

Cell Navigator™ Lysosome Staining Kit *NIR Fluorescence*

Catalog number: 22652
Unit size: 500 Tests

Component	Storage	Amount
Component A: LysoBrite™ NIR	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

Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The interior of the lysosomes is acidic (pH 4.5-4.8) compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels. Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, 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 NIR fluorescence. LysoBrite™ NIR, the proprietary lysotropic dye used in the kit, selectively accumulates in lysosomes probably via 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 reduces its staining background and makes it useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. LysoBrite™ dyes significantly outperform the equivalent LysoTracker™ dyes (from Invitrogen). LysoBrite™ dyes can stay in live cells for more than a week with very minimal cell toxicity while the LysoTracker dyes can only be used for a few hours. LysoBrite™ dyes can survive a few generations of cell division. In addition, LysoBrite™ dyes are much more photostable than the LysoTracker dyes.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add LysoBrite™ NIR working solution
3. Incubate at 37°C for 30 minutes
4. Wash the cells
5. Analyze the cells under fluorescence microscope at Ex/Em = 630/650 nm (Cy5 filter set)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	630 nm
Emission:	650 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Cy5 filter set

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X LysoBrite™ NIR stock solution (Component A) to 10 mL of Live Cell Staining Buffer (Component B) to make LysoBrite™ NIR working solution. Protect from light.

Note 20 µL of 500X LysoBrite™ NIR (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

For adherent cells:

1. Grow cells either in a 96-well black wall/clear bottom plate (100 µL/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture medium. When cells reach the desired confluence, add equal volume of LysoBrite™ NIR working solution.
2. Incubate the cells in a 37°C, 5% CO₂ incubator for 30 minutes.
3. Wash the cells twice with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice, then fill the cell wells with HBSS or growth medium.
4. Observe the cells using a fluorescence microscope with Cy5 filter set (Ex/Em = 630/650 nm).

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.

For suspension cells:

1. Add equal volume of LysoBrite™ NIR working solution into the cells.
2. Incubate the cells in a 37°C, 5% CO₂ incubator for 30 minutes.
3. Wash the cells twice with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice, then fill the cell wells with HBSS or growth medium.
4. Observe the cells using a fluorescence microscope with Cy5 filter set (Ex/Em = 630/650 nm).

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

EXAMPLE DATA ANALYSIS AND FIGURES

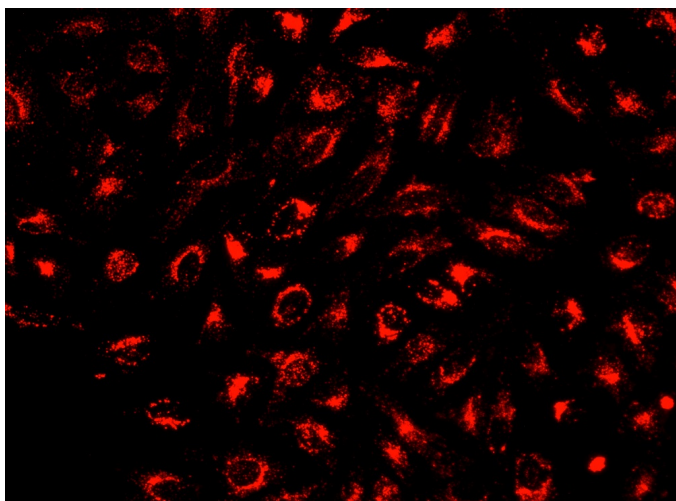


Figure 1. Image of HeLa cells stained with Cell Navigator™ Lysosome Staining Kit in a Costar black wall/clear bottom 96-well plate.

DISCLAIMER

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