

PureBlu™ Hoechst 33342 Nuclear Staining Dye

Catalog #	Description
135-1304	PureBlu Hoechst 33342 Nuclear Staining Dye , 5 x 56 µg vials

For research purposes only.

Introduction

PureBlu Hoechst 33342 Nuclear Staining Dye is a highly pure formulation of Hoechst 33342 fluorescent dye (Figure 1) packaged in a user-friendly format.

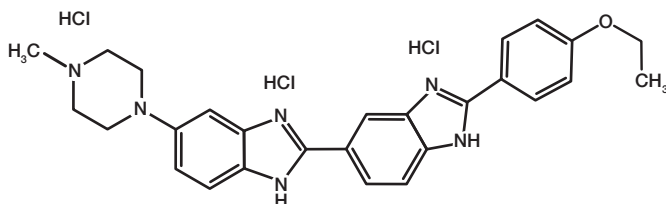


Fig. 1. Molecular structure of PureBlu Hoechst 33342 Nuclear Staining Dye.

Hoechst 33342 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 Hoechst 33342 is bound to DNA and excited by an ultraviolet light source, blue fluorescence emission can be detected with maximum emission at 461 nm. PureBlu Hoechst 33342 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). The use of Hoechst dyes is reported in scientific literature for various applications including fluorescence microscopy, chromosome sorting, and cell cycle analysis using flow cytometry.

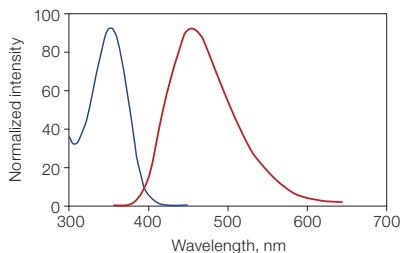


Fig. 2. Excitation and emission spectra of PureBlu Hoechst 33342 Dye.

PureBlu Hoechst 33342 Nuclear Staining Dye is compatible with fixed and unfixed cells. It exhibits a higher permeability for live cell membranes than 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) and is optimal for live cell DNA staining.

PureBlu Hoechst 33342 Nuclear Staining Dye is provided in an easy-to-reconstitute format. Each vial contains 56 µg of PureBlu Hoechst 33342 powder to generate 50 ml of 1.1 µg/ml (2 µM) working solution.

Specifications

Property	Description
Formula	$C_{27}H_{28}N_6O \cdot 3HCl$
Molecular weight	561.93
Maximum excitation/emission	350 nm/461 nm
CAS	23491-52-3
Purity	>95% (high-performance liquid chromatography)
Solubility	Soluble in deionized water (DI H ₂ O) and dimethyl sulfoxide (DMSO)
Long-term storage	-20°C
Storage and stability	Stable for 2 years at -20°C. Upon resuspension, PureBlu Hoechst 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. Add 500 µl of DI H₂O to one tube of lyophilized PureBlu Hoechst 33342 Dye, then vortex briefly to make the 100x stock solution (1.1 µg/ml [2 µM]).
2. Dilute the stock solution 1:100 with growth media (for live cells) or 1x phosphate buffered saline (PBS) (for fixed cells) to make the 1x staining solution.

Staining Live Cells

1. Grow cells of interest under conditions specific for the cell type.
2. Replace growth media with 1x staining solution (diluted in fresh growth media) and incubate at 37°C for 15 min.
3. Rinse cells with 1x PBS (prewarmed to 37°C).
4. Aspirate PBS and add fresh growth media to cells (prewarmed to 37°C).
5. Image cells.

Staining Fixed Cells

1. Grow cells of interest under conditions specific for the cell type.
2. Rinse cells with 1x PBS.
3. Fix cells with 4% formaldehyde at room temperature (RT) 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 RT for 5 min.
5. Rinse cells with 1x PBS.
6. Stain with 1x staining solution (diluted with PBS) at RT for 15 min.
7. Rinse cells with 1x PBS.
8. Optional: Remove PBS and mount cells in antifade-mounting media.
9. Image cells.

Visit [bio-rad.com/PureBluHoechst33342](https://www.bio-rad.com/PureBluHoechst33342) for more information.

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