Cell Navigator™ Lysosomal Staining Kit *Green Fluorescence*

Ordering Information:
Product Number: 22656

Instrument Platform:
Fluorescence Microscope

Storage Conditions:
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 and 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 green fluorescence. The kit uses a proprietary lysotropic dye that selectively accumulates in lysosomes probably vial 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 increases its selectivity for lysosomes. The labeling protocol is robust, requiring minimal hands-on time. It can be readily adapted for a wide variety 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. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

Kit Components

Material	Amount
Component A: Lysolite™ Green	100 µL (500 x DMSO stock solution)
Component B: Live cell staining buffer	25 mL

Assay Protocol

Brief Summary

Prepare cells → Add dye working solution→ Incubate at 37°C for 1/2-2 h→Analyze under fluorescence microscope at Ex 485/Em 505 nm (FITC filter set)

1. Prepare Lysosomal-staining solution.

1.1 Warm up component A (Lysolite™ Green) to room temperature.
1.2 Prepare dye working solution by diluting 20 µL Component A (Lysolite™ Green) to 10 mL of Component B (Live cell staining buffer).

Note1: 20 µl of component A is enough for 1 96-well plate, aliquoted and stored un-used component A at ≤ -20°C, avoiding light and repeated freeze-thaw cycles.

Note2: The optimal concentration of the fluorescent lysosome indicator varies depending on the specific application. The staining conditions may be modified depending upon 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 or on coverslips 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 pre-warmed (37°C) dye-working solution (from step 1.2). Incubate the cells at 37°C and 5% CO_2 incubator for 30 minutes to 2 hours. Observe the cells using a fluorescence microscope fitted with a FITC filter set.
Note: We recommend increasing the labeling concentration or increasing the incubation time 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 pre-warmed (37°C) dye-working solution (from step 1.2). Incubate the cells at 37°C and 5% CO_2 incubator for 30 minutes to 2 hours. Observe the cells using a fluorescence microscope fitted with a FITC filter set.
Note 1: We recommend increasing the labeling concentration or increasing the incubation time if the cells do not appear to be sufficiently stained.

Note 2: Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see step 2.1).

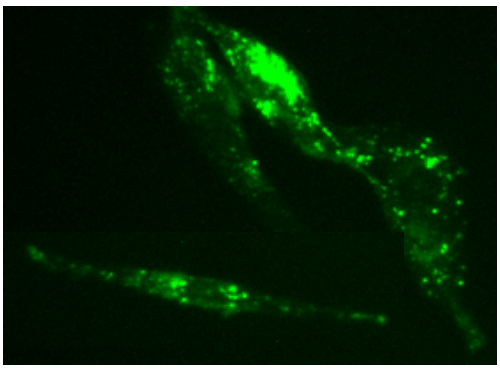

Figure 1. Image of U2OS cells in 96-well Costar black plate were stained with 2 µM of Cell Explorer™ Live Cell Labeling Kit *Green Fluorescence*.

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.

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