INSTRUCTIONS

Hoechst 33342

62249

2245.1

Number	Description
62249	**Hoechst 33342,** 5 ml

Molecular Weight: 615.99

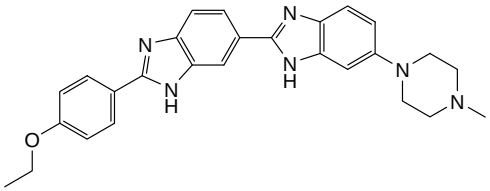
Molar Extinction Coefficient: 47,000 M^{-1} cm^{-1} at 343 nm in methanol

Excitation Wavelength: 361 nm

Emission Wavelength: 486 nm

Supplied: 12.3 mg/ml in aqueous solution (20mM)

CAS Number: 23491-52-3



Storage: Upon receipt store at 4°C protected from light. Product is shipped at ambient temperature.

Introduction

Thermo Scientific Hoechst 33342 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole trihydrochloride trihydrate) is a cell-permeable DNA stain that is excited by ultraviolet light and emits blue fluorescence at 460-490 nm. Hoechst 33342 binds preferentially to adenine-thymine (A-T) regions of DNA. This stain binds into the minor groove of DNA and exhibits distinct fluorescence emission spectra that are dependent on dye:base pair ratios.

Hoechst 33342 is used for specifically staining the nuclei of living or fixed cells and tissues. This stain is commonly used in combination with 5-bromo-2'-deoxyuridine (BrdU) labeling to distinguish the compact chromatin of apoptotic nuclei, to identify replicating cells and to sort cells based on their DNA content. A combination of Hoechst 33342 and propidium iodide have been extensively used for simultaneous flow cytometric and fluorescence imaging analysis of the stages of apoptosis and cell-cycle distribution.

Important Product Information

- The blue fluorescence of Hoechst 33342 can be efficiently excited with the UV spectral lines of the argon-ion laser, xenon or mercury-arc lamps and detected using the common DAPI filter, blue GFP filters or the Semrock BrightLine® Alexa Fluor® 350 Dye filter set. Hoechst 33342 exhibits a relatively large Stokes shift, making it suitable for multicolor labeling experiments.
- Hoechst 33342 may be added directly to cells after diluting it into the appropriate culture medium or balanced salt solutions. Nuclei are often brightly labeled by submicromolar concentrations and can be clearly visualized with or without washing.
- Optimal concentration for DNA staining varies for different cell types and should be determined for each application. Time of incubation varies with cell type, normally ranging from 5-30 minutes at room temperature or 37°C. Staining intensity may increase with time if samples are imaged without washing.

Counterstaining Procedure

A. Material Preparation

DPBS (Wash Buffer)	Modified Dulbecco's PBS (Product No. 28374): 8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM sodium chloride, 10 mM potassium chloride, pH 7.4.
Hoechst 33342 Intermediate Stock Solution	Prepare a dilution of Hoechst 33342 in DPBS for a final concentration of 1 mg/ml.
Hoechst 33342 Working Solution	Dilute Hoechst Intermediate Stock Solution to the target concentration for staining in DPBS. A typical starting concentration for validation experiments is 1 µg/ml.

B. Counterstaining Procedure

1. Follow standard procedures to fix sample and then probe with specific fluorescent-labeled antibodies.
2. Carefully wash sample with DPBS to remove nonbound probe.
3. Add a sufficient volume of Hoechst 33342 Working Solution to completely cover the sample. Place aluminum foil over the sample to protect it from light and incubate at room temperature for 5-10 minutes. Optimize incubation time to minimize signal bleeding through to other channels.
4. (Optional) Wash sample with DPBS to remove excess Hoechst 33342.
5. Mount sample with an appropriate medium and detect fluorescence according to standard protocols.

DNA Assay Procedure

Hoechst 33342, like DAPI, can be used to quantitate DNA in solution. The method is relatively insensitive at pH 5-10 but is sensitive to temperature and ionic strength changes and fluorescence quenching by divalent or heavy metal cations.[1] Fluorescence from Hoechst 33342 is not linear over broad ranges of DNA concentration; therefore, an internal standard must be used each time the assay is performed.

A. Material Preparation

Assay Buffer	0.1 M NaCl, 10 mM EDTA, 10 mM Tris, pH 7.0.
DNA Standards	Dilute known amount of calf thymus DNA with Assay Buffer to make a series of DNA standards with concentrations ranging from 0 to 5 µg/ml (0 to 250 ng/50 µl). Prepare replicates of each dilution to calculate error statistics.
Hoechst 33342 Intermediate Stock Solution	Prepare a dilution of Hoechst 33342 in DPBS for a final concentration of 1 mg/ml.
Hoechst 33342 Working Solution (0.1 µg/ml)	Prepare 0.1 µg/ml Hoechst 33342 Working Solution by 1:10,000 dilution of the Intermediate Stock Solution in Assay Buffer.

B. DNA Assay Procedure

1. Add 50 µl of each unknown sample or DNA Standard to a disposable fluorometer cuvette.
2. Add 1.5 ml Hoechst 33342 Working Solution to each cuvette.
3. Cover cuvettes with foil and incubate at room temperature for 10 minutes.
4. Measure fluorescence of each solution.
5. Prepare a standard curve by plotting the mean values of the standards plotted against their concentrations.
6. Determine concentration of each unknown sample based on its fluorescence measurement relative to the standard curve.

Troubleshooting

Problem	Possible Cause	Solution
Resulting final signal bleeds through to other fluorescent channels	Stain incubation time was too long	Decrease incubation time
	Exposure time is too long	Adjust the exposure time to obtain an optimal contrast
Signal is saturated (too bright) even at short exposure times	Concentration of the stain is too high	Reduce concentration of stain added to the cells
Cannot acquire image or quantitate signal	Different microscopes, cameras and filters may make some signals appear too bright	Adjust the exposure time to obtain an optimal contrast

Related Thermo Scientific Products

62247 **DAPI**, 10 mg

62248 **DAPI**, 1 mg/ml

Visit our website for information about other fluorescent protein labeling reagents, including fluorescein (Product No. 46409, 46410 and 53029), rhodamine (Product No. 46406 and 53031), and amine-reactive or sulfhydryl-reactive Thermo Scientific DyLight Fluors, which cover the entire color spectrum. We also offer a variety of labeled secondary antibodies.

Cited References

1. Brunk, C.F., *et al.* (1979). Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* **92**:497-500.

General References

1. Mocharla, R., *et al.* (1987). A novel, sensitive fluorometric staining technique for the detection of DNA in RNA preparations. *Nucleic Acids Res.* **15(24)**:10589.
2. Latt, S.A. and Stetten, G. (1976). Spectral studies on 33258 Hoechst and related bisbenzimidazole dyes useful for fluorescent detection of deoxyribonucleic acid synthesis. *J. Histochem. Cytochem.* **24**:24.
3. Latt, S.A. (1977). Fluorometric detection of deoxyribonucleic acid synthesis; possibilities for interfacing bromodeoxyuridine dye techniques with flow fluorometry. *J. Histochem. Cytochem.* **25**:913.
4. Sandhu, L.C., *et al.* (1985). Fluorescence studies of Hoechst 33342 with supercoiled and relaxed plasmid pBR322 DNA. *Cytometry* **3**:191-4.