

## Product Data Sheet

Product Name: Hoechst 33342 analog 2 trihydrochloride

Cat. No.: GC36246

### Chemical Properties

Cas. No. 155815-98-8

SMILES OC1=CC=C(C2=NC3=CC(C4=NC5=CC(N6CCN(C)CC6)=CC=C5N4)=CC=C3N2)C=C1I  
.[H]Cl.[H]Cl.[H]Cl

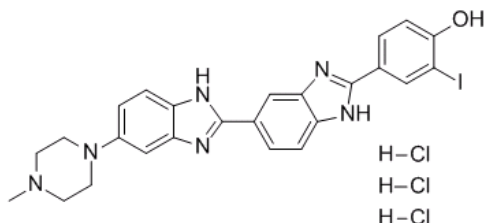
Formula  $C_{25}H_{26}Cl_3IN_6O$  M.Wt 659.78

Solubility Water: 15.83 mg/mL (23.99 mM and warming) Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



### Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Prepare Hoechst staining solution

(1) Prepare Hoechst dye stock solution: Use DMSO to dissolve solid and prepare a 10 mg/mL Hoechst dye stock solution.

Note: Hoechst stock solution is recommended to be aliquoted and stored in the dark at -4°C or -20°C

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

to avoid repeated freezing and thawing.

(2) Working solution preparation: Use preheated serum-free medium or buffer (such as HBSS or PBS) to dilute the stock solution and prepare a Hoechst working solution with a concentration of 10 µg/mL.

Note: Please adjust the concentration of Hoechst working fluid according to the actual situation and prepare it immediately.

### 2. Cell staining

#### 2.1 Suspension cells (taking 6-well plate as an example)

(1) Centrifuge suspended cells at 1000g for 3-5 minutes. Discard the supernatant and wash twice with PBS for 5 minutes each time.

(2) Add 1 mL of Hoechst 33342 dye working solution and incubate at room temperature in the dark for 5-10 minutes.

(3) After the incubation, centrifuge at 1000g for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(4) Use serum-free cell culture medium or PBS to resuspend the cells and observe them through fluorescence microscopy or flow cytometry.

#### 2.2 Adherent cells

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100µL of Hoechst dye working solution from one corner of the coverslip, shake gently to evenly cover all cells with the dye, and incubate at room temperature in the dark for 5-15 minutes.

(4) Aspirate away the dye working solution, wash the coverslip 2 to 3 times with culture solution, and observe with a fluorescence microscope.

3. Microscope detection: The excitation/emission light of Hoechst 33342 is 350/461nm respectively.

### Precautions:

① For staining of fixed cells or tissue samples, the fixative needs to be removed by rinsing after fixation;

② Hoechst 33342 staining is usually performed after other staining. If no other staining is required, Hoechst 33342 staining is performed directly;

③ To slow down fluorescence quenching, it is recommended to use anti-fluorescence quenching mounting agent;

④ Fluorescent dyes all have quenching problems, so please try to avoid light;

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

## Product Data Sheet

---

⑤ Hoechst 33342 is irritating to the human body. For your safety and health, please wear a lab coat and disposable gloves when operating.

### References:

[1]. Lisa C Crowley, Brooke J Marfell , Nigel J Waterhouse. Analyzing Cell Death by Nuclear Staining with Hoechst 33342. 2016 Sep 1;2016(9). doi: 10.1101/pdb.prot087205.

### Background

Hoechst 33342 analog 2 trihydrochloride is an analog of Hoechst 33342 dye. Hoechst 33342 is a nuclear dye that binds to the grooves in the DNA double strands. Hoechst 33342 dye is also used to differentiate between normal cells, apoptotic cells, and necrotic cells. The nuclei of normal cells are round and uniformly stained, but during mitosis, the nuclei are also condensed, and DNA forms two parallel lines when chromosomes are separated; in apoptotic cells, due to the condensation of DNA, the nuclei are usually fragmented and stained more Strong; the DNA in necrotic cells is not concentrated, and the edges of the nucleus are unclear[1].

Hoechst dyes can also be used to monitor cell viability by tracking changes in their emission spectra. As slightly groove-binding DNA stains with AT selectivity, Hoechst dyes are able to bind to all nucleic acids, but they show greater fluorescence enhancement for AT-rich double-stranded DNA strands compared to GC-rich strands[2]. This property has been used to identify Q bands in chromosomes, which are AT base pair-rich regions that fluoresce brightly when stained with quinacrine dye[3].

### References:

[1]. Lisa C Crowley, Brooke J Marfell , Nigel J Waterhouse. Analyzing Cell Death by Nuclear Staining with Hoechst 33342. 2016 Sep 1;2016(9). doi: 10.1101/pdb.prot087205.

[2]. Portugal J, Waring MJ. Assignment of DNA binding sites for 4', 6-diamidine-2-phenylindole and bisbenzimidazole (Hoechst 33258). A comparative footprinting study. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression. 1988 Feb 28;949(2):158-68.

[3]. Weisblum B, Haenssler E. Fluorometric properties of the bibenzimidazole derivative Hoechst 33258, a fluorescent probe specific for AT concentration in chromosomal DNA. Chromosoma. 1974 Sep;46(3):255-60.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA