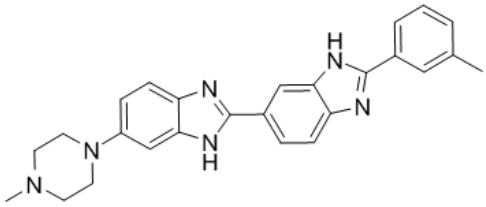
Product Data Sheet

Product Name: Hoechst 33258 analog 2
Cat. No.: GC36240

Chemical Properties

Cas. No.	23491-54-5		
SMILES	CN1CCN(C2=CC=C3N=C(C4=CC=C5N=C(C6=CC=CC(C)=C6)NC5=C4)NC3=C2)CC1		
Formula	$C_{26}H_{26}N_6$	M.Wt	422.52
Solubility	Soluble in DMSO	Storage	4°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.
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

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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 1000g 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 1000g 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.

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

Background

Hoechst 33258 analog 2 are part of a family of blue fluorescent dyes used to stain DNA.IC50 Value:Target: These Bis-benzimides were originally developed by Hoechst AG, which numbered all their compounds so that the dye Hoechst 33342 is the 33342nd compound made by the company. There are three related Hoechst stains: Hoechst 33258, Hoechst 33342, and Hoechst 34580. The dyes Hoechst 33258 and Hoechst 33342 are the ones most commonly used and they have similarexcitation/emission spectra. Both dyes are excited by ultraviolet light at around 350 nm, and both emit blue/cyan fluorescent light around anemission maximum at 461 nm. Unbound dye has its maximum fluorescence emission in the 510-540 nm range. Hoechst dyes are soluble in water and in organic solvents such as dimethyl formamide or dimethyl sulfoxide. Concentrations can be achieved of up to 10 mg/mL. Aqueous solutions are stable at 2-6 °C for at least six months when protected from light. For long-term storage the solutions are instead frozen at ≤-20 °C.The dyes bind to the minor groove of double-stranded DNA with a preference for sequences rich in adenine andthymine. Although the dyes can bind to all nucleic acids, AT-rich double-stranded DNA strands enhance fluorescence considerably.Hoechst dyes are cell-permeable and can bind to DNA in live or fixed cells. Therefore, these stains are often called supravital, which means that cells survive a treatment with these compounds. Cells that express specific ATP-binding cassette transporter proteins can also actively transport these stains out of their cytoplasm.

[1]. 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. [2]. a b c "Hoechst Stains". Invitrogren (Molecular Probes). [3]. Portugal J, Waring MJ. Assignment of DNA binding sites for 4',6-diamidine-2-phenylindole and bisbenzimide (Hoechst 33258). A comparative footprinting study. Biochimica et Biophysica Acta 949 (2): 158-68.