Hoechst 33258

Cat #: BMD0061 Size: 10 mg/100 mg

	Hoechst 33258		
REF	**Cat #:** BMD0061	LOT	**Lot #:** Refer to product label
	Application range: Nuclear staining reagents for DNA staining		**Recommended working concentrations:** 0.5-10 μg/mL
	Excitation/Emission wavelengths: Ex/Em=352/461 nm (unbound DNA), Ex/Em=346/460 nm (bound DNA)		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Hoechst 33258, also known as bis Benzimide H 33258 or HOE 33258, DAPI has the molecular formula $C_{25}H_{24}N_6O \cdot 3HCl$, molecular weight 533.9 and CAS number 23491-45-4, is a non-embedded bright blue fluorescent dye. Dyes have weak fluorescence in solution, but they become bright after binding to DNA in the groove of DNA poly AT sequence enriched region in living cells, so such dyes are also called DNA probes. Due to the low background staining cells do not need washing and the staining is very stable, non-toxic to living cells, and the fluorescence can last for several days or longer after combining with DNA staining. Hoechst33258 has higher solubility in water than Hoechst 33342, but both dyes have high cell membrane permeability and are widely used for apoptosis detection. After staining, fluorescence microscopy or flow cytometry can be used for detection. In the case of adherent cells (96-well plates), 100 μL of the staining solution was used in each well, and the concentration of the staining solution was calculated as 5 μg/mL. 10 mg of the staining solution could be used in 20,000 wells.

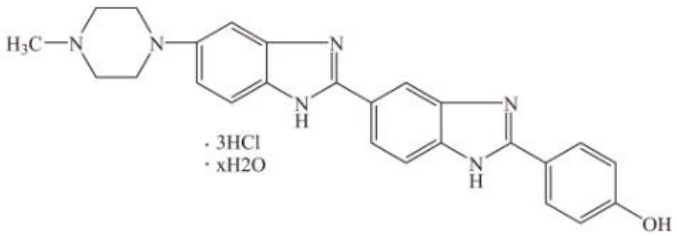

Figure 1. Molecular diagram

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
Hoechst 33258	10 mg	100 mg	-20℃, protected from light

Materials Required but Not Supplied

• Fluorescent microscope, precision pipettes, disposable pipette tips, deionized water, PBS

Assay Procedure

1. Preparation of Hoechst 33258 staining solution

(1) Add 1 mL of deionized water to the EP tube to prepare a 10 mg/mL Hoechst 33258 storage solution.

(2) Hoechst 33258 storage solution was diluted with PBS at a ratio of 1:2,000 to Hoechst 33258 working solution at a final concentration of 5 μg/mL.

2. Staining

(1) For fixed cells or tissues

a. For cell or tissue samples, after fixation, the fixative was removed by appropriate washing. If immunofluorescence staining was to be performed, Hoechst 33258 staining was performed after the completion of staining.

b. For adherent cells or tissue sections, a small amount of Hoechst 33258 staining solution was added to cover the sample. For suspended cells, at least three times the volume of the sample to be stained was added and mixed.

c. Remove Hoechst 33258 staining solution, wash the cells with TBST, PBS or normal saline for 2-3 times, 3-5 min each time.

Note: Washing is optional but not required, dyeing will not be affected after washing.

d. Observe directly under fluorescence microscope or observe under fluorescence microscope after mounting. When apoptosis occurs, the nuclei of apoptotic cells are dense and densely stained, or fragmented and densely stained.

(2) For living cells or tissues

a. Appropriate amount of Hoechst 33258 staining solution was added to cover the sample. Generally, 1 mL was added to 6-well plate per well, 100 μL to 96-well plate per well.

b. Incubate at room temperature 10-30 min, protected from light.

c. Remove Hoechst 33258 staining solution, wash the cells with PBS for 2-3 times, then add 50 μL PBS to cover the cells. Observe directly under fluorescence microscope

Note: Washing is optional but not required, dyeing will not be affected after washing.

Precautions

1. Please immediately centrifugal the product to the bottom of the tube before use, and then conduct the subsequent experiments.

2. Hoechst dye is commonly used to stain mammalian cells, but it can also be used to stain dead and living bacteria. For the staining of dead and living bacteria, it is recommended to dissolve the solution in PBS or 150 mM NaCl with the final concentration of 12-15 μg/mL for 30 min at room temperature. The staining of yeast is weak. Usually the staining of dead cells is brighter than that of living cells.

3. The Hoechst 33258 dye has a solubility of 10 mg/mL when dissolved in water. The recommended working concentration of Hoechst 33258 for nuclear staining is 0.5-10 μg/mL. The dyeing solution concentration and dyeing time can be explored according to the actual dyeing situation

4. Fluorescent dyes all have quenching problems, please try to avoid light to slow down the quenching.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.