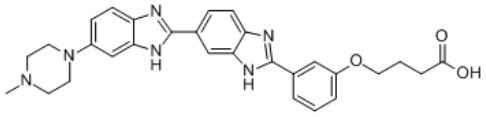
Product Data Sheet

Product Name: Hoechst 33258 analog
Cat. No.: GC36239

Chemical Properties

Cas. No.	258843-62-8		
SMILES	O=C(O)CCCOC1=CC=CC(C2=NC3=CC=C(C4=NC5=CC=C(N6CCN(C)CC6)C=C5N4)C=C3N2)=C1		
Formula	$C_{29}H_{30}N_6O_3$	M.Wt	510.59
Solubility	DMSO: ≥ 51 mg/mL (99.88 mM)	Storage	Store at 2-8°C,protect from light
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Prepare Hoechst staining solution

(1) Prepare Hoechst dye stock solution: Use DMSO to dissolve solid and prepare a 10 mg/mL Hoechst dye stock solution.

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Note: Hoechst stock solution is recommended to be aliquoted and stored in the dark at -4°C or -20°C to avoid repeated freezing and thawing.

(2) Working solution preparation: Use preheated serum-free medium or buffer (such as HBSS or PBS) to dilute the stock solution and prepare a Hoechst working solution with a concentration of 10 μg/mL.

Note: Please adjust the concentration of Hoechst working fluid according to the actual situation and prepare it immediately.

2. Cell staining

2.1 Suspension cells (taking 6-well plate as an example)

(1) Centrifuge suspended cells at 1000 *g* for 3-5 minutes. Discard the supernatant and wash twice with PBS for 5 minutes each time.

(2) Add 1 mL of Hoechst dye working solution and incubate at room temperature in the dark for 5-10 minutes.

(3) After the incubation, centrifuge at 1000 *g* for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(4) Use serum-free cell culture medium or PBS to resuspend the cells and observe them through fluorescence microscopy or flow cytometry.

2.2 Adherent cells

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100μL of Hoechst dye working solution from one corner of the coverslip, shake gently to evenly cover all cells with the dye, and incubate at room temperature in the dark for 5-15 minutes.

(4) Aspirate away the dye working solution, wash the coverslip 2 to 3 times with culture solution, and observe with a fluorescence microscope.

3. Microscope detection: The excitation/emission light of Hoechst 33258 is 365⁄458nm respectively.

Precautions:

① For staining of fixed cells or tissue samples, the fixative needs to be removed by rinsing after fixation;

② Hoechst 33258 staining is usually performed after other staining. If no other staining is required, Hoechst 33258 staining is performed directly;

③ To slow down fluorescence quenching, it is recommended to use anti-fluorescence quenching mounting agent;

④ Fluorescent dyes all have quenching problems, so please try to avoid light;

⑤ Hoechst 33258 is irritating to the human body. For your safety and health, please wear a lab coat and disposable gloves when operating.

References:

[1]. K D Harshman, P B Dervan. Molecular recognition of B-DNA by Hoechst 33258. 1985 Jul 11;13(13):4825-35. doi: 10.1093/nar/13.13.4825.