ab112137

CytoPainter Lysosomal Staining Kit - Red Fluorescence

Instructions for Use

For staining Lysosomes in suspension and adherent cells using our proprietary red 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.

ab112137 is designed to label lysosomes of live cells in red fluorescence. The kit uses a proprietary lysotropic dye that selectively accumulates in lysosomes probably via the lysosome pH gradient. The red lysosomal stain used in the kit has Ex/Em = 575/600 nm. The lysotropic indicator is a hydrophobic compound that easily permeates intact live cells, and becomes trapped inside lysosomes after it gets into cells. Its fluorescence is strongly enhanced upon entering lysosomes. This key feature significantly

reduces its staining background. The labeling protocol is robust, requiring minimal hands-on time. The Red dye used in the kit has extremely high photostability as well as excellent cellular retention 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.

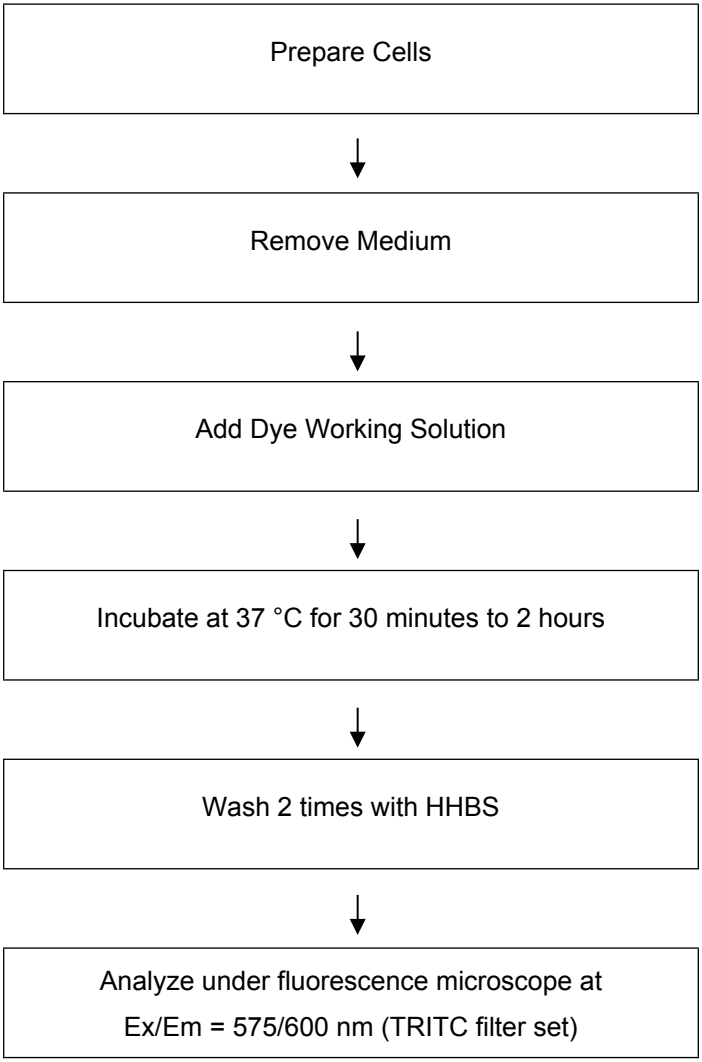
Key Features:

Increased signal intensity:
10 times brighter.

Extraordinarily high photostability:
No fading observed after 2 minutes exposure.

Excellent cellular retention:
More than 5 passages for cell tracking in Hela cells

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: LysoRed Indicator (500X DMSO Stock)	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 Lysosome Staining Solution

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

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

   Note 1: 20 µL of LysoRed Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused LysoRed 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, remove the medium from the dish and add 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. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice, fill the cell wells with HBSS. Observe the cells using a fluorescence microscope fitted with a TRITC 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: Add equal volume of dye-working solution (from Step A.2) into the cells. Incubate the cells in a 37 °C, 5% CO_2 incubator for 30 minutes. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HBSS) or buffer of your choice, fill the cell wells with HBSS or growth medium. Observe the cells

using a fluorescence microscope equipped with a TRITC 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

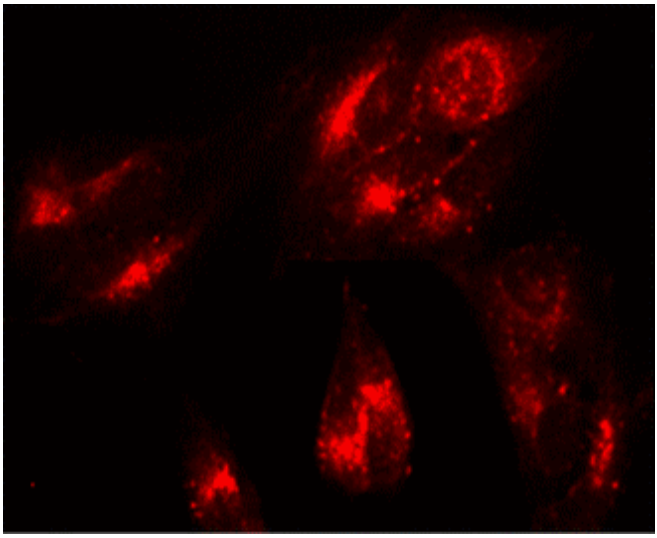


Figure 1. Image of U2OS cells stained with the ab112137 in a black 96-well plate

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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