DAPI [4,6-Diamidino-2-phenylindole, dihydrochloride]

Ordering Information	Storage Conditions
Product Numbers: 17507 (2 mL), 17510 (10 mg),	Keep at -20 °C and desiccated
17511 (100 mg), 17513 (25 mg)	Expiration date is 6 months from the date of receipt

Chemical and Physical Properties



Molecular Weight: 350.25 Solvent: water Spectral Properties: Excitation = 358 nm; Fluorescence = 461 nm.

Biological Applications

DAPI is a fluorescent stain that binds strongly to DNA. It is used extensively in fluorescence microscopy. Since DAPI passes through an intact cell membrane, it can be used to stain live cells besides fixed cells. For fluorescence microscopy, DAPI is excited with ultraviolet light. When bound to double-stranded DNA, its absorption maximum is at 358 nm and its emission maximum is at 461 nm. One drawback of DAPI is that its emission is fairly broad. DAPI also binds to RNA although it is not as strongly fluorescent as it is when it binds to DNA. Its emission shifts to around 500 nm when bound to RNA. DAPI's blue emission is convenient for multiplexing assays since there is very little fluorescence overlap between DAPI and green-fluorescent molecules like fluorescein and green fluorescent protein (GFP), or red-fluorescent stains like Texas Red. Besides labeling cell nuclei, DAPI is also used for the detection of mycoplasma or virus DNA in cell cultures.

Sample Protocol for Staining Cells

Use the fixation protocol appropriate for your sample. DAPI staining is normally performed after all other staining.

The following procedure can be adapted for most cell types. Growth medium, cell density, the presence of other cell types and other factors may influence staining. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present.

Pellet cells by centrifugation and resuspend the cells in buffered salt solutions or media, with optimal dye binding at pH 7.4. Adherent cells in culture may be stained *in situ* on cover slips or in the cell culture wells. Add DAPI stain using the concentrations between 0.5 and 5 μ M and incubate it for 15 to 60 minutes as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining.

References

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