ab176829 Lysosomal Staining Reagent - Deep Red - Cytopainter

Instructions for Use

For staining lysosomes in live cells with our proprietary Deep Red probe.

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

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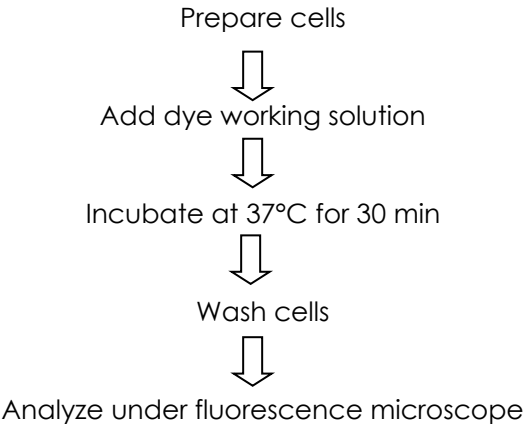
1. Introduction

Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waster 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 the pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels.

Abcam's CytoPainter LysoDeep Red Indicator Reagent (ab176829) is part of a series of new fluorogenic probes to label lysosomes of live cells. The proprietary lysotropic dye selectively accumulates in lysosomes probably via the lysosome pH gradient. The lysotropic indicator is a hydrophobic compound that easily permeates intact live cells, and is trapped in lysosomes after it gets into the cells. Its fluorescence is significantly enhanced upon entering lysosomes. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. CytoPainter LysoDeep Red Indicator Reagent (ab176829) has extremely high photostability as well as excellent cellular retention making 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.

CytoPainter LysoDeep Red Indicator Reagent can be detected at Ex/Em = 596/619 nm.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
LysoDeep Red Indicator (500X DMSO solution)	500 tests

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light. Reagent is stable for at least 6 months if stored properly. Avoid repeated freeze/ thaw cycles.

Store reagent in 20 µL aliquots. Each aliquot is enough to stain 1x96-well plate.

5. Materials Required, Not Supplied

- HHBS Buffer (Hanks and 20 mM HEPES buffer) pH=7
- Pipettes and pipette tips
- Coverslips, petri dishes or well plates to grow cells

6. Assay Protocol

1. Reagent Preparation:

a) Warm LysoDeep Red Indicator to room temperature.

b) For a 1 x 96-well plate assay, prepare dye working solution by diluting 20 µL LysoDeep Red Reagent in 10 mL of HHBS buffer.

Note: *The optional concentration of the fluorescent lysosome indicator may vary depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability if the cells or tissues to the probe.*

2. Sample Staining and Analysis:

2.1 Adherent cells:

a) Grow cells either in a 96-well back wall/clear bottom plate (100 µL/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture media.

b) When cells reach the desired confluence, add equal volume of the dye-working solution (Step 1b).

c) Incubate the cells in a 37°C, 5% CO2 incubator for 30 min.

d) Wash the cells twice with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice.

e) Fill the cell wells with HHBS or growth medium.

f) Observe cells using a fluorescence microscope or FACS machine equipped with a desired 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.*

2.2 Suspension cells:

a) Add equal volume of the dye-working solution (from Step 1b).
b) Incubate the cells in a 37 °C, 5% CO2 incubator for 30 minutes.
c) Wash the cells with pre-warmed (37°C) Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice.
d) Fill the cell wells with HHBS or growth medium.
e) Observe cells using a fluorescence microscope machine equipped with a desired 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 that have been treated, and stained as adherent cells (see Step 2.1).

7. Data Analysis

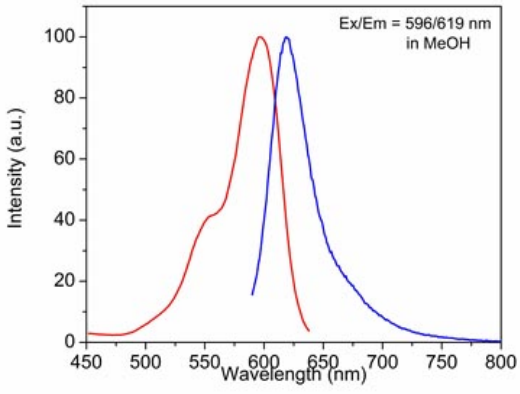


Figure 1. Spectrum of CytoPainter LysoDeep Red Indicator Reagent (ab176829).

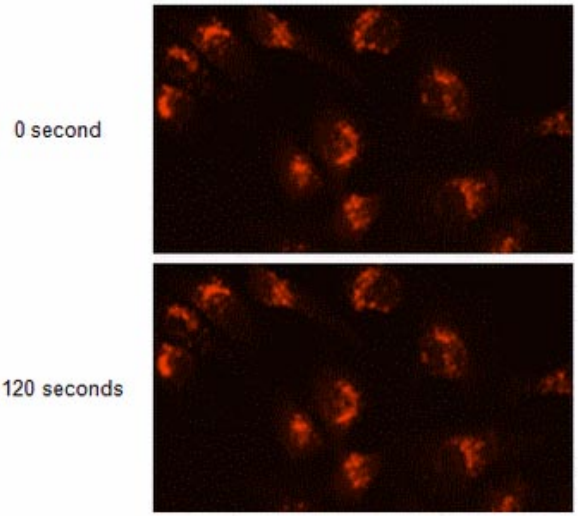


Figure 2. Image of HeLa cells stained with CytoPainter LysoDeep Red Indicator Reagent (ab176829) in a Costar black 96-well plate. The TRTIC signals were compared at 0 and 120 seconds exposure time by using an Olympus fluorescence microscope.

8. Troubleshooting

Problem	Reason	Solution
Lysosomes not sufficiently stained.	Too low dye concentration or incubation time insufficient	Increase concentration or incubation time
	Cells observed at incorrect wavelength	Ensure you are using appropriate filter settings
Cells do not appear healthy	Cells require serum to remain healthy	Add serum to stain and wash solutions. Try range 2 – 10% serum.
Nuclear counterstain is too bright	Different microscopes, cameras and filters may make some signals appear very bright	Reduce concentration of nuclear counterstain or shorten exposure time.

9. Technical Contacts

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)