Dihydroethidium

Cat. No.: HY-D0079

CAS No.: 104821-25-2

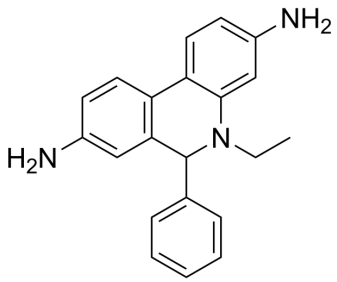
Molecular Formula: $C_{21}H_{21}N_3$

Molecular Weight: 315.41

Target: Fluorescent Dye

Pathway: Others

Storage: 4°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 50 mg/mL (158.52 mM)
H_2O : < 0.1 mg/mL (ultrasonic) (insoluble)
* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	3.1705 mL	15.8524 mL	31.7048 mL
	5 mM	0.6341 mL	3.1705 mL	6.3410 mL
	10 mM	0.3170 mL	1.5852 mL	3.1705 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
Solubility: ≥ 2.5 mg/mL (7.93 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Dihydroethidium, also known as DHE, is a peroxide indicator. Dihydroethidium penetrates cell membranes to form a fluorescent protein complex with blue fluoresces. After entering the cells, Dihydroethidium is mainly localized in the cell membrane, cytoplasm and nucleus, and the staining effect is the strongest in the nucleus. Dihydroethidium produces inherent blue fluorescence with a maximum excitation wavelength of 370 nm and a maximum emission wavelength of 420 nm; after dehydrogenation, Dihydroethidium combines with RNA or DNA to produce red fluorescence with a maximum excitation wavelength of 300 nm and a maximum emission wavelength of 610 nm. 535 nm can also be used as the excitation wavelength for actual observation[1].

In Vitro

General Protocol
Preparation of Dihydroethidium working solution
1.1 Preparation of the stock solution
Dissolve 1 mg of Dihydroethidium in 0.31 mL of DMSO to obtain 10 mM of Dihydroethidium.

Note: It is recommended to store the stock solution at -20°C or -80°C away from light and avoid repetitive freeze-thaw cycles.
1.2 Preparation of Dihydroethidium working solution
Dilute the stock solution in serum-free cell culture medium or PBS to obtain 1-10 μM of Dihydroethidium working solution.
Note: Please adjust the concentration of Dihydroethidium working solution according to the actual situation.
Cell staining
2.1 For suspension cells: Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.
For adherent cells: Discard the cell culture medium, and add trypsin to dissociate cells to make a single-cell suspension. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.
2.2 Add 1 mL of Dihydroethidium working solution, and then incubate at room temperature for 30 minutes.
2.3 Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.
2.4 Wash twice with PBS, 5 minutes each time.
2.5 Resuspend cells with serum-free cell culture medium or PBS, and then detect by fluorescence microscope or flow cytometer.

Storage
-20°C, 1 year
Protect from light

Precautions
1. It is recommended to store the stock solution at -20□ or -80□ away from light and avoid repetitive freeze-thaw cycles.
2. Please adjust the concentration of Dihydroethidium working solution according to the actual situation.
3. This product is for R&D use only, not for drug, household, or other uses.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- ACS Nano. 2024 Jan 8.
- Redox Biol. 2022: 102588.
- Acta Biomater. 2023 Feb 21;S1742-7061(23)00105-8.
- Int J Biol Sci. 2023 Mar; 19(6):1831-1845.
- Cell Death Dis. 2020 Apr 20;11(4):256.

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REFERENCES

[1]. Zielonka J, et al. Global profiling of reactive oxygen and nitrogen species in biological systems: high-throughput real-time analyses. J Biol Chem. 2012 Jan 27;287(5):2984-95.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA