Instruction Manual

Hoechst 33258 Staining Solution

Introduction

Hoechst 33258 is a blue fluorescent dye that can penetrate the cell membrane and specifically intercalates into AT-rich regions of double-stranded DNA. It exhibits relatively low cytotoxicity and is commonly used for nuclear staining. In its free state, unbound Hoechst 33258 emits very weak fluorescence, almost undetectable (maximum excitation wavelength: 346 nm; maximum emission wavelength: 460 nm). However, once intercalated into double-stranded DNA, its fluorescence properties change: upon excitation, it emits bright blue fluorescence (maximum excitation wavelength: 352 nm; maximum emission wavelength: 461 nm), and the fluorescence intensity increases proportionally with DNA content, allowing for quantitative analysis. Hoechst 33258 is often used in apoptosis detection, and the staining results can be observed under a fluorescence microscope or analyzed by flow cytometry.

Product Information

Hoechst 33258 Staining Solution	
Ingredient	Hoechst 33258 trihydrochloride
CAS	23491-45-4
Conc.	5 mg/mL
Solvent	ddH2O

Features

1. Strong DNA labeling specificity: with significantly higher affinity for DNA than RNA, resulting in minimal RNA background interference.
1. Good cell membrane permeability: no permeabilization required.
2. Low cytotoxicity: suitable for short-term live-cell tracking experiments.
3. High compatibility: allowing co-staining with multiple fluorescent probes.
4. Simple operation and rapid detection.

Applicatioin

Observation and counting of nuclear morphology;
Live-cell dynamic tracking;
Apoptosis detection;
Drug toxicity evaluation;
Chromosome karyotyping;
Nuclear localization signal studies.

Preparation of Working Solution

Dilute the Hoechst 33258 stock solution with PBS to prepare a working solution of Hoechst 33258 at 0.5-10 μg/mL (protect from light). The specific concentration should be adjusted according to the sample and experimental requirements.

Instructions

Staining of Adherent Cell

(1) Remove the cell coverslip from the incubator and wash with PBS 3 times, 3 min each.

(2) Add 4% paraformaldehyde and fix at room temperature for 10 min. Wash with PBS 3 times, 3 min each.
(3) If immunofluorescence staining is required, perform immunofluorescence staining first, followed by Hoechst 33258 staining. If no other staining is needed, proceed directly with Hoechst 33258 staining.
(4) Add Hoechst 33258 staining working solution and incubate at room temperature in the dark for 10-20 min. Wash with PBS 3 times, 3 min each.
(5) Drop an appropriate amount of antifade mounting medium onto a glass slide, place the coverslip with the cell side facing the medium, and seal around the coverslip with mounting medium.
(6) Observe under a fluorescence microscope and record images. Excitation=352 nm, Emission=461 nm.

Staining of Suspension Cell

(1) Collect suspension cells by centrifugation at 1,000 rpm, 4 °C for 5 min, discard the supernatant. Resuspend in PBS and wash twice.
(2) If immunofluorescence staining is required, perform immunofluorescence staining first, followed by Hoechst 33258 staining. If no other staining is needed, proceed directly with Hoechst 33258 staining.
(3) Add Hoechst 33258 staining working solution and incubate at room temperature in the dark for 15-30 min. Centrifuge at 1000 rpm, 4 °C for 5 min, discard the supernatant, and wash once with PBS.
(4) Resuspend in PBS for flow cytometry detection or prepare cytospin slides for fluorescence microscopy observation. Excitation=352 nm, Emission=461 nm.

Storage

Store at –20 °C, protected from light for 12 months.

Precautions

1. Hoechst 33258 is lipophilic and can passively diffuse through the lipid bilayer of the cell membrane into live cells, so no additional permeabilization is required during the experiment. When staining live cells with Hoechst 33258, fixation is not necessary.
2. Since all fluorescent dyes are subject to photobleaching, it is recommended to perform detection on the same day after staining whenever possible.
3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.