

Product Data Sheet

Product Name: DAPI (hydrochloride)

Cat. No.: GC43378

Chemical Properties

Cas. No. 28718-90-3

SMILES NC(C(C=C1)=CC=C1C2=CC3=CC=C(C(N)=N)C=C3N2)=N.Cl.Cl

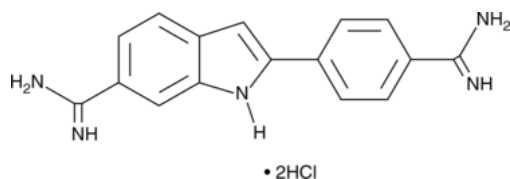
Formula $C_{16}H_{15}N_5 \cdot 2HCl$ M.Wt 350.3

Solubility Insoluble in EtOH; ≥ 10 mg/mL in Water; ≥ 53.3 mg/mL in DMSO Storage Store at $-20^{\circ}C$, protect from light

General tips For obtaining a higher solubility, please warm the tube at $37^{\circ}C$ and shake it in the ultrasonic bath for a while. Stock solution can be stored below $-20^{\circ}C$ 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.

1. Preparation of staining solution

(1) Prepare dye stock solution Use ddH₂O to dissolve DAPI and prepare a stock solution with a concentration of 1-10mg/ml.

Notice

- ① Do not use buffer to dissolve the powder directly;
- ② The prepared stock solution needs to be filtered and sterilized using a 0.22 μ m filter membrane;

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Product Data Sheet

③ Unused stock solution should be aliquoted according to single dosage and stored in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare dye working solution Use ddH₂O or PBS to dilute the stock solution and prepare a working solution with a concentration of 0.5-10µg/ml.

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 1000g 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 1000g for 3-5 minutes after digestion is completed.

(3) Add 1mL of DAPI 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 1000g for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(5) Resuspend the cells in pre-warmed serum-free cell culture medium or PBS. Observe by fluorescence microscopy 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.

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(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 3-5 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.

4. Microscope detection The maximum excitation/emission light of DAPI is 364/454nm respectively.

Precautions

- ① DAPI is suitable for staining fixed cells or tissues, usually after other staining;
- ② To slow down fluorescence quenching, anti-fluorescence quenching mounting agent can be used;
- ③ Fluorescent dyes all have quenching problems. Please try to avoid light to slow down fluorescence quenching;
- ④ DAPI is irritating to the human body. For your safety and health, please wear a lab coat and disposable gloves when operating.

Background

DAPI (hydrochloride), as a DNA-specific probe, can form a fluorescent complex by attaching in the minor groove of A-T rich sequences of DNA and nonfluorescent intercalative complexes with double-stranded nucleic acids. DAPI, as a chromosome and nuclear stain, has maximum excitation ultraviolet (UV) light wavelength with 358nm and emission in the blue range with 461nm^[1]. DAPI is usually used for flow cytometry, chromosome staining, DNA visualization and quantitation in histochemistry and biochemistry^[2]. DAPI is a good substrate of hOCT1 with a Michaelis constant of 8.94µM^[3]. 1.43µM DAPI can measure mitochondrial permeability transition and mitochondrial membrane depolarization by combining with Annexin V (FITC) and the potentiometric fluorescent dye, tetramethylrhodamine methyl ester (TMRM)^[4].

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References:

- [1] Karg TJ, et al. Photoconversion of DAPI and Hoechst dyes to green and red-emitting forms after exposure to UV excitation. *Chromosoma*. 2018 Jun;127(2):235-245.
- [2] Kapuscinski J. DAPI: a DNA-specific fluorescent probe. *Biotech Histochem*. 1995 Sep;70(5):220-33.
- [3] Yasujima T, et al. Characterization of human OCT1-mediated transport of DAPI as a fluorescent probe substrate. *J Pharm Sci*. 2011 Sep;100(9):4006-12.
- [4] Wallberg F, et al. Analysis of Apoptosis and Necroptosis by Fluorescence-Activated Cell Sorting. *Cold Spring Harb Protoc*. 2016 Apr 1;2016(4):pdb.prot087387.

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