SYTOX® Green Nucleic Acid Stain

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYTOX® Green dye	250 µL	5 mM solution in DMSO	• ≤–20°C * • Store vial upright • Protect from light	When stored as directed, product is stable for at least 1 year.

* Before refreezing, seal the vial tightly. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation.

Number of labelings: Each vial contains enough reagent to stain >1,500 samples when using a 96-well microplate assay format.

Approximate fluorescence excitation/emission maxima: 504/523 nm, bound to DNA

Introduction

SYTOX® Green nucleic acid stain (S7020) 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 with both gram-positive and gram-negative bacteria, where an exceptionally bright signal is required.[1,2] After brief incubation with SYTOX® Green nucleic acid stain, the nucleic acids of dead cells fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser, or any other 450–490 nm source. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Green stain a simple and quantitative single-step dead-cell indicator for use with fluorescence microscopes, fluorometers, fluorescence microplate readers, and flow cytometers (Figure 1).

This dead-cell stain may be used in conjunction with blue- and red-fluorescent surface labels for multiparameter analyses. It may also be possible to combine SYTOX® Green nucleic acid stain with DAPI or one of the cell-permeant SYTO® 17 or SYTO® 59–64 red-fluorescent nucleic acid stains for two-color visualization of dead and live cells. SYTOX® Green nucleic acid stain is also an excellent DNA counterstain for chromosome labeling and for fixed cells and tissues.

Spectral Characteristics

The excitation and emission spectra of the SYTOX® Green dye are given in Figure 2. These spectra were obtained in the presence of DNA; upon binding DNA, the SYTOX® Green dye exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX® Green/DNA complex has excitation and emission maxima of 504 nm and 523 nm, respectively, and a fluorescence quantum yield of 0.53. Spectral characteristics of the SYTOX® Green dye in bacteria or eukaryotic cells may vary.

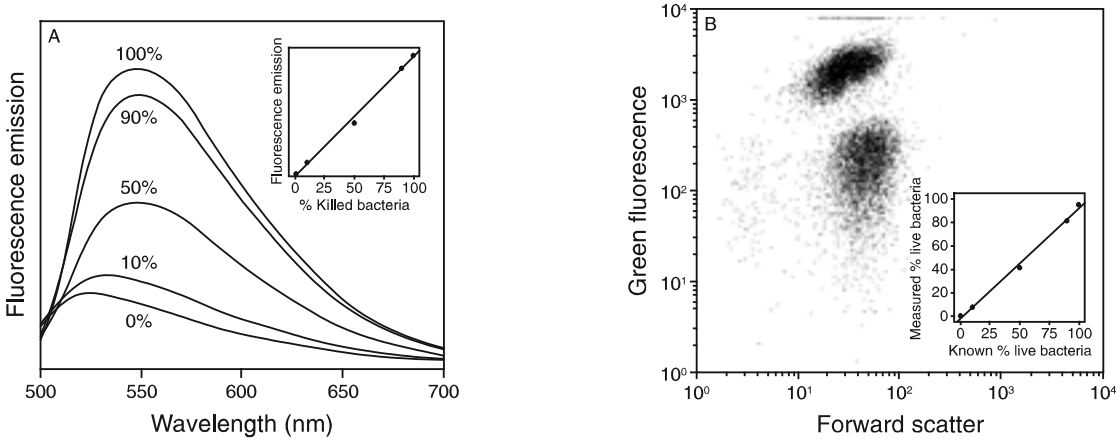

Figure 1. Evaluation of the proportion of viable bacteria in a suspension of *Escherichia coli*. This figure shows the results obtained from two different methods for assessing bacterial viability with the SYTOX® Green nucleic acid stain. (A) Suspensions containing different proportions of live and 70% isopropyl alcohol–killed bacteria were stained with the SYTOX® Green dye and analyzed by fluorometry. A linear relationship between the proportion of live and dead bacteria and the integrated fluorescence emission between 500 nm and 700 nm is shown (see inset) (B) Flow cytometry was used to differentiate live from killed bacteria on the basis of SYTOX® Green staining intensity. The SYTOX® Green–stained suspensions used in the fluorometry experiment were subjected to fluorescence analysis using 488 nm excitation in a Becton Dickinson FACS Vantage flow cytometer. When forward scatter is plotted against log fluorescence intensity (collected with a 510 nm longpass filter) for a bacterial suspension containing an equal number of live and killed cells, two distinct populations are apparent. A linear relationship exists between the distribution of cells in the two regions and the actual percentage of live cells in the sample (see inset).

Guidelines for Use

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. As with all nucleic acid stains, solutions containing this reagent should be disposed of according to local regulations.

Suggested Protocol

The following procedure can be adapted for any cell type. Note that different concentration ranges for the SYTOX® Green dye are suggested depending on the cell type (Table 2). 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® Green 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® Green stain using the concentrations listed in Table 2 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® Green dye can be viewed with a fluorescence microscope equipped with a standard fluorescein filter set. Stained eukaryotic cells will generally have bright green 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.

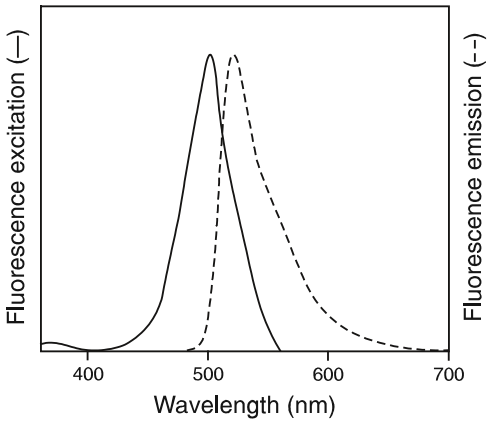

Figure 2. Fluorescence excitation and emission spectra of the SYTOX® Green nucleic acid stain bound to DNA. These spectra were obtained using a ratio of 1 dye molecule to 50 base pairs of DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.

Table 2. Recommended conditions for staining cells with SYTOX® Green dye.

Cell Type	SYTOX® Green 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.

References

1. J Appl Bacteriol 81, 411 (1996); **2.** J Appl Environ Microbiol 63, 2421 (1997).

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

Cat #	Product Name	Unit Size
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL

Contact 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

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.

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 No. 223: Labeling and Detection Technology
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) 335-0354.

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.