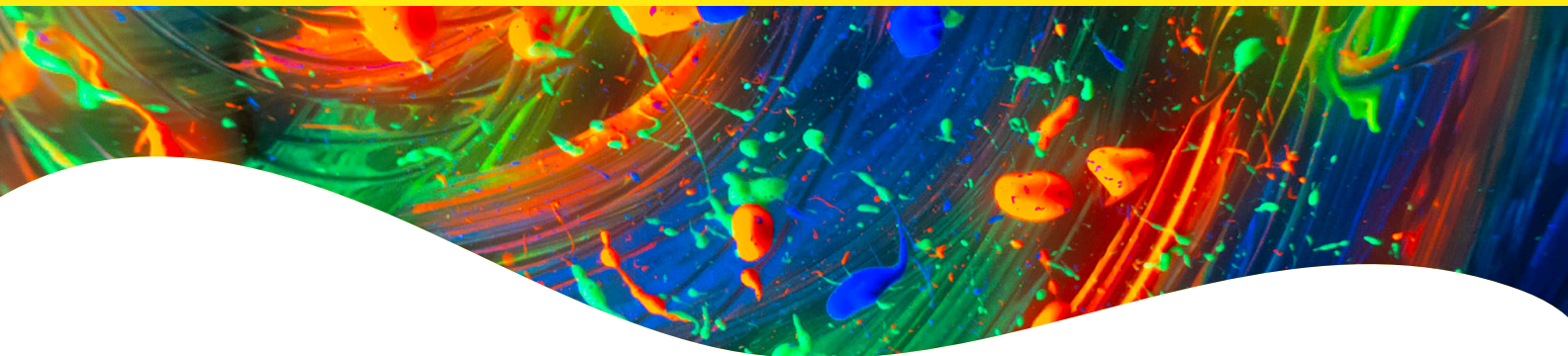
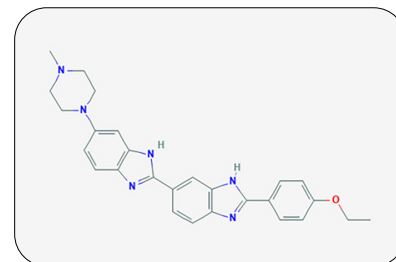


# PhenoVue Hoechst 33342 Nuclear Stain



## Overview

PhenoVue™ Hoechst 33342 nuclear stain (trihydrochloride form) is a cell-permeable organic molecule that binds preferentially to adenine-thymine (A-T) regions of minor groove of DNA. When excited by ultraviolet light, Hoechst 33342 exhibits strong fluorescence at 455 nm. It is commonly used to stain cells' nuclear compartment.



Structure of PhenoVue Hoechst 33342 nuclear stain (trihydrochloride form).  
Source: PubChem CID 137195726

## Product information

| Product name                                | Part no. | Number of vials per unit | Quantity per vial              | Format                               | Shipping conditions |
|---|----------|--------------------------|--------------------------------|--------------------------------------|---------------------|
| PhenoVue Hoechst 33342 nuclear stain 10 mg  | CP71     | 1                        | 10 mg<br>(10 mg/mL, 16.23 mM)  | Solution in 1 mL ddH <sub>2</sub> O  | Dry ice             |
| PhenoVue Hoechst 33342 nuclear stain 100 mg | CP72     | 1                        | 100 mg<br>(10 mg/mL, 16.23 mM) | Solution in 10 mL ddH <sub>2</sub> O | 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 Hoechst 33342 nuclear stain 10 mg  | 615.99 g/mol     | Already reconstituted in 1 mL ddH <sub>2</sub> O to give a stock concentration of 10 mg/mL (16.23 mM)  | 1 µg/mL - 5 µg/mL (1.62 µM - 8.1 µM) |
| PhenoVue Hoechst 33342 nuclear stain 100 mg | 615.99 g/mol     | Already reconstituted in 10 mL ddH <sub>2</sub> O to give a stock concentration of 10 mg/mL (16.23 mM) | 1 µg/mL - 5 µg/mL (1.62 µM - 8.1 µM) |

## 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 Hoechst 33342 nuclear stain 10 mg  | 2 µg/mL (3.24 µM)                      | Approx. 170 to 520                            | Approx. 140 to 520                           | Approx. 270 to 800                           |
| PhenoVue Hoechst 33342 nuclear stain 100 mg | 2 µg/mL (3.24 µM)                      | Approx. 1740 to 5200                          | Approx. 1450 to 5200                         | Approx. 2700 to 8140                         |

View our full range of high-quality imaging microplates at [Revvity.com](https://www.revvy.com)

## Spectral and photophysical properties

| Product name                                | Maximum excitation wavelength (nm) | Maximum emission wavelength (nm) | Common filter set | Quantum yield* (Φ)         | Epsilon** (ε in M <sup>-1</sup> .cm <sup>-1</sup> at λ. max) | Brightness (Φ x ε) |
|---|------------------------------------|----------------------------------|-------------------|----------------------------|--|--------------------|
| PhenoVue Hoechst 33342 nuclear stain 10 mg  | 357                                | 455                              | DAPI              | dsDNA: 0.38<br>ssDNA: 0.22 | 43000  | nd***              |
| PhenoVue Hoechst 33342 nuclear stain 100 mg | 357                                | 455                              | DAPI              | dsDNA: 0.38<br>ssDNA: 0.22 | 43000  | nd***              |

\* Cosa et al. Photochemistry and Photobiology, 2001 \*\* In 0.2 M HCl/Methanol \*\*\* Not determined

## Live- and fixed-cell compatibility

| Product name                                | Live-cell staining | Fixation/permeabilization steps post live-cell staining | Fixed-cell staining |
|---|--------------------|---|---------------------|
| PhenoVue Hoechst 33342 nuclear stain 10 mg  | Yes                | Yes   | Yes                 |
| PhenoVue Hoechst 33342 nuclear stain 100 mg | Yes                | 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<sub>2</sub> until 50-70% confluency.

PhenoVue Hoechst 33342 nuclear stain is compatible with live as well as fixed and permeabilized cells.

### Fixed-cell imaging

#### 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:

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 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min at RT.

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

#### 7. Acquire images on an imaging device.

### Live-cell imaging

1. Rinse briefly in HBSS.
2. Incubate with 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min at RT.
3. Rinse in HBSS.
4. Acquire images on a live-cell imaging device.

*Note that cytotoxicity of staining reagents such as Hoechst 33342 is usually observed in long term imaging.*

## 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
- Microscopy
- Cytometry

## Validation data

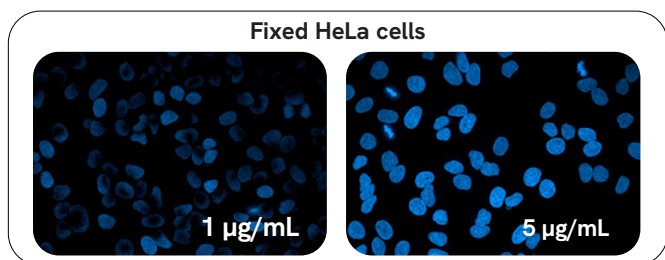


Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then stained with 1 or 5 µg/mL of **PhenoVue Hoechst 33342 nuclear stain** for 30 min at RT. Images were acquired on the Operetta CLS™ high-content analysis system.



Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were stained with 1 or 5 µg/mL of **PhenoVue Hoechst 33342 nuclear stain** for 30 min at RT, then fixed. Images were acquired on the Operetta CLS™ high-content analysis system.