Revised: 13–January–2001

SYTOX® Orange Nucleic Acid Stain (S-11368)

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Abs/Em: 547/570 nm, bound to DNA

Introduction

SYTOX® Orange nucleic acid stain (S-11368) 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. After brief incubation with SYTOX Orange nucleic acid stain, the nucleic acids of dead cells fluoresce bright orange when excited with the 543 nm spectral line of the He-Ne laser, or any other 520–550 nm source. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX Orange stain a simple and quantitative single-step dead-cell indicator for use with fluorescence microscopes, fluorometers, fluorescence microplate readers and flow cytometers.

This dead-cell stain may be useful in conjunction with blue and green fluorescent surface labels for multiparameter analyses. It may also be possible to combine SYTOX Orange nucleic acid stain with a cell permeant nucleic acid stain such as DAPI or Hoechst 33258 for two-color visualization of dead and live cells.

Contents, Storage and Handling

The SYTOX Orange 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, desiccated, 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 sufficient 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. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions containing this reagent should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Spectral Characteristics

The absorption and fluorescence emission spectra of the SYTOX Orange dye are given in Figure 1. These spectra were obtained in the presence of DNA; upon binding DNA, the SYTOX Orange dye exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX Orange/DNA complex has absorption and fluorescence emission maxima of 547 nm and 570 nm, respectively, and a fluorescence quantum yield of 0.9. Spectral characteristics of the SYTOX Orange dye in bacteria or eukaryotic cells may vary.

Experimental Guidelines

The following procedure can be adapted for any cell type. Note that different concentration ranges for the SYTOX Orange dye are suggested depending on the cell type (Table 1). Staining can be affected by the growth medium used, the cell density, the presence of other cell types and other factors. 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 water.

Pellet cells by centrifugation and resuspend in buffered saline.

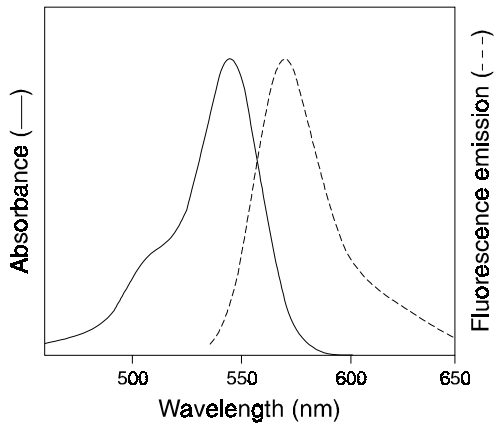

Figure 1. *Absorption and fluorescence emission spectra of the SYTOX Orange nucleic acid stain bound to DNA.*

Table 1. *Recommended conditions for staining cells with SYTOX Orange nucleic acid stain.*

Cell Type	SYTOX Orange Concentration	Incubation Conditions
Bacteria	0.01–0.1 µM	Vortex to mix, then incubate for >5 minutes.
Yeast	0.05–0.5 µM	Incubate with periodic agitation for >10 minutes.
Other Eukaryotes	0.1–5 µM	Incubate for >10 minutes

The binding of SYTOX Orange 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 Orange 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 SYTOX Orange dye can be viewed with a fluorescence microscope equipped with a filter set appropriate for tetramethylrhodamine.

Stained eukaryotic cells will generally have bright orange nuclei as well as some low-level 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
S-11368	SYTOX® Orange 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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

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

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl