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Product Information

Hoechst Stains

Catalog Number	Product Name	Unit Size
C003	Hoechst 33258	10 mg
C004	Hoechst 33258	1 mL
C005	Hoechst 33342	10 mg
C006	Hoechst 33342	1 mL

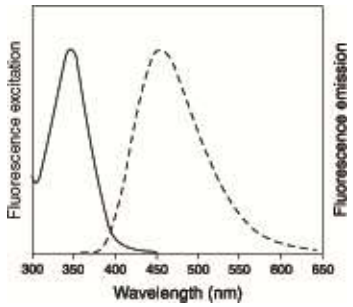
Storage upon receipt:
- -20°C
- Protect from light

Ex/Em: 350/461 nm, bound to DNA

Product Description

The blue fluorescent Hoechst dyes are cell permeable nucleic acid stains that have multiple applications, including sensitive detection (>3 ng) of DNA in the presence of RNA in agarose gels, automated DNA determination, sensitive determination of cell number and chromosome sorting. The fluorescence of these dyes is very sensitive to DNA conformation and chromatin state in cells. Consequently, they can detect gradations of nuclear damage. The Hoechst dyes are useful vital stains for the flow cytometric recognition of DNA damage and other viability measurements by monitoring the emission spectral shifts of the dyes. These bisbenzimidazole derivatives are supravital minor groove–binding DNA stains with AT selectivity. The dyes bind to all nucleic acids, but AT-rich dsDNA strands enhance fluorescence ~2-fold greater than GC-rich strands. This property has been used to identify Q-bands in chromosomes (Q-bands: AT-rich chromosome regions that fluoresce brightly when stained with the dye quinacrine). Hoechst 33258 is slightly more water soluble than Hoechst 33342, but both have been used extensively to stain live cells. The products may be used in fluorescence microscopy, microplate, cuvette, and flow cytometry applications.

Spectral Characteristics



Fluorescence excitation and emission profiles of Hoechst bound to dsDNA.

Basic Protocol for Staining Cells

The solid dyes may be dissolved in either water, DMF, or DMSO to make concentrated stock solutions up to 10 mg/mL. Stock solutions may be stored refrigerated or frozen, protected from light.

The following procedure can be adapted for most cell types. Note that different concentration ranges for the Hoechst dyes are suggested depending on the cell type (see Table 1). 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. Glassware should be washed in a mild detergent and rinsed with hot tap water followed by several rinses with deionized, distilled water.

Pellet cells by centrifugation and resuspend in buffered salt solutions or media, with optimal dye binding at pH 7.4. Adherent cells in culture may be stained *in situ* on coverslips. Add Hoechst stain using the concentrations listed in Table 1 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. Unbound dye has its maximum fluorescence emission in the 510–540 nm range, this green fluorescence may be observed on samples using too high a concentration of dye.

Table 1. Recommended conditions for staining cells with Hoechst stains.

Cell Type	Dye Concentration	Incubation Conditions
Bacteria	0.1 to 12 µg/mL	10 to 30 minutes
Live animal cells	0.2 to 5 µg/mL	20 to 30 minutes
Fixed animal cells	0.2 to 2 µg/mL	1 to 15 minutes