BIOLUMINOR

LysoSensor-Green DND-153

Table 1 Contents and storage

Material	Amount	Concentration	Storage	stability
LysoSensor-Green DND-153	20 vials Each：50 μL	1 mM stock solution in anhydrous DMSO	♦ ≤-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 442 nm/Ex 505 nm, Suggested Filter Set: O-5709, O-5711				

Introduction

For researchers studying the dynamic aspects of lysosome biogenesis and function in live cells, we have introduced Lyso-Sensor probes — fluorescent pH indicators that partition into acidic organelles. The Lyso-Sensor 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 Lyso-Sensor reagents exhibit a pH-dependent increase in fluorescence intensity upon acidification, in contrast to the Lyso-Tracker probes, which exhibit fluorescence that is largely independent of pH.

The probe 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[1], 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 Lyso-Sensor probes may prove useful in studying these aberrations. As in LysoTracker- stained cells, the lysosomal fluorescence in LysoSensor-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.

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 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.

1.1 Dilute the 1 mM probe stock solution to the final working concentration in the growth probes, we recommend working concentrations at least 1 μM.

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 cover slips 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℃) 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.

NOTE A： Kinetic studies on the internalization of the Lyso-Sensor Green DND-153 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 the probe is useful pH

indicators only when it is incubated with cells for 1-5 minutes at 37℃.

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.

1.3 For suspension cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in pre warmed (37℃) 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 the probe). 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. 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).

Fluorescence spectrum

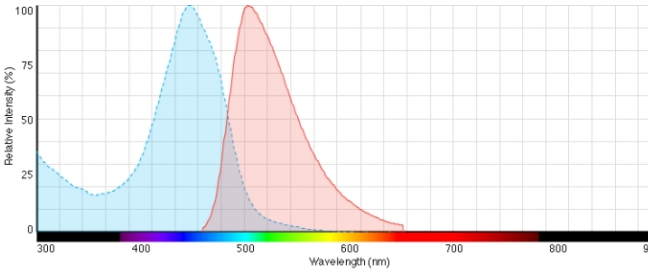


Fig 1 Fluorescence Ex/Em spectra of Lyso-Sensor- Green DND-153 in pH 5 buffer.

Further information

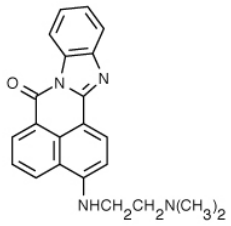
Name：LysoSensor Green DND-153

molecular formula：$C_{22}H_{20}N_4O$

molecular weight：356.4262

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.

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