DiD

Cat No. ID5560

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

Introduction：

DiD dyes are members of a family of lipophilic fluorescent dyes that can be used to stain cell membranes and other lipid-soluble biological structures. The fluorescence intensity of DiD is greatly enhanced when it binds to cell membranes, and these dyes have high quenching constants and excited state lifetimes. Once the cells are stained, the dye spreads across the entire cell membrane and at optimal concentrations can stain the entire cell membrane. DiD (far-red fluorescence) can be used to image and flow analyze living cells. DiD can be excited with a 633 nm He-NE laser and has a longer excitation and emission wavelength than DiI (a common cell fluorescent dye), making it more valuable in cell and tissue staining.

Fixation with paraformaldehyde (no other reagents such as methanol may be used) can be performed after DiD staining, but the process of permeabilization after staining is not recommended. In addition, plasma membrane staining can be performed well after fixed permeabilization (permeabilization with 0.1% TritonX-100 at room temperature).

DiD staining of fixed cell or tissue samples is usually performed using 4% paraformaldehyde formulated in PBS, and the use of other inappropriate fixatives can result in a high fluorescence background.

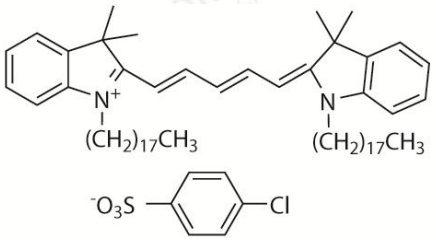
Parameter：

Ex/Em: 644/663nm

CAS No: 362596-00-7

Molecular Formula: $C_{67}H_{103}ClN_2O_3S$

Molecular Weight: 1052.1



Purity: ≥98%

Appearance: Solid

Solubility: Soluble in DMSO ≥5mg/mL

Application: Cell membrane fluorescent dyes, neuronal prograde and retrograde tracing, cellular long-term tracing

Protocols (only for reference)

Preparation of storage solution

Prepare 1~10 mM stock solution in DMSO. For example, 1 mg of DiD powder was dissolved in 0.9505 mL of DMSO to obtain 1 mM of DiD stock solution.

Note:

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

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

Preparation of working fluid

Dilute the reservoir solution with a suitable buffer (e.g. serum-free medium or PBS, etc.) to make a working solution of 1~10 μM.

Note:

a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.

b. If it is found to be difficult to dissolve, it can be sonicated appropriately to promote dissolution.

c. Please adjust the concentration of the working liquid according to the actual situation, and use immediately after dissolution

Staining of suspension cells

(1) Resuspend the cells by adding an appropriate volume of Staining Working Solution to a density of 1×10^6/mL.

(2) Incubate the cells at 37℃ for 5~20 min, the optimal incubation time was different for different cells. The optimal incubation time varied from cell to cell. The 20 min can be used as the starting incubation time, after which the system can be optimized to obtain uniform labeling effect.

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

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

Staining of adherent cells

(1) Adherent cells were cultured 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 so that the dye evenly covers all cells.

(4) Incubate the cells at 37℃ for 5~20 min, the optimal incubation time is different for different cells. The optimal incubation time varied from cell to cell. The 20 min can be used as the starting incubation time, after which the system can be optimized to obtain uniform labeling effect.

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

Results testing

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

Note：

1. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
2. For your safety and health, please wear lab coat and disposable gloves.
3. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.

Related Literature：

[1]. Yu H, Fan J, Shehla N, Qiu Y, Lin Y, Wang Z, Cao L, Li B, Daniyal M, Qin Y, Peng C, Cai X, Liu B, Wang W. Biomimetic Hybrid Membrane-Coated Xuetongsu Assisted with Laser Irradiation for Efficient Rheumatoid Arthritis Therapy. ACS Nano. 2022 Jan 25;16(1):502-521. doi: 10.1021/acsnano.1c07556. Epub 2021 Dec 29. PMID: 34965104.（IF：15.88）

Note: More literature is available on the Solarbio website.

Related Products：

ID3750 DiI
ID5550 DiA
ID5560 DiD