Cell Navigator™ Lysosome Staining Kit *Orange Fluorescence*

Catalog number: 22657
Unit size: 500 Tests

Component	Storage	Amount
Component A: LysoBrite™ Orange	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 orange fluorescence. LysoBrite™ Orange, 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™ Orange working solution
3. Incubate at 37°C for 30 minutes
4. Wash the cells
5. Analyze the cells under fluorescence microscope at Ex/Em = 540/570 nm (TRITC filter set)

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

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	TRITC filter
Emission:	TRITC filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

20 µL of 500X LysoBrite™ Orange (Component A) to 10 mL of Live Cell Staining Buffer (Component B) to make LysoBrite™ Orange working solution.

Note 20 µL of 500X LysoBrite™ Orange (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.
2. When cells reach the desired confluence, add equal volume of LysoBrite™ Orange working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes.
4. Wash the cells twice with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice, fill the cell wells with HBSS or growth medium.
5. Observe the cells using a fluorescence microscope with TRITC filter set (Ex/Em = 540/570 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™ Orange working solution into the cells.
2. Incubate the cells in a 37°C, 5% CO_2 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, fill the cell wells with HBSS or growth medium.
4. Observe the cells using a fluorescence microscope with TRITC filter set (Ex/Em = 540/570 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

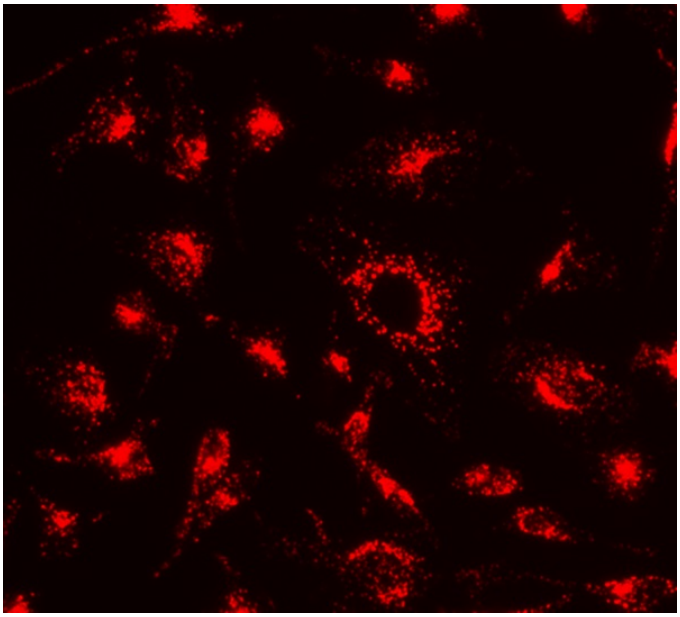

Figure 1. Image of HeLa cells stained with Cell Navigator™ Lysosomal Staining Kit in a Costar black wall/clear bottom 96-well plate using an Olympus fluorescence microscope TRITC channel.

DISCLAIMER