Cell Navigator™ Lysosome Staining Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22658 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

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.

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 red fluorescence at Ex/Em = 575/600 nm. 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. LysoBrite™ Red dye used in the kit has extremely high photostability as well as excellent cellular retention 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.

Kit Key Features

Increased signal intensity:	*10 times brighter than LysoTracker® Red (Invitrogen)*
Extraordinarily high photostability:	*No fading observed after 2 minutes exposure*
Excellent cellular retention:	*More than 5 passages for cell tracking in Hela cells*

Kit Components

Components	**Amount**
Component A: LysoBrite™ Red	100 µL (500X DMSO stock solution)
Component B: Live Cell Staining Buffer	50 mL

Assay Protocol

Brief Summary

Prepare cells → Add dye working solution → Incubate at 37 °C for 30 minutes → Wash the cells → Analyze under fluorescence microscope at Ex/Em = 575/600 nm (TRITC filter set)

1. Prepare Lysosome-staining solution:

1.1 Warm LysoBrite™ Red (Component A) to room temperature.

1.2 Prepare dye working solution by diluting 20 µL of LysoBrite™ Red (Component A) to 10 mL of Live Cell Staining Buffer (Component B).

Note 1: 20 µL of LysoBrite™ Red (Component A) is enough for one 96-well plate. Aliquot and store unused LysoBrite™ Red (Component A) at < -20 °C. Protect it from light and avoid repeated freeze-thaw cycles.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
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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.

2. Prepare and stain cells:

2.1 For adherent cells: 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). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes. 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. Observe the cells using a fluorescence microscope fitted with a TRITC 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: 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. 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. Observe the cells using a fluorescence microscope equipped with a TRITC 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).

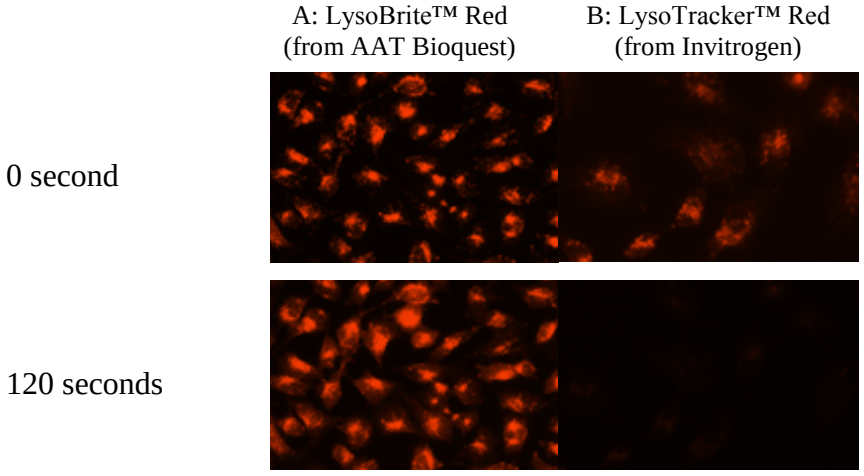

Figure 1. Image of Hela cells stained with the A: Cell Navigator™ Lysosomal Staining Kit or B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black 96-well plate. The TRTIC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

References

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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.