

## Product Data Sheet

Product Name: Cy7 DiC18

Cat. No.: GC35773

### Chemical Properties

Cas. No. 100068-60-8

SMILES CCCCCCCCCCCCCCCCC[N+]1=C(/C=C/C=C/C=C/C=C/C=C2N(CCCCCCCCCCCCCCCC(CC)C3=C(C=CC=C3)C/2(C)C)C(C)(C)C4=C1C=CC=C4.[I-]

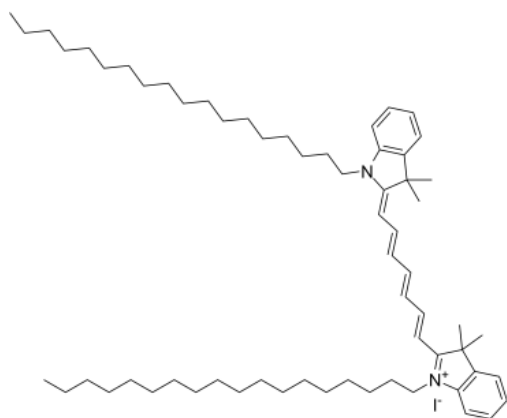
Formula  $C_{63}H_{101}IN_2$  M.Wt 1013.39

Solubility Soluble in DMSO Storage 4°C, protect from light

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



### Protocol

This plan only provides a guide, please modify it to meet your specific needs. 1. Preparation of cell membrane staining solution

(1) Prepare DMSO or EtOH storage solution: The storage solution is prepared with DMSO or EtOH, with a concentration of 1~5mM.

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Note: Unused storage solution should be stored in aliquots at -20°C in the dark and avoid repeated freezing and thawing.

(2) Preparation of working solution: Dilute the storage solution with an appropriate buffer (such as serum-free medium, HBSS or PBS) to prepare a working solution with a concentration of 0.5~5  $\mu$ M.

Note: The final concentration of the working solution is prepared based on experience with different cells and experiments. Optimum conditions can be found from more than ten times the recommended concentration.

### 2. Suspension cell staining

(1) Centrifuge the suspended cells at 1000 *g* for 3-5 minutes at 4°C and discard the supernatant. Wash twice with PBS for 5 minutes each time.

(2) Add 1 mL of dye working solution and incubate at room temperature in the dark for 5-30 minutes.

Note: The optimal culture time for different cells is different and can be adjusted according to specific experimental needs.

(3) After the incubation, centrifuge at 1000-1500 *g* for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(4) Resuspend the cells in pre-warmed serum-free cell culture medium or PBS. Observe by fluorescence microscopy or flow cytometry.

### 3. Staining of adherent cells

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100 $\mu$ L of dye working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye.

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(4) Incubate at room temperature in the dark for 5-30 minutes. The optimal culture time for different cells is different.

(5) After the incubation, discard the dye working solution and wash the coverslip 2 to 3 times with pre-warmed culture solution.

### 4. Exosome staining

(1) Take an appropriate volume of Di dye stock solution and add it to the collected exosome filtration supernatant to reach a final concentration of 5  $\mu$ M, and mix gently using a pipette;

(2) Incubate at room temperature in the dark for 30 minutes, turning gently every 5 minutes.

(3) Transfer the incubated sample to a centrifuge tube and centrifuge it in an ultracentrifuge at 100000g and 4°C for 30 minutes;

(4) After centrifugation, carefully tilt and discard the supernatant in a clean bench. Use a micropipette with a sterile tip to remove the liquid that forms in the test tube but does not drip. This is the marked outer layer. secretion body;

Note: During this step, the free dye disappears with the decanted supernatant, so the solution can be decanted, but always be careful not to lose the precipitate.

5. Microscope detection: The excitation/emission light of Cy7 DiC18 (DiR) is 750/780nm respectively.

### Precautions:

1) Fluorescent dyes all have quenching problems. Please avoid light as much as possible to slow down fluorescence quenching.

2) For your safety and health, please wear a lab coat and disposable gloves.

### Background

Cy7 DiC18 (DiIC 18(7); DiR) is a lipophilic long-chain carbocyanine dye that produces near-infrared

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fluorescence and is widely used as Di to label cells, organelles, liposomes, viruses and lipoproteins. The two long 18-carbon chains of DiR insert into the cell membrane, resulting in specific and stable cell staining with little dye transfer between cells. DiR exhibits pronounced infrared fluorescence, which facilitates multicolor imaging and flow cytometry analysis of living cells. DiR is of great significance in in vivo imaging or tracking. The emitted infrared light can efficiently pass through cells and tissues, and the background fluorescence level in the infrared range is very low<sup>[1]</sup>.

### References:

[1]. Shiyang Fu, Ruihao Yang, Junjie Ren, Jiahui Liu, Lei Zhang, Zhigang Xu, Yuejun Kang, and Peng Xue. Catalytically Active CoFe<sub>2</sub>O<sub>4</sub> Nanoflowers for Augmented Sonodynamic and Chemodynamic Combination Therapy with Elicitation of Robust Immune Response. ACS Nano Article ASARDOI: 10.1021/acsnano.1c03128.

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