# Product Information

## DiD

Catalog Number: D4019

Product Size: 10 mg

Application Scope: Cell tracing, tracking, cellular imaging

## Parameters

Appearance: Dark blue solid soluble in DMSO, DMF or EtOH

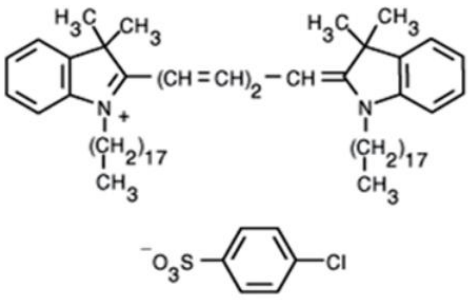
Ex/Em (MeOH): 644/663 nm

CAS No.: 127274-91-3

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

Molecular Weight: 1052.1

Molecular Structure:



## Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 12 months.

## Description

DiD is cyanine fluorescent dye with hydrophobic hydrocarbon tails, which can be used to stain cell membranes and other fat-soluble biological structures. It is weakly fluorescent in water but highly fluorescent and quite photostable when incorporated into membranes. It has an extremely high extinction coefficient and short excited-state life times (~1 nanosecond) in lipid environments. Once applied to cells, the dye diffuses laterally within the plasma membrane.

DiD is similar to DiI, but with longer absorption and emission wavelengths which is more valuable in cell and tissue staining. Cells can be fixed with formaldehyde either before or after staining, but not recommended for permeabilization.

## Protocol

**1. Dyeing liquid preparation**

(1) Stock solution: Use DMSO or EtOH to make a stock solution with a concentration of 1 to 5 mM.

Note: It is recommended to store the storage solution at -20 ℃, and aliquot it in small quantities to avoid repeated freeze-thaw cycles.

(2) Preparation of working solution: Dilute the storage solution with a suitable buffer (such as serum-free medium, HBSS or PBS) to prepare a working solution with a concentration of 1 to 5 μM.

Note: You may need to optimize the staining procedure for each particular cell type by varying the dye concentration, staining volume, labeling time, or wash steps.

**2. Suspension cell staining**

(1) Suspend cells at a density of $1\times10^6$/mL in working solution.

(2) Incubate for 20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 20 minutes and optimize as needed for uniform labeling.

(3) Pellet the cells by centrifugation at 1000～1500 rpm for 5 minutes.

(4) Remove the supernatant and wash the cells by gently



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resuspending them in warm (37°C) medium.

(5) Repeat the centrifugation and wash steps (Steps 3 and 4) two more times.

(6) Image fluorescence. Cells can be imaged in culture medium.

**3. Adherent cell staining**

(1) Remove growth medium from the cells.

(2) Add enough working solution to completely cover the cells.

(3) Incubate the cells at 37°C. The optimal incubation time will vary depending on the cell type. Start with 20 minutes and optimize as needed for uniform labeling.

(4) Remove the working solution.

(5) Wash the cells by adding fresh warm growth medium and incubating at 37°C for 5 minutes. Repeat this wash step two more times.

(6) Image fluorescence. Cells can be imaged in culture medium.

## Notes

1. When DiD stains fixed cell or tissue samples, the samples should be fixed with 4% paraformaldehyde in PBS. The use of other improper fixing solutions will result in a high fluorescence background.

2. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

3. For your safety and health, please wear lab coats and disposable gloves.



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