ab138895

CytoPainter Lysosomal Staining Kit – Orange Fluorescence

Instructions for Use

For staining Lysosomes in live cells using our proprietary orange fluorescence probe

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

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1. Introduction

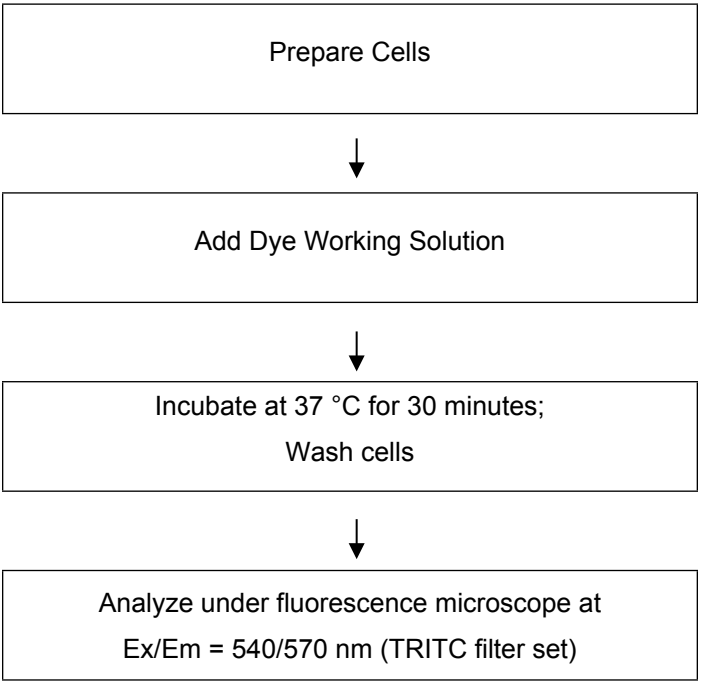
Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The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

ab128895 is designed to label lysosomes of live cells in orange fluorescence at Ex/Em = 540/560 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. The LysoOrange Indicator

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.

2. Protocol Summary



3. Kit Contents

Components	Amount
Component A: LysoOrange Indicator (500X DMSO Stock)	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

A. Prepare Lysosome Staining Solution

1. Warm all the components to room temperature.

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

   Note 1: 20 µL of 500X LysoOrange Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused 500X LysoOrange Indicator at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

   Note 2: The optimal concentration of the fluorescent lysosomal 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 (e.g. 100 µL for a 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. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells by using a fluorescence microscope fitted with a TRITC filter set.

   Note: It's 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 pellets gently in pre-warmed (37 °C) growth medium, and 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. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice (e.g. the buffer with growth medium at 1:1 concentration). Observe the cells by using a fluorescence microscope fitted 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

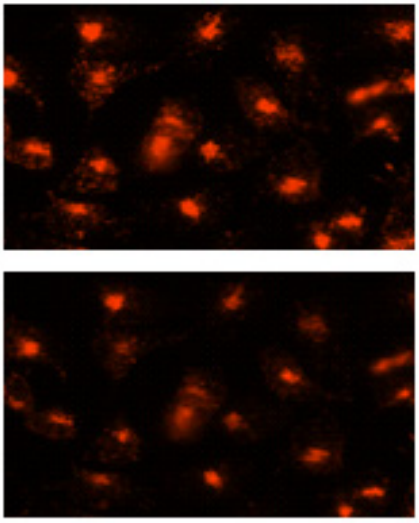


Figure 1. Image of HeLa cells stained with ab138895 in a black 96-well plate. The TRTIC signals were compared at 0 seconds (upper image) and 120 seconds (lower image) exposure time by using a fluorescence microscope.

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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