

Product Name: Hoechst 33258 Live Cell DNA Dye

Catalog Number: RA20040



Basic Information

Product Name	Hoechst 33258 Live Cell DNA Dye
Size	10mg
Storage	-20 °C, protected from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em(Unbound DNA)	346/460 nm
Ex/Em(Bound DNA)	352/461 nm

Product Introduction

Hoechst 33258, also known as bis Benzimide H 33258 or HOE 33258, is a non-intercalating bright blue fluorescent dye. The fluorescence of the dye is weak in solution, but it becomes bright when it binds to the minor groove of DNA in AT-rich regions in living cells. Therefore, these dyes are also referred to as DNA probes. Due to the low background, there is no need for a washing step after staining, and the staining is very stable, non-toxic to live cells, and can last for several days or even longer after binding to DNA. Compared with Hoechst 33342, Hoechst 33258 has higher solubility in water, but both dyes have high cell membrane permeability and are widely used for apoptosis detection. After staining, they can be observed with a fluorescence microscope or detected by flow cytometry.

Reagent preparation

- (1) Add 1 mL of ddH₂O to the EP tube to prepare a 10 mg/mL stock solution.
- (2) Dilute the Hoechst 33258 stock solution 1:2000 in PBS to a final working concentration of 5 µg/mL.

Experimental procedures

For fixed cells or tissues

- (1) For cell or tissue samples, wash appropriately after fixation to remove the fixative. If immunofluorescence staining is required, perform immunofluorescence staining first, and then perform Hoechst 33258 staining according to the subsequent steps after staining.
- (2) For adherent cells or tissue sections, add a small amount of Hoechst 33258 working solution to cover the sample. For suspended cells, add at least 3 times the volume of the sample to be stained, mix well, and leave at room temperature for 3-5 minutes.
- (3) Hoechst removal 33258 staining solution, wash 2-3 times with TBST, PBS or saline, each time for 3-5 min.

Note: The washing step is optional but not necessary, and washing does not affect staining.

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(4) Observe directly under a fluorescence microscope or observe under a fluorescence microscope after sealing. When cells undergo apoptosis, the nuclei of apoptotic cells will be densely stained or densely stained in fragments.

For living cells or tissues

(1) Add an appropriate amount of Hoechst 33258 working solution to fully cover the sample to be stained. Usually, 1 mL of staining solution should be added to each well of a six-well plate, and 100 μ L of staining solution should be added to each well of a 96-well plate.

(2) Incubate at room temperature in the dark for 10-30 min.

(3) Discard the staining solution, wash 2-3 times with PBS or culture medium, and then add 50 μ L PBS for microscopic photography.

Note: The washing step is optional but not necessary, and washing does not affect staining.

Note: This reagent is for scientific research use only!