LysoBrite™ Deep Red

Catalog number: 22646
Unit size: 500 Tests

Component	Storage	Amount
LysoBrite™ Deep Red	Freeze (< -15 °C), Minimize light exposure	500 Tests

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. LysoBrite™ Deep Red 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™ Deep Red working solution
3. Incubate at 37 °C for 30 minutes
4. Wash cells
5. Analyse using fluorescence microscope with Cy3/Texas Red filter set

Important
Thaw LysoBrite™ stock solution to room temperature. The LysoBrite™ stock solutions provided are 500X in DMSO. They should be stable for at least 6 months if store at - 20 °C. Protect the fluorescent conjugates from light, and avoid freeze/thaw cycles.

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3/Texas Red filter set
Emission	Cy3/Texas Red filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

LysoBrite™ Deep Red working solution
Prepare dye working solution by diluting 20 µL of LysoBrite™ Deep Red (500X) to 10 mL of Hanks and 20 mm Hepes buffer (HHBS) or buffer of your choice.

Note 20 µL of LysoBrite™ dye is enough for one 96-well plate. Aliquot and store unused LysoBrite™ dye stock solutions at < -15 °C. Protect it from light and avoid repeated freeze-thaw cycles./note][note]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.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol only provides a guideline, and should be modified according to your specific needs.

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 the LysoBrite™ Deep Red 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 (HHBS) or buffer of your choice, fill the cell wells with HHBS or growth medium.

4. Observe the cells using a fluorescence microscope with Cy3/Texas Red 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.

For suspension cells

1. Add equal volume of LysoBrite™ Deep Red working solution into the cells. Incubate the cells in a 37 °C, 5% CO2 incubator for 30 minutes.

2. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, fill the cell wells with HHBS or growth medium.

3. Observe the cells using a fluorescence microscope with Cy3/Texas Red 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 cover-slips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.

EXAMPLE DATA ANALYSIS AND FIGURES

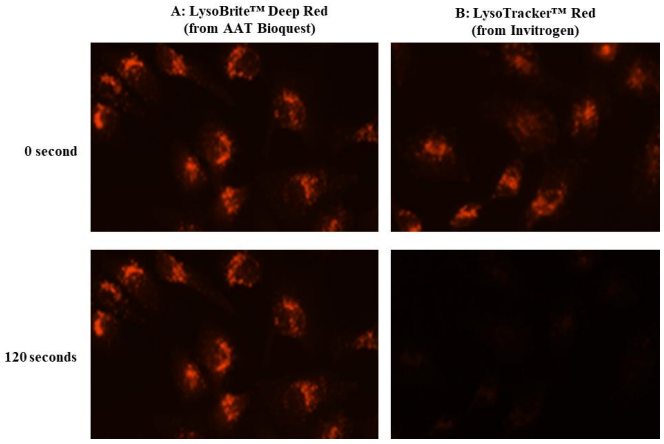

Figure 1. Image of Hela cells stained with the A: LysoBrite™ Deep Redor B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black 96-well plate. The TRTIC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

DISCLAIMER

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