# Cell Navigator™ Lysosome Staining Kit *Blue Fluorescence*

Catalog number: 22655
Unit size: 500 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: LysoBrite™ Blue | Freeze (<-15 °C), Minimize light exposure | 100 µL (500X DMSO stock solution) |
| Component B: Live Cell Staining Buffer | Freeze (<-15 °C), Minimize light exposure | 50 mL |

## OVERVIEW

Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria and nuclei etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to label lysosomes of live cells in blue fluorescence. The kit uses a proprietary lysotropic dye that selectively accumulates in lysosomes probably vial the lysosome pH gradient. The lysotropic indicator is a hydrophobic compound that easily permeates intact live cells, and trapped in lysosomes after it gets into cells. Its fluorescence is significantly enhanced upon entering lysosomes. This key feature significantly increases its selectivity for lysosomes. The labeling protocol is robust, requiring minimal hands-on time. It can be readily adapted for a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

## AT A GLANCE

**Protocol summary**

1. Prepare cells
2. Add dye working solution
3. Incubate at 37°C for 30 minutes to 2 hours
4. Analyze under fluorescence microscope at Ex/Em = 360/445 nm (DAPI filter set)

**Important** Warm LysoBrite™ Blue (Component A) to room temperature.

## KEY PARAMETERS

| | |
|---|---|
| Instrument: | Fluorescence microscope |
| Excitation: | 360 nm |
| Emission: | 445 nm |
| Instrument specification(s): | DAPI filter set |
| Recommended plate: | Black wall/clear bottom |

## PREPARATION OF WORKING SOLUTION

Dilute 20 µL of LysoBrite™ Blue (Component A) into 10 mL of Live Cell Staining Buffer (Component B). Protect from light.

***Note*** 20 µL of LysoBrite™ Blue (Component A) is enough for one 96-well plate. The optimal concentration of the fluorescent lysosome indicator varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

## PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

## SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare samples and cells.

   **For adherent cells:** Grow cells either in a black wall/clear bottom 96-well plate (100 µL/well/96-well plate) or on coverslips inside a petri dish filled with the appropriate culture medium. When cells reach the desired confluence, add equal volume (such as 100 µL/well/96-well plate) of the dye-working solution.

   **For suspension cells:** Centrifuge the cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellet gently in pre-warmed growth medium, and then add equal volume of the dye-working solution.

2. Incubate the cells in a 37°C, 5% $CO_2$ incubator for 30 minutes to 2 hours.

3. Observe the cells using a fluorescence microscope fitted with a DAPI filter set.

   ***Note*** It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

   ***Note*** Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.

## EXAMPLE DATA ANALYSIS AND FIGURES

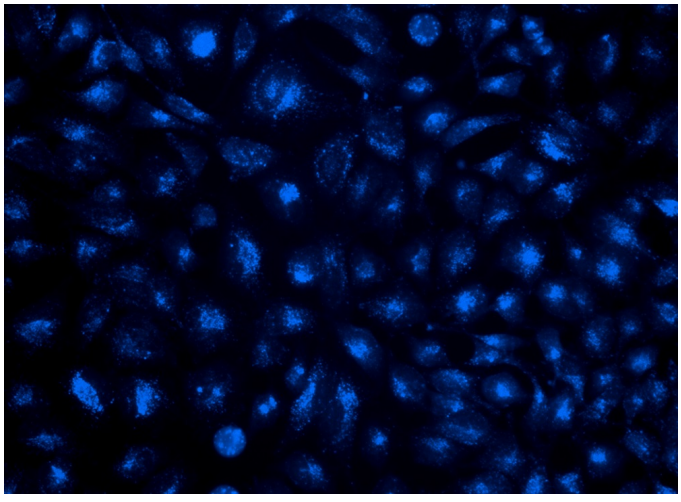

**Figure 1.** Image of HeLa cells stained with Cell Navigator™ Lysosomal Staining Kit in a Costar black wall/clear bottom 96-well plate.

## DISCLAIMER