DiD (DiIC18(5))

Cat #: BMD0073 Size: 10 mg

	DiD (DiIC18(5))		
REF	**Cat #:** BMD0073	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:** 1-5 μM
	Excitation/Emission wavelengths: Ex/Em(MeOH)=644/663 nm		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The molecular formula of DiD (DiIC18(5)) is $C_{67}H_{103}ClN_2O_3S$, the molecular weight is 1052.1, and the CAS number is 362596-00-7. DiD dye is a member of the lipophilic fluorescent dye family, which can be used to stain cell membranes and other lipid-soluble biological structures. When DiD is bound to the cell membrane, its fluorescence intensity is greatly enhanced, and these dyes have a high quenching constant and excited state lifetime. Once the cell is stained, such dyes diffuse throughout the cell membrane and at optimal concentrations stain the entire cell membrane. DiD (far red fluorescence) can be used to image live cells and flow analysis. DiD can be excited by 633 nm He-Ne laser and has longer excitation wavelength and emission wavelength than DiI (a common cell fluorescent dye), which is more valuable in cell and tissue staining. After DiD staining, the fixation of paraformaldehyde (no other reagents such as methanol) 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. Based on the calculation of using 100 μL of staining working solution at a concentration of 5 μM each time, 10 mg of working solution can be used about 19,009 times.

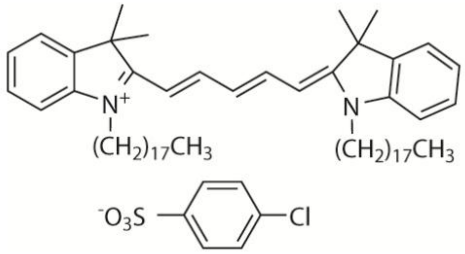

Figure 1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
DiD (DiIC18(5))	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 1-5 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 1-5 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 2-20 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 2-20 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 DiD 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.