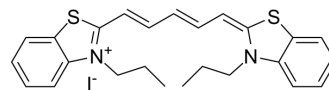


## DiSC3(5)

|                    |  |
|--------------------|--|
| Cat. No.:          | HY-D0085   |
| CAS No.:           | 53213-94-8   |
| Molecular Formula: | C <sub>25</sub> H <sub>27</sub> IN <sub>2</sub> S <sub>2</sub>   |
| Molecular Weight:  | 546.53   |
| Target:            | Fluorescent Dye  |
| Pathway:           | Others   |
| Storage:           | 4°C, sealed storage, away from moisture and light<br>* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light) |



### SOLVENT & SOLUBILITY

|   |  |                                      |                 |                 |                 |                  |
|---|--|--------------------------------------|-----------------|-----------------|-----------------|------------------|
| In Vitro  | DMSO : 16.67 mg/mL (30.50 mM; ultrasonic and warming and heat to 60°C)   |                                      |                 |                 |                 |                  |
|   | Preparing Stock Solutions  | <div>Solvent<br/>Concentration</div> | <div>Mass</div> | <div>1 mg</div> | <div>5 mg</div> | <div>10 mg</div> |
|   |  | 1 mM                                 |                 | 1.8297 mL       | 9.1486 mL       | 18.2973 mL       |
|   |  | 5 mM                                 |                 | 0.3659 mL       | 1.8297 mL       | 3.6595 mL        |
|   |  | 10 mM                                |                 | 0.1830 mL       | 0.9149 mL       | 1.8297 mL        |
| Please refer to the solubility information to select the appropriate solvent. |  |                                      |                 |                 |                 |                  |
| In Vivo   | 1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline<br>Solubility: 1.25 mg/mL (2.29 mM); Suspended solution; Need ultrasonic |                                      |                 |                 |                 |                  |
|   | 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)<br>Solubility: 1.25 mg/mL (2.29 mM); Suspended solution; Need ultrasonic            |                                      |                 |                 |                 |                  |

### BIOLOGICAL ACTIVITY

|             |  |
|-------------|--|
| Description | DiSC3(5) is a fluorescent probe commonly used as a tracer dye to evaluate mitochondrial membrane potential. The excitation/emission wavelength of DiSC3(5) is up to 622/670 nm. DiSC3(5) can inhibit the respiratory system associated with mitochondrial NAD, and the IC <sub>50</sub> value is 8 μM. DiSC3(5) in the presence of Na <sup>+</sup> /K <sup>+</sup> -ATPase inhibitor ouabain 2 can induce membrane hyperpolarization of Ehrlich ascites tumor cells <sup>[1][2][3]</sup> .   |
| In Vitro    | 1. Preparation of DiSC3(5) membrane staining solution:<br>1.1 Preparation of DMSO or EtOH reserve solution: The reserve solution should be prepared in DMSO or EtOH at 1-5 mM.<br>Note 1) : The unused portion of the reserve solution should be stored at -20 °C. Avoid repeated freezing/thawing cycles.<br>1.2 Prepare the working solution: Dilute the reserve solution into a suitable buffer, such as serum-free medium, HBSS or PBS, to make a working solution of 1 to 5 μM.<br>Note 2) : For different cell types and/or experimental conditions, the concentration of the working solution should be |

determined according to experience. It is recommended to test at concentrations at least in excess of the ten-fold range.

2. Dye the cells into a suspension:

2.1 The suspended cell density in the dye working solution was  $1 \times 10^6$ / mL.

2.2 Incubate at 37°C for 2-20 minutes. The culture time depends on the cell type. It is incubated first for 20 minutes and then optimized as needed to obtain even labeling.

2.3 Centrifuge the labeled suspension tubes at 1000 to 1500rpm for 5 minutes.

2.4 Remove the supernatant and gently re-suspend the cells in a preheated (37°C) growth medium.

2.5 Wash twice according to steps 2.3 and 2.4.

3. Staining adherent cells:

3.1 The adherent cells were cultured on a sterile glass cover slide.

3.2 Remove the cover glass from the growth medium and gently drain the excess medium. Place the cover glass in the humidity box.

3.3 Transfer the 100  $\mu$ L dye working solution to the corner of the cover glass and stir gently until all cells are covered.

3.4 Incubate the cover glass at 37°C for 2-20 minutes. The culture time varies according to cell type. Incubation is started for 20 minutes and then optimized as needed to obtain even labeling.

3.5 Drain the dye working solution and clean the cover glass with growth medium two to three times. For each wash cycle, cover the cells with preheated growth medium, incubate for 5-10 minutes, and then drain the medium.

4. Microscope inspection.

5. Flow cytometry detection:

Dis-labeled cells can be analyzed using conventional FL3 flow cytometry detection channels.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## REFERENCES

[1]. Sims PJ, et al. Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. *Biochemistry*. 1974 Jul 30;13(16):3315-30.

[2]. Smith TC, et al. The effect of the fluorescent probe, 3,3'-dipropylthiodicarbocyanine iodide, on the membrane potential of Ehrlich ascites tumor cells. *Biochem Biophys Res Commun*. 1980 Jul 31;95(2):722-7.

[3]. Yamamoto T, et al. Multiple effects of DiS-C3(5) on mitochondrial structure and function. *Eur J Biochem*. 2004;271(17):3573-3579.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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