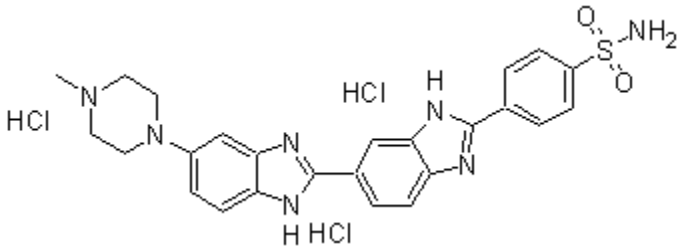
Nuclear Yellow [Hoechst S769121]

Ordering Information	*Storage Conditions*
Product Numbers: 17539	Avoid exposure to light Keep at -20 °C and desiccated Expiration: 24 months upon receipt

Chemical and Physical Properties



Molecular Weight: 651.01
Appearance: Light yellow powder
Solvents: water or dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 355 nm; Emission = 495 nm.

Biological Applications

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. 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.

The long-wavelength tracer nuclear yellow is often combined with the popular retrograde tracer True Blue for two-color neuronal mapping. In neuronal cells, nuclear yellow primarily stains the nucleus with yellow fluorescence, whereas true blue is a UV light–excitable, divalent cationic dye that stains the cytoplasm with blue fluorescence. Both Nuclear Yellow and True Blue are stable when subjected to immunohistochemical processing and can be used to photoconvert DAB into an insoluble, electron-dense reaction product. Hoechst 33258, Hoechst 33342 and Nuclear Yellow, when transported retrogradely through axons to their parent cell bodies, may migrate out of the axons and the cell bodies, as indicated by fluorescence of adjoining glial nuclei. This migration was found to take place both in vivo and in vitro during storage of the sections in water. When using the tracers in 1% concentrations the in vivo migration may be controlled by restricting the survival time. The in vitro migration may be prevented by rapid histological processing of the material.

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.

1) Pellet cells by centrifugation
2) Resuspend the cells in buffered solutions or media with optimal dye binding at pH 7.4.
3) Stain adherent cells *in situ* on cover slips or in the cell culture wells.
4) Add Hoechst stain using the concentrations between 0.5 and 5 □M.
5) Incubate the cells for 15 to 60 minutes.

Note: The incubation time and dye concentration need experimentally tested. 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.

References

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