INSTRUCTIONS

# DAPI

## (4′, 6-diamidino-2′-phenylindole, dihydrochloride)

62247 62248

2244.1

| Number | Description |
|---|---|
| **62247** | **DAPI,** 10 mg |
| **62248** | **DAPI** (1 mg/ml in water)**,** 1 ml |

Molecular weight: 350.25

Molar extinction coefficient: 30,600 $M^{-1}$ $cm^{-1}$ at 347 nm in methanol

Excitation wavelength: 341 nm

Emission wavelength: 452 nm

CAS Number: 28718-90-3

**Storage:** Upon receipt store product at 2-8°C protected from light. Product is shipped at ambient temperature.

## Introduction

DAPI (4′, 6-diamidino-2′-phenylindole, dihydrochloride) is a dye that fluoresces blue (455 nm) when bound to double-stranded DNA and excited by exposure to 345 nm light. DAPI binds selectively to the minor groove of adenine-thymine (A-T) regions of DNA, where its fluorescence is approximately 20-fold greater than in the nonbound state.[1-3] These properties make DAPI useful for assaying DNA in solution,[4] diagnosing mycoplasmal infection of cell cultures,[5] measuring nuclear content and sorting chromosomes in flow cytometry,[6] assessing apoptosis,[7] and detecting nuclei and organellar DNA in immunofluorescent and *in situ* hybridization procedures.[2,8] DAPI is also used as a replacement for ethidium bromide to stain double-stranded DNA in agarose gels.[5,9] Because DAPI is cell permeable and fluoresces blue, it is commonly used to counterstain nuclei in histochemical methods when red-fluorescent antibodies have been used to detect specific targets.[8] Reports also indicate that DAPI binds to polyphosphates and other polyanions,[10] dextran sulfate[11] and SDS.[12]

## General Procedure for Immunofluorescent Staining

### A. Reagent Preparation

| | |
|---|---|
| PBS (Wash Buffer) | Modified Dulbecco's PBS (Product No. 28374): 8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM sodium chloride, 10 mM potassium chloride; pH 7.4. |
| DAPI Stock Solution | Dissolve DAPI in ultrapure water to 1 mg/ml. Stock solution is stable for several months and repeated use if stored protected from light at -20°C. |
| DAPI Working Solution | Dilute the DAPI Stock Solution 1:1,000 in ultrapure water or PBS (1 µg/ml DAPI). Filter the Working Solution to remove dye aggregates that can result in punctate signal. |

### B. Procedure

1. Follow standard procedures to fix sample and then probe with specific fluorescent-labeled antibodies.
2. Thoroughly wash sample with PBS to remove nonbound probe.
3. Add a sufficient volume of DAPI Working Solution to completely cover the sample. Place aluminum foil over the sample to protect it from light and incubate at room temperature for 2-10 minutes. If resulting final signal bleeds through to other fluorescent channels, decrease incubation time in next experiment.
4. Wash sample thoroughly with PBS to remove excess DAPI.
5. Mount sample with an appropriate medium and detect according to standard protocols.

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## Procedure for Assaying DNA

DAPI can be used to quantitate DNA in solution. The method is relatively insensitive to pH 5-10 but is sensitive to changes in temperature and ionic strength, as well as to fluorescence quenching by divalent or heavy metal cations.[4] The fluorescence is not linear over broad concentration ranges of DNA; therefore, use an internal standard each time the assay is performed.

### A. Reagent Preparation

| | |
|---|---|
| Assay Buffer | 0.1 M NaCl, 10 mM EDTA, 10 mM Tris; pH 7.0. |
| DAPI Stock Solution | Dissolve DAPI in ultrapure water to 1 mg/ml. Stock solution is stable for several months and repeated use if stored protected from light at -20°C. |
| DAPI Working Solution | 0.1 µg/ml DAPI in Assay Buffer. |
| DNA Standards | Dilute known amount of calf thymus DNA with Assay Buffer to make a series of DNA standards with concentrations ranging from 0 to 5 µg/ml (0 to 250 ng/50 µl). Prepare replicates of each dilution so error statistics can be calculated. |

### B. Assay

1. Add 50 µl of each unknown sample or DNA Standard to a disposable fluorometer cuvette.
2. Add 1.5 ml DAPI Working Solution to each cuvette.
3. Cover cuvettes with foil and incubate at room temperature for 10 minutes.
4. Measure fluorescence of each solution.
5. Prepare a standard curve by plotting the mean values of the standards plotted against their concentrations.
6. Determine concentration of each unknown sample based on its fluorescence measurement relative to the standard curve.

## Related Thermo Scientific Products

Visit our web site for information about other fluorescent protein labeling reagents, including fluorescein (Product No. 46409, 46410 and 53029), rhodamine (No. 46406 and 53031), and amine-reactive or sulfhydryl-reactive Thermo Scientific DyLight Fluors. We also offer a variety of labeled secondary antibodies.

### References


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