DiO

Cat No. ID5570

Storage: Powder: -20℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year (protect from light)

Introduction

DiO, also known as DiOC18(3), is one of the most commonly used cell membrane fluorescent probes, showing green fluorescence. DiO is a lipophilic membrane dye that enters the cell membrane and gradually stains the entire cell membrane by lateral diffusion.DiO fluoresces very weakly before it enters the cell membrane, but its fluorescence intensity is greatly enhanced when it binds to the membrane, and it can emit green fluorescence after excitation with a high quenching constant and excited state lifetime. It has a high quenching constant and excited state lifetime, and can be detected with a standard FITC filter.

DiO is widely used as a tracer or long-term tracer in forward or reverse direction, in living or fixed cells or tissues such as nerves, etc. DiO usually does not significantly affect the viability of the cells. In addition to fluorescent labeling of cell membranes, DiO can be used for detecting cell fusion and adhesion, cell migration during development or transplantation, detecting lipid diffusion across cell membranes by FRAP (Fluorescence Recovery After Photobleaching), detecting cytotoxicity, and labeling lipoproteins. After Photobleaching) to detect lipid diffusion across cell membranes, detect cytotoxicity and label lipoproteins.

DiO staining can be followed by fixation with paraformaldehyde (no other reagents such as methanol may be used), but the process of permeabilization after staining is not recommended. In addition, plasma membrane staining can be performed well after fixation permeabilization (permeabilization with 0.1% TritonX-100 at room temperature). DiO staining intensity is usually lower than DiI and is sometimes completely lost in fixed tissues.

Note: When DiO staining fixed cell or tissue samples, 4% paraformaldehyde formulated in PBS is usually used for fixation, and the use of other inappropriate fixatives will result in a higher fluorescence background.

Parameters

Ex/Em: 484/501 nm

CAS No: 34215-57-1

Molecular Formula: $C_{53}H_{85}ClN_2O_6$

Molecular Weight: 881.70

Solubility: Soluble in DMSO/Ethanol

Application: Cell membrane fluorescent dyes, mainly used for cell imaging, cell tracing and tracking.

Protocols (only for reference)

1. Preparation of staining solution

(1) Preparation of stock solution: The stock solution is prepared with DMSO at a concentration of 1~5 mM.

Note:a. Unused storage solution is recommended to be stored at -20℃ to avoid repeated freezing and thawing.

b. When it is found to be difficult to dissolve, it can be treated with ultrasonic treatment or appropriate heating to promote dissolution.

c. Moisture-absorbing DMSO has a significant effect on the solubility of the product, please use freshly opened DMSO.

(2) Working solution preparation: Dilute the reservoir solution with a suitable buffer (e.g. serum-free medium, HBSS or PBS) and prepare a working solution with a concentration of 1-30 μM. The most commonly used working solution concentration is 5-10 μM.

Note: The final concentration of the working solution is recommended to be optimized for different cell lines and experimental systems. It is recommended to start with 10 times the recommended concentration to find the optimal concentration.

2. Staining of suspended cells

(1) Resuspend the cells by adding the appropriate volume of staining working solution to a density of 1×10^6/mL.

(2) Incubate the cells at 37°C for 2~20 min, the optimal incubation time varies for different cells.

(3) At the end of incubation, centrifuge the cells at 1000~1500 rpm for 5 min, decant the supernatant, and resuspend the cells by slowly adding 37℃ preheated growth medium again.

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

3. Staining of adherent cells

(1) Culture the adherent cells on sterile coverslips.

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

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

(4) Incubate the cells at 37℃ for 2~20 min, the optimal incubation time is different for different cells.

(5) Absorb the dye working solution, wash the coverslip with culture solution 2~3 times, cover all the cells with pre-warmed medium each time, incubate for 5~10 min, and then absorb the medium. However, keep the surface moist.

4. Results testing

Samples can be assayed in culture media and can be analyzed by fluorescence microscope imaging or flow cytometry.

Note

1. Please centrifuge the product instantaneously to the bottom of the tube before use and

subsequent experiments.

2. DiO staining of fixed cell or tissue samples is usually done with 4% paraformaldehyde formulated in PBS, and the use of other inappropriate fixatives will result in a higher fluorescence background.

3. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.

4. For your safety and health, please wear lab coat and disposable gloves.

Related Literature

[1] Kuang J, Rao ZY, Zheng DW, Kuang D, Huang QX, Pan T, Li H, Zeng X, Zhang XZ. Nanoparticles Hitchhike on Monocytes for Glioblastoma Treatment after Low-Dose Radiotherapy. ACS Nano. 2023 Jul 25;17 (14) :13333-13347. doi: 10.1021/acsnano.3c01428. Epub 2023 Jul 5. PMID: 37404077. (IF:17.1)

Related Products

ID5550 DiA
ID5560 DiD
ID5580 DiOC6(3) iodide
ID5590 DiOC2(3) iodide
ID5600 Di-8-ANEPPS
IR1840 RH 237
IR1850 RH 421