

ab176746

**CytoPainter Lysosomal
Staining Kit - NIR
Fluorescence**

Instructions for Use

For staining lysosomes in live cells with our proprietary NIR 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 the pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels.

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

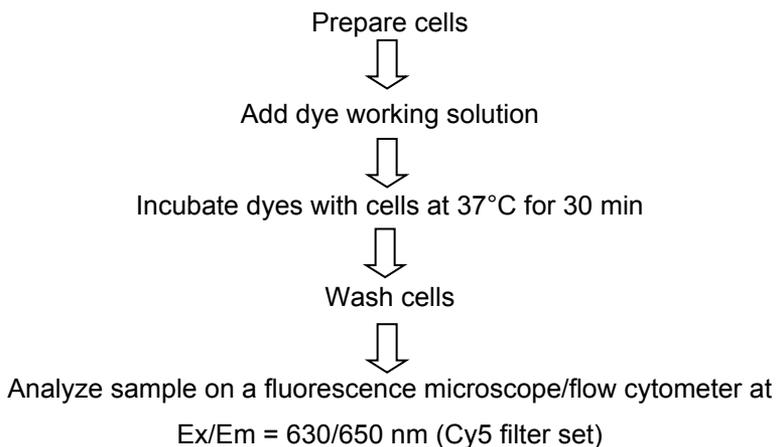
Abcam's CytoPainter Lysosomal Staining Kit – NIR Fluorescence (ab176746) is designed to label lysosomes of live cells in near infrared red fluorescence at Ex/Em = 630/650nm. The proprietary lysotropic dye used in the kit selectively accumulates in lysosomes probably via the lysosome pH gradient. The 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 LysoNIR

Indicator dye used in the kit 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.

Kit Key Features

- **Increased signal intensity** – 5 times brighter than the other common lysosomal dyes available in the market.
- **Good photostability** – much better photostability compared to other common lysosomal dyes available in the market.
- **Excellent cellular retention** – more than 4 passages for cell tracking in HeLa cells.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
LysoNIR Indicator Indicator (500X DMSO solution)	100 μ L
Live Cell Staining Buffer	50 mL

4. Storage and Stability

Upon receipt, store kit at -20°C. Avoid exposure to light.

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

LysoNIR Indicator: aliquot and store at -20°C. Protect from light and avoid repeated freeze/ thaw cycles.

5. Materials Required, Not Supplied

- MilliQ water or other type of double distilled water (ddH₂O)
- HHBS Buffer (Hanks and 20 mM HEPES buffer) pH=7
- Microcentrifuge
- Pipettes and pipette tips
- Coverslips, petri dishes or well plates to grow cells

6. Assay Protocol

1. Reagent Preparation:

- a) Warm LysoNIR Indicator to room temperature.
- b) For a 1 x 96-well plate assay, prepare dye working solution by diluting 20 μ L LysoNIR Indicator in 10 mL of Live Cell Staining Buffer.

NOTE1 : 20 μ L of LysoNIR Indicator is enough for one 96-well plate. Aliquot and store unused LysoNIR Indicator at < -20 °C. Protect it 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.

2. Sample Staining and Analysis:

2.1 Adherent cells:

- a) Grow cells either in a 96-well back wall/clear bottom plate (100 μ L/well/96-well plate) or on cover-slips inside a petri dish filled with the appropriate culture media.
- b) Optional: If needed for your experiment, treat cells with test compounds for a desired period of time.

- c) When cells reach the desired confluence, add equal volume of the dye-working solution (Step 1b).
- d) Incubate the cells in a 37°C/ 5% CO₂ incubator for 30 min – 2 hours.
- e) Wash cells twice with pre-warmed HBSS buffer at 37°C.
- f) Fill the cell wells with HBSS or growth medium.
- g) Observe cells using a fluorescence microscope fitted with a Cy5 filter set (Ex/Em = 630 – 650 nm).

2.2 Suspension cells:

- a) Grow cells to desired number/ cell density.
- b) Optional: If needed for your experiment, treat cells with test compounds for a desired period of time.
- c) Add equal volume of the dye-working solution (Step 1b).
- d) Incubate the cells in a 37°C/ 5% CO₂ incubator for 30 min – 2 hours.
- e) Wash cells twice with pre-warmed HBSS buffer at 37°C.
- f) Fill the cell wells with HBSS or growth medium.
- g) Observe cells using a fluorescence microscope fitted with a Cy5 filter set (Ex/Em = 630 – 650 nm).

7. Data Analysis

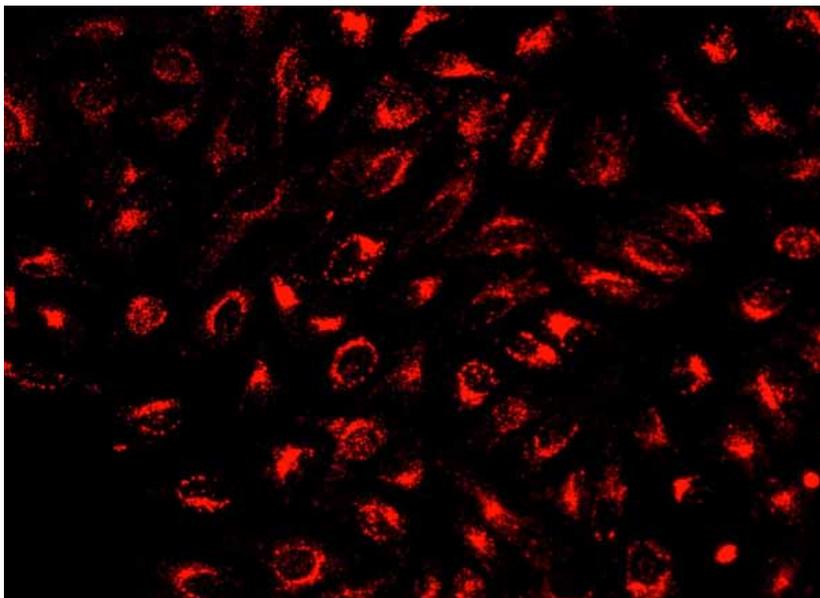


Figure 1. Image of HeLa cells stained with Abcam's CytoPainter Lysosomal Staining Kit – NIR Fluorescence (ab176746) in a Costar black 96-well.

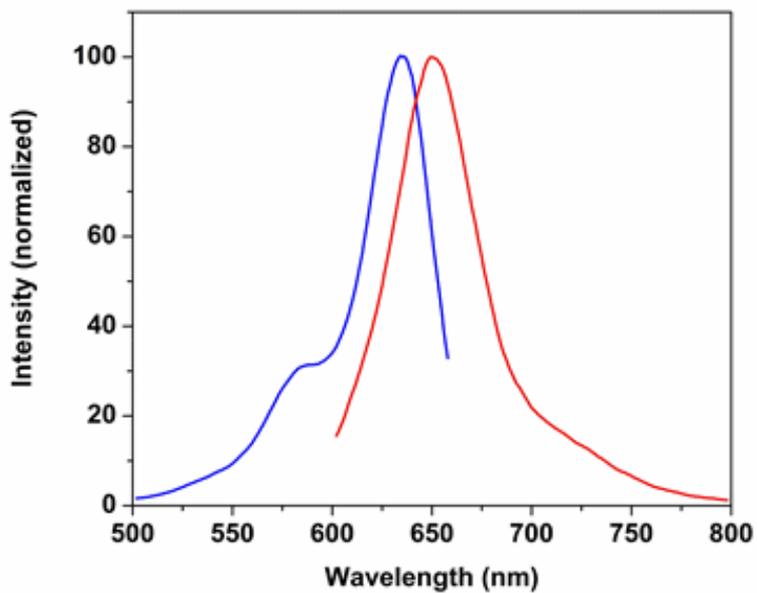


Figure 2. Spectrum for ab176746.

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.



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