DAPI

4',6-Diamidine-2-phenylindole dihydrochloride
Product No. A1001

Description

Formula:	$C_{16}H_{15}N_5 \cdot 2HCl$
Molecular weight:	350.25 g/mol
CAS-No.:	[28718-90-3]
Assay (HPLC):	min. 98 %
Melting point:	approx. 330°C
Chloride:	min. 18.2 %
Nitrogen:	min. 18.0 %
UV-Spectrum:	$\lambda_{max.}$ 223 nm, 261 nm and 342 nm $\lambda_{min.}$ 246 nm and 282 nm
Storage:	+4°C, protected from light
recommended stock solution:	1 - 5 mg/ml in water (insoluble in PBS!)
recommended working solution:	0.1 µg/ml - 200 µg/ml (diluted in methanol)

Comment

DAPI is an excellent dye for the staining of DNA. Originally, only the specific binding to AT-base pairs without an intercalation was known (2), but later on, the intercalation into GC-base pairs was shown (3). The most popular application of DAPI is its use as a reagent **to detect mycoplasma or virus DNA** (e. g. vaccinia infection or 'unwanted' viral contamination of cell culture cells) in the cell culture.

AppliChem recommends the following staining procedure:

Grow cells on a coverslip in a cell culture dish to reach approx. 70 % confluence. Pour off the medium from the cells. Wash the coverslip once with 1 µg/ml DAPI in methanol. Incubate the cells on the coverslip at 37°C for 15 minutes in 1 µg DAPI/ml in methanol. Pour off the staining solution and wash the coverslip once with methanol. Put it up-side-down on a slide with PBS or glycerol as mounting medium. Do not use water. Examine the cells under a microscope (excitation: 365 nm; emission maximum at 450 nm). Prolonged incubation with DAPI increases the nuclear fluorescence, shorter incubation time leads to a weaker nuclear staining, which facilitates the examination of the cytoplasmic fluorescence.

Solubility / Stability

Dissolve DAPI in double-distilled water to a final concentration of 1 - 5 mg. The maximum solubility in water is approx. 25 mg/ml. DAPI is insoluble in PBS. Do not use any buffers. Dilute the stock solution with methanol to a final concentration of 1 µg/ml. Solutions are stable at room temperature for 1 - 2 weeks (4), at +4°C up to 6 months and frozen between 6 and 12 months (1 ml aliquots). If the solution becomes turbid, DAPI is hydrolyzed. DAPI bleaches quickly in contact with light, even if it is quite stable against UV-light. Incubate your samples in the dark. If your samples are stored at +4°C for one day, fluorescence is stabilzed.

Application and Literature

(1)A simple cytochemical technique for demonstration of DNA in celles infected with mycoplasmas and viruses. (Russel, W.C. *et al.* (1975) *Nature* 253, 461-462)

(2)Interaction of DAPI with synthetic polynucleotides. (Kapuscinski, J. & Szer, W. (1979) *Nucleic Acids Res.* 6, 3519-3534)

(3)Binding of DAPI to GC and Mixed Sequences in DNA: Intercalation of a Classical Groove-Binding Molecule. (Wilson, W.D. et al. (1989) J. Am. Chem. Soc. 111, 5008-5010)

(4)DAPI Staining of Fixed Cells for High-Resolution Flow Cytometry of Nuclear DNA. (Otto, F. (1990) Methods Cell Biol. 33, 105-110)

(5)Reverse Fluorescent Chromosome Banding with Chromomycin and DAPI. (Schweizer, D. (1976) Chromosoma 58, 307-324)

(6)Quantitative Fluorescent Analysis of Different Conformational Forms of DNA Bound to the Dye DAPI, and Separated by Gel Electrophoresis. (Naimski, P. et al. (1980) Anal. Biochem. 106, 471-475)

(7)Spectrofluorometry of Dyes with DNAs of Different Base Composition and Conformation. (Daxhelet, G.A. et al. (1989) Anal. Biochem. 179, 401-403)

(8)Detection of Mycoplasma Infection of Mammalian Cells. (Xia, H. et al. (1997) BioTechniques 22, 934-936)

Caution: DAPI is a potential carcinogen, like all chemicals binding selectively to DNA.