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DAPI (Hydrochloride)

DNA-labeling dye

Catalog #75004 10 mg

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 used 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 been used for studying apoptosis and at low concentrations, the dye is excluded from live cells but penetrates dead or damaged cells. DAPI has also been used for studying 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)
Stability and Storage:	Store at -20°C. Product stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Product Format:	A crystalline solid

Verified Applications:	FC (imaging and non-imaging), Fluorescence microscopy
Reported Applications:	Electrophoresis, FISH, Fluorescence microscopy, Fluorometry, Genomic in situ hybridization, NMR
Special Applications:	This dye has been verified for analyzing cells cultured in mTeSR™1 (Catalog #85850) and TeSR™E8™ (Catalog #05990).

Abbreviations: CellSep: Cell separation; ChIP: Chromatin immunoprecipitation; FA: Functional assay; FACS: Fluorescence-activated cell sorting; FC: Flow cytometry; FCXM: Flow cytometric crossmatch assay; FISH: Fluorescence in situ hybridization; ICC: Immunocytochemistry; IF: Immunofluorescence microscopy; IHC: Immunohistochemistry; IHC-F: Immunohistochemistry (frozen-tissue); IHC-P: Immunohistochemistry (paraffin-embedded); IP: Immunoprecipitation; NMR: Nuclear magnetic resonance spectroscopy; RIA: Radioimmunoassay; WB: Western blotting

Directions for Use

A stock solution may be made by dissolving DAPI in the solvent of choice. DAPI is soluble in organic solvents. Guidelines for the solubility of DAPI are as follows:

- Ethanol ≤ 0.2 mg/mL
- Dimethyl sulfoxide (DMSO) ≤ 3 mg/mL
- Dimethyl formamide (DMF) ≤ 0.2 mg/mL

DAPI is sparingly soluble in aqueous buffers. For maximum solubility, DAPI should first be dissolved in an 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 it may have physiological effects at low concentrations.

Whenever possible, prepare and use stock solution on the same day. Protect stock solution from prolonged exposure to light. If stock solution must be made in advance, aliquot and store in tightly sealed vials at -20°C and protect from prolonged exposure to light. Generally these will be stable for up to 1 month.

FLOW CYTOMETRY OF FIXED CELLS

1. To prepare a 1 mg/mL (2.85 mM) stock solution of DAPI, add 10 mL of DMSO to 10 mg of DAPI (Hydrochloride).
   NOTE: If not used immediately, aliquot and store stock solution at -20°C. After thawing the aliquots, use immediately; do not re-freeze.
2. To prepare DAPI working solution, dilute DAPI stock solution prepared in step 1 in a suitable staining buffer (e.g. EasySep™ Buffer, Catalog #20144) to reach a 1 µg/mL solution.
   NOTE: Although 1 µg/mL is the recommended final concentration, it is recommended to titrate the dye for optimal performance in each application.
3. Use a fixation protocol appropriate for the sample.
4. Prepare a cell pellet containing 1 x 10^5 - 1 x 10^6 fixed cells by centrifugation.
5. Stain cells in 1 mL of 1 µg/mL DAPI on ice for 15 minutes.
   Cells are now fluorescently labeled and ready to be analyzed by flow cytometry, which can be performed in the presence of the dye.
   To view the labeled cells by fluorescence microscopy, 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 OF FIXED ADHERENT CELLS

1. To prepare a 1 mg/mL (2.85 mM) stock solution of DAPI, add 10 mL of DMSO to 10 mg of DAPI (Hydrochloride).
   NOTE: If not used immediately, aliquot and store stock solution at -20°C. After thawing the aliquots, use immediately; do not re-freeze.
2. To prepare DAPI working solution, dilute DAPI stock solution prepared in step 1 in phosphate-buffered saline (PBS) to reach a 0.5 µg/mL solution.
   NOTE: For microscopy applications, DAPI will work at a wide range of concentrations (0.1 - 10 µg/mL). For applications such as whole-mount organoid staining or thin-section staining, a higher final concentration of DAPI, such as 2 - 5 µg/mL, should be used. For any application, it is recommended to titrate the dye for optimal performance.
3. Use a fixation protocol appropriate for the adherent cell 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 ~0.5 mL of 0.5 µg/mL DAPI to each well of a 24-well plate.
   NOTE: If using other cultureware, adjust volumes accordingly. Ensure enough DAPI solution is added to cover the wells.
6. Incubate at room temperature (15 - 25°C) for 5 minutes, then rinse several times with PBS. Cells are now fluorescently labeled and ready to be mounted (if using glass coverslips) and viewed using a fluorescence microscope equipped with appropriate filters.
   NOTE: If samples are also stained for other nuclear antigens, it is recommended that these antigens be examined in red orfar-red channels if possible, to minimize any bleed-through from DAPI.

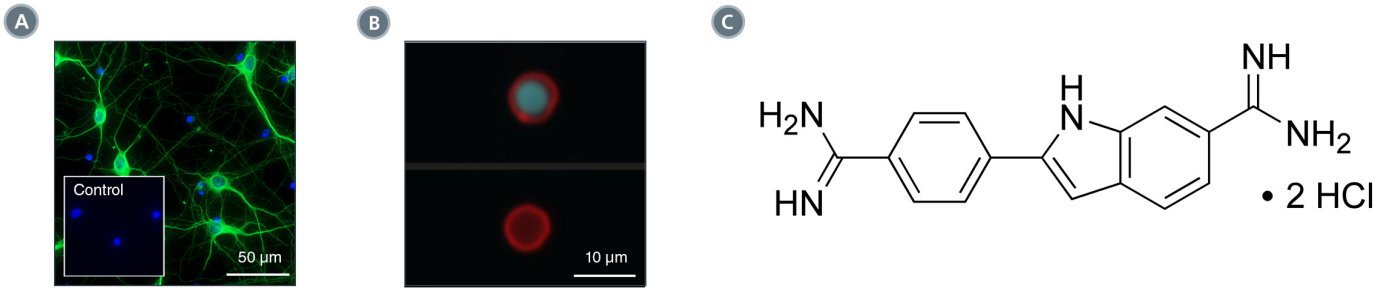

Figure 1. Staining Profiles of Various Cell Populations

(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).

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