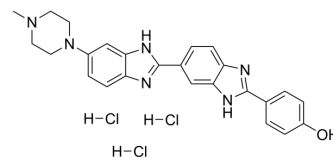


Hoechst 33258 trihydrochloride

| | |
|--------------------|--|
| Cat. No.: | HY-15558A |
| CAS No.: | 23491-45-4 |
| Molecular Formula: | C ₂₅ H ₂₇ Cl ₃ N ₆ O |
| Molecular Weight: | 533.88 |
| Target: | Fluorescent Dye |
| Pathway: | Others |
| Storage: | 4°C, sealed storage, away from moisture and light * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light) |



SOLVENT & SOLUBILITY

| | | | | | |
|---|--|--|-----------|-----------|------------|
| In Vitro | DMSO : 100 mg/mL (187.31 mM; Need ultrasonic) | | | | |
| | H ₂ O : 12.5 mg/mL (23.41 mM; ultrasonic and warming and heat to 60°C) | | | | |
| | Preparing Stock Solutions | <div>Solvent Concentration</div> <div>Mass</div> | 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. | | | | | |
| In Vivo | 1. Add each solvent one by one: PBS Solubility: 9.09 mg/mL (17.03 mM); Clear solution; Need ultrasonic and warming and heat to 60°C | | | | |

BIOLOGICAL ACTIVITY

| | |
|---------------------------|---|
| Description | Hoechst 33258 trihydrochloride 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] . |
| IC ₅₀ & Target | IC50: 51.31±4.56 μM (HeLa cell), 32.43±3.27 μM (HL60 cell), 15.42±2.16 μM (U937 cell) ^[1] |
| In Vitro | Preparation of Hoechst working solution 1.1 Preparation of stock solution Take 10 mg and dissolve in 5 mL DMSO Note: It is recommended that the stock solution be stored at 4℃ or - Store at 20℃ in the dark and avoid repeated freezing and thawing. |

1.2 Preparation of Hoechst working solution

The diluted stock solution was dissolved in serum-free cell culture medium or PBS to obtain Hoechst working solution with a final concentration of 10 µg/mL.

Note: Please adjust the concentration of Hoechst working solution according to the actual situation.

1. Cell staining

2.1 Suspension cells (6-well plate)

- Centrifuge at 1000 g for 3-5 min at 4°C and discard the supernatant. Wash 2 times with PBS, 5 minutes each. The cell density was 1×10^6 /mL.
- Add 1 mL of working solution and incubate at room temperature for 3-10 minutes.
- Centrifuge at 400 g for 3-4 minutes at 4°C and discard the supernatant.
- Wash 2 times with PBS, 5 min each.
- Resuspend cells in serum-free cell culture medium or PBS. Fluorescence microscopy or flow cytometry observation.

2.2 Adherent cells

- Adherent cells were cultured on sterile coverslips.
- Remove the coverslip from the medium and aspirate excess medium. c. Add 100 µL of working solution, shake gently to completely cover the cells, and incubate at room temperature for 3-10 minutes.
- Wash 2 times with medium, 5 min each. Fluorescence microscope or flow cytometry observation.

Store

4°C, 1 year. Avoid light

Precautions

- Please adjust the concentration of Hoechst working solution according to the actual situation.
 - This product is for R&D use only, not for medicine, household or other purposes.
 - For your safety and health, please wear a lab coat and disposable gloves for operation.
- 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/dm³ 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 2×10^4 cells/mL and left overnight in the CO₂ 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 GI₅₀ 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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