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LysoTracker® Red Lysosomal Probe

Cat. No. PA-3015

Storage upon receipt: -20°C
Avoid freeze-thaw cycles
Desiccate
Protect from light
Note: Do not store in a frost-free freezer.

Instructions for Use

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]. LysoTracker® Red is a Fluorescent Acidotropic Probe for labeling and tracking acidic organelles in live cells.[3, 4] This probe has several important features, including high selectivity for acidic organelles and effective labeling of live cells at nanomolar concentrations. LysoTracker® Red, which consists of a fluorophore linked to a weak base that is only partially protonated at neutral pH, is freely permeant to cell membranes and typically concentrates in spherical organelles. Its 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. It should be noted 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.

Storage and Handling

Upon receipt, these products should be stored desiccated at ≤-20°C and protected from light 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

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.

Method

1.1 Dilute the 1 mM probe stock solution to the final working concentration in the growth medium or buffer of choice. We recommend initial working concentrations of 50–75 nM. To reduce potential artifacts from overloading, the concentration of dye should be kept as low as possible.

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.

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. 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.
Alternatively, suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (Becton Dickinson; Franklin Lakes,

NJ) and stained as if they were adherent cells (see step 1.2). 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. Cytometry suppl 7, 77 abstract #426B (1994);
4. Mol Biol Cell 5, 113a abstract #653 (1994)