Lysosomal Acidic pH Detection Kit-Green/Deep Red

L268 Product 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.
This kit includes lysosome staining dyes, pHLys Green (pH dependent) and LysoPrime Deep Red (pH-independent). pHLys Green and LysoPrime Deep Red accumulate in the intact lysosomes. The fluorescence intensity of pHLys Green is enhanced as the acidity increases, and weak fluorescence is observed when lysosomes are neutralized due to the lysosomal dysfunction. On the other hand, LysoPrime Deep Red gives stable emissions even lysosomes are neutralized. Lysosomal pH and lysosomal mass can be measured by combining these two dyes.

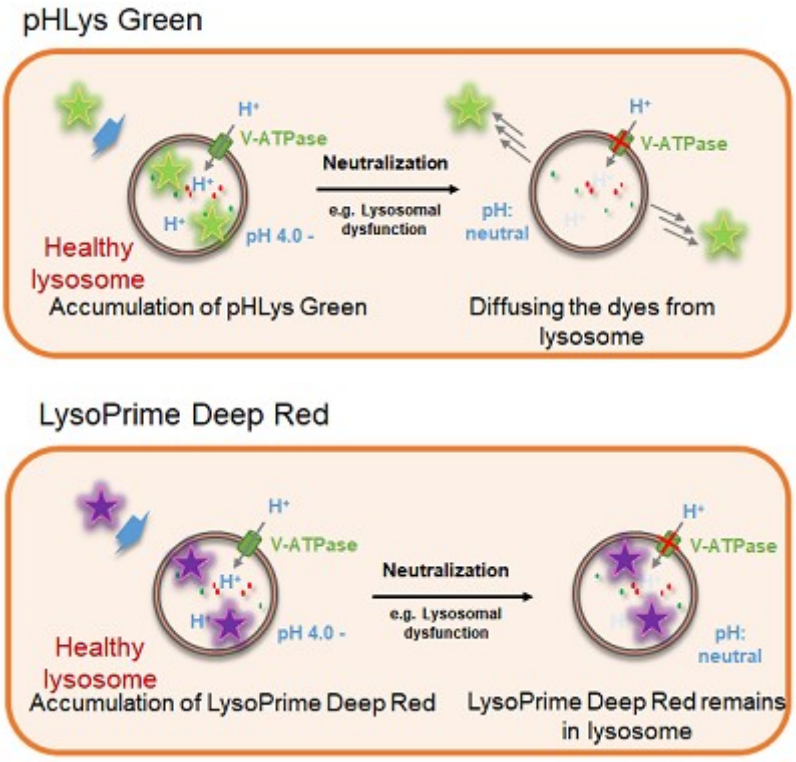
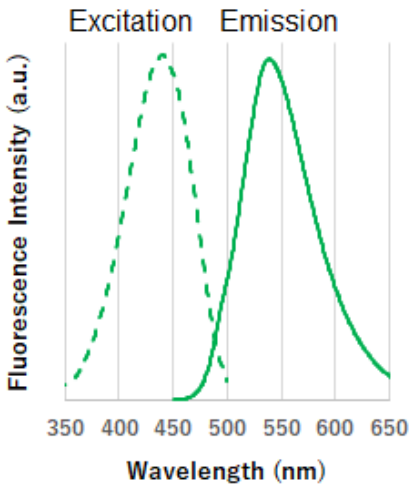


Fig 1. The difference between pHLys Green (pH-dependent) and LysoPrime Deep Red (pH-independent)

-Fluorescent Property

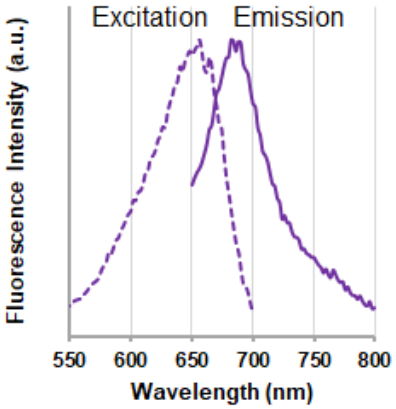
Excitation and emission spectra of pHLys Green and LysoPrime Deep Red

pHLys Green



λ ex: 440 nm
λ em: 539 nm
< Filter setting >
Ex: 488 nm
Em: 500–600 nm

LysoPrime Deep Red



λ ex: 656 nm
λ em: 682 nm
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Ex: 633 nm
Em: 640–700 nm

–Contents

- pHLys Green x 1
- LysoPrime Deep Redx 1
- Bafilomycin A1 x 1

＊Bafilomycin A1 in each tube may be barely visible due to the small amount. Please handle it carefully.

–Storage Condition

Store at –20℃ and protect from light.

–Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Growth medium
- Hanks' Balanced Salt Solution (HBSS) or serum-free medium
- Micropipettes
- Microtubes

–Preparation of Solution

Preparation of pHLys Green DMSO stock solution

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

* Store the reconstituted pHLys Green DMSO stock solution at −20° C until use. The solution is stable at −20° C for 1 month.
* The dye might deposit in the tube due to the storage and shipping conditions, but this does not affect the performance and experimental results, so please use it as it is.

Preparation of pHLys Green working solution

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

* The final concentration of pHLys Green should be optimized depending on the cell line (dilution range: 250 – 2,000 times).
* pHLys Green working solution should be used up on the day it is prepared.

Preparation of LysoPrime Deep Red DMSO stock solution

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

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

Preparation of LysoPrime Deep Red working solution

Dilute the LysoPrime Deep Red solution 1,000 times with HBSS or serum-free medium to prepare LysoPrime Deep Red working solution.

* The final concentration of LysoPrime Deep Red should be optimized depending on the cell line (dilution range: 500 – 5,000 times).
* Use HBSS or serum-free medium for the dilution because serum in medium interferes with LysoPrime Deep Red.
* LysoPrime Deep Red working solution should be used up on the day it is prepared.

Preparation of Bafilomycin A1 DMSO stock solution

Add 24 μl of DMSO to a tube of Bafilomycin A1 and dissolve by pipetting.

* Store the reconstituted Bafilomycin A1 DMSO stock solution at −20° C until use.The solution is stable at −20° C for 1 month.

Preparation of Bafilomycin A1 working solution

Dilute the Bafilomycin A1 DMSO stock solution 2,000 times with HBSS to prepare the Bafilomycin A1 working solution.

* The final concentration of Bafilomycin A1 should be optimized depending on the cell line (dilution range: 1,000 – 5,000 times).
* Bafilomycin A1 working solution should be used up on the day it is prepared.

-General Protocol

1. Seed cells in a dish and culture them overnight at 37℃ in an incubator equilibrated with 95% air and 5% CO_2
2. Discard the culture medium and wash the cells twice with serum-free medium or HBSS.
3. Add LysoPrime Deep Red working solution to the dish containing the cells and incubate them for 30 minutes at 37℃ in an incubator equilibrated with 95% air and 5% CO^2.*1
4. Discard the supernatant and wash the cells twice with HBSS.*2
5. Add pHLys Green working solution (HBSS) to the dish, and incubate them for 30 minutes at 37℃ in an incubator equilibrated with 95% air and 5% CO_2.*3
6. Discard the supernatant and wash the cells twice with HBSS or growth medium.
7. Add growth medium to the dish, then observe the stained cells under a fluorescence microscope.

*1 Please be sure to stain LysoPrime Deep Red before drug stimulation.

*2 Please be sure to stain pHLys Green after staining with LysoPrime Deep Red.

*3 For stimulation with Bafilomycin A1 (lysosomal acidification inhibitor), please add Bafilomycin A1 to pHLys Green working solution, or Bafilomycin A1 stimulation after staining with pHLys Green.

-Usage Example

1. HeLa cells were seeded (1.0 x 10^4 cells/well) on a μ-slide 8 well plate (ibidi) and cultured overnight at 37℃ in an incubator equilibrated with 95% air and 5% CO_2.
2. After washing twice with HBSS, 200 μl of LysoPrime Deep Red working solution (1,000 times dilution) and the cells were incubated at 37℃ for 30 min.
3. The supernatant was discarded, and the cells were washed twice with HBSS.
4. Two hundred (200 μl) of pHLys Green working solution (HBSS, 1,000 times dilution) containing Bafilomycin A1 (1,000 times dilution), an inhibitor of lysosomal acidification, was added to the plate, and the cells were incubated at 37℃ 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.

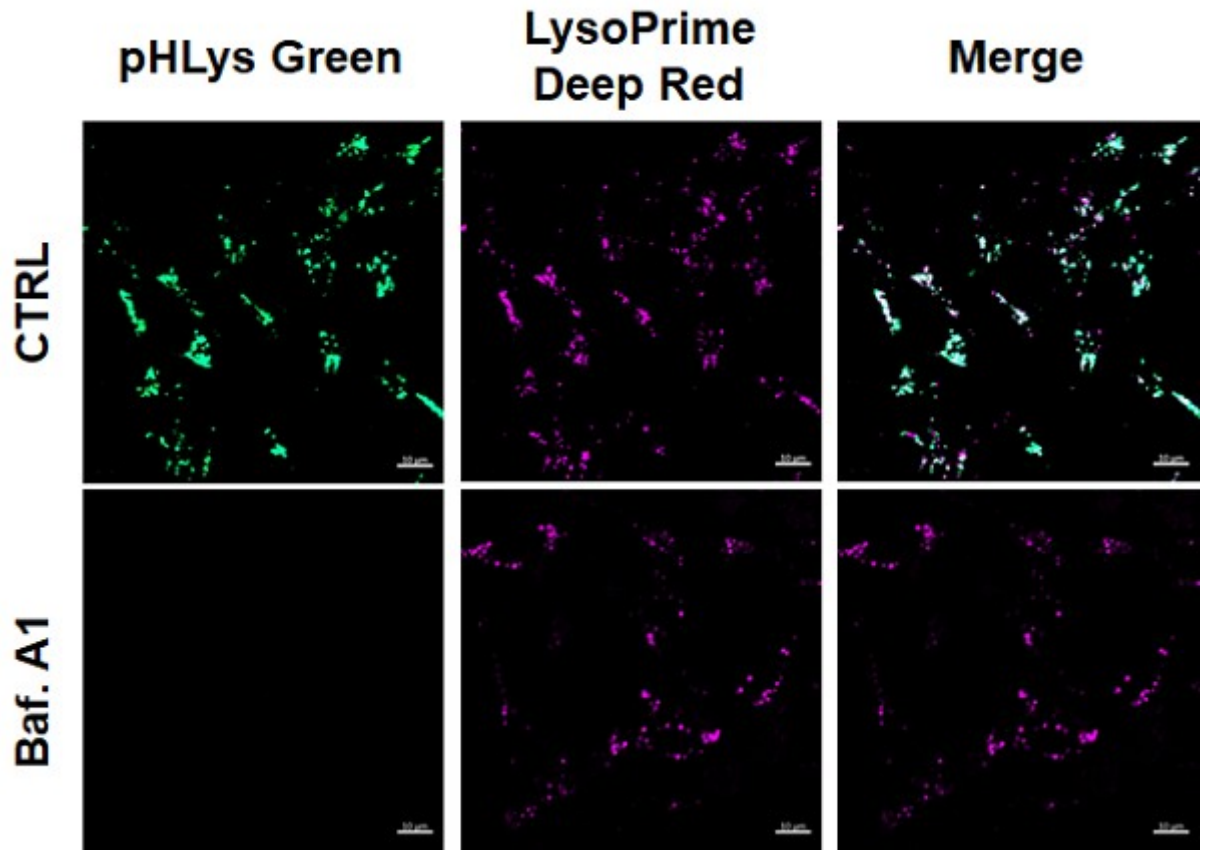

Figure 2. The effect of Bafilomycin A1 on lysosomal pH

CTRL: Normal condition, Baf. A1: Inhibition of lysosomal acidification
pHLys Green filter sets: 488 nm (Ex), 500–600 nm (Em)
LysoPrime Deep Red filter sets: 633 nm (Ex), 640–700 nm (Em)