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Hoechst 33342, trichloride, trihydrate

Cat. No. PA-3014
Storage upon receipt: 2°to 6℃
Protect from light
Concentration: 10 mg/ml

Introduction

Hoechst 33342 is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA. This dye is often used to distinguish condensed pycnotic nuclei in apoptotic cells and for cell-cycle studies in combination with BrdU.
The Hoechst 33342 dye has been used widely for staining the nuclei of living cells. Hoechst dyes preferentially bind to AT regions, making them quite selective (but not specific) for DNA; Hoechst dye–stained cells and tissues show virtually no cytoplasmic staining. The Hoechst 33342 dye is commonly used in combination with labeling by 5-bromo-2'-deoxyuridine (BrdU) to distinguish the compact chromatin of apoptotic nuclei, to identify replicating cells and to sort cells based on their DNA content.
Hoechst 33342 has high membrane permeability, is quite soluble in water (up to 2% solutions can be prepared), and relatively nontoxic. It can be excited with the UV spectral lines of the argon-ion laser and by most conventional fluorescence excitation sources and exhibits a relatively large Stokes shift (excitation/emission maxima ~350/460 nm), making it suitable for multicolor labeling experiments.
Hoechst 33342 dyes has a complex, pH-dependent spectra when not bound to nucleic acids, with a much higher fluorescence quantum yield at pH 5 than at pH 8. Its fluorescence is also enhanced by surfactants such as sodium dodecyl sulfate (SDS). The dye appears to show a wide spectrum of sequence-dependent DNA affinities and bind with sufficient strength to poly(d(A-T)) sequences that they can displace several known DNA intercalators.

It also exhibit multiple binding modes and distinct fluorescence emission spectra that are dependent on dye:base pair ratios. Hoechst dyes are used in many cellular applications, including in cell-cycle and apoptosis studies and they are common nuclear counterstains.

Hoechst 33342 is readily taken up by cells during the initial stages of apoptosis, whereas cell-impermeant dyes such as propidium iodide and ethidium bromide are excluded. Later stages of apoptosis are accompanied by an increase in membrane permeability, which allows propidium iodide to enter cells. Thus, a combination of Hoechst 33342 and propidium iodide has been extensively used for simultaneous flow cytometric and fluorescence imaging analysis of the stages of apoptosis and cell-cycle distribution. Hoechst 33342, which selectively stains nuclei of apoptotic cells blue fluorescent, has also been used in combination with calcein AM, which labels all cells that have intact membranes — even apoptotic cells — green fluorescent. Presumably the dead-cell population could be selectively detected using propidium iodide to make this a three-color assay.

Instructions for Use

Hoechst 33342 is provided as a 10 mg/ml aqueous solution. It may be added directly to cells following dilution into appropriate culture medium or balance salt solutions.
Nuclei are often brightly labeled by submicromolar concentrations and can be clearly visualized with or without washing.
Optimal concentration for nucleic acid staining varies for different cell types and should be determined for each application. Time of incubation at room temperature or 37℃ varies for cells and may range from 10-30 min. Staining intensity may increase with time if samples are viewed without washing.