LysoBrite™ Deep Red

Catalog number: 22646
Unit size: 500 Tests

Component	Storage	Amount (Cat No. 22646)
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.

Chemical and Physical Properties of LysoBrite™ Dyes

Cat#	Dye	Unit	Mol. Wt.	Ex/Em (nm)
22641	LysoBrite™ NIR	500 tests	~700	636/651 nm
22642	LysoBrite™ Blue	500 tests	~350	434/480 nm
22643	LysoBrite™ Green	500 tests	~450	501/510 nm
22644	LysoBrite™ Orange	500 tests	~700	543/565 nm
22645	LysoBrite™ Red	500 tests	~700	576/596 nm
22646	LysoBrite™ Deep Red	500 tests	~800	597/619 nm
22647	LysoBrite™ Red DND-99	500 tests	~400	573/595 nm
22648	LysoBrite™ Green DND-26	500 tests	~400	501/509 nm

AT A GLANCE

Assay Protocol with LysoBrite™ Deep Red

1. Prepare cells.
2. Add dye working solution.
3. Incubate at 37 °C for 30 minutes.
4. Wash the cells.
5. Analyze under a fluorescence microscope.

Storage and Handling Conditions

The LysoBrite™ Deep Red stock solution provided is 500X in DMSO. It should be stable for at least 6 months if stored at -20°C and protected from light. Avoid freeze/thaw cycles.

KEY PARAMETERS

Fluorescence microscope

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

Flow cytometer

Emission	630/30 nm filter
Excitation	561 nm laser

PREPARATION OF WORKING SOLUTION

Prepare LysoBrite™ Deep Red Working Solution

1. Warm LysoBrite™ Deep Red dye to room temperature.
2. Dilute 20 µL of 500X LysoBrite™ Deep Red with 10 mL of Hanks and 20 mM HEPES buffer (HBSS) or buffer of your choice.

   Note: 20 µL of LysoBrite™ Deep Red 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: 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.

Protocol for Preparing and Staining Adherent Cells

1. Grow cells in a 96-well black wall/clear bottom plate (100 µL/well/96-well plate) or on coverslips inside a petri dish filled with the appropriate culture medium.
2. When cells reach the desired confluence, add an equal volume of the dye-working solution (from Preparation of Working Solution Step 2).
3. Incubate the cells in a 37 °C, 5% CO2 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. Then fill the cell wells with HBSS or growth medium.
5. Observe the cells using a fluorescence microscope fitted with the

desired 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.

Protocol for Preparing and Staining Suspension Cells

1. Add an equal volume of the dye-working solution (from Preparation of Working Solution Step 2).

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 fitted with the desired 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 treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Protocol for Preparing and Staining Adherent Cells).

EXAMPLE DATA ANALYSIS AND FIGURES

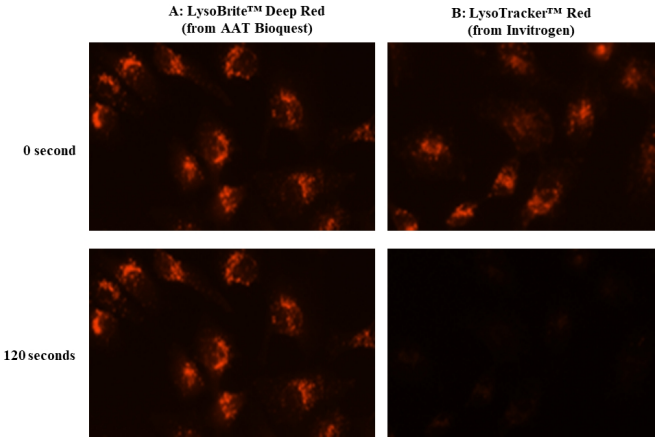

Figure 1. Image of Hela cells stained with the A: LysoBrite™ Deep Redor B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black 96-well plate. The TRTIC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

DISCLAIMER

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