

Specification

DAPI BioChemica

A1001

Physical Description:	Solid
Product Code:	A1001
Product Name:	DAPI BioChemica
Specifications:	<p>Assay (HPLC): min. 98 %</p> <p>Solubility (1 %; H₂O): clear</p> <p>N: min. 18 %</p> <p>UV spectrum</p> <p>$\lambda_{\text{max.}}$: 223 nm, 261 nm, 342 nm</p> <p>$\lambda_{\text{min.}}$: 246 nm, 282 nm</p>
WGK:	1
Storage:	<p>2-8°C</p> <p>protected from light</p>
Molecular Formula:	C ₁₆ H ₁₅ N ₅ · 2HCl
M:	350.25 g/mol
CAS:	28718-90-3
EINECS:	249-186-7
CS:	29339980

AppliChem GmbH

Ottoweg 4 • D-64291 Darmstadt • Phone +49 6151 9357 0 • Fax +49 6151 9357 11 • info.de@itwreagents.com • www.itwreagents.com
 CEO Joan Roget • Commerzbank Darmstadt • Bank 508 800 50 • Account 0186989900 IBAN DE24 5088 0050 0186 9899 00 • Swiftcode DRESDEFF508 • Finanzamt Darmstadt 07 228 16476 • Register court Darmstadt HRB Nr. 7340

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 simple 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 stabilized.

Bibliography

(1) Russel, W.C. *et al.* (1975) *Nature* **253**, 461-462 A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. (2) Kapuscinski, J. & Szer, W. (1979) *Nucleic Acids Res.* **6**, 3519-3534 Interaction of 4',6-diamidino-2-phenylindole with synthetic polynucleotides. (3) Wilson, W.D. *et al.* (1989) *J. Am. Chem. Soc.* **111**, 5008-5010 Binding of 4',6-Diamidino-2-phenylindole (DAPI) to GC and mixed sequences in DNA: Intercalation of a classical groove-binding molecule. (4) Otto, F. (1990) *Methods Cell Biol.* **33**, 105-110 DAPI-staining of cells fixed for "flow cytometry". (5) Schweizer, D. (1976) *Chromosoma* **58**, 307-324 'Reverse Fluorescent Chromosome Banding' with Chromomycin and DAPI. (6) Naimski, P. *et al.* (1980) *Anal. Biochem.* **106**, 471-475 Quantitative Fluoreszenzanalyse verschiedener Konformationen von DAPI-gebundener DNA. (7) Daxhelet, G.A. *et al.* (1989) *Anal. Biochem.* **179**, 401-403 Spectrofluorometry of Dyes with DNAs of Different Base Composition and Conformation. (8) Xia, H. *et al.* (1997) *BioTechniques* **22**, 934-936 Detection of Mycoplasma infections of mammalian cells.