GeneCopoeia™
Expressway to Discovery

9620 Medical Center Drive, Suite 101
Rockville, MD 20850, USA
Web: www.genecopoeia.com

Product Information

LysoBeacon™ Probes

Catalog Number	Product Name	Unit Size
C047	LysoBeacon™ Blue	1 mL
C048	LysoBeacon™ Green	1 mL

Storage upon receipt:
- -20°C
- Protect from light

Product Description

LysoBeacon™ probes are fluorescent pH indicators that partition into acidic organelles, and allow for studying the dynamic aspects of lysosome biogenesis and function in live cells. The LysoBeacon™ 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 LysoBeacon™ reagents exhibit a pH-dependent increase in fluorescence intensity upon acidification.

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, while other tumor cells contain lysosomes with higher pH. In addition, cystic fibrosis and other diseases result in defects in the acidification of some intracellular organelles, and the LysoBeacon™ probes may prove useful in studying these aberrations.

Table. Spectral characteristics of the LysoBeacon™ probes

Product Name	E_x (nm)	E_m (nm)	pKa
LysoBeacon™ Blue	373	425	5.1
LysoBeacon™ Green	443	505	5.2

Guidelines for Use

Cell Preparation and Staining

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.

1.1 Dilute the 1 mM probe stock solution to the final working concentration in the growth medium or buffer of choice. We recommend working concentrations 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. 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 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. 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).

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