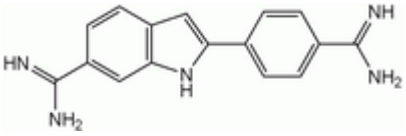
DAPI

Blue nuclear counterstain

Product Information

Name :	**DAPI,** 4',6-diamidino-2-phenylindole, dihydrochloride salt
	CAS 28718-90-3
Catalog Number :	FP-37186B, 10 mg FP-37186D, 25 mg
	FP-99963A, 10 mg (FluoProbes Pure grade)
	FP-CJF800, 2 ml (10mM solution in water)
	CAS: 28718-90-3
Molecular Weight :	350.25
Solubility:	Soluble in water
Absorption / Emission :	λ_{exc}\$\lambda_{em}$ (no DNA, water) = 344 / 450 nm λ_{exc}\$\lambda_{em}$ (DNA-bound) = 358 / 461 nm.
Extinction Coefficient :	ε (no DNA, water)=21 000 $M^{-1}cm^{-1}$



Name :	**DAPI,** dilactate
Catalog Number :	FP-66034A, 10 mg FP-66034D, 25 mg
	CAS: 28718-90-3
Molecular Weight :	457.49
Solubility:	Soluble in water or MetOH
Absorption / Emission :	λ_{exc}\λ_{em} (hydrolysed, DNA) = 358 / 461 nm.

Storage: +4°C(K) (or –20°C for long term) Protect from light and moisture

Introduction

DAPI (4',6-diamidino-2-phenylindole) is a popular blue counterstain fluorescent DNA probe for microscopy imaging.

- DAPI can also serve to fluorescent labelling cells for analyzing multicolor flow cytometry experiments, and for detection of dsDNA in electrophoresis gels (compared with Ethidium bromide , it shows superior sensitivity, and better selectivity toward dsRNA).

- DAPI also stains chromosomes, yeast, phytoplasmas, dsDNA and RNA. DAPI binds to minor grooves of DNA (preferentially dsDNA) with a selectivity for AT clusters. Fluorescence ($\lambda_{abs.}$:$\lambda_{em.}$: 358/461 nm) is increased 15-20 folds. A RNA staining is also reported with λ emission shifted to ca 500 nm and a low quantum yield of 20% (r).

- DAPI is mutagenic, and should thus be handled with suitable precautions (wear gloves). Disposal should respect local regulations, i.e; aqueous solution may be filtered through activated charcoal.

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- DAPI dilactate is a water soluble derivate of DAPI, cell-impermeant. Higher concentrations are however necessary in living cells. It is better used for microinjection.than DAPl dihydrochloride.

Directions for use

Handling and Storage

DAPI can be solubilized in water or DMF

Preparation of Stock Solution [r]

Prepare a solution of 5 mg/ml or 14.3 mM in DMF.
Mix to dissolve and it may take sometime to completely dissolve.
Aliquote and store in –20 ºC.

Preparation of Working Solution [r]
Prepare a solution of 100 ng/ml or 300 nM in water. Solubility is not good in presence of salts.
Working solution may be kept at +4°C for some days in brown bottle or wrapped with aluminum foil to protect from light or aliquoted and frozen for some months
Incubate sections in dark for 30 minutes at room temperature.

Detergents (usually Triton® X100 0.1%) are added to increase the permeability of cells.
Suitable excitation light sources are xenon-, mercury-arc lamps, and UV laser (including FCM systems). Addition of antifading agent may be required.

Protocol (1) – staining nucleus material in solution

Nuclei extracted and fractionated on a sucrose gradient can be controlled in solution:

1- 1- Incubate the nuclei solution with DAPI 0.1 mg/ml during 10 minutes protected from light.
2- Mount between cover and coverslip, and observe by fluorescence microscopy.

Protocol (2) – staining nucleus material in adherent cells and tissues

3- Incubate adherent cells for 1-5 min with a 300 nM solution of DAPI in PBS at room temperatures (staining)
4- Incubate tissue samples for 15-30 min with a 30 nM solution of DAPI in PBS (counterstaining)
5- Wash cells with PBS then mount.

Protocol (3) – staining nucleus material from cells suspension - cytometry

1- Cells in suspension should first be fixed prior to staining.
2- Washed cells may be fixed in neat ethanol at –20°C for 10 min (add cells slowly to twirling ethanol to avoid clumps formation), then re-hydrated (PBS for 15 min).
3- Stain ~10^5 pelleted cells with 1ml of DAPI 3 µM in Tris 100 mM, NaCl 150 mM, $CaCl_2$ 1mM, $MgCl_2$ 0.5 mM, Nonidet® P40 0.1%, pH 7.4 for 15 min at room temperature. Wash cells (optional).
4- Analyzed cells by flow cytometry.

Protocol (3b) – staining nucleus material from cells suspension – microscopy/apoptosis study

1- Wash harvested cells with PBS, resuspend in PBS containing Triton® X 0.1 % (to increase permeability) and incubate for 10 min on ice.
2- Spin cells down and resuspend them at 5000 cells/µl in 4% PBS buffered paraformaldehyde solution containing 10 µg/ml DAPI. Place 10 µl on a slide and observe using a fluorescence microsocope.
3- Nuclei are considered to have the normal phenotype (morphology) when glowing bright and homogenously. Apoptotic nuclei can be identified by the condensed chromatin gathering at the periphery of the nuclear membrane or a total fragmented morphology of nuclear bodies. More than 150 cells are counted and the percentage of apoptotic nuclei determined.

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Protocol [4] – staining yeast

1- Fixation: 1ml yeast + 0.1ml 36% formaldehyde. Incubate 1-2H at room temperature. Wash 2x 1ml H2O (Spin at 10 000, 5"). May leave at +4°C

2- Fixation for staining: Spin down and resuspend in 300 µl H2O. Add 700 µl 100% EtOH. Leave for 30 min at room temperature.

3- Spin down, resuspend in 500 µl H2O and sonicate 5 sec.

4- Spin down (10 000, 5") and remove top 300 µl. Vortex.

5- Staining: mix 4 µl cells and 4 µl Eileen's premade DAPI then mount media on slide. Microscope immediately or seal with nail polish & keep dark. Must seal if you want to take pictures.

EILEEN'S Premade DAPI/mounting media (final concentration is 45 µg/ml):

• 50 mg p-phenylenediamine in 5 ml 1X PBS; adjust to pH 9.0 (NaOH)
• add 45 ml glycerol and stir to homogeneity
• add 2.25 µl 1mg/ml DAPI. Store –70°C/dark

References

- **Bertho N**. *et al*., Efficient migration of dendritic cells toward lymph node chemokines and induction of TH1 responses require maturation stimulus and apoptotic cell interaction, *Blood*, Vol. **106**, No. 5, pp. 1734-1741 (2005) Article
- **Brent J.F**, *et al*., « Functional Nucleotide Receptor Expression and Sarcoplasmic Reticulum Morphology in Dedifferentiated Porcine Coronary mooth Muscle Cells » , *J Vasc Res*, **8**, 432 (2001) Article
- **Brent J.F**, *et al*., « Enhanced Endothelin$_A$ Receptor-Mediated Calcium Mobilization and Contraction in organ Cultured Porcine Coronary Arteries », *The Journal of pharmacology and experimental therapeutics*, **295**, Issue 2, 484 (2000) Article
- **Kapuscinski J**., *et al*., « DAPI: A DNA-specific fluorescent probe », *Biotechnic Histochem.* **70**, 220 (1995) Abstract
- **Li H.** *et al*., Role of neural-cadherin in early osteoblastic differentiation of human bone marrow stromal cells cocultured with human umbilical vein endothelial cells, *Am J Physiol Cell Physiol*, **299**: C422 - C430 (2010) Abstract
- **Masson J.** *et al*., Mice Lacking Brain/Kidney Phosphate-Activated Glutaminase Have Impaired Glutamatergic Synaptic Transmission, Altered Breathing, Disorganized Goal-Directed Behavior and Die Shortly after Birth, *J. Neurosci.*, **26**: 4660 - 4671 (2006) Article
- **Peneau S**. *et al*., First Evidence of Division and Accumulation of Viable but Nonculturable *Pseudomonas fluorescens* Cells on Surfaces Subjected to Conditions Encountered at Meat Processing Premises, *Applied and Environmental Microbiology*, p. 2839-2846, Vol. 73, No. 9 (2007) Article

Additional technical notes (on inquire)

Photoconversion of DAPI and Hoechst [NT-37186n]
Staining chromososmes protocol [ask+]
Phytoplasma stain protocol [ask+]
DNA and RNA stains [ask+]
Use of DAPI for nucleic acidss quantitation: see Nucleic Acids Quantitation [NT-NuAcQt]

Related products

- Fluoro-Gel mounting medium with DAPI, FP-DT094A
- Hoechst 33342, 20 mM, FP-BB1340
- Hoechst 33258, 20 mM, FP-BB1330
- Stain-all (RNA in blue, DNA in purple, protein in Red), JQ6531
- Goat anti-Mouse IgG, FluoProbes® 547H, FP-SB4000
- Goat anti-Rabbit IgG, FluoProbes® 647H, FP-SC4000

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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