ab138896

CytoPainter Lysosomal Staining Kit – Deep Red Fluorescence

Instructions for Use

For staining Lysosomes in live cells using our proprietary deep red fluorescence probe

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

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1. Introduction

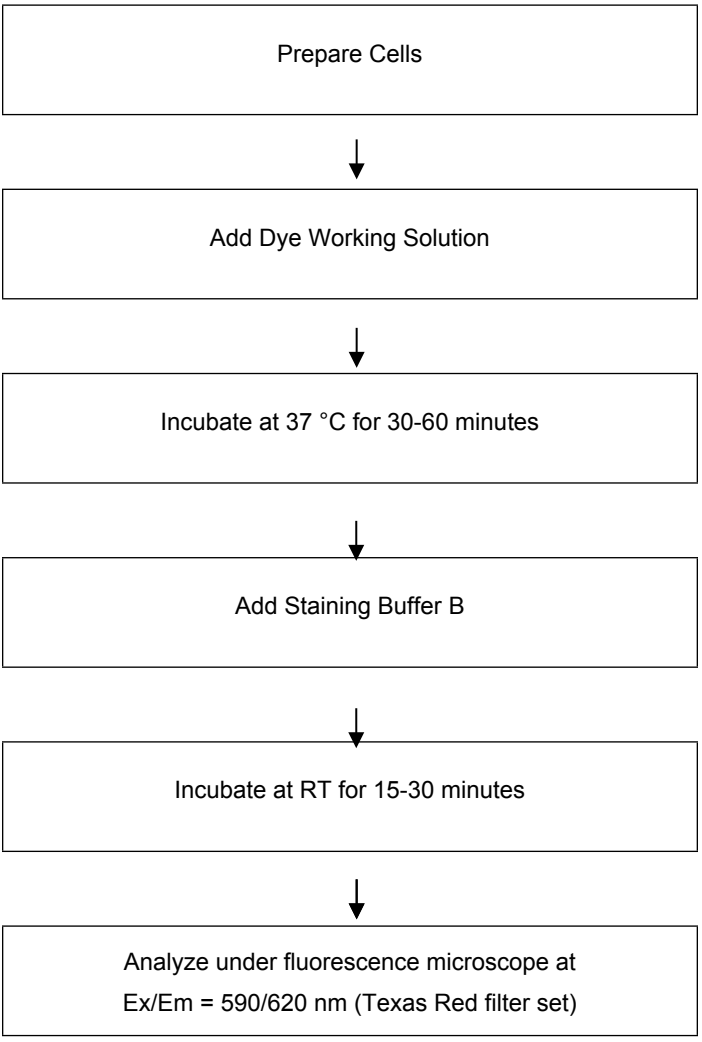
Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The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

ab128896 is designed to label lysosomes of live cells in deep red fluorescence at Ex/Em = 590/620 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 LysoDeep Red 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: LysoDeep Red Indicator	50 µL (500X DMSO Stock)
Component B: Staining Buffer A	25 mL
Component C: Staining Buffer B	25 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 10 µL of LysoDeep Red Indicator (Component A) into 5 mL of Staining Buffer A (Component B).

   Note 1: 10 µL of 500X LysoDeep Red Indicator (Component A) is enough for one 96-well plate. Aliquot and store unused 500X LysoDeep Red 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 1/2 volume (such as 50 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 30-60 minutes. Add 50 µL/well (96-well plate) of Assay Buffer B (Component C) into the dye-loading plate, incubate at room temperature for 15-30 minutes. Observe the cells by using a fluorescence microscope fitted with a Texas Red filter set.

   Note 1: The LysoDeep Red Indicator has minimum or no cell toxicity; it can be used for cell tracking. For cell tracking purpose simply skip the addition of Assay Buffer B and replace the dye-working solution to growth medium, and then observe under a fluorescence microscope

   Note 2: 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, add 1/2 volume (50 µL/tube) of the dye-working solution (from Step A.2). Incubate the cells in a 37°C, 5% CO_2 incubator for 30-60 minutes. Add 1/3 volume (50 µL/tube) of Assay Buffer B (Component C) into the dye-loading cells, incubate at room temperature for 15-30 minutes. Observe the cells using a fluorescence microscope fitted with a Texas red filter set (Ex/Em = 590/620 nm).

   Note 1: LysoDeep Red Indicator has minimum or no cell toxicity; it can be used for cell tracking. For cell tracking purpose simply skip the addition of Assay Buffer B and replace the dye-working solution to growth medium, and then observe under a fluorescence microscope.

   Note 2: 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 3: Suspension cells may be attached to cover-slips and stained as adherent cells (see Step B.1).

6. Data Analysis

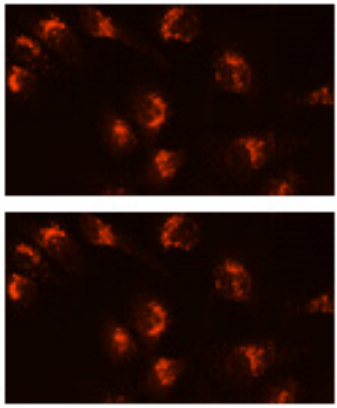


Figure 1. Image of HeLa cells stained ab138896 in a black 96-well plate. The Texas Red signals were compared at 0 (upper image) and 120 seconds (lower image) exposure time by using a fluorescence microscope.

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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