

Product Data Sheet

Product Name: DiIC1(5)
Cat. No.: GC43463

Chemical Properties

Cas. No. 36536-22-8

SMILES CC1(C)C2=CC=CC=C2N(C)/C1=C/C=C/C=C/C3=[N+](C)C4=C(C=CC=C4)C3(C)C.[I-]

Formula C₂₇H₃₁N₂•I M.Wt 510.5

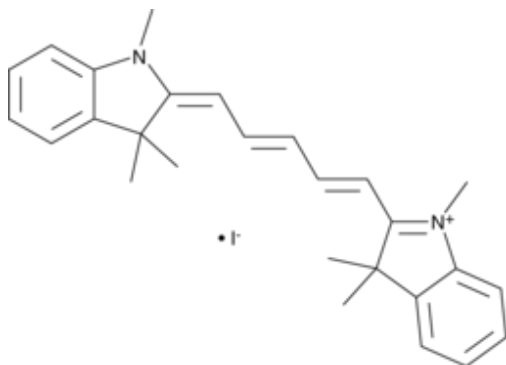
Solubility DMF: 20 mg/ml, DMSO: 20 mg/ml, Ethanol: 2 mg/ml, PBS (pH 7.2): 0.3 mg/ml

Storage Store at -20°C

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

Determination of mitochondrial membrane potential using DiIC1(5) [1]

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Preparation of cell membrane staining solution

(1) Prepare the DiIC1(5) stock solution: The DiIC1(5) stock solution is prepared with DMSO, at a concentration

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of 1-5mM.

Note: Unused stock solution should be aliquoted and stored at -20°C in a dark place to avoid repeated freezing and thawing.

(2) Prepare the working solution: Dilute the stock solution with a suitable buffer (such as: serum-free medium or PBS), to prepare a working solution at a concentration of 0.1-5μM.

Note: The final concentration of the working solution is determined based on the experience of different cells and experiments. You can search for the optimal conditions from ten times the recommended concentration upwards.

2. Staining of suspended cells

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

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

Note: The optimal incubation time for different cells varies. You can adjust working solution according to the

specific experimental requirements.

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

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

3. Staining of adherent cells

(1) Cultivate adherent cells on a sterile cover glass.

(2) Remove the cover glass from the culture medium, aspirate the excess medium, and place the cover glass in a humid environment.

(3) Add 100μl of the dye working solution from one corner of the cover glass, gently shake to evenly cover all cells.

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

(5) After incubation, discard the dye working solution, and wash the cover glass twice with prewarmed culture medium.

4. Microscopy detection: The excitation/emission wavelengths of the DiIC1(5) solution are 638/675nm.

Notes:

1) Fluorescent dyes have quenching problems. Please try to avoid light exposure to reduce fluorescence quenching.

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

References:

[1] Bucher K, Malama E, Siuda M, et al. Multicolor flow cytometric analysis of cryopreserved bovine sperm: a tool for the evaluation of bull fertility[J]. Journal of dairy science, 2019, 102(12): 11652-11669.

Background

DiIC1(5) is a signal-off fluorescent probe for the detection of mitochondrial membrane potential disruption, with excitation/emission of 638/675nm, respectively [1]. DiIC1(5) penetrates the plasma membrane of eukaryotic cells and accumulates primarily in mitochondria with active membrane potentials, and a breakdown of the mitochondrial membrane potential reflects cell death and is detected by a drop in the DiIC1(5) signal [2]. DiIC1(5) has been widely used in multichannel flow cytometry to study the activation of apoptosis in sperm[3]. DiIC1(5) can be used to develop the novel flow cytometry assay to measure mitochondrial reactive oxygen intermediates (ROI) production and to study the functional integrity of respiratory chain complexes at the single-cell level[4].

References:

[1] Munoz L E, Maueröder C, Chaurio R, et al. Colourful death: Six-parameter classification of cell death by flow cytometry—Dead cells tell tales[J]. Autoimmunity, 2013, 46(5): 336-341.

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- [2] Janko C, Munoz L, Chaurio R, et al. Navigation to the graveyard-induction of various pathways of necrosis and their classification by flow cytometry[M]//Necrosis: Methods and Protocols. Totowa, NJ: Humana Press, 2013: 3-15.
- [3] Kim H H, Funaro M, Mazel S, et al. Flow cytometric characterization of apoptosis and chromatin damage in spermatozoa[J]. Reproductive BioMedicine Online, 2013, 26(4): 393-395.
- [4] Pham N A, Robinson B H, Hedley D W. Simultaneous detection of mitochondrial respiratory chain activity and reactive oxygen in digitonin-permeabilized cells using flow cytometry[J]. Cytometry: The Journal of the International Society for Analytical Cytology, 2000, 41(4): 245-251.

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