Product Name: DiO Plus

Catalog Number: RA20019



Basic Information

Product Name	DiO Plus
Size	5mg
Storage conditions	Store at 4 °C, away from light
Shipping	Shipped with ice pack
Validity	12 months

Reagent preparation

Staining solution preparation

(1) Stock Solution Preparation:

The stock solution should be prepared using DMSO or EtOH, with a concentration of 1–5 mM. Note: Unused stock solution should be aliquoted and stored at -20°C to avoid repeated freeze-thaw cycles.

(2) Working Solution Preparation:

Dilute the stock solution with an appropriate buffer (such as serum-free culture medium, HBSS, or PBS) to prepare a working solution with a concentration of 1–5 μ M.

Note: The final concentration of the working solution is recommended to be optimized based on different cell lines and experimental systems. It is suggested to start exploring the optimal concentration within a range of 10 times the recommended concentration.

Experimental procedures

Suspension cell staining

- (1) Add an appropriate volume of staining working solution to resuspend the cells to a density of 1×10^6 /mL.
- (2) Incubate the cells at 37°C for 20 min. Different cells have different optimal incubation times. You can use 20 min as the initial incubation time, and then optimize the system to obtain a uniform labeling effect.
- (3) At the end of incubation, centrifuge at 1000-1500 rpm for 5 min. Pour off the supernatant and slowly add 37°C preheated growth medium to resuspend the cells.
- (4) Repeat step (3) two more times.

Adherent cell staining

- (1) Adherent cells were cultured on sterile coverslips.
- (2) Remove the coverslip from the medium, aspirating any excess, but leaving the surface wet.
- (3) Add at one corner of the coverslip Add 100 $\,\mu L$ of dye working solution and gently shake to allow the dye to evenly cover all cells.

Product Name: DiO Plus Catalog Number: RA20019



(4) Incubate the cells at 37°C for 2-20 min. The optimal incubation time varies for different cells. You can use 20 min as the initial incubation time, and then optimize the system to obtain a uniform labeling effect.

(5) Aspirate the dye working solution and wash the coverslip with culture medium. 2~3 times, each time covering all cells with pre-warmed culture medium, incubating for 5~10 min, and then aspirating the culture medium, but keeping the surface moist.

DiO Plus excitation/emission wavelength: 484/501nm

Note: This reagent is for scientific research use only!

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