

## Hoechst 33258, blue fluorescent nucleic acid stain

<http://www.lumiprobe.com/p/hoechst-33258>

Hoechst 33258 (bisbenzimidazole, HOE 33258) is a cell-permeant blue-emitting fluorescent dye that binds strongly to adenine-thymine-rich regions in the minor groove of double-stranded DNA. Although Hoechst 33258 can bind to all nucleic acids, AT-rich dsDNA strands enhance its fluorescence considerably.

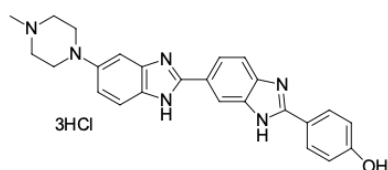
Hoechst 33258 bound with DNA has excitation/emission maxima at 351/463 nm, respectively. The fluorescence intensity of Hoechst 33258 increases with the pH of the solvent. The unbound dye fluoresces in the 510–540 nm range. The green fluorescence of unbound dye may be observed when an excessive dye concentration is used or the sample is insufficiently washed out. The dye has a considerable Stokes shift between the excitation and emission spectrum, making it worthwhile in multicolor labeling experiments.

Hoechst 33258 is able to penetrate living cells, but its cellular permeability is 10 times less than [Hoechst 33342](#). Like all Hoechst family dyes, Hoechst 33258 is less toxic than [DAPI](#), which ensures a higher viability of stained cells.

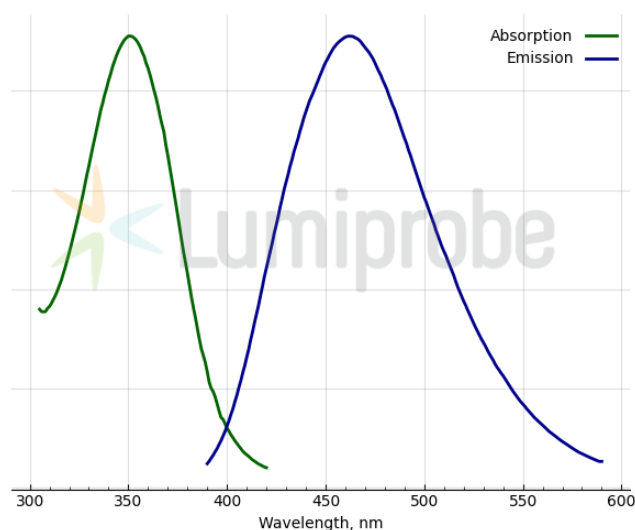
Hoechst 33258 is used extensively in fluorescence microscopy and flow cytometry for staining chromosomes and nuclei in live and fixed cells. The dye is often used to distinguish condensed pycnotic nuclei in apoptotic cells and cell sorting.

Hoechst 33258 is quenched by [bromodeoxyuridine \(BrdU\)](#), commonly used to detect dividing cells. When BrdU is integrated into DNA, the bromine is supposed to deform the minor groove so that Hoechst dyes cannot reach their optimal binding site. This property of Hoechst 33258 is used to study cell-cycle progression.

The commonly used dye concentration to stain bacteria or eukaryote cells is 0.1–10 µg/mL.



**Structure of Hoechst 33258**



**Absorption and emission spectra of Hoechst 33258 (DNA-dye complex)**

### General properties

Appearance:	dark yellow solid
Molecular weight:	533.89
Molecular formula:	C <sub>25</sub> H <sub>27</sub> Cl <sub>3</sub> N <sub>6</sub> O
Solubility:	water, DMSO, DMF
Quality control:	NMR <sup>1</sup> H and HPLC-MS (95+%)
Storage conditions:	24 months after receipt at -20°C in the dark. Transportation: at room temperature for up to 3 weeks. Desiccate.

Legal statement:

Product is offered and sold for research purposes only. Product is not tested for safety and efficacy in food, drug, medical device, cosmetic, no express or implied authorization to use for any other purpose, including, without limitation, in vitro diagnostic purposes, for humans or animals or for commercial purposes.

**Spectral properties**

Excitation/absorption maximum, nm: 351 (complex)

Emission maximum, nm: 463 (complex)