

— PROTOCOL —

HTD™ Cytoplasm Stains

Catalog numbers: HTD323-C1ML

Contents and storage

Amount	Storage	Stability
1mL (10 mg/mL, 1mL in water)	<ul style="list-style-type: none"> ◇ 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 / \geq 420 nm
 Ex/Em: 460-490 / \geq 520 nm
 Ex/Em: 525-545 / \geq 575 nm
 See Figure 1 for spectra

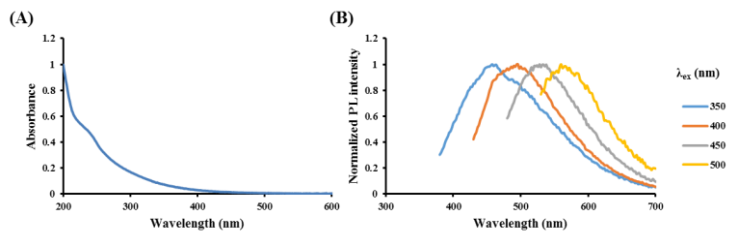
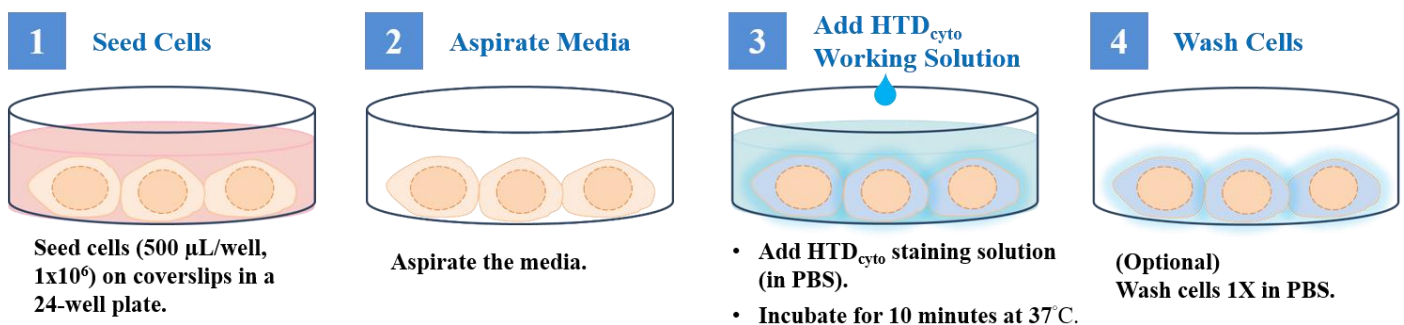


Figure 1. Absorbance (A) and fluorescence (B) spectra of HTD™ cytoplasm stains.

Description

The hydrophilic HTD™ cytoplasm targets stains label cytoplasm efficiently. The stains are used for labeling the cytoplasm of live adherent and suspension cells, as tracers for cell migration and for multi-generational cellular tracing. HTD™ cytoplasm stains have full-color fluorescence characteristics, which emit blue, green, and red fluorescence under UV, blue, and green light excitations, respectively.

Cytoplasm Staining Protocol



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 200 μ g/mL. To do this, add 20 μ L of the HTD™ cytoplasm stains per 1 mL of cell suspension. Then, mix well by low-speed vortexing or flicking the tube.
3. Incubate for 10 minutes at 37°C. The optimal incubation time will vary depending on the 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 slides, 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 200 μ g/mL by adding 20 μ L of HTD™ cytoplasm stains to 1 mL of PBS.
3. Remove the medium and add staining solution to completely cover cells. (We recommend 300 μ L/well in 24 wells cell culture plate).
4. Incubate for 10 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™ cytoplasm 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 cytoplasm, and the staining pattern in cytoplasm is are well-retained days after labeling.

Fixation After Staining

Live cells stained with HTD™ cytoplasm stains can be fixed with 4% paraformaldehyde (PFA) or methanol. (Note: Staining pattern of cytoplasm can withstand permeabilization with 0.1% Triton® X-100.

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.

— PROTOCOL —

2. Wash cells with PBS after fixation.
3. Make a staining solution of 200 µg/mL. To do this, add 20 µL of HTD™ cytoplasm 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 10 minutes at 37°C.
6. Wash the cells with PBS once, twice or as needed.
7. Image the cells with fluorescence microscope.

Materials are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

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Related Products

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