Dyes and Stains

DAPI (Hydrochloride)

DNA-labeling dye

Catalog # 75004

10 mg

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Product Description

DAPI (4',6-diamidino-2-phenylindole dihydrochloride) is a blue-fluorescent dye that binds to AT-rich regions of double-stranded DNA. Binding is accompanied by an ~20-fold enhancement in fluorescence, which is directly proportional to the amount of DNA present and has an emission maximum at ~454 nm. The complex is stable for several hours at room temperature and over the pH range 4 - 11. DAPI can also bind to RNA, evidently through AU-selective intercalation, though the DAPI/RNA complex emits at a longer wavelength (500 nm) and with only an ~20% increase in quantum yield. DAPI has been widely employed as a counterstain to detect nuclei in multicolor fluorescence applications, where its blue fluorescence vividly contrasts with red, yellow or green fluorescent dyes used to stain other structures. It has also been used for studying apoptosis (at low concentrations the dye is excluded from live cells but penetrates dead or damaged cells), and in quantitative DNA assays, in situ hybridization, chromosome sorting, and mycoplasma detection assays.

Chemical Name:	2-(4-carbamimidoylphenyl)-1H-indole-6-carboximidamide, dihydrochloride
Alternative Names:	2-(4-Amidinophenyl)-1H-indole-6-carboxamidine, dihydrochloride; 4',6-Diamidino-2-phenylindole dihydrochloride; DAPI; DAPI dihydrochloride
CAS Number:	28718-90-3
Chemical Formula:	$C_{16}H_{15}N_5 \cdot 2HCl$
Molecular Weight:	350.3 g/mol
Excitation Wavelength:	358 nm (DNA complex); 340 nm (free form)
Emission Wavelength:	454 nm (DNA complex); 488 nm (free form); 500 nm (RNA complex)

Properties

Storage:	Store at -20°C.
Shelf Life:	Product stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Format/Formulation:	A crystalline solid

Applications

Verified:	FC (Imaging and non-imaging), Fluorescence microscopy
Reported:	Electrophoresis, FC, FISH, Fluorescence microscopy, Fluorometry, Genomic in situ hybridization (GISH), NMR
Special Applications:	This dye has been verified for analyzing cells cultured in several types of media, including mTeSR™1 (Catalog #05850), TeSR™2 (Catalog #05860), TeSR™-E8™ (Catalog #05940) and NeuroCult™ SM1 Neuronal Culture Kit (Catalog #05712).

Abbreviations: CellSep: Cell separation; ChIP: Chromatin immunoprecipitation; FA: Functional assay; FC: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence microscopy; IHC: Immunohistochemistry; IP: Immunoprecipitation; RIA: Radioimmunoassay; WB: Western blotting

Handling/Directions for Use

PREPARATION
A stock solution may be made by dissolving the DAPI (Hydrochloride) in the solvent of choice. DAPI (Hydrochloride) is soluble in organic solvents. Guidelines for the solubility of DAPI (Hydrochloride) are as follows:
· Ethanol ≤ 0.2 mg/mL
· DMSO ≤ 14 mg/mL
· Dimethyl formamide ≤ 0.2 mg/mL
DAPI (Hydrochloride) is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, DAPI (Hydrochloride) should first be dissolved in organic solvent and then diluted with the aqueous buffer of choice. If performing biological experiments, ensure the residual amount of organic solvent is insignificant, as organic solvents may have physiological effects at low concentrations.

Whenever possible, prepare and use the stock solutions on the same day. Protect stock solutions from prolonged exposure to light. If stock solutions must be made in advance, it is recommended that they are stored in aliquots in tightly sealed vials at -20°C and protected from prolonged exposure to light. Generally these will be stable for up to 1 month.

FLOW CYTOMETRY (fixed cells)
It is recommended to use DAPI (Hydrochloride) at a final concentration of 3 µM, though the dye should be titrated for optimal performance for each cell type and application.
1. Prepare a stock solution of DAPI (e.g. 5 mg/mL in DMSO).
2. Dilute the DAPI to 3 µM in the staining buffer of choice (e.g. EasySep™ Buffer, Catalog #20144).
3. Use a fixation protocol appropriate for the sample and prepare a cell pellet containing 1 x 10^5 - 1 x 10^6 fixed cells by centrifugation.
4. Stain the cells in 1 mL of 3 µM DAPI for 15 minutes on ice.

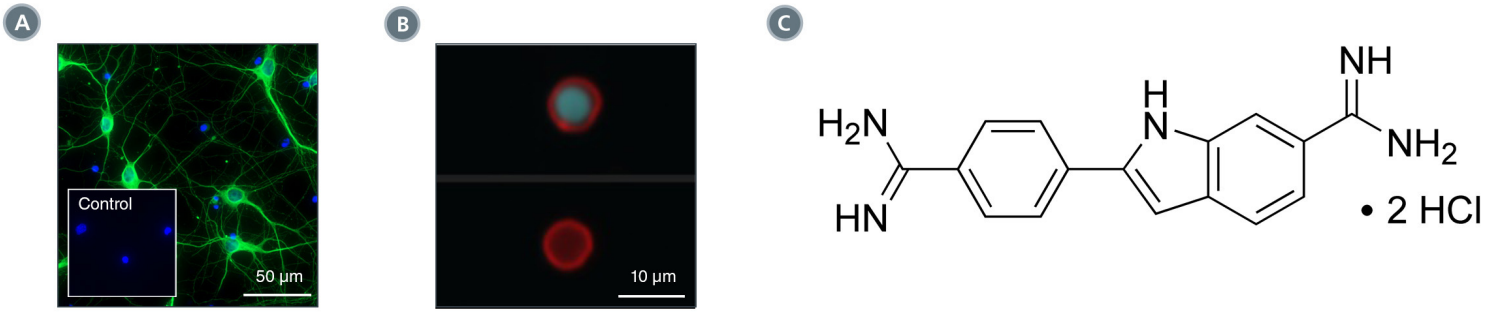
Cells are now fluorescently labeled and ready to be analyzed by flow cytometry, which can be performed in the presence of the dye.

The labeled cells may also be viewed by fluorescence microscopy. In that case, centrifuge the sample, remove the supernatant and resuspend cells in fresh buffer. Apply to a microscope slide, overlay with a coverslip, and view using a fluorescence microscope with appropriate filters.

FLUORESCENCE MICROSCOPY (fixed adherent cells)
It is recommended to use DAPI (Hydrochloride) at a final concentration of 0.3 µM, though the dye should be titrated for optimal performance for each cell type and application.
1. Prepare a stock solution of DAPI (e.g. 5 mg/mL in DMSO).
2. Dilute the DAPI stock solution to 0.3 µM in phosphate-buffered saline (PBS).
3. Use a fixation protocol appropriate for the sample.
   Note: If DAPI is to be used as a counterstain, it is usual to perform all other staining steps first.
4. Rinse the sample with PBS.
5. Add ~0.3 mL of 0.3 µM DAPI to the cells, ensuring that they are completely covered.
6. Incubate for 5 minutes, then rinse several times in PBS.

Cells are now fluorescently labeled and ready to be mounted and viewed using a fluorescence microscope with appropriate filters.

Data/Structure



(A) E18 cortical rat neurons were cultured using the NeuroCult™ SM1 Neuronal Culture Kit on poly-lysine-coated glass coverslips, then fixed and labeled with Anti-Beta-Tubulin III Antibody, Clone AA10, Alexa Fluor® 488 (Catalog #60100AD; green), and counterstained with DAPI (blue). Inset shows cells incubated with a mouse IgG2a, kappa isotype control antibody, Alexa Fluor® 488, and counterstained with DAPI.
(B) Imaging flow cytometry analysis of human peripheral blood mononuclear cells (PBMCs) labeled with Anti-Human CD45 Antibody, Clone HI30, PE (red; Catalog #60018PE) and counterstained with DAPI (blue). Staining of a non-viable leukocyte is shown in the top panel and staining of a viable leukocyte in the bottom panel.
(C) Chemical structure of DAPI (Hydrochloride).

References

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