

## Data Sheet

# BioTracker™ 490 Green Cytoplasmic Membrane Dye

Live Cell Dye

**SCT106**

**Pack Size: 1 mL**

**Store at 2–8 °C.**

**FOR RESEARCH USE ONLY**

**Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

## 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 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 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.

## Spectral Properties

**Absorbance:** 484 nm

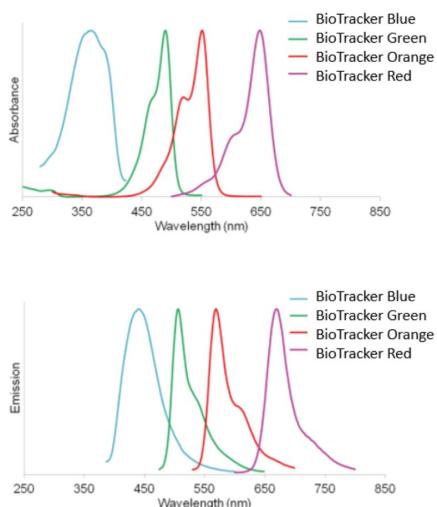
**Emission:** 501 nm

## Storage and Handling

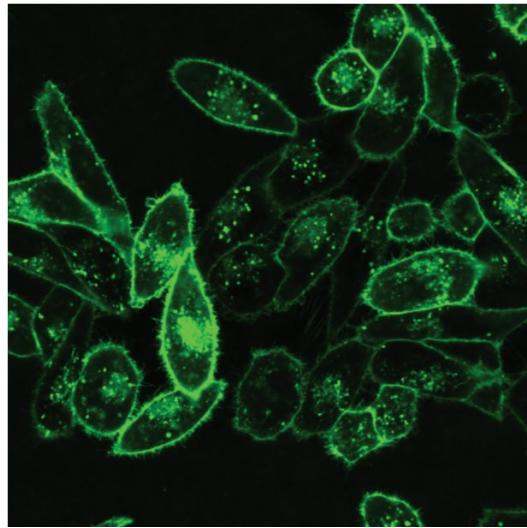
Store BioTracker™ 490 Green Cytoplasmic Membrane Dye at 2–8 °C. Protect from light.

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

## Presentation



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



**Figure 2.** Live cell staining of HeLa cells using BioTracker™ 490 Green Cytoplasmic Membrane Dye.

## Protocols

### Labeling of Cells in Suspension

1. Suspend cells at a density of  $1 \times 10^6$  /mL in normal growth medium.
2. Add 5  $\mu$ L of the cell labeling solution per 1 mL 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 300  $\times g$  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 4 and 5) twice more.
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 5  $\mu$ L of the cell labeling solution to 1 mL 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 5  $\mu$ L 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.

**Note:** 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  $\mu$ g/mL–1 mg/mL) has been reported to be compatible with carbocyanine dye staining. Avoid mounting medium containing glycerol.

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