DiBAC4(3)

Cat: ID3780
Storage: Powder: 2-8℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year（protect from light）

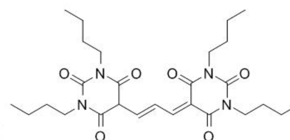
Introduction

DiBAC4(3) is a voltage-sensitive, lipophilic anionic fluorescent dye that can be used to detect the membrane potential of cells; it is non-fluorescent itself and fluoresces when it enters the cell and binds to proteins in the cytoplasm. When DiBAC4(3) enters the cell, the intracellular fluorescence intensity increases, i.e., an increase in the membrane potential indicates that the cell is depolarized, and, conversely, a decrease in the intensity of the intracellular fluorescence, i.e., a decrease in the membrane potential, indicates that the cell is Hyperpolarization.

DiBAC4(3) is a sensitive slow response probe for measuring cell membrane potential. Typically, slow response probes show potentially dependent changes in their transmembrane distribution accompanied by changes in fluorescence. The magnitude of their optical response is much larger than that of fast response probes and is suitable for detecting changes in the mean membrane potential of non-excitable cells induced by respiratory activity, ion channel permeability, drug binding and other factors.

Parameter

Ex/Em: 490/505nm
CAS: 70363-83-6
Molecular Formula: $C_{27}H_{40}N_4O_6$
Molecular Weight: 516.63
Purity: HPLC≥98%
Appearance: Solid
Solubility: Soluble in DMSO ≥5mg/mL



Protocols (*only for reference*)

Preparation of storage solution

Prepare a 10 mM stock solution in DMSO. For example, 1 mg DiBAC4(3) powder was dissolved in 0.1936 mL DMSO.

Note:

a. Unused storage solution is recommended to be stored in portions at -20℃ to avoid repeated freezing and thawing.
b. Moisture-absorbing DMSO has a significant effect on the solubility of the product, use freshly opened DMSO.

Determination of membrane potential by fluorescence microscopy

1. Transfer the digested cells to DMEM.

2. Add FBS and control the concentration in the range of: 2~15%.
3. DiBAC4 (3) was added and the concentration was controlled in the range of: 1 to 5 μM.
4. Observe the change in fluorescence intensity after stimulation with a fluorescence microscope at 37℃. Since the potential of the cell membrane changes, changes in fluorescence intensity within the cytoplasm can be observed.
   Note: Laser confocal microscopy with an Ar laser (488 nm) is recommended.

Determination of membrane potential by fluorescence enzyme labeling method

1. Assay Dilute the stock solution with buffer and prepare 5 μM of DiBAC4(3) working solution.
   Note: Buffer formulation for assay: 20 mmol/L HEPES; 120 mmol/L NaCl; 2 mmol/LKCl; 2 mmol/LCaCl2; 1 mmol/LMgCl2; 5 mmol/L glucose, pH 7.4, filtered to remove bacteria.
2. Cultivate the cells in microtiter plates.
3. Wash the cultured cells in the microtiter plate twice with the working solution containing 5 μM DiBAC4(3).
4. 180 μL of 5 μM DiBAC4(3) working solution was added to the well plate and incubated for 30 min in 5% CO_2, 37°C incubator.
5. Put into the enzyme detector. Add 20 μL of 5 μM DiBAC4(3) working solution and measure the fluorescence change every 30 s at 37°C.

Standard Curve for Membrane Potential

1. Principle: The distribution of pigments changes when the membrane potential changes, so fluorescence and absorption also change. The extent of this change depends on the ratio of the number of molecules of pigment to the number of cells, the concentration of pigment, and the type of cell. The standard curve for membrane potential is made by directly measuring the membrane potential of the target cell with an electrode, and the intensity of fluorescence and absorption measured at that potential.
2. Cells were cultured with Eagle-Dulbecco medium.
3. Take the cells in PBS and incubate them in a CO_2 incubator at 37°C for 30-60 minutes, then change the incubation time to prepare samples with different CO_2 concentrations.
4. Add DiBAC4(3) to a final concentration of 2 μM.
5. After 20 minutes, the fluorescence intensity of each concentration was measured at 517 nm and the potential difference at this point was measured directly with an electrode.
6. Use the fluorescence intensity and the corresponding potential difference to make a standard curve.

Note

1. Since the fluorescence of DiBAC4(3) changes with temperature, it must be measured at 37 °C.
2. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.
3. When it is found to be more difficult to dissolve, it can be properly sonicated to promote dissolution.

4. Please adjust the concentration of the working fluid according to the actual situation, and use it now.
5. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
6. For your safety and health, please wear lab coat and disposable gloves.
7. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.

Related Literature

[1]. Chen S, Ma J, Xiao Y, Zhou D, He P, Chen Y, Zheng X, Lin H, Qiu F, Yuan Y, Zhong J, Li X, Pan X, Fang Z, Wang C. RNA Interference against ATP as a Gene Therapy Approach for Prostate Cancer. Mol Pharm. 2023 Oct 2;20(10):5214-5225. doi: 10.1021/acs.molpharmaceut.3c00587. Epub 2023 Sep 21. PMID: 37733628.

[2]. Jiao C, Gong S, Shi M, Guo L, Jiang Y, Man C. Depletion of reactive oxygen species induced by beetroot (Beta vulgaris) extract leads to apoptosis-like death in Cronobacter sakazakii. J Dairy Sci. 2023 Jun;106(6):3827-3837. doi: 10.3168/jds.2022-22425. Epub 2023 Apr 25. PMID: 37105876.

Note: For more literature, please visit the Solarbio official website.