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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

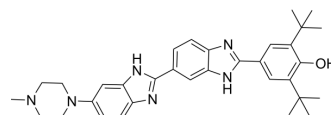
mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Hoechst 33258 analog 6

Cat. No.:	HY-15631
CAS No.:	129244-66-2
Molecular Formula:	C ₃₃ H ₄₀ N ₆ O
Molecular Weight:	536.71
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro	DMSO : 25 mg/mL (46.58 mM; Need ultrasonic)						
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	
				1 mM	1.8632 mL	9.3160 mL	18.6320 mL
				5 mM	0.3726 mL	1.8632 mL	3.7264 mL
				10 mM	0.1863 mL	0.9316 mL	1.8632 mL
Please refer to the solubility information to select the appropriate solvent.							
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (4.66 mM); Clear solution						
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (4.66 mM); Suspended solution; Need ultrasonic						

BIOLOGICAL ACTIVITY

Description	Hoechst 33258 analog 6 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 ^[1] .
In Vitro	<p>General Protocol</p> <p>Preparation of Hoechst working solution</p> <p>1.1 Preparation of the stock solution</p> <p>Dissolve 10 mg of in 5 mL DMSO</p> <p>Note: It is recommended to store the stock solution at 4° or -20° away from light and avoid repetitive freeze-thaw cycles.</p> <p>1.2 Preparation of Hoechst working solution</p>

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 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 1×10^6 /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 µL 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.

CUSTOMER VALIDATION

- Redox Biol. 2019 Sep;26:101254.
- Cell Death Dis. 2023 Mar 17;14(3):200.
- Free Radical Bio Med. 2020 May 20;152:632-649.

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REFERENCES

[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". Invitrogen (Molecular Probes).

[3]. 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.

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA