LysoBrite™ Dyes

Introduction

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™ reagents are a series new fluorogenic probes to label lysosomes of live cells. The proprietary lysotropic dyes selectively accumulates in lysosomes probably via the lysosome pH gradient. The lysotropic indicators are hydrophobic compounds that easily permeate intact live cells, and trapped in lysosomes after they get into cells. The fluorescence of LysoBrite™ reagents is significantly enhanced upon entering lysosomes. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. LysoBrite™ Orange, Red, Deep Red, and NIR reagents have extremely high photostability as well as excellent cellular retention make them useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. They are suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

Chemical and Physical Properties

Catalog Number	LysoBrite™ Dyes	Unit	Molecular Weight	Excitation	Emission
22641	LysoBrite™ NIR	500 tests	~ 700	636 nm	650 nm
22642	LysoBrite™ Blue	500 tests	~ 350	433 nm	480 nm
22643	LysoBrite™ Green	500 tests	~ 450	501 nm	509 nm
22644	LysoBrite™ Orange	500 tests	~ 700	542 nm	556 nm
22645	LysoBrite™ Red	500 tests	~ 700	575 nm	597 nm
22646	LysoBrite™ Deep Red	500 tests	~ 800	596 nm	619 nm

Storage and Handling Conditions

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.

Assay Protocol with LysoBrite™ Dyes

Brief Summary

Prepare cells → Add dye working solution → Incubate at 37 °C for 30 minutes → Wash the cells → Analyze under fluorescence microscope

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

1. Prepare Lysosome-staining solution:

1.1 Warm LysoBrite™ dyes to room temperature.

1.2 Prepare dye working solution by diluting 20 µL of 500 X LysoBrite™ dyes to 10 mL of Hanks and 20 mm Hepes buffer (HBSS) or buffer of your choice.
Note 1: 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 2: 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.

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2. Prepare and stain cells:

2.1 For adherent cells: a). 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 dye-working solution (from Step 1.2). b). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes. c). 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. d). Observe the cells using a fluorescence microscope fitted with a 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.

2.2 For suspension cells: a). Add equal volume of dye-working solution (from Step 1.2) into the cells. Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes. b). 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. c). Observe the cells using a fluorescence microscope equipped with a desired filter set.
Note 1: 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 2: Suspension cells may be attached to cover-slips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Step 2.1).

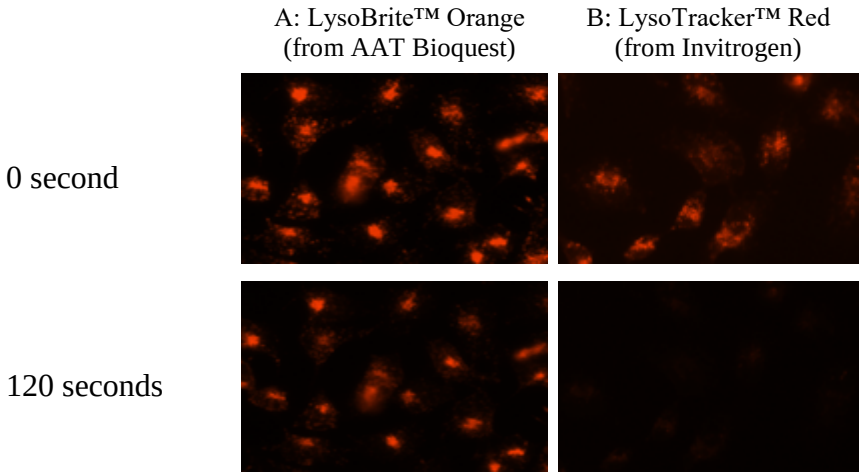

Figure 1. Image of Hela cells stained with the A: LysoBrite™ Orange or B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black 96-well plate. The TRTIC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

References

1. Hung, H; **Deerinck, TJ; Ellisman, MH; and Spector, DL. (**1994) In vivo analysis of the stability and transport of nuclear poly(A)+ RNA. J Cell Biol 126, 877-899.
2. Barasch J, Kiss B, Prince A, Saiman L, Gruenert D, al-Awqati Q. (1991) Defective acidification of intracellular organelles in cystic fibrosis. Nature 1991; 352:70-73.
3. **Jiang, LW; Maher,VM; McCormick, JJ and Schindler, M. (1990)** Alkalinization of the lysosomes is correlated with ras transformation of murine and human fibroblasts. J Biol Chem 265, 4775-4777.
4. Griffiths, G; Hoflack, B; Simons, K; Mellman, I; Kornfeld, S. (1988) The mannose 6-phosphate receptor and the biogenesis of lysosomes. *Cell.* 12;52(3):329–341.