BioTracker™ 400 Blue Cytoplasmic Membrane Dye

Live Cell Dye

Cat. # SCT109

pack size: Kit

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.

Kit Components

1. 1 vial of 250µL Cell Labeling Solution (CS224586)
2. 1 vial of 250µL Loading Buffer (CS224587)

Storage

Store BioTracker™ 400 Blue 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: 366nm
Emission: 441nm

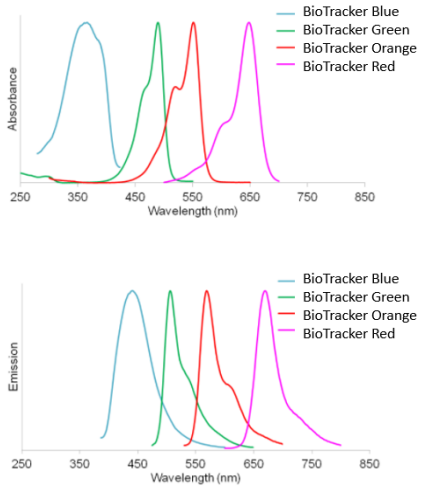

Figure 1. Excitation and emission spectra of BioTracker™ Cytoplasmic Membrane Dyes

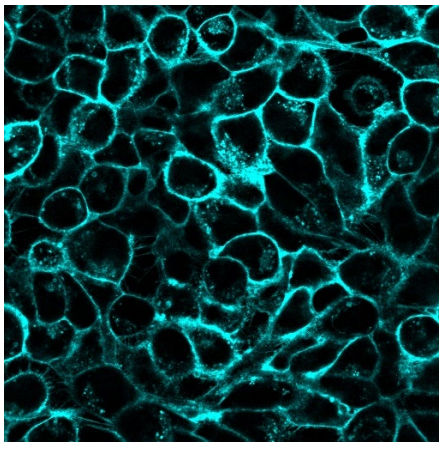

Figure 2. Live cell staining of Hela cells using BioTracker™ 400 Blue Cytoplasmic Membrane Dye

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Assay Protocol

Preparation of Working Labeling Solution

If Loading Buffer has solidified, heat the solidified gel to 50-60C for 5-10 minutes and vortex periodically until it has formed a clear liquid. Loading Buffer is viscous, so pipet it slowly to ensure the correct volume is added.

Just before use, prepare a 1:1 mixture of Cell Labeling Solution and Loading Buffer in a clean tube. Mix 5uL of labeling solution with 5uL of loading buffer per mL of staining medium required. Pipette the mixture up and down to mix thoroughly. This is the working labeling solution. Add 10uL of the working labeling solution per mL of cell suspension or staining medium as described below.

Labeling of Cells in Suspension

1. Suspend cells at a density of 1×10^6/mL in normal growth medium.
2. Add 10uL of the working labeling solution per 1mL of cell suspension and mix well.
3. Incubate for 1-20 minutes at 37°C. We recommend starting with 20 minutes and optimizing the incubation time for even labeling.
4. Pellet the cells by centrifuging at 1500 rpm for 5 minutes.
5. Remove the supernatant and gently resuspend the cells in warm medium.
6. Repeat the wash procedure (Steps 4 and 5) two more times.
7. Resuspend the cells in medium and 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 10uL of the working labeling solution to 1mL of normal growth medium. Vortex to mix well.
4. Pipet the staining medium onto the cells. Alternatively, 10uL of working labeling solution can be added directly to the cell culture and mixed well by shaking or swirling the plate, but this may result in uneven labeling.
5. Incubate the cells at 37°C for 20 minutes. Staining time may need to be optimized for even 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. Add fresh medium to the cells and 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.*
- *If cells are returned to culture after staining, membrane internalization will occur, resulting in more intracellular staining and less surface staining over time.*
- *Cells stained with carbocyanine dyes can be fixed with formaldehyde, but detergent permeabilization may adversely affect staining. Digitonin permeabilization (10 ug/mL - 1 mg/mL) has been reported to be compatible with carbocyanine dye staining. Avoid mounting medium containing glycerol.*
- *Formaldehyde fixed cells can be stained using the same protocols above. Staining of fixed cells can be performed in PBS or other buffer at room temperature instead of culture medium at 37°C.*

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.