Product Manual

LYSO-ID® Red Detection Kit (GFP CERTIFIED®)

Catalog #: ENZ-51005

ENZ-51005-500

500 assays

ENZ-51005-0100

100 assays

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

The LYSO-ID® Red Detection Kit (GFP CERTIFIED®) contains a novel acidic organelle-selective dye suitable for live-cell staining. Conventional fluorescent stains for acidic organelles, such as Acridine Orange (Catalog No. 52405), form metachromatic artifacts that interfere with multicolor imaging applications. LYSO-ID® Red dye generates emission profiles that can be multiplexed with other fluorophores. The dye accumulates in acidic compartments, such as endosomes, lysosomes, and secretory vesicles. Low micromolar concentrations of LYSO-ID® Red dye are sufficient for staining mammalian cells. This has been validated with a human cervical carcinoma cell line, HeLa, a human T-lymphocyte cell line, Jurkat, and human bone osteosarcoma epithelial cell line, U2OS.

One important application of LYSO-ID® Red dye is in fluorescence co-localization imaging with green fluorescent protein (GFP)-tagged proteins. This is a powerful approach for determining the targeting of molecules to intracellular compartments, and for screening of associations and interactions between these molecules. For example, dual-color fluorescence detection may be employed for the identification of vesicular compartments and the investigation of their dynamics and fusion during exocytosis. However to date, photoconversion of red fluorescent dyes to green has been problematic as observed with Lysotracker® Red DND-99 dye. In addition, metachromatic artifacts wherein fluorescent dyes emit both in the red and green regions of the spectrum are evident, as observed with Acridine Orange dye. These observations have led to spurious results in GFP co-localization experiments.[1,2] Additionally, many organelle-targeting probes photobleach rapidly, are subject to quenching when concentrated in organelles, are highly toxic, or only transiently associate with the target organelle, requiring imaging within a minute or two of dye addition.[3,4]

The LYSO-ID® Red dye, a new red-emitting, cell-permeable small organic probe molecule that spontaneously localizes to live cell acidic organelles, was developed to overcome the above problems. The LYSO-ID® Red dye can be readily used in combination with other common UV and visible light excitable fluorescent dyes and various fluorescent proteins in multicolor imaging and detection applications. The dye is suitable for both short-term and long-term tracking studies.

LYSO-ID® Red dye emits in the Texas Red region of the visible light spectrum, and is highly resistant to photobleaching, concentration quenching and photoconversion.

The LYSO-ID® Red Lysosomal Detection Kit (GFP-CERTIFIED®) is specifically designed for use with GFP-expressing cell lines, as well as cells expressing blue, cyan or yellow fluorescent proteins (BFPs, CFPs, YFPs). A lysosome perturbation agent, chloroquine, is provided as a positive control for monitoring changes in lysosome number and volume. A nuclear counterstain is also provided in the kit to highlight this organelle as well.

REAGENTS PROVIDED AND STORAGE

All reagents are shipped on dry ice. Upon receipt, the kit should be stored at ≤-20°C, protected from light. When stored properly, these reagents are stable for at least twelve months. Avoid repeated freezing and thawing.

Reagents provided in the ENZ-51005-500 kit are sufficient for approximately 500 assays using either live, adherent cells or cells in suspension.

Reagents provided in the ENZ-51005-100 kit are sufficient for approximately 100 assays using either live, adherent cells or cells in suspension.

Reagent	ENZ-51005-500	ENZ-51005-0100
LYSO-ID® Red Detection Reagent	50 µL	13 µL
Hoechst 33342 Nuclear Stain	50 µL	13 µL
Chloroquine Control	7.5 µmol	7.5 µmol
10X Assay Buffer	15 mL	3 mL

OTHER MATERIALS NEEDED

- Standard fluorescence microscope
- Calibrated, adjustable precision pipetters, preferably with disposable plastic tips
- Adjustable speed centrifuge with swinging buckets (for suspension cultures)
- Glass microscope slides
- Glass cover slips
- Deionized water
- Anhydrous DMSO (optional)
- Growth medium (e.g., Dulbecco's Modified Eagle Medium, D-MEM)

SAFETY WARNINGS AND PRECAUTIONS

Handle with care

- This product is for research use only and is not intended for diagnostic purposes.
- The LYSO-ID® Red Detection Reagent contains DMSO which is readily absorbed through the skin. It is harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Observe appropriate precautions when handling
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

Protect from light

- To avoid photobleaching, perform all manipulations in low light environments or protected from light by other means.

METHODS AND PROCEDURES

***NOTE**: Allow all reagents to warm to room temperature before starting with the procedures. Upon thawing of solutions, gently hand-mix or vortex the reagents prior to use to ensure a homogenous solution. Briefly centrifuge the vials at the time of first use, as well as for all subsequent uses, to gather the contents at the bottom of the tube.*

A. REAGENT PREPARATIONS

1. **Positive Control**

   Chloroquine is a lysosomotropic agent. It accumulates preferentially in the lysosomes of cells.[5] The pKa for the quinoline nitrogen of chloroquine is approximately 8.5. At physiological pH, it is roughly 10% deprotonated as calculated using the Henderson-Hasselbalch equation. This decreases to roughly 0.2% at a lysosomal pH of 4.6. Since the deprotonated form of the compound is more membrane permeable than the protonated form, chloroquine becomes quantitatively "trapped" in lysosomes.

   The chloroquine provided in the kit may be used as a positive control for increasing lysosome number and volume. It is supplied lyophilized (7.5 µmol) and should be centrifuged briefly to gather the material at the bottom of the tube. Reconstitute the lyophilized material in 125 µL deionized water for a 60 mM stock solution. It is recommended that treatment with the agent be performed using 10-300 µM final concentration in order to observe changes in lysosomal morphology. Unused stock chloroquine may be stored in small aliquots at -20°C for several weeks.

2. **1X Wash Buffer**

   Allow the 10X Assay Buffer to warm to room temperature. Make sure that the reagent is free of any crystallization before dilution. Prepare enough 1X Assay Buffer for the number of samples to be assayed by diluting 1 mL of the 10X Assay Buffer with 9 mL of deionized water.

3. **Dual Detection Reagent**

   The concentration of LYSO-ID® Red dye for optimal staining will vary depending upon the application. Suggestions are provided to use as guidelines, though some modifications may be required depending upon the particular cell type employed and other factors such as the permeability of the dye to the cells or tissues. To reduce potential artifacts from overloading of the cells, the concentration of the dye should be kept as low as possible.

   Prepare sufficient amount of Dual Detection Reagent for the number of samples to be assayed as follows: To each mL of 1X Assay Buffer (see preparation in step A-2) or cell culture medium, add 1 µL of LYSO-ID® Red Detection Reagent and 1 µL of Hoechst 33342 Nuclear Stain. Serum may be included, if preferred.

B. CELL PREPARATIONS

Cells should be maintained via standard tissue culture practices. Positive control cells should be pretreated with the chloroquine control for 2-8 hours. Response to chloroquine is time and concentration dependent and may also vary significantly depending upon cell type and cell line. Negative control cells should be treated with a vehicle (DMSO, media or other solvent used to reconstitute or dilute an inducer or inhibitor) for an equal length of time under similar conditions.

C. STAINING LIVE, ADHERENT CELLS

1. Grow cells on cover slips inside a Petri dish filled with the appropriate culture medium. When the cells have reached the desired level of confluence, carefully remove the medium.
2. Dispense sufficient volume of Dual Detection Reagent (see section A-3) to cover the monolayer cells (~100 µL of labeling solution for cells grown on an 18 X 18 mm coverslip).
3. Protect samples from light and incubate for 15-30 minutes at 37°C.
4. Wash the cells with 100 µL 1X Assay Buffer. Remove excess buffer and place coverslip on slide.

5. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard Rhodamine or Texas Red filter set for imaging the lysosomes. Optionally, image the nucleus using a DAPI filter set and the GFP-tagged protein using a GFP/FITC filter set.

D. STAINING LIVE CELLS GROWN IN SUSPENSION

1. Centrifuge cells for 5 minutes at 400 x g at room temperature (RT) to obtain a cell pellet.
2. Carefully remove the supernatant by aspiration and dispense sufficient volume of Dual Detection Reagent (see section A-3) to cover the dispersed cell pellet (~10^6 cells/mL).
3. Protect samples from light and incubate for 15 to 30 minutes at 37°C.
4. Wash the cells with 100 µL 1X Assay Buffer. Remove excess buffer. Resuspend cells in 100 µL 1X Assay Buffer, then apply the cells to a glass slide and overlay with a coverslip.
5. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard Rhodamine or Texas Red filter set for imaging the lysosomes. Optionally, image the nucleus using a DAPI filter set and the GFP-tagged protein using a GFP/FITC filter set.

APPENDICES

FILTER SET SELECTION

The selection of optimal filter sets for a fluorescence microscopy application requires matching the optical filter specifications to the spectral characteristics of the dyes employed in the analysis. Consult the microscope or filter set manufacturer for assistance in selecting optimal filter sets for your microscope.

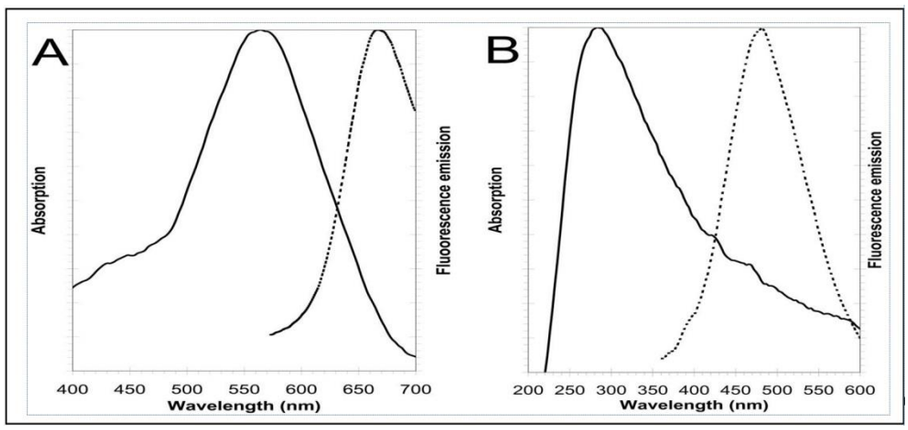

Figure 1. Absorption and fluorescence emission spectra for LYSO-ID® Red (A) and Hoechst 33342 (B) dyes. All spectra were determined in 1X Assay Buffer.

RESULTS

Lysosomes are membrane-bound organelles involved in the degradation of macromolecules and pathogens in diverse processes including endocytosis, phagocytosis and autophagy. Lysosomal morphology varies with the state of the cell and its degree of degradative activity, and the vesicles can have pieces of membranes, vacuoles, granules and even parts of mitochondria within them. They are typically spherical vesicles, ranging in size from 0.2 to 2 µm in diameter. The lumen of lysosomes and other acidic organelles is characterized by low pH generated via proton-pumping vacuolar ATPases. LYSO-ID® Red dye is selectively sequestered in acidic organelles by a mechanism that likely involves protonation and retention within the membranes of the organelles. However, staining is even feasible in cells pretreated with weakly basic cell-permeant compounds, such as chloroquine, and the fluorescence emission of the dye itself is largely independent of pH. HeLa and U2OS cells treated with 300 µM chloroquine for 4 hours show a dramatic increase in lysosome-like vesicle number and volume, confirming LYSO-ID® Red dye is associated with this subcellular

compartment. Thus, LYSO-ID® Red stain can be employed to highlight lysosome-like organelles under certain conditions, such as chloroquine drug treatment, wherein cells produce lysosome-like bodies that contain most of the degradative enzymes of the lysosome, but are not as acidic as this organelle.

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6. A.S. Kwok, et al. (2011) “HspB8 Mutation Causing Hereditary Distal Motor Neuropathy Impairs Lysosomal Delivery of Autophagosomes” J. Neurochem. 119:1155.
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TROUBLESHOOTING GUIDE

Problem	Potential Cause	Suggestion
Acidic organelles are not sufficiently stained.	Very low concentration of LYSO-ID® Red dye was used or dye was incubated with the cells for an insufficient length of time.	Either increase the labeling concentration or increase the time allowed for the dye to accumulate in the lysosome once the cells have been transferred to fresh medium.
The fluorescence of LYSO-ID® Red dye appears to spread laterally out of the acidic organelles and dissipate within the cell during prolonged observation by fluorescence microscopy.	Too intense incident illumination is being employed with the dye. Other commercial dyes will rapidly photobleach under similar conditions, but LYSO-ID® Red dye is very photostable.	Insert neutral density filters into the optical illumination path or reduce duration of exposure to the incident light.
LYSO-ID® Red dye fails to stain acidic organelles in fixed and/or permeabilized cells.	The dye is only suitable for live-cell staining.	Dye has been shown to work in live-cell staining and subsequent fixing.[6,7]
Precipitate is seen in the 10X Assay Buffer.	Precipitate forms at low temperatures.	Allow solution to warm to room temperature or 37°C, then vortex to dissolve all precipitate.
Blue nuclear counterstain is too bright compared to the red lysosomal stain.	Different microscopes, cameras and filters may make some signals appear very bright.	Reduce the concentration of the nuclear counterstain or shorten the exposure time.
Cells do not appear healthy.	Some cells require serum to remain healthy.	Add serum to stain and wash solutions. Serum does not affect staining. Normal amounts of serum added range from 2% to 10%.
Chloroquine treated cells apper dead or are no longer attached to the surface.	The EC_{50} of chloroquine may be different with different cell lines.	Try lowering the dose of Chloroquine, or shortening the time of exposure.

NOTES

NOTES

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