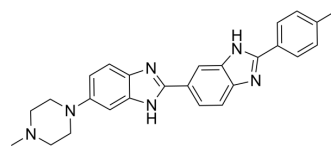


## Hoechst 33258 analog 3

Cat. No.:	HY-15625
CAS No.:	23554-98-5
Molecular Formula:	C <sub>26</sub> H <sub>26</sub> N <sub>6</sub>
Molecular Weight:	422.52
Target:	DNA Stain
Pathway:	Cell Cycle/DNA Damage
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : 3.57 mg/mL (8.45 mM; ultrasonic and warming and heat to 70°C)

Concentration	Mass		
	1 mg	5 mg	10 mg
1 mM	2.3668 mL	11.8338 mL	23.6675 mL
5 mM	0.4734 mL	2.3668 mL	4.7335 mL
10 mM	---	---	---

Please refer to the solubility information to select the appropriate solvent.

### BIOLOGICAL ACTIVITY

#### Description

Hoechst 33258 analog 3 is a marker dye in Hoechst series. Hoechst is A live nuclear marker dye. Hoechst binds to the grooves in the DNA double strand, which tends to be A/ T-rich DNA strand. Although it binds to all nucleic acids, the A/ T-rich double strand DNA significantly enhances fluorescence intensity Therefore, Hoechst dye can be used for living cell labeling. The fluorescence intensity of Hoechst dye increases with the increase of pH of solution<sup>[1]</sup>.

#### In Vitro

General Protocol  
 Preparation of Hoechst working solution  
 1.1 Preparation of the stock solution  
 Dissolve 10 mg of in 5 mL DMSO  
 Note: It is recommended to store the stock solution at 4°C or -20°C away from light and avoid repetitive freeze-thaw cycles.  
 1.2 Preparation of Hoechst working solution  
 Dilute the stock solution in serum-free cell culture medium or PBS to obtain final concentration 10 µg/mL Hoechst working solution.  
 Note: Please adjust the concentration of Hoechst working solution according to the actual situation.  
 1. Cell staining  
 2.1 Suspension cells in 6-well plate  
 a. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.

The cell density is  $1 \times 10^6$ /mL.

- b. Add 1 mL of working solution, and then incubate at room temperature for 3-10 minutes.
- c. Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.
- d. Wash twice with PBS, 5 minutes each time.
- e. Resuspend cells with serum-free cell culture medium or PBS. Observation by fluorescence microscopy or flow cytometry.

#### 2.2 Adherent cells

- a. Culture adherent cells on sterile coverslips.
- b. Remove the coverslip from the medium and aspirate excess medium.
- c. Add 100  $\mu$ L of working solution, gently shake it to completely cover the cells, and then incubate at room temperature for 3-10 minutes.
- d. Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy or flow cytometry.

#### Precautions

1. Please adjust the concentration of Hoechst working solution according to the actual situation.
2. This product is for R&D use only, not for drug, household, or other uses.
3. For your safety and health, please wear a lab coat and disposable gloves to operate.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## REFERENCES

[1]. Portugal J, Waring MJ. Assignment of DNA binding sites for 4',6-diamidino-2-phenylindole and bisbenzimidazole (Hoechst 33258). A comparative footprinting study. *Biochimica et Biophysica Acta* 949 (2): 158-68.

[2]. Latt SA, Stetten G, Juergens LA, Recent developments in the detection of deoxyribonucleic acid synthesis by 33258 Hoechst fluorescence. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 23 (7): 493-505.

[3]. a b c "Hoechst Stains". *Invitrogen (Molecular Probes)*.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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