— PROTOCOL —

HTD™ Plasma Membrane Stains

Catalog numbers: HTD323-M1ML

Contents and storage

Amount	Storage	Stability
1mL (10 mg/mL, 1mL in water)	✧ Room temperature ✧ Cap the vials tightly after each use to avoid evaporation	The product is stable for at least 12 months from date of receipt

Spectral Properties : Full Color Characteristic:

Ex/Em: 360-370 / ≥ 420 nm

Ex/Em: 460-490 / ≥ 520 nm

Ex/Em: 525-545 / ≥ 575 nm

See Figure 1 for spectra

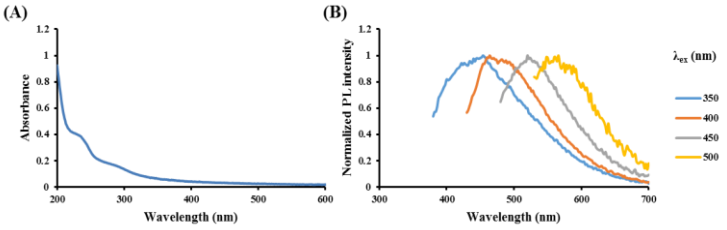


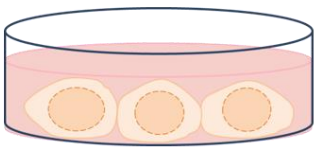
Figure 1. Absorbance (A) and fluorescence (B) spectra of HTD™ plasma membrane stains.

Description

The amphiphilic HTD™ plasma membrane stains can label not only plasma membrane but also other membranous structures, including nuclear membrane. The stains could be used for labeling the membranous structures of live adherent and suspension cells, as tracers for the dynamics of membrane and for multi-generational cellular tracing. HTD™ plasma membrane stains have full-color fluorescence characteristic, which emit blue, green, and red fluorescence under UV, blue, and green light excitations, respectively.

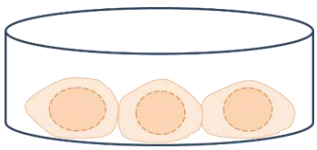
Plasma Membrane Staining Protocol

1 Seed Cells



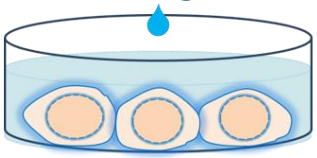
Seed cells (500 µL/well, 1x10^6) on coverslips in a 24-well plate.

2 Aspirate Media



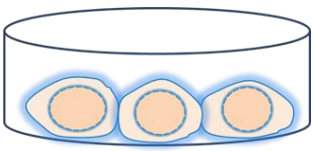
Aspirate the media.

3 Add HTD_{mem} Working Solution



- Add HTD_{mem} staining solution (in PBS).
- Incubate for 30 minutes at 37°C.

4 Wash Cells



(Optional)
Wash cells 1X in PBS.

Staining Protocols

For homogenization, this 10 mg/mL stock solution should be pre-warmed for 10 minutes at 37°C prior to application. (Note: It is recommended to optimize the staining procedure for each particular cell type. In some cases, it may be necessary to change or alter the staining volume and time.)

— PROTOCOL —

Labeling Live Cells in Suspension

1. Prepare suspension cells at a density of 1×10^6/mL in PBS.
2. Make a staining solution of 50 µg/mL. To do this, add 5 µL of the HTD™ plasma membrane stains per 1 mL of cell suspension. Then, mix well by low-speed vortexing or flicking the tube.
3. Incubate for 30 minutes at 37°C. The optimal incubation time will vary depending on cell type.
4. Pellet the cells by centrifugation at 350 x g for 5 minutes.
5. Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) PBS.
6. Place the cells on microscopic slide, and then cover the cells with coverslip.
7. Image the cells with fluorescence microscope with oil immersion lens.

Labeling Live Adherent Cells

1. Grow cells to 80% density on coverslips or chamber slides.
2. Make a staining solution of 50 µg/mL by adding 5 µL of HTD™ plasma membrane stains to 1 mL of PBS.
3. Remove the medium and add staining solution to completely cover cells. (We recommend 300ul/well in 24 wells cell culture plate).
4. Incubate for 30 minutes at 37°C. The optimal incubation time will vary depending on the cell type.
5. Remove the staining solution.
6. Wash the cells by adding fresh PBS and then aspirate it. Wash the cells once, twice or as needed.
7. Fix the cells and then mount the cells. (see "Fixation After Staining")
8. Image the cells with fluorescence microscope with oil immersion lens.

Long Term Cell Staining

The HTD™ plasma membrane stains are very stable, and can be used to stain live cells for days in culture. Immediately after labeling cells, the dyes predominantly stain the plasma membrane, and the staining pattern in plasma membrane is are well-retained days after labeling.

Fixation After Staining

Live cells stained with HTD™ plasma membrane stains can be fixed with 4% paraformaldehyde (PFA) or methanol. (Note: Staining pattern will change after permeabilization with 0.1% Triton® X-100, which should be avoided.)

1. Fix the cells with 4% paraformaldehyde (PFA) or methanol for 15 minutes at 37°C.
2. Wash the cells with PBS. (Once)
3. Image the cells with fluorescence microscope.

Labeling Fixed Cells

1. Fix the cells with 4% paraformaldehyde (PFA) or methanol for 15 minutes at 37°C.
2. Wash cells with PBS after fixation.

3. Make a staining solution of 50 µg/mL. To do this, add 5 µL of HTD™ plasma membrane stains to 1 mL of PBS.
4. Remove the PBS from the cells and add the staining solution to completely cover the cells.
5. Incubate for 30 minutes at 37°C.
6. Wash the cells with PBS, once, twice or as needed.
7. Image the cells with fluorescence microscope.

Related Products

Catalog number	Product
HTD323-N1ML	HTD™ Nucleolus Stains
HTD323-C1ML	HTD™ Cytoplasm Stains
HTD323-M1ML	HTD™ Plasma Membrane Stains