

**Product Name:** DiD Membrane Probe (Red)

**Product number:** RA20003

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## Basic Information

Product name	DiD Membrane Probe (Red)
Size	10 mg
Storage	Store at -20 °C, protected from light
Shipping	Shipped with ice pack
Validity	12 months

## Reagent preparation

( 1 ) Preparation of stock solution: The stock solution is prepared with anhydrous DMSO or EtOH, with a concentration of 1~5 mM.

Note: Unused stock solution should be stored in aliquots at -20°C to avoid repeated freezing and thawing.

( 2 ) Preparation of working solution: Dilute the stock solution with an appropriate buffer (e.g. serum-free medium, HBSS or PBS) to prepare a working solution with a concentration of 1~5  $\mu$ 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 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  $1 \times 10^6$  cells/mL.  
(2) 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.

( 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) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium and aspirate away any excess medium, but keep the surface moist.

(3) Add 100  $\mu$ L of dye working solution to one corner of the coverslip and gently shake to ensure that the dye evenly covers 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, wash the coverslip with culture medium 2-3 times, cover all cells with pre-warmed culture medium each time, incubate for 5-10 min, and then aspirate the culture medium. However, keep the surface moist.

DiD excitation/emission wavelength: 644/663 nm

**Note: This reagent is for scientific research use only!**