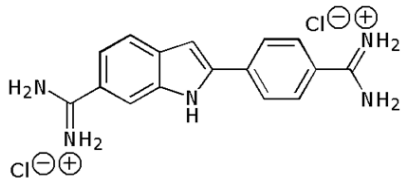
# Instructions For Use

# DAPI

## (4',6-diamidino-2-phenylindole)

**Product Code:** F-0408 / F-0410



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| **CAS Number** | [28718-90-3] |
| **Molecular Formula** | $C_{16}H_{17}N_5Cl_2$ |
| **Molecular Weight** | 350.25 g/mol |
| **Shipping** | Ambient |
| **Storage** | +2-8 °C. Keep away from light! |

## Introduction

DAPI (4',6-diamidino-2-phenylindole) is a membrane-permeable fluorescent dye that binds strongly to A-T-rich regions in DNA. Upon UV excitation (~358 nm), it emits bright blue fluorescence between 461 - 470 nm, making it a reliable nuclear stain. It is commonly used in fluorescence microscopy of fixed cells and tissues. Its sharp nuclear localization and compatibility with other fluorescent dyes make it a staple in multicolor fluorescence microscopy.

This protocol outlines procedures for staining fixed cells with DAPI in aqueous solution.

*emp BIOTECH* portfolio for cellular stains offers two DAPI formats. Both F-0408 and F-0410 are suitable for use in fixed cell imaging applications.

- The aqueous 1 mg/mL formulation is ready-to-use or can be diluted for working solutions.
- The powder form offers maximum shelf-life and customizable stock concentrations.

## Manual for Fixed-Cell-Staining

**1. Cell Fixation**

- Remove culture medium.
- Rinse cells gently with PBS.
- Add 4 % paraformaldehyde in PBS and incubate for 10 - 15 minutes at room temperature.
- Wash cells 2 - 3 times with PBS to remove residual fixative.

**2. Cell Permeabilization**

- Add permeabilization buffer (e.g., 0.1 % Triton X-100 in PBS).
- Incubate for 5 - 10 minutes at room temperature.
- Wash cells 2 - 3 times with PBS.

**3. Preparation of DAPI working solution**

- Dilute DAPI to a final concentration of 0.1 - 1 µg/mL in PBS.

- Protect from light to avoid photobleaching.

**4. Cell Staining**

- Add DAPI staining solution to the fixed, permeabilized cells.
- Incubate for 1 - 5 minutes at room temperature in the dark.

**5. Washing and Mounting**

- Wash cells 2 - 3 times with PBS to remove excess dye.
- If using coverslips, mount with antifade mounting medium.
- Keep samples protected from light until imaging.

**6. Imaging**

- Visualize cells using appropriate UV filters (excitation: 350 - 360 nm, emission: 460 - 470 nm) or use the DAPI filter set (configuration: excitation: 352 - 402 nm, emission: 417 - 477 nm).

## Frequently Asked Questions

**1. How does DAPI work?**

It binds to the minor groove of DNA, especially adenine-thymine-rich regions, and fluoresces blue when excited by UV light (~358 nm), with emission around 461 nm.

**2. Can DAPI stain live cells?**

DAPI is typically avoided in live-cell-staining due to its limited ability to cross intact cell membranes. At high concentrations, it can be cytotoxic, so it's best to use minimal amounts with brief incubation times and confirm cell viability afterward. For live-cell imaging, Hoechst 33342 is a more permeable and safer alternative.

**3. Is DAPI suitable for staining fixed cells?**

DAPI is ideal for staining fixed cells and integrates well into most immunofluorescence workflows. It performs reliably with various fixatives, especially paraformaldehyde, which tends to produce consistent nuclear staining. While methanol or acetone can be used, they may lead to uneven staining or artifacts. Because DAPI readily enters cells with compromised membranes, fixation alone is typically sufficient for effective nuclear labeling.

**4. Can DAPI be used with other dyes?**

DAPI fluoresces bright blue (~461 nm) under UV light, making it compatible with green, red, and far-red dyes. It's commonly used as a nuclear counterstain with fluorophore-labeled antibodies like Alexa Fluor 488, Cy3, or DyLight 550.

**5. Can I combine DAPU staining with antibody staining?**

DAPI emits blue fluorescence (~461 nm) that does not overlap with common antibody fluorophores like Alexa Fluor 488, 555, or 647. It's usually added after antibody staining, and both methods work well together on fixed, permeabilized cells.

**6. Do I need to wash the cells after staining?**

Washing is optional. It can reduce background but may also lower signal. Some protocols recommend imaging directly in the staining solution.

**7. How long does the stain last?**

DAPI-stained samples are stable for several days if mounted properly and stored in the dark at 4 °C. Fluorescence may fade over time, so imaging soon is best. Protect DAPU from light to prevent photodegradation.

**8. How should I store DAPI?**

Store stock solution at 2 - 8 °C for short-term or ≤ -20 °C for long-term. Protect from light to prevent degradation. Use amber vials or wrap containers in foil to prevent photobleaching. Ideally, prepare fresh DAPI working solutions to maintain staining consistency. Avoid repeated freeze-thaw cycles of stock solutions to prevent dye degradation.

**9. Is DAPI toxic?**

DAPI is classified as a known mutagen by some manufacturers, meaning it can cause DNA mutations under certain conditions. It's more toxic to live cells than alternatives like Hoechst dyes, which is why DAPI is typically reserved for fixed-cell imaging.

**10. How does DAPI compare with other nuclear stains?**

- Hoechst is ideal for live-cell nuclear staining due to its membrane permeability and low toxicity.
- DAPI is excellent for fixed cells, offering strong fluorescence and stability, but it cannot cross intact membranes.
- PI is used to identify dead or dying cells, as it only enters cells with compromised membranes.
- DRAQ5 is a versatile, far-red DNA stain that works well in live-cell imaging and avoids UV excitation, making it ideal for multiplexing with other fluorophores.

**11. How do you solubilize DAPI?**

Use ultrapure water or dimethylformamide (DMF). Avoid PBS, as DAPI is poorly soluble in phosphate-buffered saline. For stock concentrations dissolve to a final concentration of 1 - 5 mg/mL. The maximum solubility in water is approximately 25 mg/mL. The stock can be used directly or diluted in water or PBS to prepare working concentrations (typically 0.1 - 1 µg/mL) for fixed-cell staining.

**12. What challenges or limitations can arise when working with DAPI?**

- Excess DAPI can lead to high background fluorescence and obscure nuclear detail. Optimize incubation time and concentration. Shorter staining with lower doses often yields cleaner results.
- Avoid combining DAPI with other blue-emitting dyes like Alexa Fluor 405 or DyLight 405, as their emission spectra can overlap and cause signal confusion
- Under intense UV exposure, DAPI may shift emission into the green channel, potentially interfering with GFP or FITC signals. To avoid artifacts, image DAPI after other fluorophores and use low concentrations with proper mounting media to minimize this effect.
- At high concentrations or with improper dilution, DAPI can crystallize, creating bright, non-specific artifacts that resemble contamination. Recommended concentrations are typically 0.1 - 1 µg/mL. Ensure proper mixing and filtration of staining solutions.