

Product Data Sheet

Product Name: Lyso-Tracker Green

Cat. No.: GC20101

Chemical Properties

Cas. No.

Formula $C_{18}H_{26}BClF_2N_4O$

M.Wt 398.7

Solubility 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 Green staining solution

(1) Take out the frozen dyeing solution, return it to room temperature, and concentrate the dyeing solution at the bottom of the tube by instant centrifugation.

(2) Preparation of working solution: Dilute the stock solution (1mM) with a suitable buffer (such as serum-free medium or PBS) to prepare a working solution with a concentration of 50-75nM.

Caution: Product has not been fully validated for medical applications. For research use only.

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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 vacuolization of cells will be observed.

2. Cell suspension staining

- (1) Suspension cells: Centrifuge the suspended cells at 4°C and 1000 *g* 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 1000 *g* for 3-5 minutes after digestion is completed.
- (3) Resuspend approximately 10^6 cells in 0.5-1mL working solution and incubate at 37°C in the dark for 30 minutes to 2 hours. 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 1000 *g* for 5 minutes, remove the supernatant, and add PBS to wash 2-3 times, 5 minutes each time.
- (5) Use pre-warmed cell culture medium to resuspend and incubate the cells.

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.

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(4) Incubate at 37°C in the dark for 30 minutes to 2 hours. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(5) After the incubation, discard the dye working solution, add PBS to wash 2-3 times, and use pre-warmed culture medium to incubate the cells.

4. Fluorescence detection: The maximum absorption/emission light of Lyso-Tracker Green is 504/511nm.

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;

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

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