



# SZABO SCANDIC

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## Produktinformation



Forschungsprodukte & Biochemikalien



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Diagnostik & molekulare Diagnostik



Laborgeräte & Service

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### Lieferung & Zahlungsart

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### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

### SZABO-SCANDIC HandelsgmbH

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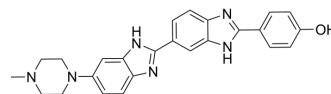
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## Hoechst 33258

Cat. No.:	HY-15558
CAS No.:	23491-44-3
Molecular Formula:	C <sub>25</sub> H <sub>24</sub> N <sub>6</sub> O
Molecular Weight:	424.5
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-20°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 41.67 mg/mL (98.16 mM); ultrasonic and warming and heat to 60°C						
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	
				1 mM	2.3557 mL	11.7786 mL	23.5571 mL
				5 mM	0.4711 mL	2.3557 mL	4.7114 mL
				10 mM	0.2356 mL	1.1779 mL	2.3557 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: ≥ 2.08 mg/mL (4.90 mM); Clear solution						
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (4.90 mM); Clear solution						

### BIOLOGICAL ACTIVITY

Description	Hoechst 33258 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> .
IC <sub>50</sub> & Target	IC50: 51.31±4.56 μM (HeLa cell), 32.43±3.27 μM (HL60 cell), 15.42 ± 2.16 μM (U937 cell) <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 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 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.

## PROTOCOL

### Cell Assay [2]

Hoechst 33258 is prepared as stock solutions in highly pure water. Working solutions in a concentration range of  $10^{-3}$ - $10^{-6}$  mol/dm<sup>3</sup> are prepared prior to testing. Cytotoxic effects of Hoechst 33258 on tested cell lines are determined by the MTT assay. Cells are seeded in 96 micro well flat bottom plates at a concentration of  $2 \times 10^4$  cells/mL and left overnight in the CO<sub>2</sub> incubator allowing them to attach to the plate surface. Growing medium is replaced with compound supplemented or control medium and incubated for 72 h. Fresh medium with 5 mg/mL of MTT is added onto cells and incubated for 4 h at 37°C. Upon media removal, water insoluble MTT-formazan crystals formed inside the living cells are dissolved in DMSO and the absorbance at 570 nm proportional to the number of living cells is measured on an Elisa Microplate Reader. All experiments are performed at least three times in triplicates. The GI<sub>50</sub> value, defined as the compound concentration (µM) leading to cellular growth inhibition by 50%, is calculated and used as a parameter to compare cytotoxicity among the compounds [2].

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

## CUSTOMER VALIDATION

- Nat Commun. 2020 Feb 5;11(1):719.
- Chem Eng J. 365 (2019) 270-281.
- Int Immunopharmacol. 2023 Apr 17;119:110136.
- Virulence. 2022 Dec;13(1):444-457.
- Food Biosci. 11 December 2021, 101501.

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## REFERENCES

- [1]. Wang XJ, et al. Newly synthesized bis-benzimidazole derivatives exerting anti-tumor activity through induction of apoptosis and autophagy. *Bioorg Med Chem Lett.* 2012 Oct 1;22(19):6297-300.
- [2]. Stolić I, et al. Synthesis, DNA/RNA affinity and antitumour activity of new aromatic diamidines linked by 3,4-ethylenedioxythiophene. *Eur J Med Chem.* 2011 Feb;46(2):743-55.
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**Caution: Product has not been fully validated for medical applications. For research use only.**

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