

— PROTOCOL —

## HTD™ Nucleolus Stains

Catalog numbers: HTD323-N1ML

### Contents and storage

Amount	Storage	Stability
1mL (10 mg/mL, 1mL in water)	<ul style="list-style-type: none"> <li>◇ Room temperature</li> <li>◇ Cap the vials tightly after each use to avoid evaporation</li> </ul>	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

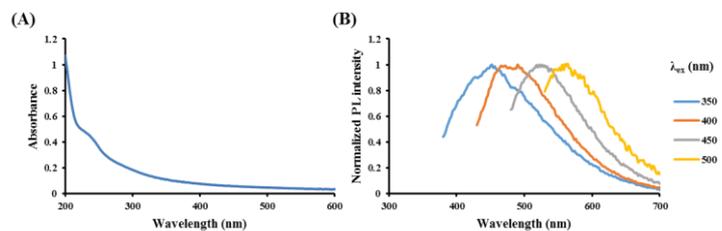
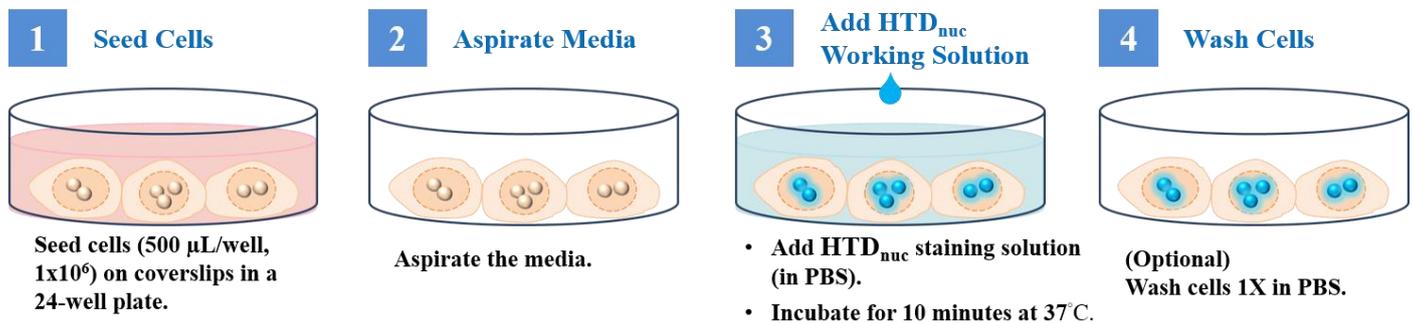


Figure 1. Absorbance (A) and fluorescence (B) spectra of HTD™ nucleolus stains.

### Description

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

### Nucleolus 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.)

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### Labeling Live Cells in Suspension

1. Prepare suspension cells at a density of  $1 \times 10^6$ /mL in PBS.
2. Make a staining solution of 200  $\mu$ g/mL. To do this, add 20  $\mu$ L of the HTD™ nucleolus 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 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 200  $\mu$ g/mL by adding 20  $\mu$ L of HTD™ nucleolus 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 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

Amphiphilic HTD™ nucleolus 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 nucleolus, and the staining pattern in nucleolus are well-retained days after labeling.

### Fixation After Staining

Live cells stained with HTD™ nucleolus stains can be fixed with 4% paraformaldehyde (PFA) or methanol. (Note: Staining pattern of nucleolus 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.

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2. Wash cells with PBS after fixation.
3. Permeabilize cells with 0.1% Triton® X-100 in PBS for 15 minutes at 37°C. (Note: Staining of nucleolus with 4% paraformaldehyde (PFA) must permeabilization with 0.1% Triton® X-100.)
4. Wash the cells 2 times with PBS to remove all traces of detergent.
5. Make a staining solution of 1 mg/mL. To do this, add 100 µL of HTD™ nucleolus stains to 1 mL of PBS.
6. Remove the PBS from the cells and add the staining solution to completely cover the cells.
7. Incubate for 10 minutes at 37°C.
8. Wash the cells with PBS once, twice or as needed.
9. 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