

# Product Information

## CytoMBrite Cytoplasmic Membrane Dyes

Catalog Number and Product Size:

C4048 CytoMBrite Green: 200 µL Neuro-DiO

C4049 CytoMBrite Orange: 200 µL DiI

C4050 CytoMBrite Red: 200 µL DiD

Application Scope: Membrane staining, cell tracing, tracking, cellular imaging

### Parameters

- NeuroDiO (C4048) Ex/Em: 484/501 nm
- DiI (C4049) Ex/Em: 549/565 nm
- DiD (C4050) Ex/Em: 644/665 nm

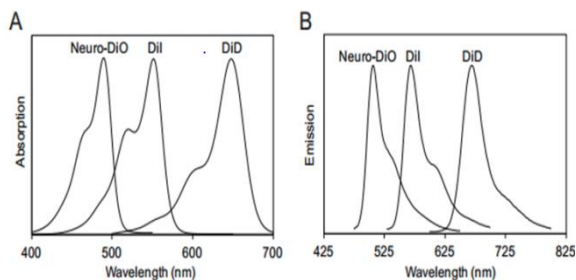


Figure 1. Absorption and emission spectra of CytoMBrite Cytoplasmic Membrane Dyes

### Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

### Description

DiI, DiO and DiD are a class of lipophilic carbon cyanine dyes. Carbocyanine dyes label cytoplasmic membrane and intracellular membrane structures efficiently and permanently. They have been used as tracers in cell–cell fusion, cellular adhesion, and migration applications due to their properties of low cytotoxicity and high resistance to intercellular transfer. The combination of CytoMBrite Cytoplasmic Membrane Dyes

and other cell membrane dyes, such as DiR, NIR680 provides an effective tool for multicolor imaging and flow cytometry.

Cells can be fixed with formaldehyde either before or after staining, but not recommended for permeabilization.

### Protocol

You may need to optimize the staining procedure for each particular cell type by varying the dye concentration, staining volume, labeling time, or wash steps.

#### 1. Suspension cell staining

- (1) Add an appropriate volume of medium to resuspend the cells to a density of  $1 \times 10^6$  / mL, and then add the staining solution at a ratio of 1: 200.
- (2) Incubate for 2~20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 2 minutes and optimize as needed for uniform labeling.
- (3) Pellet the cells by centrifugation at 1000 ~ 1500 rpm for 5 minutes.
- (4) Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) medium.
- (5) Repeat the centrifugation and wash steps (Steps 3 and 4) two more times.
- (6) Image fluorescence. Cells can be imaged in culture medium.



## 2. Adherent cell staining

- (1) Prepare staining working solution: Add 5  $\mu$ L of the dyeing stock solution to each 1 mL of medium, and vortex to mix.
- (2) Remove growth medium from the cells.
- (3) Add enough working solution to completely cover the cells.
- (4) Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start with 2 minutes and optimize as needed for uniform labeling.
- (5) Remove the working solution.
- (6) Wash the cells by adding fresh warm growth medium and incubating at 37°C for 5 minutes. Repeat this wash step two more times.

- (7) Image fluorescence. Cells can be imaged in culture medium.

## Notes

1. When CytoMBrite Cytoplasmic Membrane Dyes stain fixed cell or tissue samples, the samples should be fixed with 4% paraformaldehyde in PBS. The use of other improper fixing solutions will result in a high fluorescence background.
2. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.
3. For your safety and health, please wear lab coats and disposable gloves.

