

# Product Information

## Hoechst 33258

Catalog Number: H4046, H4078

Product Size: 10mg (H4046), 10mL (H4078)

Application Scope: Cell tracing, tracking, cellular imaging

## Parameters

Appearance: Yellow solid soluble in water

Ex/Em (with DNA): 352/461 nm

Ex/Em (no DNA): 346/460 nm

CAS No.: 23491-45-4

Molecular Formula:  $C_{25}H_{24}N_6O \cdot 3HCl$

Molecular Weight: 533.88

Molecular Structure:

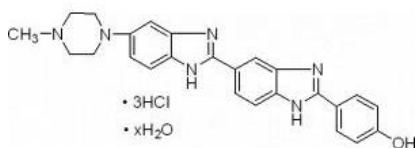


Figure 1. Hoechst 33258

## Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 6 months.

## Description

Hoechst 33258 is a cell membrane-permeant, minor groove-binding blue fluorescent DNA stains. The dye is weakly fluorescent in solution, but the fluorescence becomes bright after binding to DNA in the small grooves in the AT-rich region. The dye is widely used in cell cycle and apoptosis studies as nuclear counterstains. Hoechst 33258 is slightly more water soluble than Hoechst 33342. The dyes can be used to stain live or fixed cells with no wash step required.

Hoechst can also be used to stain live bacteria (gram-positive

and gram-negative), but in live yeast the staining is weak and not nuclear.

## Protocol

**1. Dyeing liquid preparation (For H4078 ready-to-use dyes, you can directly perform the second staining step)**

(1) Preparation of stock solution: The solid dye may be dissolved in water to make concentrated stock solutions up to 10 mg/mL.

(2) Preparation of working solution: Dilute the stock solution with PBS to prepare a working solution with a concentration of 5 µg/mL.

## 2. Cell staining

### 2.1 Staining of fixed cells or tissue samples

(1) For fixed cell or tissue samples, wash to remove the fixative. For immunofluorescence staining, first immunofluorescence staining, then Hoechst 33258 staining.

(2) For adherent cells or tissue sections, add a amount of Hoechst 33258 working solution to cover the sample. For suspended cells, add at least 3 times the volume of the staining sample to be tested and mix well. Leave at room temperature for 3-5 minutes

(3) Remove Hoechst 33258 and wash with TBST, PBS or saline for 2-3 times, each time for 3-5 minutes.

Note: The cleaning step is optional but not necessary. It does not affect the staining after cleaning.

(4) Image the samples.



## 2.2. Live cell staining

(1) Add an appropriate amount of Hoechst 33258 working solution, usually 1 mL for each well of a six-well plate and 100 µl for 96-well plate.

(2) Incubate cells at room temperature or 37°C for 5-15 minutes.

(3) Remove Hoechst 33258 and wash with TBST, PBS or saline for 2-3 times, each time for 3-5 minutes.

Note: The cleaning step is optional but not necessary. It does not affect the staining after cleaning.

(4) Image the samples.

## Notes

1. Hoechst dye is usually used to stain mammalian cells. It can also stain bacteria. It is recommended to stain with a final concentration of 12-15 µg / mL in PBS or 150 mM NaCl for 30 min at room temperature. Staining of yeast is weaker. Dead cells are usually stained more brightly than live cells.

2. The solubility of Hoechst 33258 can reach 10 mg/mL when dissolved in water. When staining nucleus, the working concentration of Hoechst 33258 is 0.5-10 µg/mL. You may need to optimize the staining procedure for each particular cell type by varying the dye concentration.

