Revised: February 21, 2019

Product Information

Hoechst 33342

Catalog number	Product	MW	Size
40046	Hoechst 33342, 10 mg/mL in H_2O	561.93	10 mL
40047	Hoechst 33342, trihydrochloride trihydrate	615.98	100 mg

Storage and Handling

Store Hoechst 33342, 10 mg/mL in H_2O at 4°C, protected from light. Store Hoechst 33342, trihydrochloride trihydrate solid desiccated at 4°C, protected from light. Product is stable for at least one year from date of receipt when stored as recommended. It is not recommended to store working solutions of Hoechst dye, because the dye will be lost to precipitation or adsorption to the container over time.

Molecular Information

$C_{27}H_{31}Cl_3N_6O$

CAS number: 23491-52-3

Color and form:

40046: Light yellow solution
40047: Light yellow solid

Solubility: Soluble in water up to 10 mg/mL

Absorption/Emission: 350/461 nm (with DNA) (Figure 2)

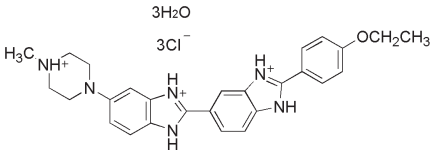

Figure 1. Hoechst 33342, trihydrochloride trihydrate.

Product Description

Hoechst dyes are widely-used cell membrane-permeant blue fluorescent DNA binding dyes that can be used for nuclear staining of live or fixed cells. The dyes have minimal fluorescence in solution, but become brightly fluorescent upon binding to DNA. Therefore, they can be used to stain cells without a wash step. The staining is very stable and non-toxic to live cells for several days or longer.

Hoechst 33342 and Hoechst 33258 are structurally similar minor-groove DNA binding dyes that perform comparably as nuclear counterstains. Hoechst 33528 is slightly more water soluble than Hoechst 33342, but both dyes are highly cell membrane permeant and widely used in cell cycle studies and as nuclear counterstains for live or fixed cells.

References

1) J. Cell Physiol. 102, 175 (1980); 2) Proc. Natl. Acad. Sci. USA 78, 363 (1981); 3) J. Histochem. Cytochem. 24, 24 (1976).

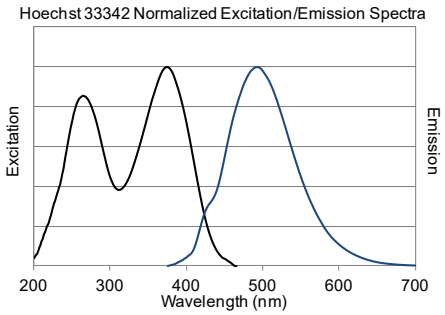

Figure 2. Normalized excitation and emission spectra of Hoechst 33342 with DNA.

Staining Protocols

Live cell staining

Below we provide two protocols for staining live cells with Hoechst. Staining by medium exchange results in uniform exposure of cells to probe. However, for some cell types, morphology or viability may be affected by medium exchange. In addition, floating dead cells may be lost during medium removal, and suspension cells must be collected by centrifugation to exchange the medium. Direct addition of 10X probe is a convenient staining method that doesn't require medium exchange, but care must be taken to mix immediately yet gently to avoid high transient probe concentration or disruption of cells by pipetting. Note that we do not recommend adding highly concentrated dye directly to cells in culture, as this will result in local areas of high dye exposure.

Live cell staining by medium exchange

1. Dilute Hoechst dye to 1 ug/mL in fresh, complete cell culture medium. Hoechst can be combined with other fluorescent probes.
2. Remove medium from the cells and replace with fresh medium containing Hoechst dye.
3. Incubate cells at room temperature or 37°C for 5-15 minutes, then image. Note: Washing is not necessary for specific staining, but nuclear staining is stable after washing.

Live cell staining by direct addition of 10X probe

1. Prepare 10X dye solution by diluting Hoechst dye to 10 ug/mL in fresh, complete culture medium. Hoechst can be combined with other fluorescent probes, which should be diluted to 10 times the final desired concentration.
2. Without removing the medium from the cells, add 1/10 volume of 10X dye solution directly to the well.
3. Immediate mix thoroughly by gently pipetting the medium up and down. For larger well sizes (e.g., 24-well to 6-well plates), the plate can be gently swirled to mix.
4. Incubate cells at room temperature or 37°C for 5-15 minutes, then image. Note: Washing is not necessary for specific staining, but nuclear staining is stable after washing.

Staining of fixed cells or tissue sections

1. Add Hoechst dye to PBS at 1 ug/mL. Hoechst can be included together with antibodies or other probes, and can be diluted in buffers with detergent or blocking agents if convenient.
2. Add the PBS with dye to cells or tissue sections and incubate at room temperature for at least 5 minutes.
3. Image the samples; washing is optional but not required. Note: Samples can be stored at 4°C after staining and before imaging.

Staining bacteria or yeast

Hoechst dyes stain bacteria more dimly than mammalian cells. Live or killed bacteria can be stained with 12-15 ug/mL Hoechst dye in PBS or 150 mM NaCl for 30 minutes at room temperature. The dyes tend to stain dead cells more brightly than live cells.

In *S. cerevisiae*, Hoechst dyes stain both the nucleus and cytoplasm. When used to stain yeast at 12-15 ug/mL in PBS, Hoechst preferentially stains dead cells, with dim staining of live cells.

Related Products

Catalog number	Product
40044	Hoechst 33258, 10 mg/mL in H_2O
40045	Hoechst 33258, pentahydrate
40083	NucSpot® 470
40081	NucSpot® Live 488
40082	NucSpot® Live 650
40085	NucSpot® Far-Red
40060	RedDot™1 Far-Red Nuclear Stain
40061	RedDot™2 Far-Red Nuclear Stain
40084	7-AAD Solution, 1 mg/mL
40048	Propidium Iodide Buffer, 50 ug/mL
40009	DAPI, dilactate
40011	DAPI, dihydrochloride
40043	DAPI, dilactate 10 mg/mL in H_2O
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
30068	ViaFluor® 405 SE Cell Proliferation Kit
30086	ViaFluor® 488 SE Cell Proliferation Kit
70065	LipidSpot™ 488 Lipid Droplet Stain
70069	LipidSpot™ 610 Lipid Droplet Stain
70066	LysoView™ 405 Lysosome Stain
70067	LysoView™ 488 Lysosome Stain
70061	LysoView™ 540 Lysosome Stain
70058	LysoView™ 633 Lysosome Stain
70059	LysoView™ 650 Lysosome Stain
70070	MitoView™ 405 Mitochondrial Stain
70054	MitoView™ Green Mitochondrial Stain
70055	MitoView™ 633 Mitochondrial Stain
70075	MitoView™ 650 Mitochondrial Stain
70068	MitoView™ 720 Mitochondrial Stain
70064	ViaFluor® 405 Live Cell Microtubule Stain
70062	ViaFluor® 488 Live Cell Microtubule Stain
70063	ViaFluor® 647 Live Cell Microtubule Stain
30090	CellBrite™ Fix 488 Membrane Stain
30088	CellBrite™ Fix 555 Membrane Stain
30089	CellBrite™ Fix 640 Membrane Stain
30092-30099	MemBrite™ Fix Cell Surface Staining Kits
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22020	10X Phosphate-Buffered Saline (PBS)

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