Product Name: **Enhanced DiO Membrane Probe (Green)**

Catalog Number: **RA20019**

Basic Information

Product Name	Enhanced DiO Membrane Probe (Green)
Size	5mg
Storage conditions	Store at 4 °C, away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	484/501nm

Product Introduction

DiO is a green fluorescent, lipophilic carbocyanine and widely used as a lipophilic tracer. It is weakly fluorescent in water but highly fluorescent and quite photostable when incorporated into membranes. It has an extremely high extinction coefficient and short excited-state lifetimes (~1 nanosecond) in lipid environments. Once applied to cells, the dye diffuses laterally within the plasma membrane.

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 μM.

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 μL of dye working solution and gently shake to allow the dye to evenly cover all cells.

(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.

Note: This reagent is for scientific research use only!