Product Information

DiBAC4(3) (Bis-(1, 3-Dibarbituric Acid)-Trimethine Oxanol)

Catalog Number: D4008

Product Size: 5 mg

Parameters

Appearance: Orange solid soluble in DMFor DMSO

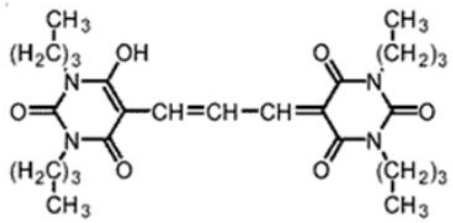
λEx/λ Em (MeOH) = 493/516 nm

CAS No.: 70363-83-6

Molecular Formula: $C_{27}H_{40}N_4O_6$

Molecular Weight: 519

Molecular Structure:



Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

The slow-response potential-sensitive probe, DiBAC4(3) can enter depolarized cells where it binds to intracellular proteins or membrane and exhibits enhanced fluorescence and a red spectral shift. Increased depolarization results in additional influx of the anionic dye and an increase in fluorescence. Conversely, hyperpolarization is indicated by a decrease in fluorescence.

Protocol

1. Preparation of stock solution: DiBAC4(3) can be dissolved in DMSO, anhydrous ethanol or pure water, and prepared with DMSO into 10 mM stock solution. If the low concentration can also meet the requirements, it can be prepared with pure water.

2. Fluorescence microscope

(1) Reagents: DiBAC4(3); Dulbecco's MEM (DMEM); FBS (fetal bovine serum)

(2) Stain

1) Transfer the digested cells to DMEM medium.

2) Add FBS to control the concentration range: 2 ~ 15%.

3) Add DiBAC4(3) to control the concentration range: 1 ～ 5 μM.

4) Observe the change in fluorescence intensity after stimulation with a fluorescence microscope, which can be detected with a laser confocal microscope using an argon ion laser (488 nm). Since the fluorescence of DiBAC4(3) changes with temperature, it must be measured at 37°C. When the cell membrane potential changes, changes in the fluorescence intensity within the cell can be observed.

3. Microplate reader

(1) Reagent: DiBAC4(3); detection buffer (pH7.4; 20 mM HEPES, 120 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose)

(2) Stain

1) Culture cells: culture cells in microplates

2) Wash the cells: Wash the cells in the microplate twice with 200 μL of 5 μM DiBAC4(3) working buffer.

3) Staining: Add 180 μL of working buffer containing 5 μM DiBAC4 (3) to the microplate, and incubate for 30 min in a

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37 °C incubator at 5% CO_2.

4) Measurement: Use fluorescence microplate reader to observe the change of intracellular fluorescence intensity after stimulation. Since the fluorescence of DiBAC4(3) changes with temperature, it must be measured at 37 °C. Add 20 μL of working buffer containing DiBAC4(3) and measure the fluorescence change every 30s.

4. Standard curve of membrane potential

(1) Principle: When the membrane potential changes, the pigment distribution also changes, so the fluorescence strength and absorption also change. The degree of variation depends on the ratio of the number of molecules of the pigment and the number of cells, the concentration of the pigment, and the type of cell. The standard curve of the membrane potential is prepared by directly measuring the membrane potential of the target cell with an electrode, and measuring the fluorescence and absorption intensity at the potential.

(2) Reagent: DiBAC4(3); Eagle-Dulbecco medium; PBS

(3) Method

1) Culture the cells with Eagle-Dulbecco medium.

2) 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.

3) DiBAC4(3) was added to a final concentration of 2 μM.

4) After 20 minutes, measure the fluorescence intensity of each concentration at 517 nm, and directly measure the potential difference at this time with the electrode.

5) Use the fluorescence intensity and the corresponding potential difference to make a standard curve.

(4) Other methods

There are methods to compare potassium diffusion potential and fluorescence or absorption intensity. Valamycin induces potassium diffusion potential. The potassium diffusion potential (V) is calculated according to the Nernst equation as follows:

V = -RT / F log [K +] in / [K +] out = -59 log [K +] in / [K +] out (mV)

In the presence of vamycin, by changing the extracellular potassium ion concentration and intracellular potassium ion concentration, the diffusion potential is calculated, the fluorescence or absorption intensity of each potential is measured, and a standard curve can be prepared after comparison.

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.

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