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 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 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, Fluorescence in situ hybridization (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 #85850), TeSR™2 (Catalog #05860), and TeSR™-E8™ (Catalog #05990).

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

For preparing a stock solution, DAPI is soluble in organic solvents, 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, first dissolve DAPI in organic solvent, then dilute 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 (fixed cells)
1. Prepare a stock solution of DAPI; e.g. 3 mg/mL (8 µM) in DMSO.
2. Dilute the DAPI stock solution to 3 µM in a suitable staining buffer (e.g. EasySep™ Buffer, Catalog #20144).
   NOTE: Although 3 µM is the recommended final concentration, 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 3 µM DAPI on ice for 15 minutes.

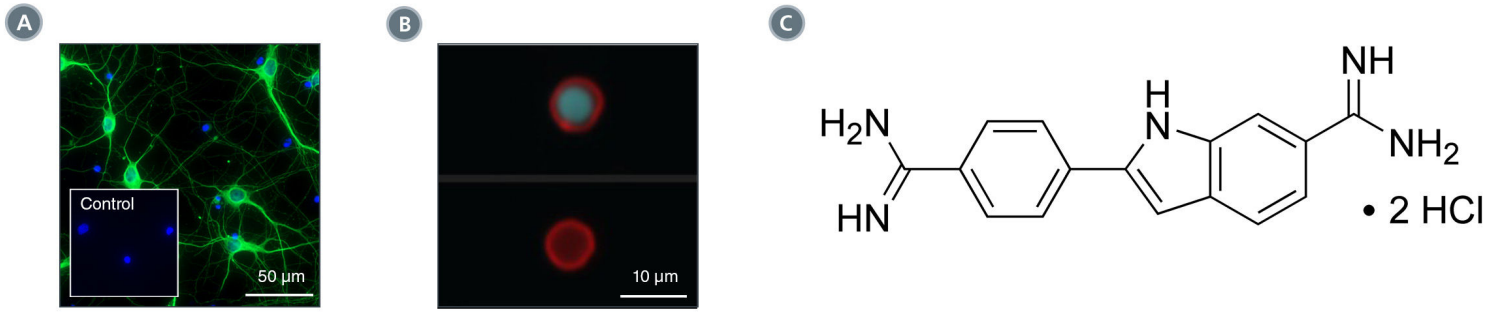
Cells are now fluorescently labeled and ready to be analyzed by flow cytometry, which can be performed in the presence of the dye.

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 (fixed adherent cells)
1. Prepare a stock solution of DAPI; e.g. 3 mg/mL (8 µM) in DMSO.
2. Dilute the DAPI stock solution to 0.3 µM in phosphate-buffered saline (PBS).
   NOTE: Although 3 µM is the recommended final concentration, titrate the dye for optimal performance in each application.
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 they are completely covered.
6. Incubate at room temperature (15 - 25°C) 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 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 labeled with Mouse IgG2a, kappa Isotype Control Antibody, Clone MOPC-173, Alexa Fluor® 488 (Catalog #60071AD), 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

1. Ogawa W et al. (2015) Characterization of MATE-type multidrug efflux pumps from Klebsiella pneumoniae MGH78578. PLoS One 10(3): e0121619. (Fluorometry)
2. Pita S et al. (2014) Distribution and evolution of repeated sequences in genomes of Triatominae (Hemiptera-Reduviidae) inferred from genomic in situ hybridization. PLoS One 9(12): e114298. (Chromosome staining/GISH, Fluorescence microscopy)
3. Banerjee D & Pal SK. (2008) Dynamics in the DNA recognition by DAPI: exploration of the various binding modes. J Phys Chem B 112(3): 1016–21. (Electrophoresis)
4. Lai J et al. (2003) Loss of HSulf-1 up-regulates heparin-binding growth factor signaling in cancer. J Biol Chem 278(25): 23107–17. (Fluorescence microscopy)
5. Soto P et al. (2003) SMAD2 and SMAD7 involvement in the post-translational regulation of Muc4 via the transforming growth factor-beta and interferon-gamma pathways in rat mammary epithelial cells. J Biol Chem 278(22): 20338–44. (Fluorescence microscopy)
6. Zink D et al. (2003) Visualizing chromatin and chromosomes in living cells. Methods 29(1): 42–50. (Chromosome staining/FISH)
7. Kapuscinski J. (1995) DAPI: a DNA-specific fluorescent probe. Biotech Histochem 70(5): 220–33. (Review)
8. Tanious FA et al. (1992) DAPI (4',6-diamidino-2-phenylindole) binds differently to DNA and RNA: minor-groove binding at AT sites and intercalation at AU sites. Biochemistry 31(12): 3103–12. (Fluorometry, NMR)
9. Kubista M et al. (1987) Characterization of interaction between DNA and 4',6-diamidino-2-phenylindole by optical spectroscopy. Biochemistry 26(14): 4545–53. (Fluorometry)
10. Lawrence ME & Possingham JV. (1986) Direct measurement of femtogram amounts of DNA in cells and chloroplasts by quantitative microspectrofluorometry. J Histochem Cytochem 34(6): 761–68. (Fluorescence microscopy, Fluorometry)
11. Morikawa K & Yanagida M. (1981) Visualization of individual DNA molecules in solution by light microscopy: DAPI staining method. J Biochem 89(2): 693–96. (Fluorescence microscopy)
12. Brunk CF et al. (1979) Assay for nanogram quantities of DNA in cellular homogenates. Anal Biochem 92(2): 497–500. (Fluorometry)
13. Russell WC et al. (1975) A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature 253(5491): 461–62. (Fluorescence microscopy)

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