# Cell Navigator™ Lysosomal Staining Kit

**Green Fluorescence**

| *Ordering Information:* | *Storage Conditions:* | *Instrument Platform:* |
|---|---|---|
| Product Number: 22656 (500 assays) | Keep in freezer and protect from light | Fluorescence microscope |

## 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 green 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 the 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 different 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™ Green | 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 min to 2 hr → Analyze under fluorescence microscope at Ex/Em = 490/525 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 of Component A (Lysolite™ Green) 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, add equal volume (such as 100 µL/well/96-well plate) of 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. Observe the cells using a fluorescence microscope fitted with a FITC 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 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% $CO_2$ incubator for 30 minutes to 2 hours. Observe the cells using a fluorescence microscope fitted with a FITC 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).*

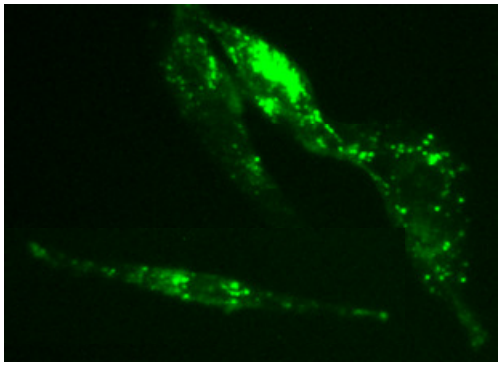

**Figure 1.** Image of U2OS cells stained with the Cell Navigator™ Lysosomal Staining Kit *Green Fluorescence* in a 96-well Costar black plate

## 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.