long-term cell labeling and tracking studies. When live cells are stained, the dyes label plasma membranes and also are taken up into endocytic compartments. Cells can be fixed either before or after staining, although permeabilization affects the staining pattern.

Unlike PKH dyes, BioTracker™ Cytoplasmic Membrane Dyes do not require a complicated hypoosmotic labeling protocol. They are ready-to-use dye delivery solutions that can be added directly to normal culture media to label suspended or adherent cells in culture.

NIR Cytoplasmic Membrane Dyes are novel near-infrared carbocyanine dyes for labeling the cytoplasmic membranes of living cells. Due to their long emission wavelengths, near-infrared cell membrane stains can be used to label cells for near-infrared small animal imaging studies for non-invasive imaging of cell migration and cell homing.

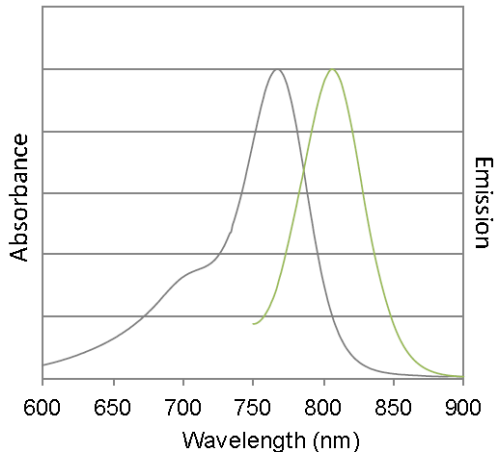

Figure 1. Excitation and emission spectra of BioTracker™ NIR770 Cytoplasmic Membrane Dyes

Storage

Store BioTracker™ NIR770 Cytoplasmic Membrane Dye at room temp. Protect From Light.

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Spectral Properties

Absorbance: 767nm
Emission: 806nm

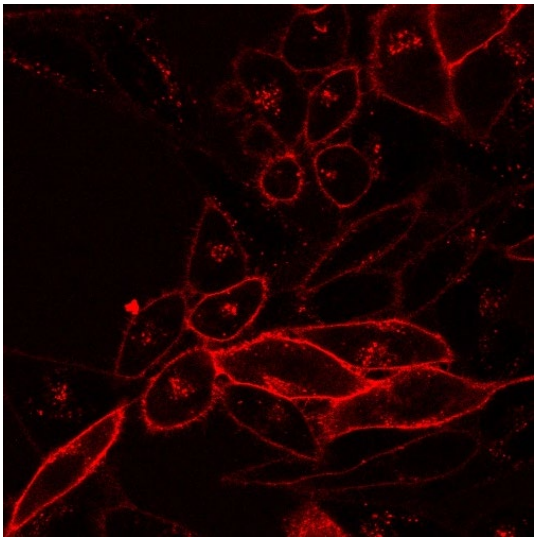

Figure 2. Live cell staining of Hela cells using BioTracker™ NIR770 Cytoplasmic Membrane Dye

Rev.1.0/2018-01-29/SCT114/NJA

Assay Protocol

Labeling of Cells in Suspension

1. Prepare staining medium by diluting dye 1:2000 in culture medium for a final dye concentration of 1uM (see notes below).
2. Pellet cells by centrifugation at 350xg for 5 minutes.
3. Remove supernatant and resuspend cells at a density of 1×10^6/mL in staining medium.
4. Incubate cells for 20 minutes at 37°C (see notes below).
5. Pellet the labeled cells at 350xg for 5 minutes, preferably at 37°C.
6. Remove supernatant and resuspend the cells in warm (37°C) medium.
7. Repeat wash steps (5 and 6) two more times.
8. Observe fluorescence by confocal microscopy, or proceed with experiment.

Labeling of Adherent Cells

1. Prepare staining medium by diluting dye 1:2000 in culture medium for a final dye concentration of 1uM (see notes below).
2. Aspirate culture medium and add sufficient staining medium to completely cover the cells.
3. Incubate cells for 20 minutes at 37°C (see notes below).
4. Aspirate the staining medium and wash the cells three times. For each wash, cover the cells with fresh, warm growth medium, and incubate at 37°C for 5 minutes.
5. Observe fluorescence by confocal microscopy, or proceed with experiment.

Notes: We recommend optimizing the staining procedure for each particular cell type. In some cases, it may be necessary to vary dye concentration (recommended range 1-5uM) or staining time to obtain optimal labeling. Higher dye concentrations may be required to detect cell staining by confocal microscopy using 640 nm excitation.

Cells stained with carbocyanine dyes can be fixed with formaldehyde. Detergent permeabilization may adversely affect staining. Permeabilization with digitonin (10-1000ug/mL) has been reported to be compatible with carbocyanine dye staining (10). Avoid mounting medium containing glycerol.

Frequently Asked Questions

1. **Q. Does BioTracker Membrane Dyes specifically stain the plasma membrane?**
   A. No, BioTracker Membrane Dyes are lipophilic carbocyanine dyes. These dyes undergo an increase in fluorescence when they insert into lipid bilayers. Lipophilic carbocyanine dyes stably label the plasma membrane and other intracellular membranes of cells. They also can be used to stain artificial lipid bilayers

2. **Q. How stable is BioTracker Membrane staining? Are the dyes toxic to cells?**
   A. Lipophilic carbocyanine dyes have been used to stain neuronal cells in culture for several weeks, and in vivo for up to a year. The dyes do not appreciably affect cell viability, and do not readily transfer between cells with intact membranes, allowing cell migration and tracking studies in mixed populations. Stability of labeling may vary between cell types, depending on rates of membrane turnover or cell division.

3. **Q. Can cells be fixed after membrane staining? Can the dye be used to stain cells or tissues after they are fixed?**
   A. Yes, cells can be fixed with formaldehyde after labeling with BioTracker Membrane dyes. Lipophilic carbocyanine dyes like the have also been used to stain cells or tissues after formaldehyde fixation.