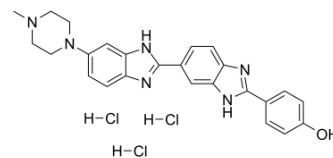


## Hoechst 33258 trihydrochloride

<b>Cat. No.:</b>	HY-15558A
<b>CAS No.:</b>	23491-45-4
<b>Molecular Formula:</b>	C <sub>25</sub> H <sub>27</sub> Cl <sub>3</sub> N <sub>6</sub> O
<b>Molecular Weight:</b>	533.88
<b>Target:</b>	Others
<b>Pathway:</b>	Others
<b>Storage:</b>	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

#### In Vitro

H<sub>2</sub>O : ≥ 72.85 mg/mL (136.45 mM)  
\* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.8731 mL	9.3654 mL	18.7308 mL
	5 mM	0.3746 mL	1.8731 mL	3.7462 mL
	10 mM	0.1873 mL	0.9365 mL	1.8731 mL

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

### BIOLOGICAL ACTIVITY

#### Description

Hoechst 33258 trihydrochloride is a fluorescent dye, which can be used as a cell dye for DNA.

#### IC<sub>50</sub> & Target

IC<sub>50</sub>: 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

Hoechst 33258, a fluorescent compound with a head-to-tail bis-benzimidazole structure, is initially found to be cytotoxic against L1210 murine leukemia. Hoechst 33258 is evaluated for their cytotoxicity against human tumor cell lines, which are cervix carcinoma cell line (HeLa), Human promyelocytic leukemia cell (HL60) and U937 cell Line. The IC<sub>50</sub> determined in the case of HeLa, HL60 and U937 is 51.31±4.56, 32.43±3.27 and 15.42±2.16 μM for Hoechst 33258, respectively<sup>[1]</sup>. The cytotoxic property of Hoechst 33258 is investigated on a panel of seven tumour cell lines of different histological origin and Madine-Darby canine kidney (MDCK) normal cells. All cell lines, except MCF-7, exposed to Hoechst 33258 exhibit GI<sub>50</sub> from 84×10<sup>-6</sup> to 191.5×10<sup>-6</sup> mol/dm<sup>3</sup>. Under the same experimental conditions, Hoechst 33258, used as a binder reference compound, stops the cell cycle in S phase and G0/G1<sup>[2]</sup>.

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.
- J Cell Biochem. 2019 Jun;120(6):9181-9192.
- Int J Biochem Cell Biol. 2017 Mar;84:75-88.
- Biochem Biophys Res Commun. 2020 May 20;S0006-291X(20)30857-3

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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]. Stoli? 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.

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

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