molecular probes®
by life technologies™

LysoTracker® and LysoSensor™ Probes

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
LysoTracker® and LysoSensor™ dyes	20 vials, each containing 50 µL	1 mM stock solution in anhydrous DMSO	• ≤–20°C • Desiccate • Protect from light • Avoid freeze-thaw cycles • Do not store in a frost-free freezer • Store in single-use aliquots, if possible	When stored as directed, products are stable for at least 6 months*
LysoTracker® Deep Red	5 vials, each containing 50 µL			
LysoSensor™ Yellow/Blue dextran	5 mg, lyophilized solid	NA	• ≤–20°C • Desiccate • Protect from light	When stored as directed, product is stable at least 1 year
* If refreezing after use, seal the vial tightly.				
Approximate fluorescence excitation and emission, in nm: See Table 2, page 2.				

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 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 2, page 2), 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. Note that in LysoTracker® dye–stained cells, the lysosomal fluorescence may constitute only a small portion of total cellular fluorescence, making it difficult to quantitate the number of lysosomes by flow cytometry or fluorometry.

Table 2 Summary of our LysoTracker® and LysoSensor™ probes.

Cat. no.	Probe	Abs * (nm)	Em * (nm)	pK_a
L7525	LysoTracker® Blue DND-22	373	422	NA
L12490	LysoTracker® Blue-White DPX	380	†	NA
L7526	LysoTracker® Green DND-26	504	511	NA
L12491	LysoTracker® Yellow-HCK-123	465	535	NA
L7528	LysoTracker® Red DND-99	577	590	NA
L12492	LysoTracker® Deep Red	647	668	NA
L7533	LysoSensor™ Blue DND-167	373	425	5.1
L7535	LysoSensor™ Green DND-189	443	505	5.2
L7534	LysoSensor™ Green DND-153	442	505	7.5
L7545	LysoSensor™ Yellow/Blue DND-160 (PDMPO)	329, 384 ‡	440, 540 ‡	4.2
L22460	LysoSensor™ Yellow/Blue dextran	335, 381 ‡	452, 521 ‡	3.9

***** Absorption (Abs) and fluorescence emission (Em) maxima, determined in aqueous buffer or methanol; values may vary somewhat in cellular environments. † 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, page 3).

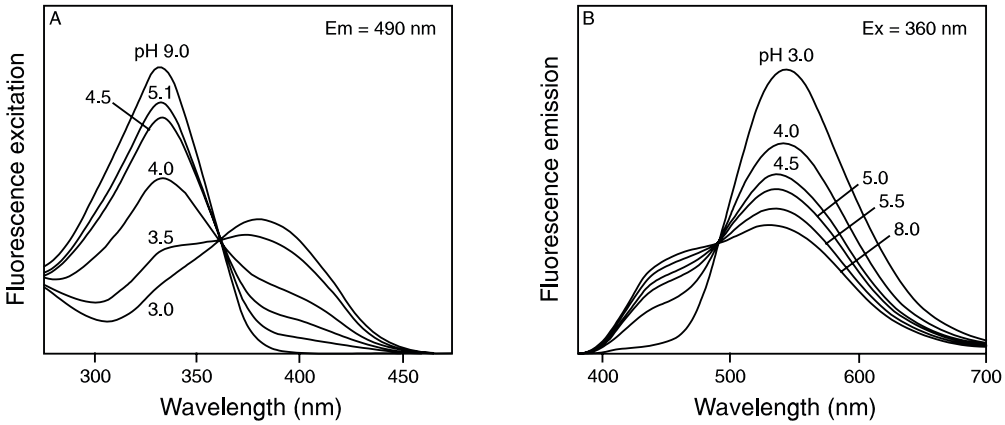
LysoSensor™ pH Indicators

For researchers studying the dynamic aspects of lysosome biogenesis and function in live cells, we offer 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 2). 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 2 and that only qualitative and semiquantitative 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™ Green DND-153 is brightly fluorescent at neutral pH. LysoSensor™ Yellow/Blue DND-160 (PDMPO) 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 (PDMPO) 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. LysoSensor™ Yellow/Blue dextran allows loading of the cells by endocytosis. This conjugate should prove useful for studying the endocytic pathway. The pK_a is somewhat lower than the pK_a of the free LysoSensor™ Yellow/Blue dye.

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® dye–stained cells, the lysosomal fluorescence in LysoSensor™ dye–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.

Figure 1 The pH-dependent spectral response of LysoSensor™ Yellow/Blue DND-160 (PDMPO, Cat. no. L7545). (A) fluorescence excitation spectra and (B) fluorescence emission spectra.



Guidelines for Use

Before opening, allow the vial to warm to room temperature and then briefly centrifuge the vial in a microcentrifuge to deposit the DMSO 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.

LysoTracker® and LysoSensor™ Dyes

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

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. Then replace the loading solution with fresh medium and observe the cells using a fluorescence microscope fitted with the correct filter set (see Table 2, page 2). 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.

Note: Kinetic studies on the internalization of the LysoTracker® Green DND-26 and LysoSensor™ Yellow/Blue DND-160 (PDMPO) 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.

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 (see note above regarding internalization rate of these probes). 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 (Table 2, page 2). 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.

Alternatively, suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as if they were adherent cells (see step 1.2).

LysoSensor™ Yellow/Blue Dextran

2.1 To prepare a stock solution, reconstitute the lyophilized dextran to 50 mg/mL in phosphate-buffered saline, pH 7.4. Store the stock solution ≤–20°C, protected from light.

2.2 Dilute the stock solution to a final working concentration in the growth medium or buffer of choice. We recommend a working concentration of 1–5 mg/mL.

2.3 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) dextran working solution. Incubate the cells for 1–24 hours under growth conditions appropriate for the particular cell type and experiment. Replace the loading solution with fresh medium and observe the cells using a fluorescence microscopy fitted with the correct filter set (Table 2, page 2).

2.4 For suspension cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in pre-warmed (37°C) dextran-containing medium. Incubate the cells for 1–24 hours under growth conditions appropriate for the particular cell type. 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 (Table 2, page 2).

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 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 website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A1301	Acridine orange	1 g
A3568	Acridine orange	10 mL
D1552	N-(3-((2,4-dinitrophenyl)amino)propyl)-N-(3-aminopropyl)methylamine, dihydrochloride	100 mg
L7533	LysoSensor™ Blue DND-167	20 × 50 µL
L7534	LysoSensor™ Green DND-153	20 × 50 µL
L7535	LysoSensor™ Green DND-189	20 × 50 µL
L22460	LysoSensor™ Yellow/Blue dextran, 10,000 MW, anionic, fixable	5 mg
L7545	LysoSensor™ Yellow/Blue DND-160 (PDMPO) *1 mM solution in DMSO*	20 × 50 µL
L7525	LysoTracker® Blue DND-22	20 × 50 µL
L12490	LysoTracker® Blue-White DPX	20 × 50 µL
L7526	LysoTracker® Green DND-26	20 × 50 µL
L7528	LysoTracker® Red DND-99	20 × 50 µL
L12491	LysoTracker® Yellow HCK-123	20 × 50 µL
L12492	LysoTracker® Deep Red	5 × 50 µL

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