

## DiD perchlorate \*Oil\*

# [1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine

V02

## perchlorate]

Cat: D8940

Specification:25mg

**Storage:** Store at -20°C, avoid light, and it is valid for 5 years.

**Product Information** 

CAS: 362596-00-7

English name: DiD perchlorate \*Oil\* [1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine

(CH<sub>2</sub>)<sub>17</sub>CH<sub>3</sub>

(CH<sub>2</sub>)<sub>17</sub>CH<sub>3</sub>

perchlorate]

Appearance (Character): Dark blue solid

Molecular Formula: C67H103ClN2O3S

**Molecular Weight:** 1052.1

Ex/Em (MeOH) = 644/663 nm

**Solubility:** Soluble in ethanol, DMF and DMSO.

**Molecular Structure:** 

### Introduction

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 binds to the cell membrane, its fluorescence intensity greatly increases. These dyes have high quenching constants and excited state lifetimes. Once the cells are stained, these dyes diffuse throughout the cell membrane, and at optimal concentrations, they can stain the entire cell membrane. DiD far-red fluorescence can be used for imaging and flow analysis of living cells. DiD can be excited by a 633 nm He–Ne laser, with longer excitation and emission wavelengths than DiI, a common cell fluorescent dye, making it more valuable for cell and tissue staining. After DiD staining, fixation with paraformaldehyde and other reagents such as methanol can be performed, but it is not recommended to perform a permeabilization process after staining. In addition, after permeabilization with 0.1% TritonX-100 at a fixed room temperature, the plasma membrane staining can also be well performed.

### **Instructions for use:**

### 1. Preparation of dyeing solution

(1) Preparation of stock solution: Stock solution is prepared with anhydrous DMSO or EtOH at a concentration of 1-5 mM.

**Note:** Unused storage solution should be stored at -20°C to avoid repeated freezing and thawing.

(2) Preparation of working solution: dilute the stock solution with a suitable buffer such as serum-free medium, HBSS, or PBS to prepare a working solution with a concentration of 1-5  $\mu$ M.

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**Note:** The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems. It is recommended to start exploring the optimal concentration within a range of 10 times the recommended concentration.

## 2. Suspension cell staining

- (1) Add an appropriate volume of staining working solution to resuspend the cells, resulting in a density of  $1\times10^6$  cells/mL.
- (2) Incubate the cells at 237°C for 2-20 minutes, and the optimal culture time varies for different cells. 20 min can be used as the initial incubation time, and then the system can be optimized to obtain uniform labeling effects.
- (3) After incubation, centrifuge at 1000-1500 rpm for 5 minutes. Pour the supernatant and slowly add the growth medium preheated at 37°C to resuspend the cells.
  - (4) Repeat step (3) more than twice.

## 3. Adherent cell staining

- (1) Cultivate adherent cells on sterile coverslips.
- (2) Remove the coverslips from the culture medium, aspirate excess culture medium, but keep the surface moist.
- (3) Add 100  $\mu$ L of dye working solution to one corner of the coverslip and gently shake to evenly cover all cells with the dye.
- (4) Incubate the cells at 37°C for 2-20 minutes, and the optimal incubation time varies for different cells. 20 minutes can be used as the initial incubation time, and then the system can be optimized to obtain uniform labeling effects.
- (5) Aspirate the dye working solution, wash the coverslip with culture medium 2-3 times, covering all cells with pre-warmed medium each time, incubate for 5-10 minutes, and then aspirate the medium. However, keep the surface moist.

### 4. Result detection

The samples can be detected in the culture medium, and can be analyzed through fluorescence microscopy imaging or flow cytometry.

#### Note

- 1. Before use, please centrifuge the product briefly to the bottom of the tube before proceeding with subsequent experiments.
- 2. When fixing DiD-stained cells or tissue samples, 4% paraformaldehyde prepared in PBS is usually used. The use of other inappropriate fixatives may result in a higher fluorescence background.
- 3. Fluorescent dyes are prone to quenching, so please try to avoid light exposure to slow down fluorescence quenching.
- 4. The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
- 5. The products are all for scientific research use only. Do not use it for medical, clinical diagnosis or treatment, food and cosmetics, etc. Do not store them in ordinary residential areas.
- 6. For your safety and health, please wear laboratory clothes, disposable gloves and masks to operate.