Hoechst 33342 (Hydrochloride)

Fluorescent stain for visualization of cell nuclei

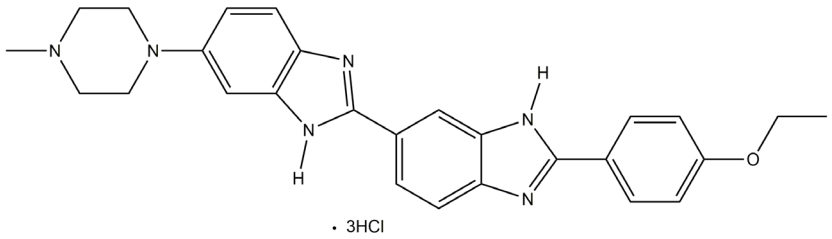
Catalog #100-1540 5 mL 20 mM

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Hoechst 33342 (Hydrochloride) is part of the Hoechst family of fluorescent stains that also includes Hoechst 33258 and 34580. Hoechst 33342 (Hydrochloride) is a cell-permeable, fluorescent dye that is recommended for staining and visualization of cell nuclei in live or fixed cells. The excitation of Hoechst 33342 (Hydrochloride) occurs at an ultraviolet wavelength of 350 nm, with maximal emission occurring at 461 nm, emitting a blue/cyan fluorescent light. Compared to the other Hoechst stains, Hoechst 33342 (Hydrochloride) is more readily cell-permeable due to the presence of an additional lipophilic ethyl group (Bucevičius et al.). For live cultures, Hoechst 33342 (Hydrochloride) is recommended to be used in place of DAPI, as it is less toxic and more capable of preserving cell viability after staining. Hoechst 33342 (Hydrochloride) may also be used as a nuclear counterstain in fixed samples.

Alternative Names:	Bisbenzimide
CAS Number:	875756-97-1
Chemical Formula:	$C_{27}H_{28}N_6O \cdot 3HCl$
Molecular Weight:	561.93 g/mol
Chemical Name:	2′-(4-Ethoxyphenyl)-6-(4-methyl-1-piperazinyl)-2,6′-bi-1H-benzimidazole trihydrochloride
Excitation Wavelength:	350 nm
Emission Wavelength:	461 nm
Extinction Coefficient:	~43000 $cm^{-1}M^{-1}$ (at OD 345 nm in methanol)
Structure:	



Stability and Storage:	Store at -20°C with a desiccant. Product stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Product Format:	A yellow solution in water
Verified Applications:	Fluorescence microscopy
Reported Applications:	Flow cytometry, Microplate reader

Directions for Use

The following protocol is for staining cells in one well of a 24-well plate (e.g. Catalog #100-0097). If using other cultureware, adjust volumes accordingly.

A. PREPARATION OF HOECHST 33342 (HYDROCHLORIDE) WORKING SOLUTION

The suggested working concentration of Hoechst 33342 (Hydrochloride) is 0.5 - 5 µM. The optimal concentration of the working solution should be determined for different cell types. High concentrations may cause non-specific staining of other cellular structures in some cell types.

NOTE: Protect solutions containing Hoechst 33342 (Hydrochloride) from light.

Live cells

Hoechst 33342 (Hydrochloride) stock solution (20 mM) should be diluted in warm (37°C) culture medium immediately before use. For example, to prepare a 5 mL working solution of 5 µM Hoechst 33342 (Hydrochloride), resuspend 1.25 µL of Hoechst 33342 (Hydrochloride) stock solution (20 mM) in 5 mL of warm culture medium.

Fixed cells

Hoechst 33342 (Hydrochloride) stock solution (20 mM) should be diluted in 1X, room temperature (15 - 25°C) phosphate-buffered saline (PBS). For example, to prepare a 5 mL working solution of 5 µM Hoechst 33342 (Hydrochloride), resuspend 1.25 µL of Hoechst 33342 (Hydrochloride) stock solution (20 mM) in 5 mL of 1X, room temperature PBS.

B. STAINING CELLS

Live cells

NOTE: For cells in suspension, pellet cells by centrifugation following your protocol of choice and then follow steps 1 - 4.

1. Aspirate culture medium completely.
2. Immediately add 1 mL of 5 µM Hoechst 33342 (Hydrochloride) working solution (prepared in section A) and incubate samples at 37°C and 5% CO_2 for 15 - 60 minutes; protect from light.
3. Wash sample three times with warm (37°C) PBS.
   Optional: For gentle washing of cells where adhesion is a concern, PBS containing Ca2+ and Mg2+ may be used.
4. Add 1 mL of fresh warm phenol-free culture medium.

Fixed cells

1. Fix and permeabilize cells as desired.
2. Wash sample three times with PBS.
3. Add 1 mL of 5 µM Hoechst 33342 (Hydrochloride) working solution (prepared in section A) and incubate samples at room temperature (15 - 25°C) for 5 - 15 minutes; protect from light.
4. Wash sample three times with PBS; leave the final PBS rinse within the well.

C. IMAGING STAINED CELLS

Observe stained cells using a fluorescence microscope equipped with a standard DAPI filter set. Monitor the fluorescence intensity using a flow cytometer with a 405 nm (violet) or 355 nm (ultraviolet) laser line and appropriate emission filters. Gate on cells of interest and exclude debris.

OPTIONAL: Cells attached to coverslips may be mounted onto glass microscope slides using an antifade mounting medium; ensure the mountant is cured completely before imaging.

References

Bucevičius J et al. (2018) The use of hoechst dyes for DNA staining and beyond. Chemosensors 6(2): 18.

Hanson KM & Finkelstein JN. (2019) An accessible and high-throughput strategy of continuously monitoring apoptosis by fluorescent detection of caspase activation. Anal Biochem 1(564-5): 96–101.

Schmid I et al. (2007) Live-cell assay for detection of apoptosis by dual-laser flow cytometry using hoechst 33342 and 7-amino-actinomycin. D. Nat Protoc 2(1) :187–90.

Related Products

For a complete list of related products available from STEMCELL Technologies, visit www.stemcell.com/dyesandstains, or contact us at techsupport@stemcell.com.