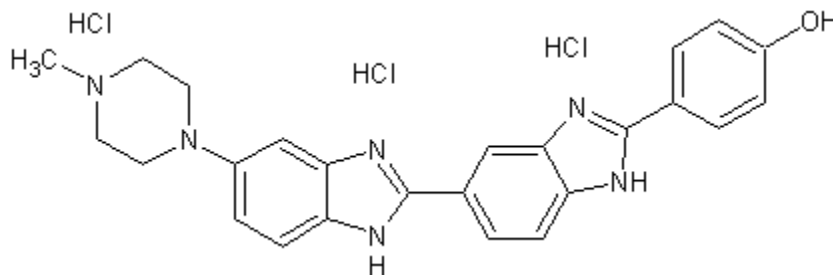


Hoechst 33258 *UltraPure grade*

Ordering Information	Storage Conditions
Product Numbers: 17520 (100 mg), 17523 (1 g), 17525 (5 mL)	Avoid exposure to light Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Chemical and Physical Properties



Molecular Weight: 533.88

Appearance: Light yellow powder

Solvents: water or dimethylsulfoxide (DMSO)

Spectral Properties: Excitation = 352 nm; Emission = 461 nm.

Biological Applications

The Hoechst stains are a family of fluorescent stains for labeling DNA in fluorescence microscopy. Because these fluorescent stains label DNA, they are also commonly used to visualize nuclei and mitochondria. Two of these closely related bis-benzimides, Hoechst 33258 and Hoechst 33342, are commonly used. Both dyes are excited by ultraviolet light at around 350 nm, and both emit blue/cyan fluorescence light with an emission maximum at 461 nm. The Hoechst stains may be used with live or fixed cells, and are often used as a substitute for another nucleic acid stain, DAPI. The key difference between them is that the additional ethyl group of Hoechst 33342 makes it more lipophilic, and thus more readily to cross intact cell membranes. In some applications, Hoechst 33258 is significantly less permeant than Hoechst 33342. These dyes can also be used to detect the contents of a sample DNA by plotting a standard emission-to-content curve.

Sample Protocol for Staining Cells

The following procedure can be adapted for most cell types. Growth medium, cell density, the presence of other cell types and other factors may influence staining. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present.

Pellet cells by centrifugation and resuspend the cells in buffered salt solutions or media, with optimal dye binding at pH 7.4. Adherent cells in culture may be stained *in situ* on cover slips or in the cell culture wells. Add Hoechst stain using the concentrations between 0.5 and 5 μ M and incubate it for 15 to 60 minutes as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining.

Disclaimer: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.