

Cell Navigator™ Lysosome Staining Kit

Green Fluorescence 405 nm Excitation

| Ordering Information | Storage Conditions | Instrument Platform |
|------------------------------------|--|-------------------------|
| Product Number: 22651 (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 (H^+ ions) 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 green fluorescence of large Stokes Shift at Ex/Em = 405/520 nm. The kit uses a proprietary lysotropic dye that selectively accumulates in lysosomes probably via the lysosome pH gradient. The lysotropic indicator, a hydrophobic compound, easily permeates intact live cells and gets trapped inside the lysosomes. 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. The kit provides all the essential components. It is suitable for both suspension and adherent cells.

Kit Components

| Components | Amount |
|--|--|
| Component A: LysoBrite™ VLG26 | 100 μ L (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 to 2 hours
→ Analyze under fluorescence microscope at Ex/Em = 405/480 nm (Violet filter set)**

1. Prepare lysosomal-staining solution:

- 1.1 Warm LysoBrite™ VLG26 (Component A) to room temperature.
- 1.2 Prepare dye working solution by diluting 20 μ L of LysoBrite™ VLG26 (Component A) to 10 mL of Live Cell Staining Buffer (Component B).

Note 1: 20 μ L of LysoBrite™ VLG26 (Component A) is enough for one 96-well plate. Aliquot and store unused LysoBrite™ VLG26 (Component A) at $\leq -20^\circ\text{C}$. 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 μ L/well/96-well plate) or on coverslips inside a petri dish filled with the appropriate culture medium. When cells reach the desired

confluence, add equal volume (such as 100 μ L/well/96-well plate) of the dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO₂ incubator for 30 minutes to 2 hours. Observe the cells using a fluorescence microscope fitted with a Violet filter set (Ex/Em = 405/480 nm).

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 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant. Resuspend the cell pellet gently in pre-warmed growth medium, and then add equal volume of the dye-working solution (from Step 1.2). Incubate the cells in a 37 °C, 5% CO₂ incubator for 30 minutes to 2 hours. Observe the cells using a fluorescence microscope fitted with a Violet filter set (Ex/Em = 405/480 nm).

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 coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Step 2.1).

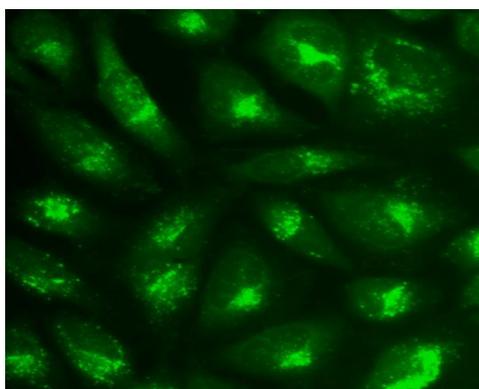


Figure 1. Image of U2OS cells stained with the Cell Navigator™ Lysosomal Staining Kit *Green Fluorescence with 405 nm Excitation* in a Costar black 96-well plate

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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.