Cell Navigator™ Lysosomal Staining Kit

Red Fluorescence

Ordering Information	*Storage Conditions*
Product Number: 22658	Keep in freezer and protect from light

Introduction

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. The kit uses a proprietary lysotropic dye that 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 strongly enhanced upon entering lysosomes. This key feature significantly increases its selectivity for lysosomes. The labeling protocol is robust, requiring minimal hands-on time. The kit can be readily adapted for many types of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. The kit provides all the essential components with an optimized cell-labeling protocol and it can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

Kit Components

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

Assay Protocol

Brief Summary

Prepare cells → Remove Medium →Add dye working solution → Incubate at 37 °C for 30 min to 2 hr → Wash 2 times with HHBS → Analyze under fluorescence microscope at Ex/Em = 540/590 nm (TRITC filter set)

1. Prepare Lysosome-staining solution:

1.1 Warm up component A (Lysolite™ Red) to room temperature.

1.2 Prepare dye working solution by diluting 20 µL of Component A (Lysolite™ Red) to 10 mL of Component B (Live cell staining buffer).
Note 1: 20 µL of component A is enough for one 96-well plate. Aliquot and store unused component A at < -20 °C. Protect 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.

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, remove the medium from the dish and add the dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes to 2 hours. Wash the cells with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice twice, fill the cell wells with HBSS. 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: Centrifuge the cells at 1000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellet gently in dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes to 2 hours. Wash the cells with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice twice, fill the cell wells with HBSS. 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).

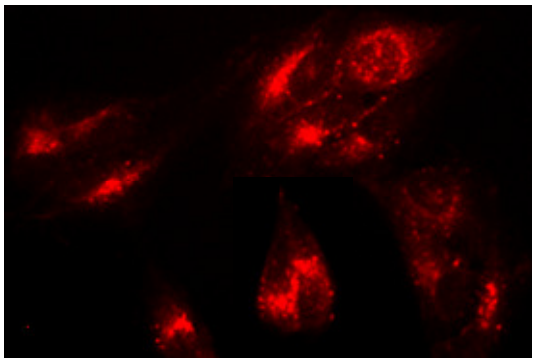

Figure 1. Image of U2OS cells stained with the Cell Navigator™ Lysosomal Staining Kit *Red Fluorescence* in a 96-well Costar black plate

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.