SYTOX® Blue Nucleic Acid Stain (S11348)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate

  Protect from light

Abs/Em: 444/480 nm, bound to nucleic acid

Introduction

SYTOX® Blue nucleic acid stain (S11348) is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes and yet will not cross the membranes of live cells. It is particularly useful for bacterial staining, rendering both gram-positive and gram-negative bacteria brightly fluorescent. After brief incubation with SYTOX® Blue nucleic acid stain, the nucleic acids of dead cells fluoresce bright blue when excited with the 436 nm spectral line of the mercury arc lamp. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Blue stain a simple and quantitative single-step dead-cell indicator for use with fluorescence microscopes, fluorometers (Figure 1), fluorescence microplate readers, and arc lamp-equipped flow cytometers.

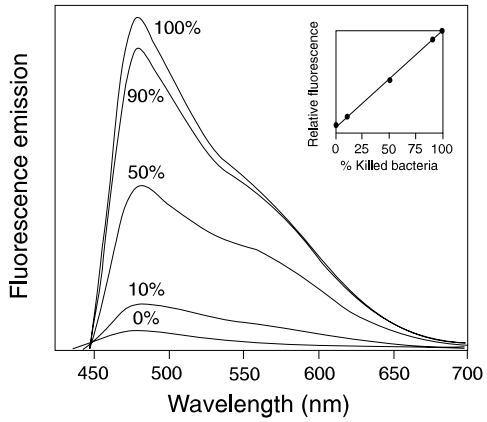

Figure 1. Evaluation of the proportion of viable bacteria in a suspension of *Escherichia coli*. Suspensions containing different proportions of live and 70% isopropyl alcohol–killed bacteria were stained with the SYTOX® Blue dye and analyzed by fluorometry. A linear relationship between the proportion of live and dead bacteria and the integrated fluorescence emission is shown (see inset).

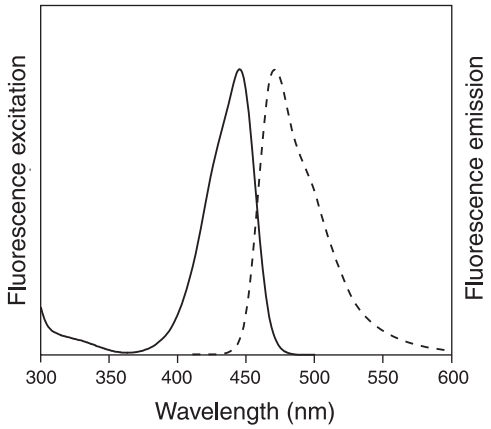

Figure 2. Absorption and fluorescence emission spectra of the SYTOX® Blue nucleic acid stain bound to DNA. These spectra were obtained using a ratio of 1 dye molecule to 50 base pairs of DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.

This dead-cell stain may be used in conjunction with fluorescent surface labels of contrasting colors for multiparameter analyses.

Materials

The SYTOX® Blue dye is supplied as a 5 mM solution in dimethylsulfoxide (DMSO) in a unit size of 250 µL. Upon receipt, this vial should be stored frozen at ≤–20°C, upright, and protected from light. Before refreezing, seal the vial tightly. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation. When stored properly, this stock solution is stable for at least one year. Each vial contains enough reagent to stain >1500 samples when using a 96-well microplate assay format.

Caution: No data are available addressing the mutagenicity or toxicity of this reagent. Because the reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Use protective equipment and practices appropriate for the hazards posed by this material and dispose of it according to all pertaining local regulations.

Spectral Characteristics

The absorption and fluorescence emission spectra of the SYTOX® Blue dye are given in Figure 2. These spectra were obtained in the presence of DNA; upon binding DNA, the SYTOX® Blue dye exhibits a fluorescence enhancement of greater than 500 fold. The SYTOX® Blue/DNA complex has absorption and fluorescence emission maxima of 444 nm and 480 nm, respectively. Spectral characteristics of the SYTOX® Blue dye in bacteria or eukaryotic cells may vary.

Table 1. Recommended conditions for staining cells with SYTOX® Blue dye.

Cell Type	SYTOX® Blue Concentration	Incubation Conditions
Bacteria	0.5–5 µM	Vortex to mix then incubate for >5 minutes.
Yeast	1–50 µM	Incubate with periodic agitation for >10 minutes.
Other Eukaryotes	10 nM–1 µM	Incubate for >10 minutes.

Experimental Guidelines

The following procedure can be adapted for any cell type. Note that different concentration ranges for the SYTOX® Blue dye are suggested depending on the cell type (Table 1). Growth medium, cell density, the presence of other cell types, and other factors may influence staining. In general, the best results are obtained in buffers that do not contain phosphate. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present. Be sure to wash glassware in a mild detergent and rinse thoroughly with hot tap water followed by several rinses with deionized, distilled water.

Pellet cells by centrifugation and resuspend in buffered salt solution or water. The binding of SYTOX® Blue stain may be reduced somewhat in solutions containing very high concentrations of monovalent or divalent cations. Adherent cells such as mammalian tissue cells may be stained *in situ* on coverslips. Add SYTOX® Blue stain using the concentrations listed in Table 1 as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining.

Cells stained with the SYTOX® Blue dye can be viewed with a fluorescence microscope equipped with a filter set matched to the dye's absorbance and emission maxima.

Stained eukaryotic cells will generally have bright blue nuclei as well as variable levels of cytoplasmic staining. Bacteria generally stain uniformly once the intracellular dye is at equilibrium with the staining solution. Allow 5 minutes or more for staining of bacteria or eukaryotic cells to reach completion.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
S11348	SYTOX® Blue nucleic acid stain *5 mM solution in DMSO*	250 µL

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — **probes.invitrogen.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Service: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License
For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2005, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.