

Product Data Sheet

Product Name: Lyso-Tracker Red (solution)

Cat. No.: GC19882

Chemical Properties

Cas. No. 231946-72-8

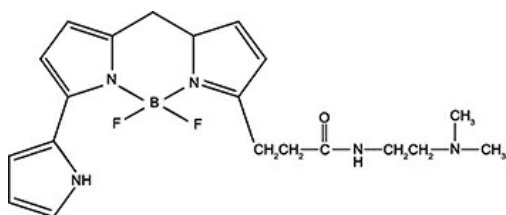
Formula $C_{20}H_{24}BF_2N_5O$ M.Wt 399.25

Solubility DMSO : 5 mg/mL (12.52 mM; Need ultrasonic) Storage Store at -20°C, protect from light

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Prepare Lyso-Tracker Red staining solution

(1) Prepare stock solution: It is recommended to use anhydrous DMSO to dissolve the powder and prepare a stock solution with a concentration of 1 mM.

Note: It is recommended to aliquot unused stock solution and freeze it at -20°C or -80°C in the dark.

(2) Preparation of working solution: Dilute the stock solution with a suitable buffer (such as serum-free medium or PBS) to prepare a working solution with a concentration of 50-100 nM.

Notes:

- ① Please adjust the concentration of the working fluid according to the actual situation and prepare it as needed.
- ② In order to reduce false positives caused by excessively high background or overload, the concentration of dyes should be as low as possible.
- ③ If cells are incubated in a buffer free of dye after staining, attenuation of fluorescence signals and

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vacuolization of cells will be observed.

2. Cell suspension staining

(1) Suspension cells: Centrifuge the suspended cells at 4°C and 1000g for 3-5 minutes, discard the supernatant, and wash twice with PBS for 5 minutes each time.

(2) Adherent cells: Wash twice with PBS, add trypsin to digest the cells, and centrifuge at 1000g for 3-5 minutes after digestion is completed.

(3) Use 0.5-1mL working solution to resuspend approximately 10⁶ cells, and incubate at room temperature in the dark for 5-30 minutes. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(4) After the incubation, centrifuge at 1000g for 5 minutes, remove the supernatant, and add PBS to wash 2-3 times, 5 minutes each time.

(5) Resuspend cells in pre-warmed serum-free cell culture medium or PBS and observe by fluorescence microscope or flow cytometry.

3. Cell adhesion staining

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, aspirate the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100μL of dye working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye.

(4) Incubate at room temperature in the dark for 5-30 minutes. The optimal culture time for different cells is different.

(5) After the incubation, discard the dye working solution and wash the coverslip 2 to 3 times with pre-warmed culture solution.

4. Fluorescence detection: The maximum absorption/emission light of Lyso-Tracker Red is 577/590nm.

Notes:

① A kinetic study on the internalization of Lyso-Tracker revealed that the rate of uptake of this probe by living cells can be completed within a few seconds. However, the probe exhibits a lysosomal "alkalization effect", and prolonged incubation can lead to an increase in lysosomal pH. Therefore, incubating the cell at 37°C for 1-5 minutes is a useful pH indicator.

② If the dyeing is not sufficient, it is recommended to increase the dye concentration or extend the dyeing time to allow the dye to aggregate on lysosomes.

③ Fluorescent dyes all have quenching problems. Please try to avoid light as much as possible to slow down fluorescence quenching.

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④ For your safety and health, please wear laboratory clothes and disposable gloves when operating.

Background

Lyso-Tracker Red is an eosinophilic red fluorescent probe that can penetrate the cell membrane at neutral pH and can be used for lysosome-specific fluorescent staining of living cells and tracing of acidic organelles. The maximum excitation and emission wavelengths of Lyso-Tracker Red are 577/590 nm^[1].

Lyso-Tracker Red is a weakly alkaline fluorescent probe fluorescently labeled with DND-99, in which only the weak base can partially provide protons to maintain the pH at neutral. In the neutral charge state, the Lyso-Tracker Red probe can freely diffuse through the intact plasma membrane of living cells. Due to the inherent acidity in lysosomes, the weakly alkaline groups will be protonated when diffusing into lysosomes. In this charged state, the probe is not easy to diffuse through the organelle membrane, and forms local accumulation in the acidic lysosomes, thereby achieving specific fluorescent labeling of the lysosomes. Lyso-Tracker Red is suitable for fluorescent staining of lysosomes in living cells, but not for fluorescent staining of lysosomes in fixed cells. If cells stained with Lyso-Tracker Red need to be fixed, 3% glutaraldehyde can be tried^[1].

Lyso-Tracker Red must be used at very low concentrations to achieve excellent selectivity. Studies of the endocytosis kinetics of the Lyso-Tracker Red probe have shown that the uptake of the dye into living cells takes only seconds. However, these lysosomal probes cause lysosomes to become alkaline, and long-term incubation can induce an increase in lysosomal pH. Therefore, it is recommended that cells should not be incubated with the probe for too long before imaging. When evaluating the number and status of lysosomes in HUVEC cells, acidic compartments in HUVEC can be visualized by staining with Lyso-Tracker Red^[2]. Lysosomal distribution can be monitored by incubating HepG2 cells with 100 nM Lyso-Tracker Red for 30 min^[3]. Furthermore, 100 nM Lyso-Tracker Red can penetrate the MDCK cell membrane at neutral pH and distribute within acidic organelles, such as late endosomes and lysosomes in living cells^[4].

References :

[1] Price O T, Lau C, Zucker R M. Quantitative fluorescence of 5-FU-treated fetal rat limbs using confocal laser scanning microscopy and Lysotracker Red[J]. Cytometry Part A: The Journal of the International Society for Analytical Cytology, 2003, 53(1): 9-21.

[2] Li G, Ma S, Tang J, et al. Lysosome-targeted two-photon fluorescent probes for rapid detection of

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H₂S in live cells[J]. New Journal of Chemistry, 2019, 43(3): 1267-1274.

[3] Wang Y, Bao D, Dong Y, et al. α -Lipoic Acid-Plus Ameliorates Endothelial Injury by Inhibiting the Apoptosis Pathway Mediated by Intralysosomal Cathepsins in an In Vivo and In Vitro Endothelial Injury Model[J]. Oxidative Medicine and Cellular Longevity, 2022, 2022(1): 8979904.

[4] Pereira L C, Duarte F V, Varela A T I F, et al. An autophagic process is activated in HepG2 cells to mediate BDE-100-induced toxicity[J]. Toxicology, 2017, 376: 59-65.

[5] Nagahama M, Itohayashi Y, Hara H, et al. Cellular vacuolation induced by Clostridium perfringens epsilon-toxin[J]. The FEBS journal, 2011, 278(18): 3395-3407.

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