

Product Data Sheet

Product Name: Dihydroethidium (Hydroethidine)

Cat. No.: GC30025

Chemical Properties

Cas. No. 104821-25-2,38483-26-0

SMILES NC1=CC=C2C3=C(C=C(N)C=C3)C(C4=CC=CC=C4)N(CC)C2=C1

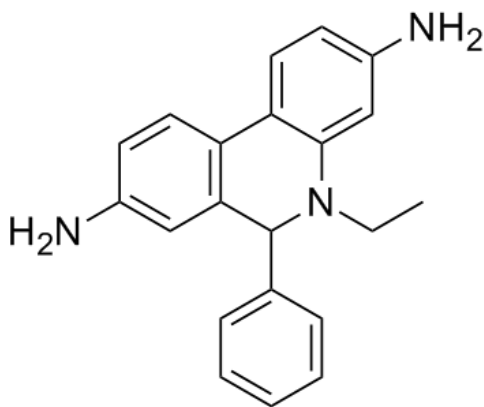
Formula $C_{21}H_{21}N_3$ M.Wt 315.41

Solubility DMSO : ≥ 50 mg/mL (158.52 mM); Water : < 0.1 mg/mL (insoluble) Storage Store at -20°C , protect from light

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

This plan only provides a guide, please modify it to meet your specific needs.

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

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1. Preparation of staining solution

(1) Prepare stock solution: Dissolve Dihydroethidium in DMSO and prepare a stock solution with a concentration of 1-10mM.

Notice:

- ① Aliquot the unused stock solution and store it in the dark at -20°C or -80°C to avoid repeated freezing and thawing;
- ② This product is easy to oxidize. Try to avoid contact with air during stock. It needs to be stored under nitrogen or argon, especially in solution.

(2) Prepare working solution: Dilute the stock solution with a suitable buffer (such as serum-free medium or PBS) and prepare a working solution with a concentration of 1-10μM.

Note: Please adjust the concentration of the working fluid according to the actual situation and prepare it now.

2. Cell suspension staining

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

(2) Adherent cells: Wash twice with PBS, add trypsin to digest the cells, and centrifuge at 1000 *g* for 3-5 minutes after digestion is completed.

(3) Add Dihydroethidium working solution to resuspend the cells, and incubate at room temperature in the dark for 5-30 minutes. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(4) After the incubation, centrifuge at 1000 *g* for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(5) Resuspend cells in pre-warmed serum-free cell culture medium or PBS and observe by fluorescence microscope or flow cytometry.

3. Cell adhesion staining

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100μL of dye working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye.

(4) Incubate at room temperature in the dark for 5-30 minutes. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(5) After the incubation, discard the dye working solution and use pre-warmed culture solution to wash the coverslip 2 to 3 times.

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4. Microscope detection: The maximum excitation/emission wavelength of Dihydroethidium is 518/616nm.

Precautions:

① Dihydroethidium is very sensitive to light, please avoid light as much as possible to slow down fluorescence quenching;

Dihydroethidium produces ethidium compounds after oxidation, which are highly toxic. For your safety and health, please wear a lab coat and disposable gloves when operating.

Background

Dihydroethidium(Hydroethidine) (DHE) oxidation is commonly used as a method for monitoring cellular production of "reactive oxygen species (ROS)". Usually changes in DHE fluorescence due to oxidation in cells and tissues are measured by microscopy, flow cytometry, and occasionally by HPLC analysis [1]. Dihydroethidium is a hydrophobic uncharged compound that can cross extra- and intracellular membranes and, upon oxidation, becomes positively charged and accumulates in cells by intercalating into DNA, primarily by electrostatic interactions with DNA phosphate groups and further via hydrophobic interactions [2]. Dihydroethidium oxidation by different oxidizing systems has been used increasingly for fluorescent analysis of ROS output in cells and tissues. Dihydroethidium - derived red fluorescence observed with a rhodamine filter (excitation 490; emission 590 nm) was attributed to ethidium compound formation, a two-electron oxidation product, and the red fluorescence was obtained more specifically with superoxide-generating systems (xanthine or glucose oxidase) rather than with oxidants such as hydrogen peroxide, peroxyxynitrite, or hydroxyl radical (generated by the Fenton reaction) [3,4].

Detection of ROS production in the liver tissues [5]

The levels of ROS production in the liver tissues were determined by dihydroethidium (DHE) staining. Frozen sections of liver tissues in each group (5-µm-thick) were prepared and incubated with DHE (7.5 mM,) for 30 min in the dark at 37 °C. After staining with DAPI, the sections were observed using a fluorescence microscope.

References:

[1]. Wagner B A, Buettner G R. Quantitative Changes in Dihydroethidium (DHE) Oxidation Products from Isolated Mitochondria While Respiring on Select Substrates and the Effects Mitochondrial Inhibitors Commonly Used in Bioenergetic Profiling[J]. Free Radical Biology and Medicine, 2016, 100:

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[2]. Garbett N C, Hammond N B, Graves D E. Influence of the amino substituents in the interaction of ethidium bromide with DNA[J]. Biophysical journal, 2004, 87(6): 3974-3981.

[3]. Benov L, Sztejnberg L, Fridovich I. Critical evaluation of the use of hydroethidine as a measure of superoxide anion radical[J]. Free Radical Biology and Medicine, 1998, 25(7): 826-831.

[4]. Biemond P, Swaak A J G, Beindorff C M, et al. Superoxide-dependent and-independent mechanisms of iron mobilization from ferritin by xanthine oxidase. Implications for oxygen-free-radical-induced tissue destruction during ischaemia and inflammation[J]. Biochemical Journal, 1986, 239(1): 169-173.

[5]. Zheng J, Chen L, Lu T, et al. MSCs ameliorate hepatocellular apoptosis mediated by PINK1-dependent mitophagy in liver ischemia/reperfusion injury through AMPK α activation[J]. Cell death & disease, 2020, 11(4): 1-19.

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