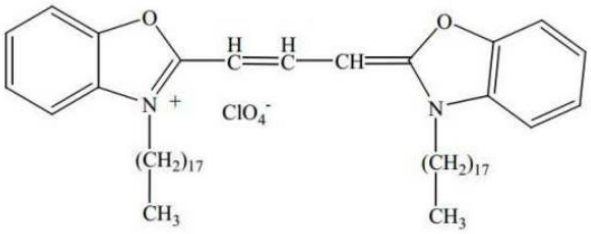
DiO

V02

Cat: D5840
Specification: 10mg
Storage: Keep valid and away from light at 2-8℃, with a shelf life of 2 years. The prepared storage solution should be kept valid, stored at -20℃ and protected from light, with a shelf life of 6 months.

Product Information
CAS： 34215-57-1
Appearance (Character)： Yellow solid
Molecular Formula: $C_{53}H_{85}ClN_2O_6$
Molecular Weight:882
Solubility: DMSO 10mg/mLDiO is soluble in absolute ethanol, DMSO, and DMF. When it is difficult to dissolve, it can be heated or treated with ultrasound.
λEx/λEm(MeOH)=484/501 nm
Purity/grade: 0.98/AR
Molecular Structure:



Introduction

DiO, also known as DiOC18(3), is one of the most commonly used fluorescent probes for cell membranes, emitting green fluorescence. It is a lipophilic membrane dye that, upon entering the cell membrane, laterally diffuses to gradually stain the entire cellular membrane. Prior to entering the cell membrane, DiO exhibits very weak fluorescence, but upon binding to the membrane, its fluorescence intensity significantly increases, enabling it to emit green fluorescence upon excitation. With a high quenching constant and excited state lifetime, DiO can be detected using standard FITC filters. As a tracer or long-term tracer, DiO is widely utilized in forward or reverse tracing of live or fixed neural and other types of cells or tissues. Typically, DiO does not significantly affect cell viability.

Apart from its use as a fluorescent membrane marker, DiO can also be employed to detect cell fusion and adhesion, cell migration during development or transplantation, monitor lipid diffusion on cell membranes through FRAP (Fluorescence Recovery After Photobleaching), assess cell toxicity, and label lipoproteins. After staining with DiO, cells can be fixed with paraformaldehyde (methanol or other reagents are not recommended). However, permeabilization post-staining is not advised. Additionally, excellent plasma membrane staining can be achieved even after fixation and permeabilization (using 0.1% TritonX-100 at room temperature). Notably, the staining intensity of DiO is generally lower than that of DiI and may be completely lost in fixed tissues in some cases.

Usage Instructions:

1. Preparation of Staining Solution

(1) Preparation of Stock Solution in Anhydrous DMSO, Anhydrous DMF, or EtOH: Prepare a stock solution with a concentration of 1–5 mM using anhydrous DMSO, anhydrous DMF, or EtOH. DiO exhibits higher solubility in DMSO and DMF compared to EtOH.

Note:

a. Unused stock solutions should be aliquoted and stored at -20°C to avoid repeated freeze-thaw cycles.

b. If difficulty is encountered in dissolution, gentle heating and ultrasonic treatment can be applied to facilitate dissolution.

(2) Preparation of Working Solution: Dilute the stock solution with an appropriate buffer (e.g., serum-free medium, HBSS, or PBS) to obtain a working solution with a concentration of 1–30 μM. The most commonly used working concentration is 5-10 μM.

Note:

The final concentration of the working solution is recommended to be optimized based on different cell lines and experimental systems. It is suggested to start the optimization process within a 10-fold range of the recommended concentration.

2. Staining of Suspension Cells

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

(2) Incubate the cells at 37°C for 2–20 minutes, as the optimal incubation time varies among different cell types. Start with an incubation time of 20 minutes and then optimize the system to achieve uniform labeling results.

(3) After incubation, centrifuge the cells at 1000–1500 rpm for 5 minutes. Discard the supernatant and gently resuspend the cells in pre-warmed growth medium at 37°C.

(4) Repeat step (3) two or more times.

3. Staining of Adherent Cells

(1) Cultivate the adherent cells on sterile coverslips.

(2) Remove the coverslips from the culture medium and aspirate excess medium, but ensure the surface remains moist.

(3) Add 100 μL of the staining working solution to one corner of the coverslip and gently shake to evenly distribute the dye over all cells.

(4) Incubate the cells at 37°C for 2–20 minutes, as the optimal incubation time varies among different cell types. Start with an incubation time of 20 minutes and then optimize the system to achieve uniform labeling results.

(5) Aspirate the staining working solution and wash the coverslips with culture medium 2–3 times. Each time, cover all cells with pre-warmed medium, incubate for 5–10 minutes, then aspirate the medium, ensuring the surface remains moist.

4. Result Detection

The samples can be examined directly in the culture medium, either by fluorescence microscopy imaging or flow cytometry analysis.

Application Range: As a fluorescent dye for cell membranes, DiO is primarily used for cell imaging, cell tracking, and tracing.

Note:

1. When staining fixed cells or tissue samples with DiO, it is recommended to use 4% paraformaldehyde dissolved in PBS for fixation. Using other inappropriate fixatives may result in a higher fluorescent background.
2. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
3. For your safety and health, please wear a laboratory coat and wear disposable gloves to operate.