Hoechst 33342 trihydrochloride

Cat. No.: HY-15559A

CAS No.: 875756-97-1

Molecular Formula: $C_{27}H_{31}Cl_3N_6O$

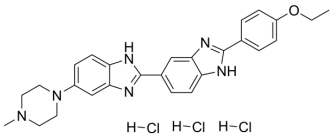
Molecular Weight: 561.93

Target: Autophagy

Pathway: Autophagy

Storage: 4°C, sealed storage, away from moisture and light

* The compound is unstable in solutions, freshly prepared is recommended.



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 46 mg/mL (81.86 mM)

H_2O : ≥ 5.6 mg/mL (9.97 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.7796 mL	8.8979 mL	17.7958 mL
5 mM	0.3559 mL	1.7796 mL	3.5592 mL
10 mM	0.1780 mL	0.8898 mL	1.7796 mL

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

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 3.33 mg/mL (5.93 mM); Clear solution; Need ultrasonic and warming and heat to 60°C

BIOLOGICAL ACTIVITY

Description

Hoechst 33342 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

Dye reagent[1]

DNA Stain[1]

In Vitro

General Protocol

Preparation of Hoechst working solution

1.1 Preparation of the stock solution

Dissolve 10 mg of in 5 mL ddH_2O

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 1×10^6/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.

Storage
4°C, 1 year. Protect from light

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 [1]

Labeling Nuclear DNA with Hoechst 33342[1]Step 1, Dilute the Hoechst stock solution 1:100 in H_2O for use in labeling. Step 2, Aspirate the cell medium from cells grown on coverslips. Rinse the cells three times with PBS^+. Step 3, Incubate the cells in the Hoechst labeling solution (from Step 1) for 10-30 min at room temperature. Step 4, Aspirate the labeling solution. Rinse the cells three times in PBS^+.Step 5, Mount the coverslips.Step 6, Image the cells (λex ~353 nm, λem ~483 nm for Hoechst 33342)[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Science. 2022 Nov 18;378(6621):eabq7361.
- Cell Host Microbe. 2023 Nov 8;31(11):1820-1836.e10.
- Bioact Mater. 2022 Mar 17;18:91-103.
- ACS Nano. 2023 Jul 23.
- Chem Eng J. 2023 Dec 2, 147850.

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REFERENCES

[1]. Chazotte B. Labeling nuclear DNA with hoechst 33342. Cold Spring Harb Protoc. 2011 Jan 1;2011(1):pdb.prot5557.

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