**Product Name:** Dil Membrane Probe (Orange-Red)

**Catalog Number: RA20004** 



#### **Basic Information**

| <b>Product Name</b> | Dil Membrane Probe (Orange-Red) |
|---------------------|---------------------------------|
| Size                | 10mg                            |
| Storage             | -20 °C, protected from light    |
| Shipping            | Shipped with ice pack           |
| Validity            | 12 months                       |
| Ex/Em               | 549/565nm                       |

#### **Product Introduction**

Dil is a lipophilic fluorescent dye that can be used to stain cell membranes and other lipophilic biological structures. Once incorporated into the cell membrane, Dil diffuses throughout the membrane, and at optimal concentrations, it can stain the entire cell membrane. The fluorescence of Dil is very weak before it enters the cell membrane, but its fluorescence intensity significantly increases upon binding to the membrane. Upon excitation, Dil emits an orange-red fluorescence, characterized by a high quenching constant, moderate photon intensity, and a short excited-state lifetime. It can be detected using standard TRITC filter sets.

Dil generally does not significantly affect cell viability and is therefore widely used as a tracer or long-term tracer for live or fixed neurons and other cells or tissues in both anterograde and retrograde tracing applications. In addition to simple cell membrane fluorescence labeling, Dil can also be used to detect cell fusion and adhesion, monitor cell migration during development or transplantation, investigate lipid diffusion in cell membranes via FRAP (Fluorescence Recovery After Photobleaching), assess cell toxicity, and label lipoproteins.

# **Reagent preparation**

#### **Staining solution preparation**

(1) Preparation of stock solution: The stock solution is prepared in anhydrous DMSO or EtOH with a concentration of  $1\sim10$  mM .

Note: Store unused stock solution in aliquots at -20°C to avoid repeated freezing and thawing. (2) Preparation of working solution: Dilute the stock solution with a suitable buffer (e.g. serum-free

culture medium, HBSS or PBS) to prepare a working solution with a concentration of 1-10  $\mu$ M. Note: The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems. It is recommended to start exploring the optimal concentration within 10 times the recommended concentration.

**Product Name: Dil Membrane Probe (Orange-Red)** 

**Catalog Number: RA20004** 



## **Experimental procedures**

### Suspension cell staining

- (1) Add an appropriate volume of staining working solution to resuspend the cells to a density of 1×10 <sup>6</sup>/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.
- (4) Incubate the cells at 37°C for 5-20 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!