PureBlu DAPI Nuclear Staining Dye

Catalog #	Description
1351303	**PureBlu DAPI Nuclear Staining Dye**, 5 x 50 µg vials

For research purposes only.

Introduction

PureBlu DAPI Nuclear Staining Dye is a highly pure formulation of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) fluorescent dye (Figure 1) packaged in a user-friendly format.

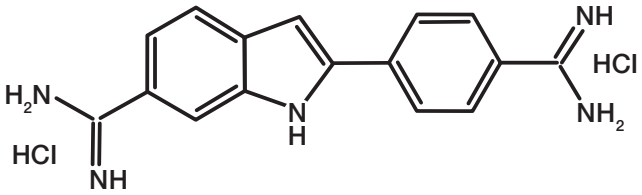

Fig. 1. Molecular structure of PureBlu DAPI Nuclear Staining Dye.

DAPI is a cell-permeable fluorescent compound that is able to stain the DNA of eukaryotic and prokaryotic cells by binding with high affinity to the minor groove of AT-rich DNA sequences.

When DAPI is bound to DNA and excited by an ultraviolet light source, blue fluorescence emission can be detected with maximum emission at 461 nm. PureBlu DAPI Dye has a characteristic Stokes shift of approximately 100 nm, which makes this dye an optimal choice when good spectral separation is desired (Figure 2).

PureBlu DAPI Nuclear Staining Dye is compatible with fixed and unfixed cells (Figure 3). While it is able to permeate the membrane of live cells, a greater amount of dye is usually required and the proper concentration should be determined experimentally. PureBlu Hoechst 33342 Nuclear Staining Dye (catalog #1351304) should be considered as a viable alternative for live cell applications.

PureBlu DAPI Nuclear Staining Dye is provided in an easy-to-reconstitute format. Each vial contains 50 µg of PureBlu DAPI Powder to generate 50 ml of 1 µg/ml working solution.

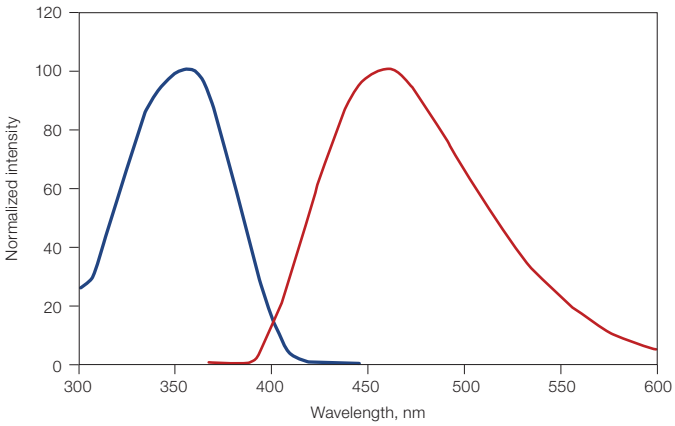

Fig. 2. Excitation and emission spectra of PureBlu DAPI Dye.

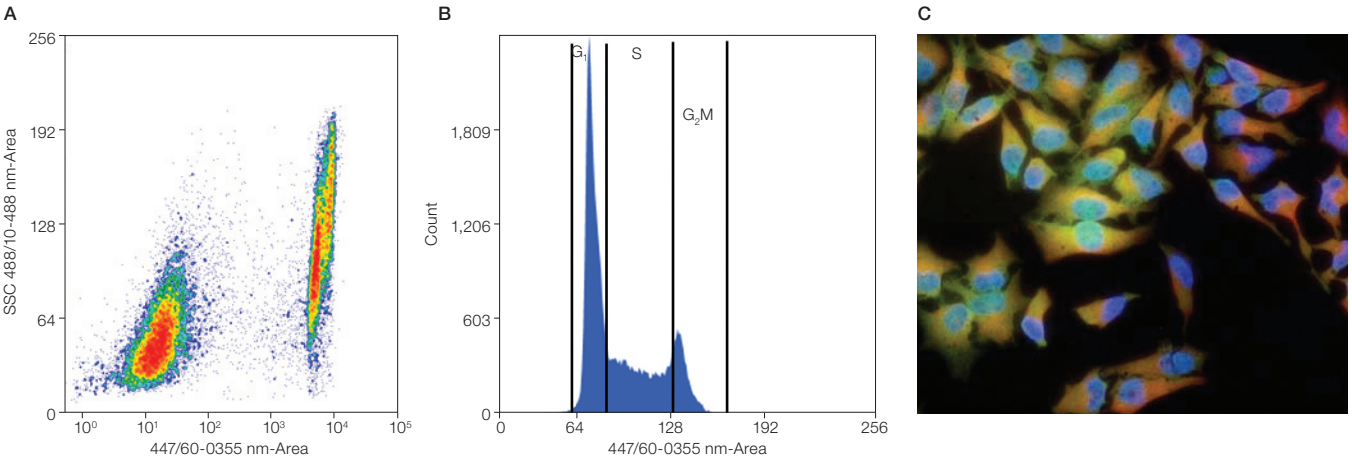

Fig.3. Analysis of Jurkat and HeLa cells. A, flow cytometric analysis of Jurkat cell viability. Jurkat cells were heat shocked at 65°C for 5 min. Dead cells show positive staining with PureBlu DAPI. **B**, analysis of DNA content of Jurkat cells. Cultured cells were harvested, washed in phosphate buffered saline (PBS), and fixed in cold 70% ethanol for 2 hr at 4°C. The Jurkat cells were then washed in Bio-Rad Staining Buffer (#BUF073) and stained with PureBlu DAPI Dye containing 0.1% Triton X-100 for 30 min. Cells were first gated to discriminate single cells and then plotted into histograms discriminating G_1, S, and G_2/M populations. All flow cytometry was carried out on a Bio-Rad ZE5 Cell Analyzer (#12004279). **C**, HeLa cells, formaldehyde fixed and permeabilized, stained with Rat Anti-Alpha Tubulin Antibody (red) (#MCA78G), Mouse Anti-Human CD49a Antibody, green (#MCA1133), and PureBlu DAPI Dye (blue).

Specifications

Property	Description
Formula	$C_{16}H_{17}Cl_2N_5$
Molecular weight	350.3
Maximum excitation/ emission	359 nm/461 nm
CAS	28718-90-3
Purity	>95% (high performance liquid chromatography)
Solubility	Soluble in deionized water (DI water) and dimethyl sulfoxide (DMSO)
Long-term storage	–20°C
Storage and stability	Stable for 2 years at –20°C. Upon resuspension, PureBlu DAPI Dye is stable for 1 year at –20°C or 6 months at 2–8°C
Handling	Protect from light

Cell Staining Protocol

Note: The optimal concentration for different cell types should be determined empirically.

Preparation of the Staining Solution

1. For a 100x solution, used in flow cytometry applications, add 500 µl of DI water to one tube of lyophilized PureBlu DAPI Dye, then vortex briefly.
2. For a 1x staining solution, used in microscopy and cell imaging, dilute the 100x stock solution 1:100 with PBS for a final concentration of 1 µg/ml.

Staining Procedure — Flow Cytometry Cell Viability

1. Grow cells of interest under conditions specific for the cell type. Harvest cells and obtain a single cell suspension.
2. Check cell numbers and viability using a Bio-Rad TC20 Automated Cell Counter (#1450102) and trypan blue.
3. Resuspend cells at 2 x 10^6 cells/ml in 0.5 ml Staining Buffer or 1x PBS.
4. Add 5 µl of 100x PureBlu DAPI Dye stock solution to 0.5 ml cells.
5. Stain at room temperature for 15 min in the dark.
6. Optional: rinse cells with Staining Buffer or 1x PBS.
7. Proceed with analysis by flow cytometry. When bound to double-stranded DNA (dsDNA) PureBlu DAPI Dye can be excited with either a 355 nm or a 405 nm laser, with optimum emission at 461 nm.

Staining Procedure — Flow Cytometry Cell DNA Content

1. Grow cells of interest under conditions specific for the cell type. Harvest cells and obtain a single cell suspension.
2. Check cell numbers and viability using a Bio-Rad TC20 Automated Cell Counter and trypan blue.
3. Wash cells in Staining Buffer or 1x PBS.
4. Fix in ice cold 70% ethanol for 2 hr at 4°C.
5. Wash cells in Staining Buffer or PBS.
6. Stain cells with 5 µl of 100x PureBlu DAPI Dye stock solution to 0.5 ml cells at a concentration of 2 x 10^6 cells/ml in cell staining buffer containing 0.1% Triton X-100.

   Note: Optimal concentrations of cells and PureBlu DAPI may vary depending on cell type and should be determined through careful titration prior to final experimental analysis.
7. Stain at room temperature for 30 min in the dark.
8. Optional: rinse cells with Staining Buffer or 1x PBS.
9. Proceed with analysis by flow cytometry. When bound to dsDNA PureBlu DAPI Dye can be excited with either a 355 nm or a 405 nm laser, with optimum emission of 461 nm.

Staining Procedure — Cell Nuclear Visualization in Microscopy and Cell Imaging

1. Grow cells of interest under conditions specific for the cell type.
2. Rinse cells with 1x PBS.
3. Optional: fix cells with 3.7% formaldehyde at room temperature for 10 min.
4. Optional: rinse cells with 1x PBS and permeabilize them with 1x PBST (0.1% Triton X-100 in 1x PBS) at room temperature for 5 min.
5. Rinse cells with 1x PBS.
6. Stain with 1x staining solution (diluted with PBS) at room temperature for 15 min.
7. Rinse cells with 1x PBS.
8. Optional: remove PBS and mount cells in antifade mounting media.
9. Image cells using the Bio-Rad ZOE Fluorescent Cell Imager (#1450031).

Visit **bio-rad.com/PureBluDAPI** for more information.

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