Cell Navigator® Lysosome Staining Kit *Green Fluorescence with 405 nm Excitation*

Catalog number: 22651
Unit size: 500 Tests

Component	Storage	Amount (Cat No. 22651)
Component A: LysoBrite™ VLG26	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL, 500X DMSO stock solution)
Component B: Live Cell Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (50 mL)

OVERVIEW

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. This kit is optimized for flow cytometers equiped with viloet laser excitation @ 405 nm and emission around 500 nm.

AT A GLANCE

Protocol Summary

1. Prepare cells
2. Add LysoBrite™ VLG26 working solution
3. Incubate at 37°C for 30 minutes to 2 hours
4. Analyze the cells under fluorescence microscope at Ex/Em = 405/480 nm (Violet filter set)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Excitation	405 nm
Emission	480 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Violet filter set

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X LysoBrite™ VLG26 stock solution (Component A) to 10 mL of Live Cell Staining Buffer (Component B) and mix well to make LysoBrite™ VLG26 working solution. Protect from light.

Note: 20 µL of 500X LysoBrite™ VLG26 stock solution (Component A) is enough for one 96-well plate. 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.

SAMPLE EXPERIMENTAL PROTOCOL

For adherent cells:

1. Grow cells either in a 96-well black wall/clear bottom plate (100 µL/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 µL/well/96-well plate) of LysoBrite™ VLG26 working solution.
2. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
3. Observe the cells using a fluorescence microscope with 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.

For suspension cells:

1. Centrifuge the cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
2. Resuspend the cell pellet gently in pre-warmed growth medium, and then add equal volume of LysoBrite™ VLG26 working solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 30 minutes to 2 hours.
4. Observe the cells using a fluorescence microscope with 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. Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.

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EXAMPLE DATA ANALYSIS AND FIGURES

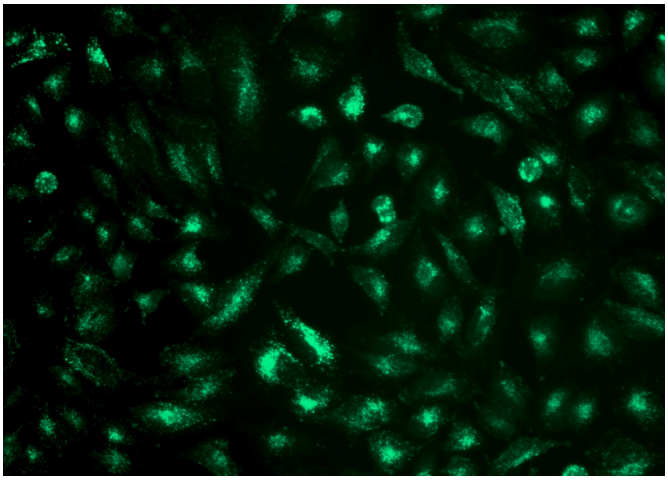


Figure 1. Image of U2OS cells stained with Cell Navigator® Lysosomal Staining Kit in a Costar black wall/clear bottom 96-well plate.

DISCLAIMER

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