BIOLUMINOR

LysoSensor DR (Blue/Orange)

Table 1 Contents and storage

Material	Amount	Concentration	Storage	stability
LysoSensor DR (Blue/Orange)	10 vials Each：100 μL	2mg/mL stock solution in anhydrous DMF	♦ ≤-20℃ ♦ Desiccate ♦ Protect from light ♦ Avoid freeze-thaw cycles ♦ Store in single-use aliquots, if possible	When stored as directed, products are stable for at least 6 months
If refreezing after use, seal the vial tightly.				
Abs 480 nm/Ex 560 nm				

Introduction

LysoSensor DR, with pH activatable rhodamine-lactam bridged with dansyl fluorophore via an acidotrophic linker, was prepared for ratiometric sensing of lysosomal pH in living cells. The bioimaging can be performed by confocal fluorescence microscopy, allowing measuring lysosomal pH at an individual organelle level. LysoSensor-DR was proved to be highly efficiency in differentiating apoptosis vs. necrosis cells. Compared with commercial lysosensors which often contain a single fluorophore, LysoSensor DR consists of dansyl group and rhodamine moiety, two widely utilized fluorophores with distinguished photophysical properties.

LysoSensor DR is a dual emissive dye that selectively accumulates in lysosomes. It emits pH independent blue light and lysosomal pH dependent orange light and thus allows the resolution of individual lysosomes of different pH values.

1. Selective staining of lysosomes
2. pH dependent ratiometric siganls (fluorescence emission@480 nm and 560 nm)

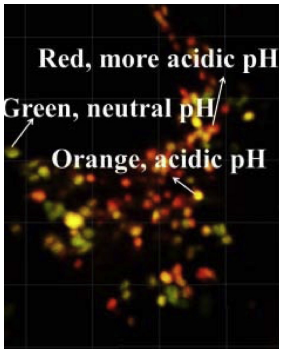


Fig. 1 Resolution of lysosomes with LysoSensor DR

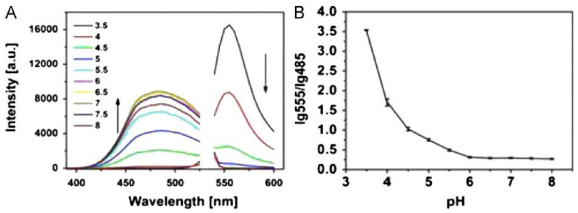


Fig. 2 pH dependent ratiometric fluorescence emissions. (A)Fluorescence emission spectra of LysoSensor-DR (10 μg/mL) at pH 3.5–8.0 under dual-wave length excitation (λex @ 300 nm for dansyl group, 532 nm for R6G-lactam).(B) pH titration curves of lg ratios between fluorescence emission of R6G-amide(I_{555} nm) and that of dansyl group (I_{485} nm).

Guidelines for Use

Before opening, allow the vial to warm to room temperature and then briefly centrifuge the vial in a micro centrifuge to deposit the DMF solution at the bottom of the vial.

The concentration of probe for optimal staining will vary depending on the application. Here we suggest some initial conditions to use as a guideline. The staining conditions may need to be modified depending upon the particular cell type and the permeability of the cells or tissues to the probe, among other factors.

1.1 Imaging of lysosomal acidity with LysoSensor DR

Cells were respectively seeded on 35 mm glass-bottom dishes and incubated in DMEM medium for 24 h, followed by addition of LysoSensor DR (1 μg/mL). The cells were further incubated for 30 min and then analyzed with a confocal fluorescence microscope. The ratio of the channel 1 (dansyl

fluorescence@ 410–470 nm) to channel 2 (R6G-amide fluorescence emission@ 565–625 nm) was calculated using the value of selected lysosomes given by the software. For single lysosome acidity determination, 120 lysosomes were collected automatically and the data was analyzed with Imaris software. For average lysosomal acidity of cells, 5 cells were randomly selected where the pH of 120 lysosomes were determined and averaged. F

1.2 Ratiometric imaging of lysosomal acidity in cells undergoing apoptosis and nercrosis

Cells were seeded on 35 mm glass-bottom dishes and incubated in DMEM medium for 24 h, then treated with 1 μM of staurosporine (STS) or 10 ng/mL of tumornecrosis factor-α (TNF) for 4 h to induce apoptosis and necrosis respectively. Cells were then incubated with LysoSensor DR (1 μg/mL) for 30 min and then analyzed on a confocal microscope. A statistical analysis was performed with pH value of 20 treated or untreated cells.

NOTE B：If the cells do not appear to be sufficiently stained, we recommend either increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the lysosomes.

Further information

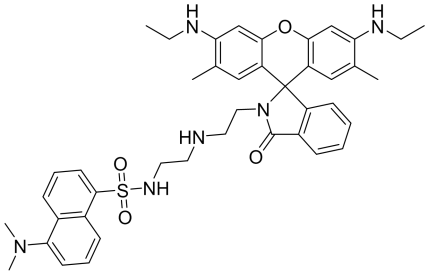
Name：LysoSensor DR (Blue/Orange)

molecular formula：$C_{42}H_{52}N_8O_S$

molecular weight：716.99

CAS NO: N/A

structural formula：



Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes.

Please visit our Web site —**www.Bioluminor.com** — for the most up-to-date information.

Reference：

1. *Talanta*, *114 (2013)* 254–260

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