pHLys Red
- Lysosomal Acidic pH Detection

Technical Manual

General Information

The lysosome is an organelle in which an acid vacuole is formed by a biomembrane. Lysosomes contain various degrading enzymes and contribute to maintaining intracellular homeostasis by acting as a waste disposal system. Recent findings reveal that lysosomal dysfunction is related to some neurodegenerative disorders. Consequently, the investigation of lysosomal function is attracting considerable interest in the scientific community.
pHLys Red accumulates in the intact lysosome, and its fluorescence intensity is enhanced as the acidity increases. On the other hand, weak fluorescence is observed when lysosomes are neutralized due to the lysosomal disfunction. Please also choose Lysosomal Acidic pH Detection Kit (Dojindo code: L266), which includes lysosome staining dye, LysoPrime Green (pH-independent). Lysosomal pH and lysosomal mass can be measured by combining these two dyes that exhibit pH-independent (pHlys Red) and pH-dependent (LysoPrime Green) in lysosomes.

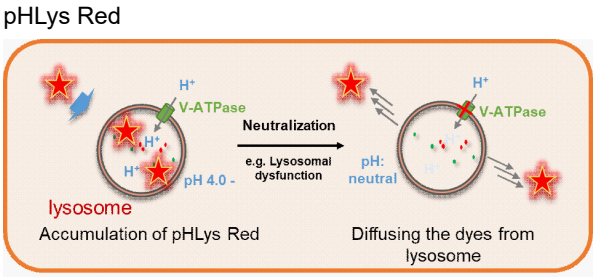
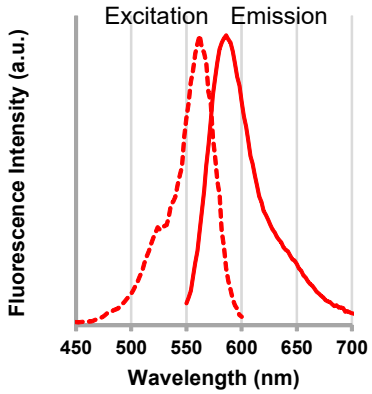


Fig 1. pHLys Red showing lysosomal pH-dependent accumulation

Fluorescent Property

Excitation and emission spectra of pHLys Red



λ_{ex}: 562 nm
λ_{em}: 586 nm

< Recommended filter settings >
Ex : 561 nm, Em : 560 – 650 nm

Contents

pHLys Red	x 1 (corresponds to 10 dishes (35 mm))
	x 3 (corresponds to 30 dishes (35 mm))

*pHLys Red in the tube may be barely visible due to the small amount. Please handle it carefully.

Storage Condition

Store in a cool and dark place.

Required Equipment and Materials

- Growth medium
- Hanks' Balanced Salt Solution (HBSS)
- Micropipettes
- Microtubes

Preparation of Solution

Preparation of pHLys Red DMSO stock solution

Add 20 µl of DMSO to the provided tube containing pHLys Red, and dissolve by vortex mixer.

*Store the reconstituted pHLys Red DMSO stock solution at −20°C until use.
The solution is stable at −20°C for 1 month.

Preparation of pHLys Red working solution

Dilute the pHLys Red DMSO stock solution 1,000 times with medium or HBSS to prepare pHLys Red working solution.

*The final concentration of pHLys Red should be optimized depending on the cell line (dilution range: 250 – 1,000 times).
*pHLys Red working solution should be used on the day it is prepared.

General Protocol

1. Seed cells in a dish and culture them overnight at 37 °C in an incubator equilibrated with 95% air and 5% CO_2.
2. Discard the culture medium and wash the cells twice with a growth medium or HBSS.
3. Add pHLys Red working solution to the dish containing the cells and incubate them for 30 minutes at 37 °C in an incubator equilibrated with 95% air and 5% CO_2.
4. Discard the supernatant and wash the cells twice with growth medium or HBSS.
5. Add growth medium to the dish, then observe the stained cells under a fluorescence microscope.

Usage Example

Observation of the lysosomal pH change by confocal fluorescence microscope

1. HeLa cells were seeded (1.0×10^4 cells/well) on a µ-slide 8 well plate (ibidi) and cultured overnight at 37 °C in an incubator equilibrated with 95% air and 5% CO_2.
2. After washing twice with HBSS, 200 µl of working solution [LysoPrime Green (Code: L261): 2,000 times dilution] or [LysoPrime Deep Red (Code: L264): 1,000 times dilution] and the cells were incubated at 37 °C for 30 min.
3. The supernatant was discard, and the cells were washed twice with HBSS.
4. Two hundred (200) µl of pHLys Red working solution (HBSS, 1,000 times dilution) containing Bafilomycin A1 (Baf. A1), an inhibitor of lysosomal acidification, was added to the plate, and the cells were incubated at 37 °C for 30 min.
5. The supernatant was discarded, and the cells were washed twice with HBSS.
6. MEM (200 µl, containing 10% fetal bovine serum) was added to the well, and the stained cells were observed under a confocal fluorescence microscope.

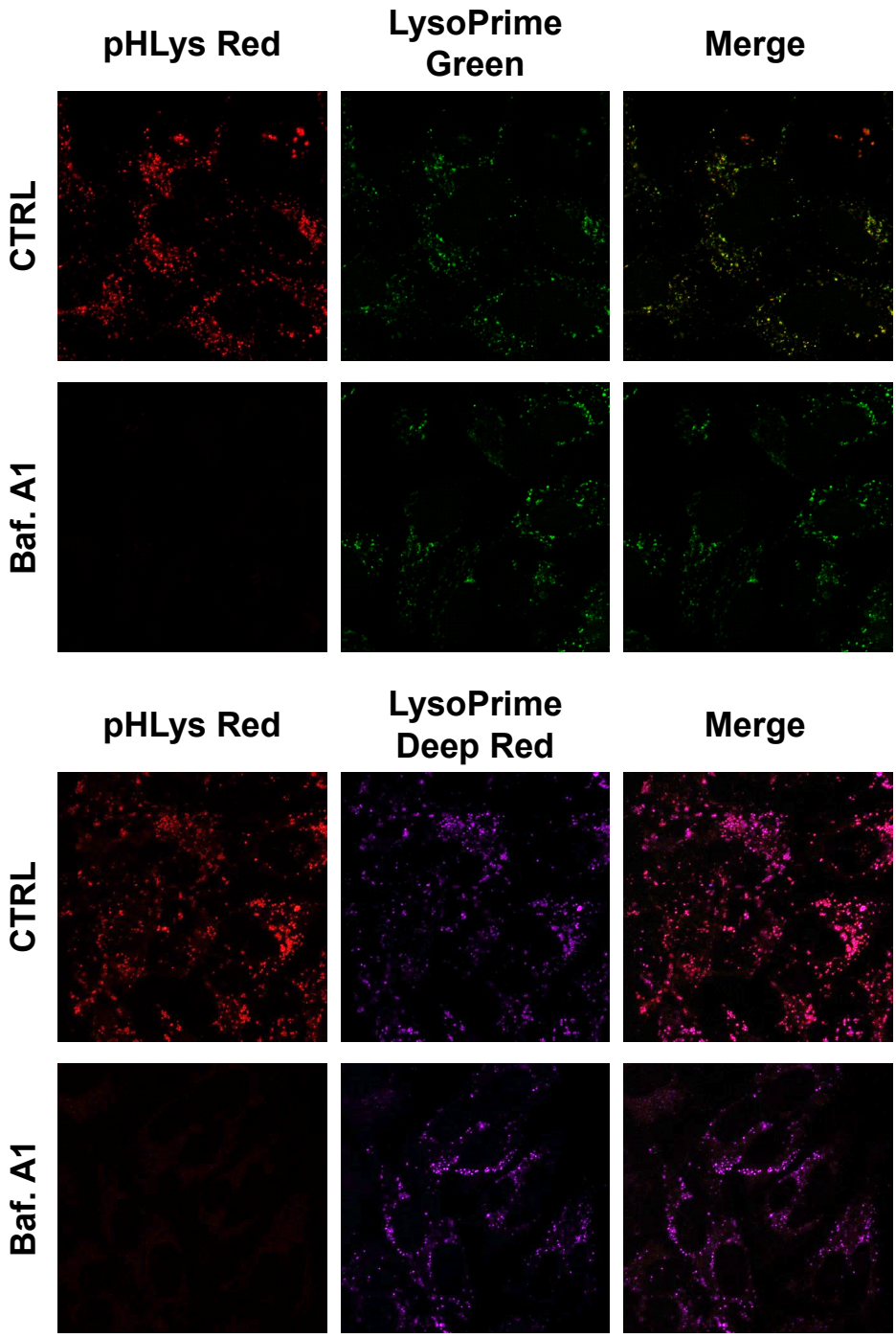


Figure 2. The effect of Bafilomycin A1 on lysosomal pH
CTRL: Normal condition, Baf. A1: Inhibition of lysosomal acidification (50 nmol/l)
pHLys Red filter sets: 561 nm (Ex), 560 – 650 nm (Em)
LysoPrime Green filter sets: 488 nm (Ex), 500 – 570 nm (Em)
LysoPrime Deep Red filter sets: 633 nm (Ex), 640 – 700 nm (Em)

If you need more information, please contact Dojindo technical service.

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