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DAPI Nucleic Acid Stain

Cat. No. PA-3013
Storage upon receipt:
• Room temperature
• Protect from light
Molecular Weight: 350.3 (dihydrochloride)
Ex/Em: 358/461 nm, bound to DNA

Introduction

DAPI is a popular nuclear and chromosome counterstain for use in multicolor fluorescent techniques. Its blue fluorescence stands out in vivid contrast to green, yellow or red fluorescent probes of other structures. Although the dye is cell impermeant, higher concentrations will enter a live cell.
The blue fluorescent DAPI Nucleic Acid Stain preferentially stains dsDNA; it appears to associate with AT clusters in the minor groove.[1] Binding of DAPI to dsDNA produces a ~20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove.[2] DAPI also binds RNA, however in a different binding mode, one thought to involve AU-selective intercalation.[3] The DAPI/RNA complex exhibits a longer-wavelength fluorescence emission maximum than the DAPI/dsDNA complex (~500 nm versus ~460 nm) and a quantum yield that is only about 20% as high.[4]
When used according to our protocols, DAPI stains nuclei specifically, with little or no cytoplasmic labeling. The counterstaining protocols are compatible with a wide range of cytological labeling techniques, direct or indirect antibody-based detection methods, mRNA in situ hybridization or staining with fluorescent reagents specific for cellular structures. DAPI can also serve to fluorescently label cells for analysis in multicolor flow cytometry experiments. The following protocols can be modified for tissue staining or for staining unfixed cells or tissues.

Instructions for Use
Storage and Handling

DAPI dihydrochloride (MW = 350.3) is supplied in a unit size of 10 mg. Upon receipt, store the vial at room temperature, protected from light. The solid should be stable for at least a year.
To make a 5 mg/ml DAPI stock solution (14.3 mM), dissolve the contents of one vial (10 mg) in 2 ml of deionized water (dH_2O) or dimethylformamide (DMF). DAPI dihydrochloride may take some time to completely dissolve in water and sonication may be necessary.

For long-term storage the stock solution can be aliquoted and stored at -20℃. For short-term storage the solution can be kept at 2.6℃, protecte d from light. When handled properly, DAPI solutions are stable for at least six months.

Caution: DAPI is a known mutagen and should be handled with care. The dye must be disposed of safely and in accordance with applicable regulations. DAPI can be removed from aqueous solutions by filtration through activated charcoal. The charcoal and adsorbed dye must then be disposed of in a safe and appropriate manner

Fluorescence Spectral Characteristics

The excitation maximum for DAPI bound to dsDNA is 358 nm, and the emission maximum is 461 nm. DAPI can be excited with a xenon or mercury-arc lamp or with a UV laser. Generally, DAPI fluorescence is detected in the FL4 channel of flow cytometers.

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Protocol for Counterstaining Adherent Cells for Fluorescence Microscopy

Sample Preparation

Use the fixation protocol appropriate for your sample. DAPI staining is normally performed after all other staining. Note that fixation and permeabilization of the sample are not necessary for counter staining with DAPI.

Counterstaining Protocol

1.1 Equilibrate the sample briefly with phosphate-buffered saline (PBS).
1.2 Dilute the DAPI stock solution to 300 nM in PBS. Add approximately 300 µl of this dilute DAPI staining solution to the coverslip preparation, making certain that the cells are completely covered.
1.3 Incubate for 1.5 minutes.
1.4 Rinse the sample several times in PBS. Drain excess buffer from the cover slip and mount, preferably using a mounting medium with an antifade reagent.
1.5 View the sample using a fluorescence microscope with appropriate filters.

Protocol for Counterstaining Cells in Suspension for Flow Cytometry

Sample Preparation

Use the fixation protocol appropriate for your sample, or use the following protocol.

2.1 Collect a cell suspension of 2×10^5 to 1×10^6 cells.
2.2 Pellet the cells by centrifugation and discard the supernatant.
2.3 Tap the tube to resuspend the pellet in the residual liquid and add 1 ml of PBS at room temperature.
2.4 Transfer the full volume of resuspended cells to 4 ml of absolute ethanol at -20℃ by pipetting the cell suspension slowly into the ethanol while vortexing at top speed. Leave the cells in ethanol at -20℃ for 5-15 minutes.
2.5 Pellet the cells by centrifugation and discard the ethanol.
2.6 Tap the tube to loosen the pellet and add 5 ml of PBS at room temperature. Allow the cells to rehydrate for 15 minutes.

Counterstaining Protocol

3.1 Dilute the DAPI stock solution to 3 µM in staining buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.1% Nonidet™ P-40). A 1 ml volume will be required for each cell sample.
3.2 Centrifuge the cell suspension (from step 2.6) and discard the supernatant. Tap to loosen the pellet and add 1 ml of DAPI diluted in staining buffer.
3.3 Incubate for 15 minutes at room temperature.
3.4 Analyze by flow cytometry in the presence of the dye. If the cells are to be viewed by fluorescence microscopy, centrifuge the sample, remove the supernatant and resuspend cells in fresh buffer. Apply a drop of the suspension to a microscope slide, cover with a coverslip and view.

Protocol for Chromosome FISH Counterstaining
Sample Preparation

Prepare the specimen according to standard procedures.[5,6] Briefly rinse the final preparations in dH_2O before counterstaining to remove residual buffer salts from the slide. This final rinse will help reduce nonspecific background staining on the glass. Allow the preparation to air dry.

Counterstaining Protocol

4.1 Dilute the DAPI stock solution to 30 nM in PBS. Pipet 300 µl of this staining solution directly onto the specimen. A plastic cover slip can be used to distribute the dye evenly on the slide.
4.2 Incubate the specimen in the dark for 30 minutes at room temperature.
4.3 Carefully remove the coverslip and rinse the specimen briefly with PBS or dH_2O to remove unbound dye.
4.4 Remove excess liquid from the slide by gently blotting around the sample with an absorbent tissue.
4.5 Place a glass coverslip on the slide and seal the edges with wax or nail polish.
4.6 View the sample using a fluorescence microscope with appropriate filters.

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References

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