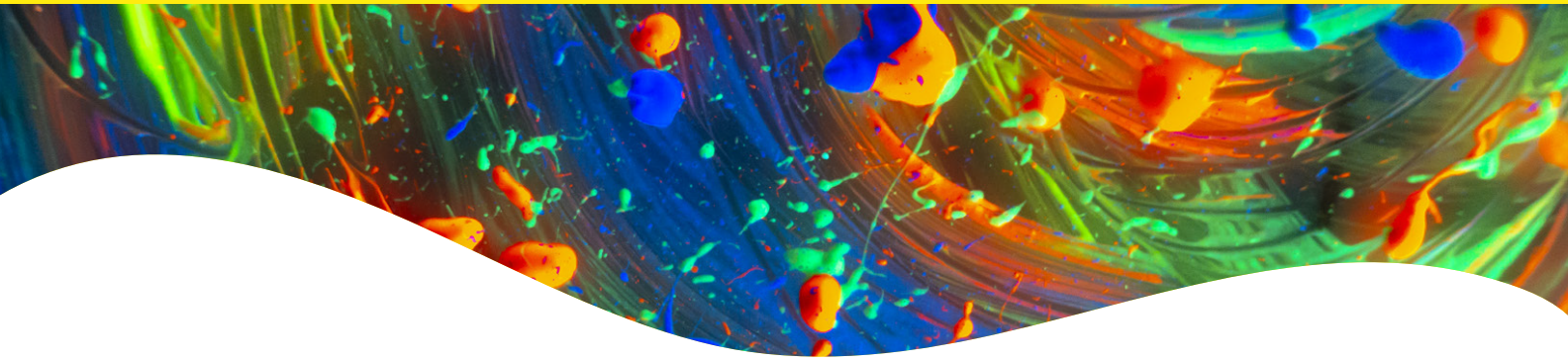
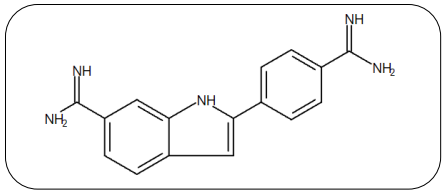
PhenoVue DAPI Nuclear Stain



Overview

PhenoVue™ DAPI nuclear stain is a cell-impermeable organic molecule that binds preferentially to adenine-thymine (A-T) regions of minor groove of DNA. When excited by ultraviolet light, DAPI exhibits strong fluorescence at 455 nm. It is commonly used to stain cells' nuclear compartment.



Structure of 4′,6-Diamidino-2-phenylindole (DAPI). Source: *PubChem CID 2954*

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue DAPI nuclear stain	CP81	1	1mg (1 mg/mL, 2.85 mM)	Solution in 1 mL ddH_2O	Dry ice

Storage and stability

- Store stock solution at 2-8°C for short term (6 months) or -16 °C or below for long term (> 6 months), protected from light.
- Avoid repeated freeze/thaw cycles.
- The stability of these products is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.

Recommended reconstitution

Product name	Molecular weight	Recommended stock concentration	Working concentration range*
PhenoVue DAPI nuclear stain	350.22 g/mol	Already reconstituted in 1 mL ddH_2O to give a stock concentration of 1 mg/mL (2.85 mM)	0.1 µg/mL – 1 µg/mL (285 nM – 2.85 µM)

* Dilutions can be done in PBS.

Equivalent number of microplates

Product name	When used at recommended concentration	96-well microplate (100 µL - 300 µL per well)	384-well microplate (25 µL - 90 µL per well)	1536-well microplate (4 µL - 12 µL per well)
PhenoVue DAPI nuclear stain	0.1 µg/mL (285 nM)	Approx. 350 - 1040	Approx. 290 - 1040	Approx. 540 - 1630

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Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)*	Maximum emission wavelength (nm)*	Common filter set	Quantum yield* (Φ)	Epsilon** (ε in $M^{-1}.cm^{-1}$ at λ max)	Brightness (Φ x ε)
PhenoVue DAPI nuclear stain	348	457	DAPI	dsDNA: 0.34 ssDNA: 0.19	31000	nd***

* Cosa et al. photochemistry and photobiology, 2001 ** In methanol *** Not determined

Live-and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue DAPI nuclear stain	No, except at concentration ≥ 10 µg/mL	Yes	Yes

Protocols

Cell culture

Seed cells in imaging microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO_2 until 50-70% confluency.

Fixed-cell imaging

Rinse briefly in phosphate-buffered saline (PBS) then proceed with cell fixation.

1. Fixation: 2 options:

1. Add ready to use PhenoVue paraformaldehyde 4% methanol-free solution (PVPFA41) for 10 min at room temperature. Note that paraformaldehyde (PFA) is the most popular fixative reagent.
   or
2. Add 100% methanol (chilled to -20 °C) at room temperature for 5 min.

2. Washing: Wash three times with PBS.

3. Permeabilization (optional):

1. For PFA fixed cells, add ready to use PhenoVue permeabilization 0.5% Triton X-100 solution (PVPERM051) for 10 min (for membrane-associated antigens, 100 µM digitonin or 0.5% saponin are preferred). Triton X-100 is the most popular detergent for improving the penetration of antibodies. However, it may be not appropriate for some imaging applications since it can destroy membranes.
2. Methanol fixed cells do not require permeabilization.

4. Washing: Wash three times with PBS for 5 min.

5. Incubate: Incubate with 0.1 - 1 µg/mL PhenoVue DAPI nuclear stain for 10 min at RT.

6. Washing: Wash once with PBS for 5 min.

7. Acquire images on an imaging device.

Tips

- PhenoVue DAPI nuclear stain may form aggregates at concentration greater than 20 µg/mL.
- Washing steps are not mandatory prior to image acquisition.

Safety information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Applications

- High-content analysis/high-content screening
- Imaging microscopy
- Flow cytometry

Validation data

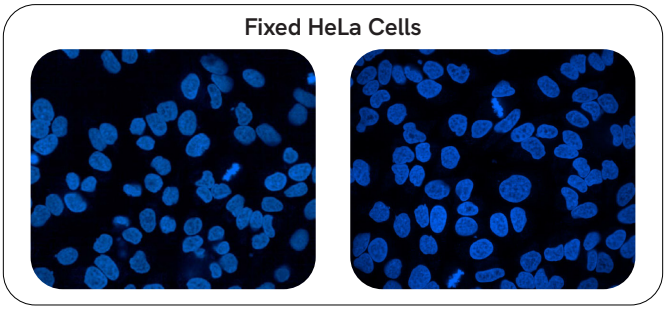

Figure 1: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then stained with 0.1 or 1 µg/mL of PhenoVue DAPI nuclear stain for 30 min at RT. Images were acquired on the Opera Phenix® high content analysis system.

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