Product Information

Neuro-DiO

Catalog Number: N4021

Product Size: 5 mg

Application Scope: Cell Tracing, Tracking, Cellular Imaging

Parameters

Appearance: Orange yellow solid soluble in ethanol, hexane, DMSO or vegetable oil

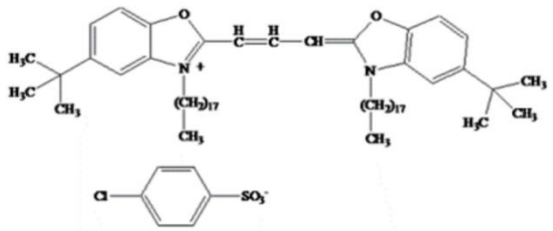
Ex/Em (MeOH): 484/501 nm

CAS No.: 127274-91-3

Molecular Formula: $C_{16}H_{17}Cl_2N_5$

Molecular Weight: 1086

Molecular Structure:



Storage

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

Description

Neuro-DiO as a replacement for the green fluorescent DiO, which has been found to be difficult to use for neurons and cell suspension due to low solubility, tendency to form aggregates and slow lateral diffusion rate. Neuro-DiO and DiO have nearly identical absorption and emission spectra, but the former has better solubility in membranes and does not form nonfluorescent aggregates, which also tend to slow down the dye diffusion rate in membranes.

Cells can be fixed with formaldehyde either before or after Neuro-DiO staining, but not recommended for permeabilization.

Protocol

1. Dyeing liquid preparation

(1) Preparation of 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 1×10^6/mL in working solution.

(2) Incubate for 5 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.

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(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 Neuro-DiO 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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