DiI (DiIC18(3))

Cat #: BMD0071 Size: 10 mg

	DiI (DiIC18(3))		
REF	**Cat #:** BMD0071	LOT	**Lot #:** Refer to product label
	Application range: Cell membrane fluorescent dyes, anterograde and retrograde tracing of neurons, and long-term tracing of cells		**Recommended working concentrations:** 1-10 μM
	Excitation/Emission wavelengths: Ex/Em(MeOH)=549/565 nm		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

DiI (DiIC18(3)) is one of the most commonly used cell membrane fluorescent probes with orange-red fluorescence, its molecular formula is $C_{59}H_{97}ClN_2O_4$, its molecular weight is 933.9, and its CAS number is 41085-99-8. DiI is a lipophilic membrane dye that can gradually stain the cell membrane of the whole cell by lateral diffusion after entering the cell membrane. The fluorescence of DiI is very weak before it enters the cell membrane, and only after it enters the cell membrane can it be excited to emit strong fluorescence. It is often used together with DiA for dual-color labeling of the cell membrane. As a tracer or long-term tracer, DiI can be widely used in forward or reverse, living or fixed nerve cells or tissues. DiI usually did not affect cell viability. Dii-labeled neurons could survive for up to 4 weeks in vitro culture and up to a year in vivo. The migration rate of DiI on the membrane of fixed neurons was 0.2-0.6 mm/day, and the migration rate of dii on the membrane of living neurons was 6 mm/day. In addition to the fluorescent labeling of cell membrane, DiI can also be used to detect cell fusion and adhesion, cell migration during development or transplantation, detect the diffusion of lipid on the cell membrane by FRAP (light decolorization fluorescence recovery technique), detect cytotoxicity and label lipoprotein. After DiI staining, the fixation of paraformaldehyde (no other reagents such as methanol can be used) can be carried out, but the process of permeabilization after staining is not recommended. In addition, plasma membrane staining was also well performed after fixed permeabilization with 0.1% TritonX-100 at room temperature. According to the calculation of using 100 μL of staining working solution at a concentration of 10 μM, 10 mg of working solution can be used 10,707 times.

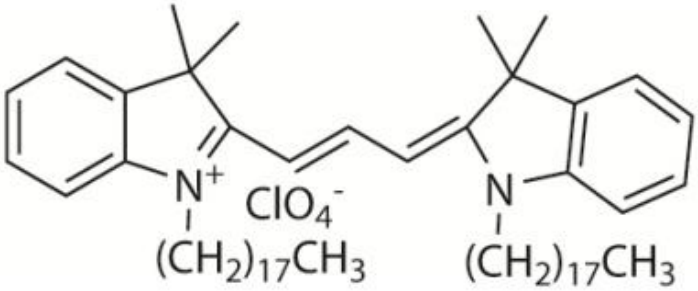

Figure 1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
DiI (DiIC18(3))	10 mg	-20℃, protected from light

Materials Required but Not Supplied

• Fluorescent microscope or flow cytometry, precision pipettes, disposable pipette tips, DMSO, EtOH

Assay Procedure

1. Preparation of staining solution

(1) Preparation of storage solution: The storage solution was prepared with anhydrous DMSO or EtOH at a concentration of 1-10 mM.

Note: Unused storage solution was stored in aliquots at -20℃ to avoid repeated freezing and thawing.

(2) Preparation of working solution: The storage solution was diluted with appropriate buffer (e.g., serum-free medium, HBSS or PBS) to prepare working solution with a concentration of 1-10 μM.

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

2. Staining of Suspension cell

(1) The appropriate volume of staining solution was added to resuspend the cells, so that the density was 1×10^6/mL.

(2) The cells were incubated at 37℃ for 5-20 min, and the optimal culture time was different for different cells. 20 min can be used as the initial incubation time, after which the system can be optimized to obtain uniform labeling effect.

(3) At the end of incubation, the cells were centrifuged at 1,000-1,500 rpm for 5 min. The supernatant was poured and cells were resuspended by slowly adding the prewarmed growth medium at 37℃ again.

(4) Repeat step (3) more than twice.

3. Staining of adherent cells

(1) Adherent cells were cultured on sterile cover slips.

(2) Remove the cover slip from the medium, sucking off excess culture, but leaving the surface moist.

(3) Add 100 μL of dye working solution to one corner of the cover slip, and gently shake to evenly cover all cells with the dye.

(4) The cells were incubated at 37℃ for 5-20 min, and the optimal culture time was different for different cells. 20 min can be used as the initial incubation time, after which the system can be optimized to obtain uniform labeling effect.

(5) Blot the dye working solution, wash the cover glass with culture solution 2-3 times, cover all cells with prewarmed medium each time, incubate for 5-10 min, and then blot the medium dry, but keep the surface moist.

4. Results testing

Samples can be examined in culture medium and can be imaged by fluorescence microscopy or analyzed by flow cytometry.

Precautions

1. Please immediately centrifugal the product to the bottom of the tube before use, and then conduct the subsequent experiments.
2. When DiI stains fixed cell or tissue samples, 4% paraformaldehyde prepared in PBS is usually used for fixation, and the use of other inappropriate fixative will result in high fluorescence background.
3. Fluorescent dyes all have quenching problems, please try to avoid light to slow down fluorescence quenching.
4. For your safety and health, please wear a lab coat and wear disposable gloves to operate.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.