LysoTracker™ and LysoSensor™ Probes

Quick Facts

Storage upon receipt:

- –20°C
- Avoid freeze-thaw cycles
- Desiccate
- Protect from light

Abs/Em: See Table 1

Note: Do not store in a frost-free freezer

Introduction

LysoTracker™ Probes

Weakly basic amines selectively accumulate in cellular compartments with low internal pH and can be used to investigate the biosynthesis and pathogenesis of lysosomes.[1,2] The most frequently used acidic organelle probe, DAMP (D-1552), is not fluorescent and therefore must be used in conjunction with anti-DNP antibodies conjugated to a fluorophore, enzyme or ferritin in order to visualize the staining pattern.[1] The fluorescent probes neutral red (N-3246) and acridine orange (A-1301, A-3568) are also commonly used for staining acidic organelles, though they lack specificity.[2,3]

These limitations have motivated us to search for alternative acidic organelle–selective probes, both for short-term and long-term tracking studies. The LysoTracker probes are fluorescent acidotropic probes for labeling and tracking acidic organelles in live cells.[4,5] These probes have several important features, including high selectivity for acidic organelles and effective labeling of live cells at nanomolar concentrations. Furthermore, the LysoTracker probes are available in several fluorescent colors (Table 1), making them especially suitable for multicolor applications.

The LysoTracker probes, which consist of a fluorophore linked to a weak base that is only partially protonated at neutral pH, are freely permeant to cell membranes and typically concentrate in spherical organelles. Their mechanism of retention has not been firmly established but is likely to involve protonation and retention in the membranes of the organelles, although staining is generally not reversed by subsequent treatment of the cells with weakly basic cell-permeant compounds. We must note that in LysoTracker dye–stained cells, the lysosomal fluorescence may constitute only a small

Table 1. *Summary of our LysoTracker and LysoSensor probes.*

Cat #	Probe	Abs * (nm)	Em * (nm)	pK_a	Suggested Filter Set †
L-7525	LysoTracker Blue DND-22	373	422	NA	O-5703, O-5704
L-12490	LysoTracker Blue-White DPX	380	‡	NA	O-5703, O-5704
L-7526	LysoTracker Green DND-26	504	511	NA	O-5715, O-5717
L-12491	LysoTracker Yellow-HCK-123	465	535	NA	O-5713, O-5716
L-7527	LysoTracker Yellow DND-68	534	551	NA	O-5722, O-5723
L-7528	LysoTracker Red DND-99	577	590	NA	O-5730, O-5731
L-7533	LysoSensor Blue DND-167	373	425	5.1	O-5703, O-5704
L-7532	LysoSensor Blue DND-192	374	424	7.5	O-5703, O-5704
L-7535	LysoSensor Green DND-189	443	505	5.2	O-5709, O-5711
L-7534	LysoSensor Green DND-153	442	505	7.5	O-5709, O-5711
L-7545	LysoSensor Yellow/Blue DND-160	329, 384 §	440 , 540 §	4.2	O-5703

***** Absorption (Abs) and fluorescence emission (Em) maxima, determined in aqueous buffer or methanol; values may vary somewhat in cellular environments. † These Omega® Optical bandpass and longpass filter sets are available directly from Molecular Probes. For more information on these and other filter sets, consult our *Handbook of Fluorescent Probes and Research Chemicals*, visit our Web site (www.probes.com) or contact our Technical Assistance Department. ‡ Emission is extremely sensitive to environment; stained lysosomes appear blue-white, although the emission maximum in methanol is 576 nm. § Dual-absorption and dual-emission maxima, sensitive to pH (see Figure 1).

portion of total cellular fluorescence, making it difficult to quantitate the number of lysosomes by flow cytometry or fluorometry.

LysoSensor™ pH Indicators

For researchers studying the dynamic aspects of lysosome biogenesis and function in live cells, we have introduced LysoSensor probes — fluorescent pH indicators that partition into acidic organelles. The LysoSensor dyes are acidotropic probes that appear to accumulate in acidic organelles as the result of protonation. This protonation also relieves the fluorescence quenching of the dye by its weak base side chain, resulting in an increase in fluorescence intensity. Thus, the LysoSensor reagents exhibit a pH-dependent increase in fluorescence intensity upon acidification, in contrast to the LysoTracker probes, which exhibit fluorescence that is largely independent of pH.

Molecular Probes offers five LysoSensor reagents that differ in color and pK_a (Table 1). Because these probes may localize in the membranes of organelles, it is probable that the actual pK_a values in cellular environments will differ from the values listed in Table 1 and that only qualitative and semi-quantitative comparisons of organelle pH will be possible. The blue and green fluorescent LysoSensor probes are available with optimal pH sensitivity in either the acidic or neutral range (pK_a ~5.2 or ~7.5). Because of their low pK_a values, LysoSensor Blue DND-167 and LysoSensor Green DND-189 are almost nonfluorescent except when inside acidic compartments, whereas LysoSensor Blue DND-192 and LysoSensor Green DND-153 are brightly fluorescent at neutral pH. LysoSensor Yellow/Blue DND-160 is unique in that it exhibits both dual-excitation and dual-emission spectral peaks that are pH-dependent (Figure 1). Nevertheless, this LysoSensor only exhibits the pH-dependent dual-emission spectra in living cells. In acidic organelles LysoSensor Yellow/Blue DND-160 has predominantly yellow fluorescence, and in less acidic organelles it has blue fluorescence. Dual-emission measurements may permit ratio imaging of the pH in acidic organelles such as lysosomes or the acrosomes of spermatozoa.

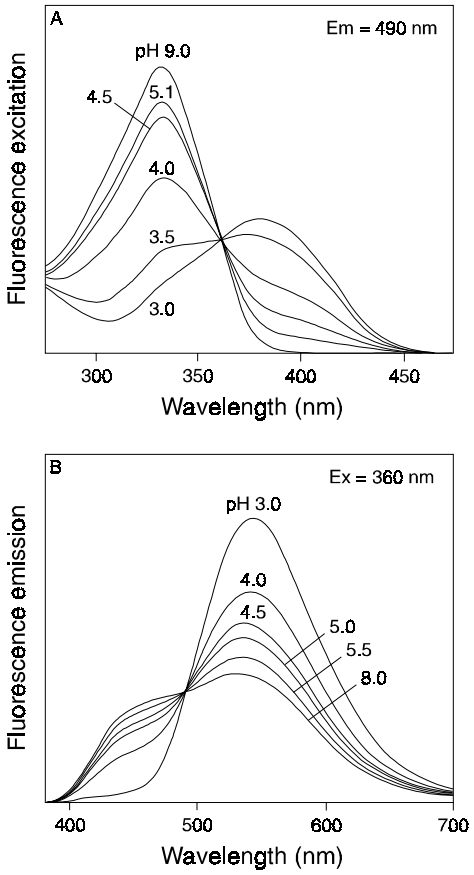

Figure 1. *The pH-dependent spectral response of LysoSensor Yellow/Blue DND-160 (L-7545): A) fluorescence excitation spectra and B) fluorescence emission spectra.*

These probes can be used singly (or potentially in combination) to investigate the acidification of lysosomes and alterations of lysosomal function or trafficking that occur in cells. For example, lysosomes in some tumor cells have a lower pH than normal lysosomes,[8] while other tumor cells contain lysosomes with higher pH.[9] In addition, cystic fibrosis and other diseases result in defects in the acidification of some intracellular organelles,[10] and the LysoSensor probes may prove useful in studying these aberrations. As in LysoTracker-stained cells, the lysosomal fluorescence in Lyso-Sensor-stained cells may constitute only a small portion of total cellular fluorescence, making it difficult to quantitate the number of lysosomes or their pH by flow cytometry or fluorometry.

Storage and Handling

The fluorescent LysoTracker and LysoSensor reagents are provided as specially packaged sets of 20 separate vials, each containing 50 µL of a 1 mM stock solution in high-quality, anhydrous dimethylsulfoxide (DMSO). Upon receipt, these products should be stored desiccated at -20°C until required for use, preferably in single-use aliquots. AVOID REPEATED FREEZING AND THAWING. DO NOT STORE IN A FROST-FREE FREEZER.

Before opening, the vial should be allowed to warm to room temperature and then briefly centrifuged in a microcentrifuge to deposit the DMSO solution at the bottom of the vial. Before refreezing, seal the vial tightly. When stored properly, these stock solutions are stable for at least six months.

Cell and Tissue Loading

Cell Preparation and Staining

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 Dilute the 1 mM probe stock solution to the final working concentration in the growth medium or buffer of choice. For the LysoTracker probes, we recommend working concentrations of 50–75 nM and for the LysoSensor probes at least 1 µM. To reduce potential artifacts from overloading, the concentration of dye should be kept as low as possible (note **A**).

1.2 For adherent cells, grow cells on coverslips inside a petri dish filled with the appropriate culture medium. When cells

have reached the desired confluence, remove the medium from the dish and add the prewarmed (37°C) probe-containing medium. Incubate the cells for 30 minutes to 2 hours under growth conditions appropriate for the particular cell type (note **B**). Then replace the loading solution with fresh medium and observe the cells using a fluorescence microscope fitted with the correct filter set (see Table 1) (note **C**).

1.3 For suspension cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) probe-containing medium. Incubate the cells for 30 minutes to 2 hours under growth conditions appropriate for the particular cell type (note **B**). Re-pellet the cells by centrifugation and resuspend in fresh prewarmed medium. Observe the cells using a fluorescence microscope fitted with the correct filter set (see Table 1) (note **C**).

Alternatively, suspension cells may be attached to coverslips that have been treated with Cell-Tak® (Collaborative Biomedical Products; Bedford, MA) and stained as if they were adherent cells (see step 1.2).

Fluorescence Microscopy

Molecular Probes offers high-quality Omega® Optical filter sets for fluorescence microscopy that are optimized to match the spectral properties of our dyes. See Table 1 for the absorption and emission maxima of the LysoTracker and LysoSensor probes and suggested filter sets. For further information on our extensive filter selection, consult our *Handbook of Fluorescent Probes and Research Chemicals* at our Web Site (www.probes.com) or call our Technical Assistance Department.

Notes

[A] If the cells are incubated in dye-free medium after staining, we often observe a decrease in fluorescent signal and cell blebbing.

[B] Kinetic studies on the internalization of the LysoTracker Green DND-26 and LysoSensor Yellow/Blue DND-160 probes indicate that the rates of uptake of these dyes into living cells can occur within seconds. Unfortunately, these lysosomal probes can exhibit an "alkalizing effect" on the lysosomes, such that longer incubation with these probes can induce an increase in lysosomal pH. We suggest that these probes are useful pH indicators only when they are incubated with cells for 1–5 minutes at 37°C.

[C] 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.

[D] After the acetone permeabilization step, only the larger acidic organelles appear to retain the fluorescent signal. Permeabilization is not always necessary when labeling with a secondary detection reagent such as an antibody or streptavidin conjugate. Because it significantly reduces the signal, the requirement for permeabilization should be tested in each particular application.

References

1. Cell 52, 329 (1988); **2.** *Lysosomes in Biology and Pathology*, J.T. Dingle *et al.*, Eds., North-Holland Publications Co. (1969); **3.** J Cell Biol 106, 539 (1988); **4.** Cytometry suppl 7, 77 abstract #426B (1994); **5.** Mol Biol of the Cell 5, 113a abstract #653 (1994); **6.** J Cell Biol 126, 877 (1994); **7.** J Cell Biol 128, 901 (1994); **8.** *Molecular Aspects of Anticancer Drug Action*, S. Neidle and M.J. Waring, Eds., Macmillian (1983) pp. 233–282; **9.** J Biol Chem 265, 4775 (1990); **10.** Nature 352, 70 (1991).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A-1301	acridine orange *≥98% by HPLC*	1 g
A-3568	acridine orange *10 mg/mL solution in water*	10 mL
D-1552	*N*-(3-((2,4-dinitrophenyl)amino)propyl)-*N*-(3-aminopropyl)methylamine, dihydrochloride (DAMP)	100 mg
L-7533	LysoSensor™ Blue DND-167 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7532	LysoSensor™ Blue DND-192 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7534	LysoSensor™ Green DND-153 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7535	LysoSensor™ Green DND-189 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7545	LysoSensor™ Yellow/Blue DND-160 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7525	LysoTracker™ Blue DND-22 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-12490	LysoTracker™ Blue-White DPX *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7526	LysoTracker™ Green DND-26 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7528	LysoTracker™ Red DND-99 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-7527	LysoTracker™ Yellow DND-68 *1 mM solution in DMSO* *special packaging*	20x50 µL
L-12491	LysoTracker™ Yellow HCK-123 *1 mM solution in DMSO* *special packaging*	20x50 µL
N-3246	neutral red *high purity*	25 mg

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