DiO (DiOC18(3))

Cat #: BMD0072 Size: 10 mg

	DiO (DiOC18(3))		
REF	**Cat #:** BMD0072	LOT	**Lot #:** Refer to product label
	Application range: Cell membrane fluorescent dye, mainly used for cell imaging, cell tracing and tracing		**Recommended working concentrations:** 5-10 μM
	Excitation/Emission wavelengths: Ex/Em (MeOH)= 484/501 nm		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

DiO (DiOC18(3)) is one of the most commonly used cell membrane fluorescent probes with green fluorescence, its molecular formula is $C_{53}H_{85}ClN_2O_6$, its molecular weight is 881.7, and its CAS number is 34215-57-1. DiO is a lipophilic membrane dye that can gradually stain the cell membrane of the whole cell by lateral diffusion after entering the cell membrane. DiO fluorescence is very weak before entering the cell membrane. When it is combined with the cell membrane, its fluorescence intensity is greatly enhanced. After being excited, DIO can emit green fluorescence, which has a high quenching constant and excited state lifetime, and can be detected by standard FITC filter. DiO is widely used as a tracer or long-term tracer in forward or reverse direction, living or fixed nerve and other cells or tissues. DiO usually does not significantly affect cell viability. In addition to cell membrane fluorescence labeling, DiO can also be used to detect cell fusion and adhesion, cell migration during development or transplantation, the diffusion of lipid on the cell membrane by FRAP (light decolorization fluorescence recovery technique), detection of cytotoxicity and labeling of lipoproteins. After DiO staining, fixation with 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. DiO staining was usually less intense than DiI and was sometimes completely lost in fixed tissues. 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 about 11,341 times.

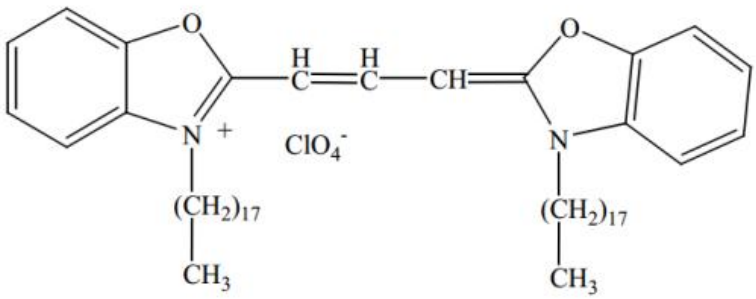

Figure 1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
DiO (DiOC18(3))	10 mg	4℃, protected from light

Materials Required but Not Supplied

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

Assay Procedure

1. Preparation of staining solution

(1) Preparation of storage solution: The storage solution was prepared with anhydrous DMSO, anhydrous DMF or EtOH at a concentration of 1-5 mM. DiO is more soluble in anhydrous DMSO and anhydrous DMF than in EtOH.

Note: The unused storage solution should be stored at -20℃ to avoid repeated freezing and thawing. If it is found to be difficult to dissolve, it can be heated appropriately and sonicated to promote dissolution.

(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 1-30 μM. The most commonly used working solution concentration was 5-10 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 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 DiO 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.