BioTracker™ 555 Orange Cytoplasmic Membrane Dye

Live Cell Dye

Cat. # SCT107

pack size: 1mL

Store at 2-8ºC

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.

Storage

Store BioTracker™ 555 Orange Cytoplasmic Membrane Dye at 2-8ºC. Protect From Light.

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

Spectral Properties

Absorbance: 549nm
Emission: 565nm

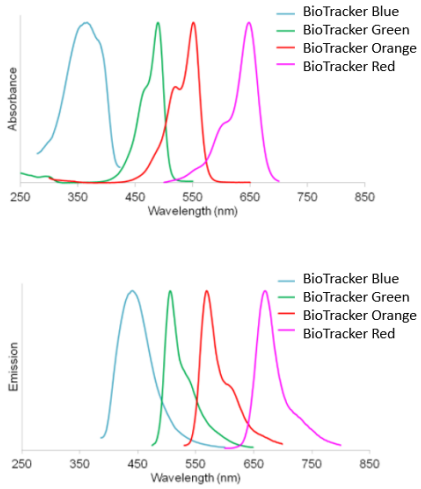

Figure 1. Excitation and emission spectra of BioTracker™ Cytoplasmic Membrane Dyes

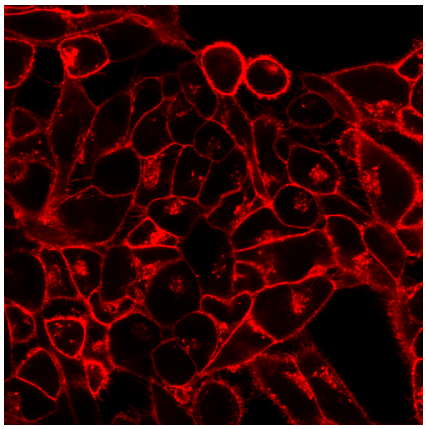

Figure 2. Live cell staining of Hela cells using BioTracker™ 555 Orange Cytoplasmic Membrane Dye

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Assay Protocol

Labeling of Cells in Suspension

1. Suspend cells at a density of 1×10^6/mL in normal growth medium.
2. Add 5uL of the cell labeling solution per 1mL of cell suspension. Mix well by flicking the tube.
3. Incubate for 1–20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
4. Centrifuge the labeled suspension tubes at 1500 rpm for 5 minutes at 37°C.
5. Remove the supernatant and gently resuspend the cells in warm (37°C) medium.
6. Repeat the wash procedure (Steps 1.4 and 1.5) two more times.
7. Proceed with fluorescence observation.

Labeling of Adherent Cells

1. Culture adherent cells in sterile glass coverslips or chamber slides as either confluent or subconfluent monolayers.
2. Remove coverslips from growth medium and gently drain off or aspirate excess medium. Then place coverslips in a humidity chamber.
3. Prepare staining medium by adding 5uL of the cell labeling solution to 1mL of normal growth medium and mixing well.
4. Pipet the staining medium onto the cells. Alternatively, cell labeling solution can be added directly to the cell culture and mixed well by shaking or swirling the plate. Add 5uL of cell labeling solution per mL of culture medium in the plate.
5. Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start by incubating for 20 minutes and subsequently optimize as necessary to obtain uniform labeling.
6. Aspirate the staining medium and wash the cells three times. For each wash cycle, cover the cells with fresh, warmed growth medium, and incubate at 37°C for 5 minutes.
7. Proceed with fluorescence observation.

Notes: It is recommended to optimize the staining procedure for each particular cell type. In some cases, it may be necessary to vary the staining volume and time. Cells stained with carbocyanine dyes can be fixed with formaldehyde. Detergent permeabilization may adversely affect staining. Digitonin permeabilization (10 ug/mL-1 mg/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.