FT-61248A

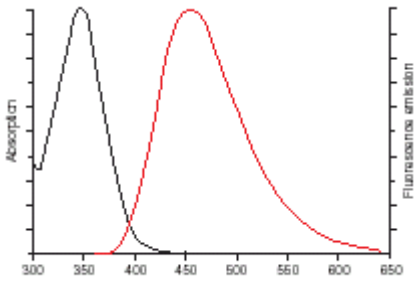
Hoechst 33258, 33342

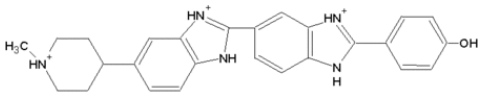
Cell membrane-permeant, minor groove binding blue fluorescent DNA stains.

Product Information

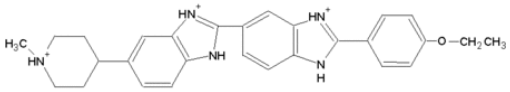
Name :	**Hoechst 33258**
Catalog Number :	FP-61248A, 100 mg FP-41387A, 10 ml at 10mg/ml solution FP-BB1330, 5 ml at 20mM solution FP-P6275A, 100 mg (FluoGrade) CAS: 23491-45-4
Molecular Weight :	MW= 623.96
Solubility:	In water, DMF
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (DNA-bound) = 352/461 nm
Extinction Coefficient :	40 000 M^{-1} cm^{-1}

Absorption and emission spectra of Hoechst 33258 bound to DNA





Name :	**Hoechst 33342** 2,5'-Bi-1H-benzimidazole, 2'-(4-ethoxyphenyl)-5-(4-methyl-1- piperazinyl)
Catalog Number :	FP-71131A, 100 mg FP-590461, 5 ml at 10mg/ml solution FP-BB1340, 5ml at 20mM solution FP-99964A, 100 mg (FluoGrade) CAS: 23491-52-3
Molecular Weight :	615.99 MW
Soluble:	In water
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (H_2O) = 350/461nm
Extinction Coefficient :	42 700 M^{-1} cm^{-1}



Storage:	Powder :	Store at ≤25°C; protect from light (stable at least 1 year)
	Solution :	Store at +4°C; protect from light (stable at least 6 months)(H) For long-term, store aliquots at ≤-20°C

Introduction

Hoechst are bisbenzimides dyes that bind to minor-grooves of DNA (multiple affinity types) with fluorescence enhancement. Fluorescence depends on pH (higher at pH5), and surfactants. Their use has been popularized thanks to their relatively non toxicity, ability to be excited by most common light sources (i.e. argon/ion laser), and suitability for multicolor imaging (large Stocke's shift).
Hoechst 33258 and Hoechst 33342 are two closely related fluorescent stains. They fluoresce strongly when bound to DNA, but are not visible under transmitted light. They are functionally very similar, and both may be used in living cells, often as a substitute for another nucleic acid stain, DAPI. Because the Hoechst stains bind to DNA, they can disrupt DNA replication during cell division. Consequently they are potentially mutagenic and carcinogenic. Care should be taken in their handling and disposal.

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Hoechst 33342 nucleic acid stain 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.

Hoechst 33258 nucleic acid stain is a popular nuclear counterstain that emits blue fluorescence when bound to dsDNA. Its uses are similar to Hoechst 33342 for counterstaining, apoptosis and cell cycle studies, but Hoechst 33258 is reportedly less cell-permeant. It also has been used to detect DNA in solution between 250ng/ml to 20µg/ml in solution.

Hoechst 33258

Counterstaining with the chromosomal dye Hoechst 33258 is a simple procedure that provides an excellent general purpose nuclear counterstain for immunofluorescent work. Benefits from its use include the ready identification and orientation of structures, differentiation of lymphoid and non-lymphoid cells and easy assessment of frequency in specifically labelled cellular subpopulations. It can be used at the same time as FITC and TRITC double-labelling immunofluorescence.

J Immunol Methods. 1983 Aug 26;62(2):193-5. Article

Hoechst as a Fluorescent Nissl Counterstain (r)

1. Combine tiny amount of Hoechst 33258 to 400 ml PBS
2. Store in brown bottle in refrigerator at 5-10°C.
3. After use, pour back into bottle. Can be reused for 3-4 months.

Apoptosis on adherent cells

After treatment, wash cell cultures twice with phosphate-buffered saline (PBS). Cellular DNA can be stained with Hoechst 33258 at 2 µM for 1 hour at 37°C in the dark. After three washes with PBS, view the cells with a fluorescence microscope equipped with a UV filter.
Apoptotic cells have condensed, fragmented nuclei consistent. In contrast, nuclear staining of non apoptotic cells is uniform.

References - Hoechst 33258

Cho J, *et al*, Specific binding of Hoechst 33258 to site 1 thymidylate synthase mRNA, *Nucleic Acids Res*., **28**, 2158(2000)
Disney MD., *et al.*, Activity of Hoechst 33258 against Pneumocystis carinii f. sp. muris, Candida albicans, and Candida dubliniensis *Antimicrob. Agents Chemother*., **49**, 1326 (2005) Abstract
Drobyshev AL, *et al.***,** Massive parallel analysis of DNA-Hoechst 33258 binding specificity with a generic oligodeoxyribonucleotide microchip, *Nucleic Acids Res*., **27**, 4100(1999) Article
Kakazu A. *et al*., HGF Protects Corneal Epithelial Cells from Apoptosis by the PI-3K/Akt-1/Bad- but Not the ERK1/2-Mediated Signaling Pathway, *Investigative Ophthalmology and Visual Science*; 45:3485-3492 (2004)
Leng S. *et al*., Insulin-like growth factor-II renders LIM 2405 human colon cancer cells resistant to butyrate-induced apoptosis: a potential mechanism for colon cancer cell survival in vivo, *Carcinogenesis*, Vol. 22, No. 10, 1625-1631 (2001)

Hoechst 33342

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.

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Hoechst dyes are used in many cellular applications, including in cell-cycle and apoptosis studies and they are common nuclear counterstains. Cells readily take up Hoechst 33342 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.

Aqueous solution 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°C varies for cells and may range from 10-30 min. Staining intensity may increase with time if samples are viewed without washing.

Protocols may be found in the literature.

Ordering information

Related products and documents:

Photoconversion of DAPI and Hoechst [NT-37186n]

FP-R1286A	Hoechst 34580 (longer-wavelength)
FP-M1477A	Hoechst S769121 (membrane impermeant)(Nuclear Yellow)
FP-371867	DAPI
FP-466251	Calcein-AM

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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