BIOLUMINOR

# Nuclear yellow (Hoechst S769121) for Labeling of nucleus

The Hoechst stains are a family of fluorescent stains for labeling DNA used in fluorescence microscopy and flow cytometry. Because these fluorescent stains label DNA, they are also commonly used to visualize nuclei and mitochondria.

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. Nuclear yellow (Hoechst S769121) is more commonly used as a neuronal retrograde tracer.

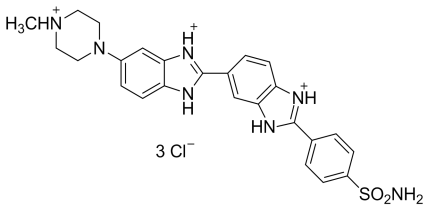


**Fig. 1** Structure of Hoechst S769121.

**Photophysical properties Hoechest S769121 bound to DNA**

The Excitation/Emission of Hoechest S769121 bound to DNA is 355/495 nm.

**Preparing Stock Solutions**

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

Note: The Hoechst stains should not be resolubilized in phosphate-buffered saline (PBS), but dilute solutions of the dye may be used with PBS or other phosphate-containing buffers. Solutions of Hoeschst dye should be stored at 2 – 6° C, protected from light. Stock solutions in water are stable for at least 6 months when refrigerated. For long-term storage the stock solution can be aliquoted and stored at ≤ – 20° C.

**Basic Protocol for Staining Cells**

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.

## Caution

The Hoechst strains are known mutagens and should be handled with care. The dye must be disposed of safely and in accordance with applicable regulations.

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

| Cell Type | Hoechst 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 |

## References


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