BIOLUMINOR

LysoTracker-Blue DND-22

Table 1 Contents and storage

Material	Amount	Concentration	Storage	stability
Lyso-Tracker-Blue DND-22	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, Stable for at least 6 months
If refreezing after use, seal the vial tightly.				
Abs 373 nm/Ex 422 nm, Suggested Filter Set: O-5703, O-5704				

Introduction

Weakly basic amines selectively accumulate in cellular compartments with low internal pH and can be used to investigate the biosynthesis and pathogenesis of lysosomes. The LysoTracker probes are fluorescent acidotropic probes for labeling and tracking acidic organelles in live cells. 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, 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 subse quent 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.

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 of 50-75 nM.

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: Kinetic studies on the internalization of the Lyso-Tracker Blue DND-22 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℃.

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 pre-warmed 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

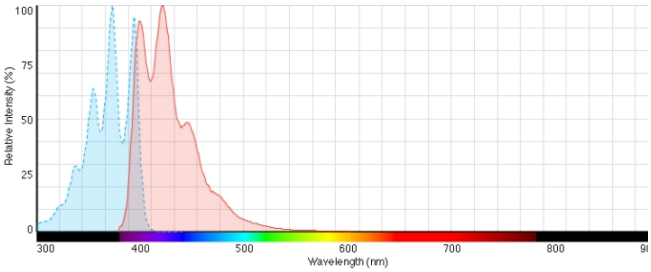


Fig 1 Fluorescence Ex/Em spectra of Lyso-Tracker- Blue DND-22 in methanol.

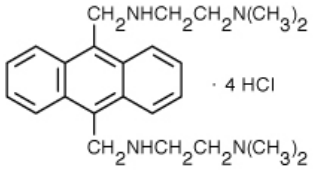
Further information

Name：Lyso-Tracker-Blue DND-22

molecular formula：$C_{24}H_{38}Cl_4N_4$

molecular weight：524.403

CAS NO: N/A



structural formula：