ab112136

CytoPainter Lysosomal Staining Kit - Green Fluorescence

Instructions for Use

For staining Lysosomes in suspension and adherent cells by using our proprietary green fluorescence probe

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

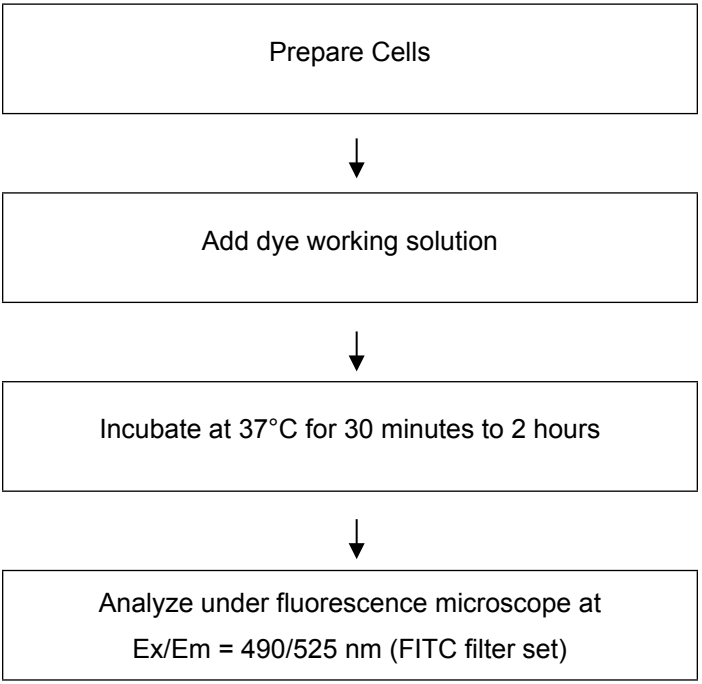
Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The 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 from the cytosol across the membrane via proton pumps and chloride ion channels.

Abcam 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.

ab112136 is designed to label lysosomes of live cells in green fluorescence at Ex/Em = 490/525 nm. The proprietary lysotropic dye used in the kit 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 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. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells.

2. Protocol Summary



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: LysoGreen Indicator	100 µL
Component B: Live Cell Staining Buffer	50 mL

4. Storage and Handling

Keep at -20°C. Protect from light.

5. Assay Protocol

A. Prepare Lysosomal Staining Solution

1. Warm LysoGreen Indicator (Component A) to room temperature.

2. Prepare dye working solution by diluting 20 µL of LysoGreen Indicator (Component A) to 10 mL of Live Cell Staining Buffer (Component B).

   Note 1: 20 µL of LysoGreen Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused LysoGreen Indicator (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.

B. Prepare and Stain Cells

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 A.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. 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 A.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 and stained as adherent cells (see Step B.1).

6. Data Analysis

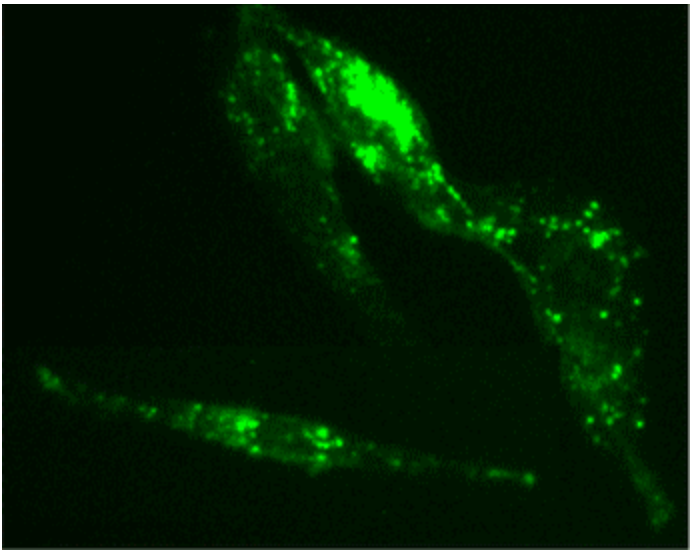


Figure 1. Image of U2OS cells stained with ab112136 in a black 96-well plate

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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