

# Product Information

## DiA (4-(4-Dihexadecylaminostyryl)-N-methylpyridinium iodide)

Catalog Number: D4059

Product Size: 50 mg

Application Scope: Cell tracing, tracking, cellular imaging

### Parameters

Appearance: Red solid soluble in DMSO

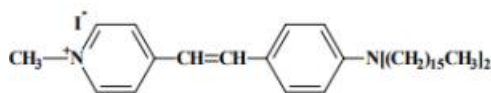
Ex/Em (MeOH): 491/613 nm

CAS NO.: 114041-00-8

Molecular Formula: C<sub>46</sub>H<sub>79</sub>IN<sub>2</sub>

Molecular Weight: 787

Molecular Structure:



### Storage

Store at 4 °C and protect from light. When stored as directed, product is stable for at least 12 months.

### Description

DiA is a green fluorescent membrane dye which diffuses much faster than DiO in cell membranes. DiA and DiI have been used together for two color membrane staining.

Cells can be fixed with formaldehyde (Do not use methanol or other fixatives) either before or after staining, but not recommended for permeabilization. The effect of fixed cells by DIA staining was better than that by DIO staining.

### Protocol

#### 1. Dyeing liquid preparation

(1) Preparation of stock solution: Use anhydrous DMSO, DMF or EtOH to make a stock solution with a concentration of 1 to

5mM. The solubility of DiA in DMSO and DMF is higher than the solubility in EtOH.

Notes: ① It is recommended to store the storage solution at -20 °C, and aliquot it in small quantities to avoid repeated freeze-thaw cycles.

② When it is difficult to dissolve, heat or sonication can be used to promote dissolution.

(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 30 μM. The most common working solution concentration is 5-10 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 1×10<sup>6</sup>/mL in working solution.

(2) Incubate for 5~20 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 5 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 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 5 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 DiA 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.

