SYTOX® Green Dead Cell Stain

for flow cytometry

Catalog no. S34860

Table 1 Contents and storage

Material	Amount	Concentration	Storage*	Stability
SYTOX® Green dead cell stain	1,000 µL	30 µM in DMSO	• ≤–20°C • Desiccate • Store vial upright • Protect from light	When stored as directed, the product is stable for at least 1 year from receipt.
*Before refreezing, seal the vial tightly. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation.				
Number of reactions: Sufficient material is supplied for 1,000 tests, based on the protocol below.				
Approximate fluorescence excitation and emission maxima: 504/523 nm, bound to DNA.				

Introduction

SYTOX® Green dead cell stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes, but will not penetrate healthy cell membranes. 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. The emission of SYTOX® Green dead cell stain easily allows dead cell discrimination with minimal compensation in neighboring channels (Figure 1, page 2). These properties, combined with its >100-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Green dead cell stain a simple and quantitative single-step dead-cell indicator (Figure 2, page 2). SYTOX® Green dead cell stain is compatible with multicolor flow cytometry and can be combined with other dyes, including cell permeant nucleic acid stains such as Vybrant® DyeCycle™ Violet, for two-color visualization of live and dead cells.

Spectral Characteristics

The excitation and emission spectra of the SYTOX® Green stain are given in Figure 1. These spectra were obtained in the presence of DNA; upon binding DNA, the SYTOX® Green stain exhibits a fluorescence enhancement of greater than 100-fold. The SYTOX® Green/DNA complex has excitation and emission maxima of 504 nm and 523 nm, respectively.

Figure 1 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.

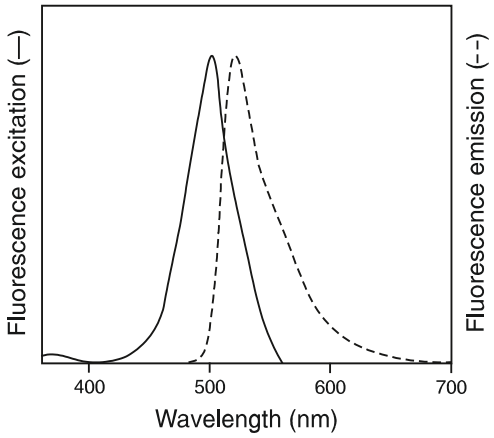
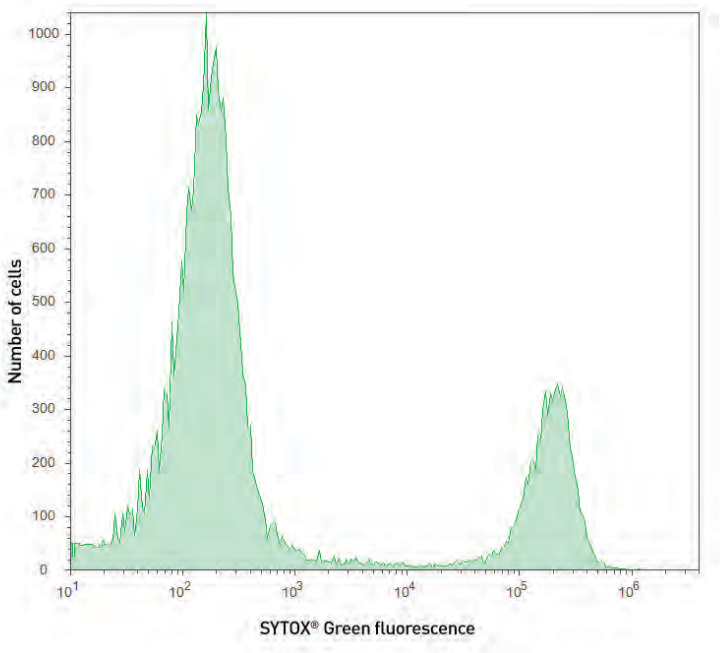


Figure 2 A mixture of heat-killed and live Jurkat cells were stained with 30 nM SYTOX® Green dead cell stain and incubated at room temperature for 20 minutes. Cells were analyzed on the Attune® Acoustic Focusing Cytometer equipped with a 488 nm laser. Fluorescence emission was collected using a 530/30 bandpass filter. Live cells are easily distinguished from the brighter dead cell population.



Before Starting

Materials Required but Not Provided

- Cells and culture medium
- Appropriate suspension buffer
- 12 × 75-mm tubes, or other flow cytometry tubes

Caution

No data are available addressing the mutagenicity or toxicity of SYTOX® Green dead cell stain. Because SYTOX® Green dead cell stain binds to nucleic acids, treat the stain as a potential mutagen and use with care. Handle the DMSO dye solution with caution, because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Storage and Handling

Upon receipt, store the vials of dye frozen at ≤–20°C, upright, and protected from light. Before opening, allow the vials to warm to room temperature and then briefly centrifuge in a microcentrifuge to bring the DMSO solution to the bottom of the vial. Before refreezing, seal all vials tightly. When stored properly, the stock solution is stable for at least one year.

Experimental Protocols

The following procedure was developed using the Jurkat T-cell leukemia cell line, but it can be adapted for any cell type. Growth medium, cell density, cell type variations, and other factors may influence staining. In initial experiments, test a range of stain concentrations to determine the optimal stain concentration for the given cell type, buffer, and experimental conditions. For using the SYTOX® Green dead cell stain in combination with other dyes for multicolor applications, see **Multicolor Staining** below.

1.1 Remove the vial containing the SYTOX® Green dead cell stain from the freezer, and allow the contents to equilibrate to room temperature.

The SYTOX® Green dead cell stain solution in DMSO may be subjected to many freeze-thaw cycles without reagent degradation and is stable for 1 year when stored at ≤–20°C.

1.2 Harvest the cell sample(s). Using an appropriate buffer, adjust the cell concentration of the sample(s) to be from 1×10^5 to 5×10^7 cells/mL.

1.3 Prepare flow cytometry tubes each containing 1 mL of cell suspension.

1.4 Add 1 µL of SYTOX® Green dead cell stain solution in DMSO to each flow cytometry tube and mix well. The final labeling concentration of stain is 30 nM.

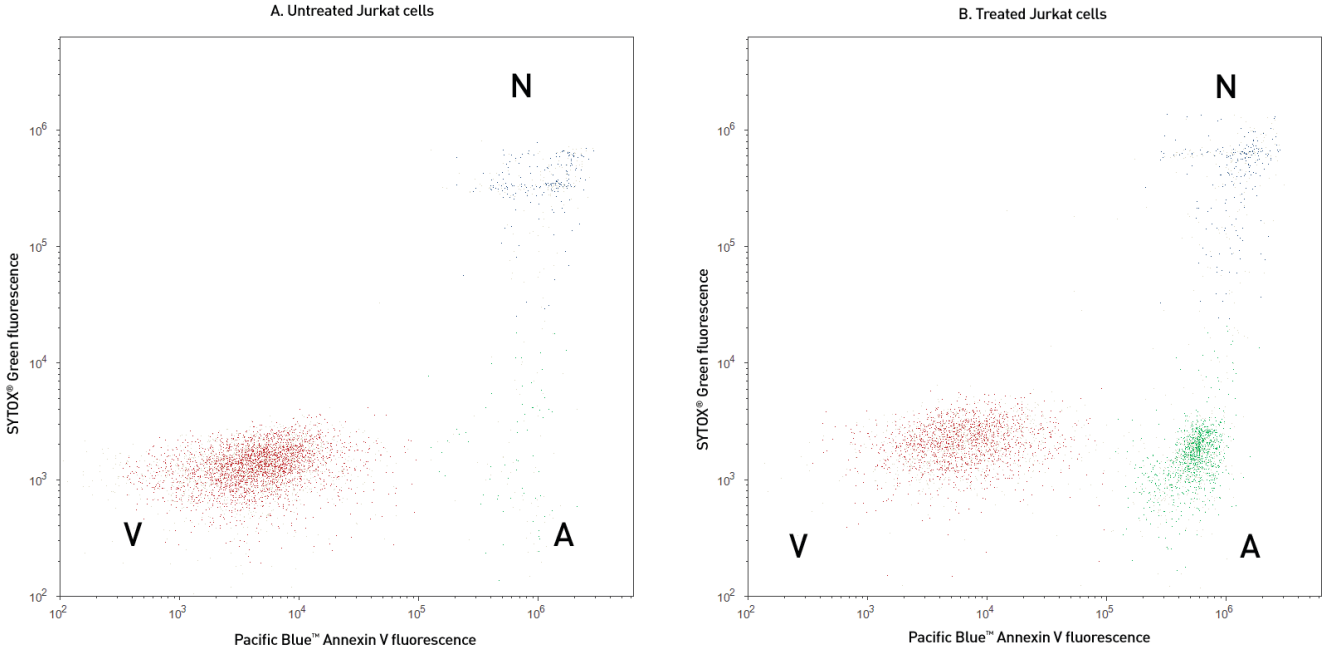
1.5 Incubate flow cytometry tubes for a minimum of 20 minutes at room temperature or 2–6°C, protected from light.

1.6 Analyze samples without washing or fixing, using 488 nm excitation and emission collected in a 530/30 bandpass filter or equivalent.

Multicolor Staining

SYTOX® Green dead cell stain has little spectral overlap with fluorophores excited by other laser lines, and it can be combined with other dyes excitable by the 488 nm laser. SYTOX® Green dead cell stain may be combined with many Annexin-V conjugates to distinguish live, apoptotic, and dead cells (Figure 3). If SYTOX® Green dead cell stain is used in combination with other dyes for multicolor applications, apply the other dyes to the sample first following manufacturer's instructions, including washes. Apply the SYTOX® Green stain as the last stain to the sample following the protocol described above, and do not wash or fix samples prior to flow cytometric analysis.

Figure 3 Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for 4 hours (panel B) or untreated control (panel A). Cells were stained with an anti-human Annexin V-Pacific Blue™ conjugate (Cat. no. A35112) and 30 nM of SYTOX® Green dead cell stain and then analyzed using the Attune® Acoustic Focusing Cytometer equipped with 488 nm and 405 nm lasers for the excitation of SYTOX® Green and Pacific Blue™ dyes, respectively. Samples were run at a collection rate of Sensitive 100 µL/minute and fluorescence emission was detected using a 530/30 bandpass filter for SYTOX® Green and 450/40 bandpass filter for the Pacific Blue™ conjugate. Note that the camptothecin-treated cells (panel B) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.



Tips and Tricks for the Attune® Acoustic Focusing Cytometer

- SYTOX® Green dead cell stain may be analyzed at any collection rate using the Attune® Acoustic Focusing Cytometer with fluorescence emission collected in the BL1 (530/30) channel using 488 nm excitation.
- Samples which are dilute (1×10^4–1×10^5 cells/mL) can be run at any collection rate without dilution.
- To analyze concentrated samples (≥1×10^6 cells/mL) at Standard 200, Standard 500, and Standard 1,000 transit times, dilute the samples in buffer containing SYTOX® Green at a final concentration of 30 nM immediately prior to analysis. Failure to include SYTOX® Green in the diluent may lead to inaccurate results.

References

1. Methods Mol Biol 521, 449 (2009); **2.** Anal Chem 81, 5517 (2009); **3.** Anal Chem 81, 6952 (2009)**; 4.** J Biol Chem 284, 15496 (2009); **5.** Inflamm Res 58, 210 (2009); **6.** J Food Prot 71, 2168 (2008); **7.** J Ind Microbiol Biotechnol 35, 1261 (2008); **8.** Exp Hematol 36, 909 (2008); **9.** J Leukoc Biol 83, 456 (2008); **10.** Nat Protoc 2, 2295 (2007); **11.** Mutat Res 630, 78 (2007); **12.** Appl Environ Microbiol 72, 7829 (2006); **13.** Environ Technol 27, 909 (2006); **14.** J Clin Endocrinol Metab 91, 4154 (2006); **15.** Mar Environ Res 62, 247 (2006); **16.** Environ Mol Mutagen 47, 56 (2006); **17.** J Microbiol Methods 64, 232 (2006); **18.** Leuk Res 29, 1029 (2005); **19.** Curr Protoc Cytom Chapter 7: Unit 7.23 (2004); **20.** Cell Cycle 1, 132 (2002); **21.** Cytometry 48, 93 (2002); **22.** J Appl Microbiol 92, 866 (2002); **23.** Mutat Res 515, 3 (2002); **24.** Photochem Photobiol 72, 28 (2000); **25.** Antimicrob Agents Chemother 44, 676 (2000); **26.** J Appl Environ Microbiol 63, 2421 (1997); **27.** J Appl Bacteriol 81, 411 (1996).

Product List **Current prices may be obtained at www.invitrogen.com or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
S34860	SYTOX® Green dead cell stain *for flow cytometry* *30 µM* *1,000 tests*	1 mL
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