Cell Navigator™ Lysosome Staining Kit *Deep Red Fluorescence*

Catalog number: 22659
Unit size: 500 Tests

Component	Storage	Amount
Component A: LysoBrite™ Deep Red	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL-500X DMSO stock solution)
Component B: Staining Buffer A	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)
Component C: Staining Buffer B	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 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™ Deep Red, 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 Lysolite™ Deep Red working solution
3. Incubate at 37°C for 30 - 60 minutes
4. Add Staining Buffer B
5. Incubate at room temperature for 15 - 30 minutes
6. Analyze the cells under fluorescence microscope at Ex/Em = 590/620 nm (Texas Red filter set)

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

KEY PARAMETERS

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

PREPARATION OF WORKING SOLUTION

Add 10 µL of 500X Lysolite™ Deep Red (Component A) into 5 mL of Live Cell Staining Buffer A (Component B) and mix well to make Lysolite™ Deep Red working solution. Protect from light.

Note 10 µL of 500X Lysolite™ Deep Red (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 1/2 volume (such as 50 µL/well/96-well plate) of Lysolite™ Deep Red working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 to 60 minutes.
4. Add 50 µL/well (96-well plate) of Staining Buffer B (Component C) into Lysolite™ Deep Red working solution plate.
5. Incubate at room temperature for 15 - 30 minutes.
6. Observe the cells using a fluorescence microscope with Texas Red filter set (Ex/Em = 590/620 nm).

   Note The LysoBrite™ Deep Red has minimal or no cell toxicity; it can be used for cell tracking. For cell tracking purpose, skip adding Staining Buffer B: Simply replace the dye-working solution with growth medium, and then observe under fluorescence microscope. 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. Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
2. Resuspend the cell pellet gently in pre-warmed growth medium (such as 100 µL/tube), and then add 1/2 volume (50 µL/tube) of Lysolite™ Deep Red working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 to 60 minutes.
4. Add 50 µL/tube of Staining Buffer B (Component C) into the Lysolite™ Deep Red working solution plate.
5. Incubate at room temperature for 15 - 30 minutes.

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6. Observe the cells using a fluorescence microscope with Texas red filter set (Ex/Em = 590/620 nm).

Note The LysoBrite™ Deep Red has minimal or no cell toxicity; it can be used for cell tracking. For cell tracking purpose, skip adding Staining Buffer B: Simply replace the dye-working solution with growth medium, and then observe under fluorescence microscope. 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

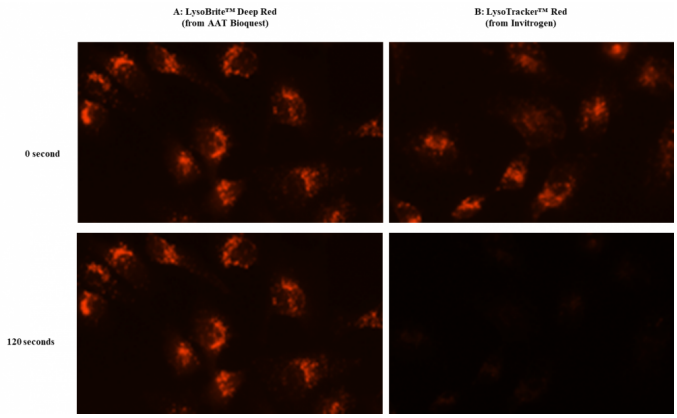

Figure 1. Image of Hela cells stained with the A: Cell Navigator™ Lysosomal Staining Kit or B: LysoTracker® Red DND99 (from Invitrogen) in a Costar black 96-well plate. The fluorescence signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope with Texas red filter set.

DISCLAIMER