ab112135

CytoPainter Lysosomal Staining Kit - Blue Fluorescence

Instructions for Use

For staining Lysosomes in suspension and adherent cells by using our proprietary blue 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 (H^+ ions) from the cytosol across the membrane via proton pumps and chloride ion channels.

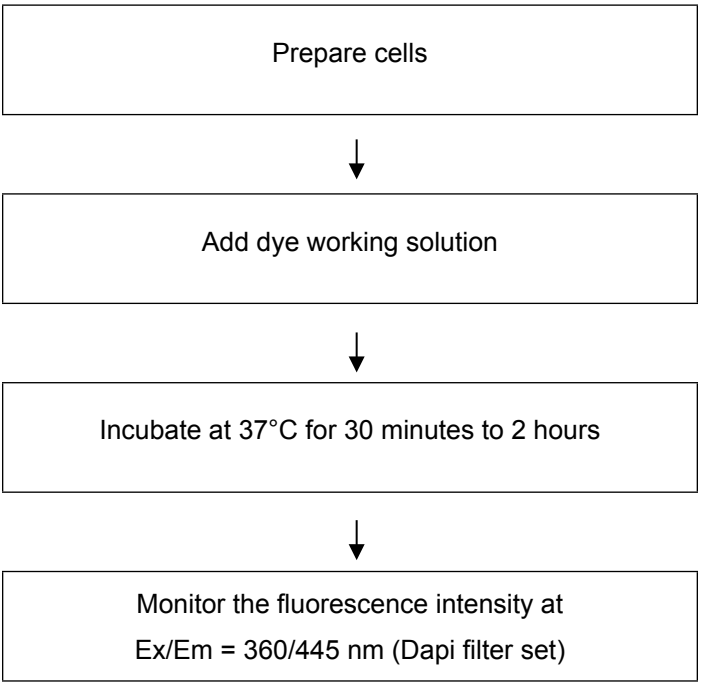
Abcam fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

ab112135 is designed to label lysosomes of live cells in blue fluorescence. ab112135 uses a proprietary lysotropic dye that selectively accumulates in lysosomes probably via the lysosome pH gradient. The stain has Ex/Em = 350/440 nm. 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 significantly enhanced upon entering lysosomes. This key feature significantly reduces its staining background. The

labeling protocol is robust, requiring minimal hands-on time. ab112135 can be readily adapted for many different types of fluorescence platforms such as microplate assays, flow cytometry and fluorescence microscope. It is useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. ab112135 provides all the essential components with an optimized cell-labeling protocol and can be used for both proliferating and non-proliferating cells (either suspension or adherent cells).

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

Components	Amount
Component A: LysoBlue Indicator (500x DMSO solution)	100 µL
Component B: Live Cell Staining Buffer	50 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Lysosomal Staining Solution

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

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

   Note 1: 20 µL of LysoBlue Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused LysoBlue 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 black wall/clear bottom 96-well 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 Dapi 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 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 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 Dapi 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

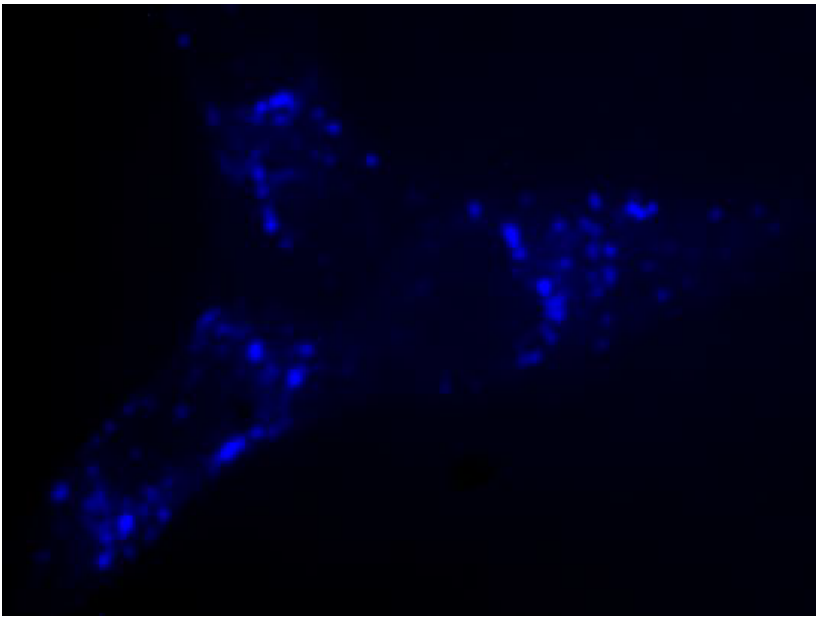


Figure 1. Image of U2OS cells stained with the ab112135 in a black 96-well plate

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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