SYTOX® Blue Dead Cell Stain

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

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

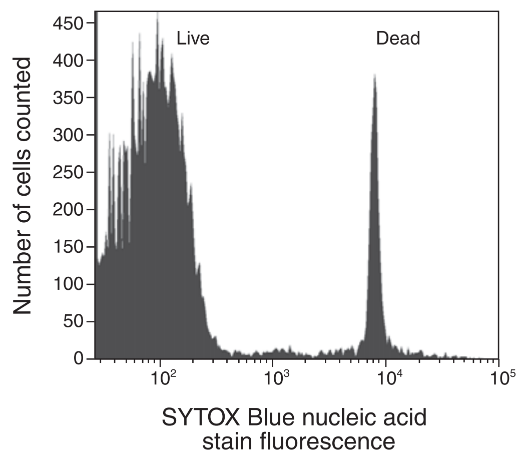

Figure 1. A mixture of heat-killed and untreated Jurkat cells were stained with 1 µM SYTOX® Blue stain for 5 minutes. Cells were analyzed on a flow cytometer equipped with a 405 nm violet diode laser and a 440/40 nm bandpass filter. Live cells are easily distinguished from the dead cell population

Introduction

SYTOX® Blue dead cell stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes but will not cross uncompromised cell membranes. After brief incubation with SYTOX® Blue stain, the nucleic acids of dead cells fluoresce bright blue when excited with 405 nm violet laser light. 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 violet laser–equipped flow cytometers (Figure 1). The violet-excited fluorescence emission of the SYTOX® Blue stain permits clear discrimination from probes excited by most other laser lines, facilitating the development of multicolor assays with minimal spectral overlap between signals.

Spectral Characteristics

The absorption and fluorescence emission spectra of the SYTOX® Blue stain are shown in Figure 2. These spectra were obtained from samples of the dye bound to DNA. The SYTOX® Blue stain exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX® Blue stain/DNA complex has fluorescence excitation and emission maxima of 444 nm and 480 nm, respectively.

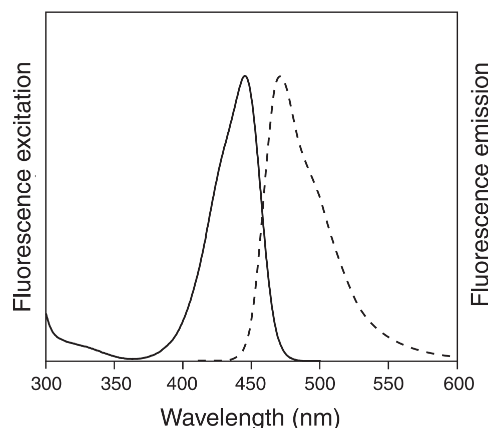

Figure 2. Fluorescence excitation and emission spectra of the SYTOX® Blue stain bound to DNA. These spectra were obtained using a ratio of 1 dye molecule to 50 base pairs of DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.

Materials

Storage and Handling

The SYTOX® Blue stain is supplied as a 1 mM solution in dimethylsulfoxide (DMSO) in a unit size of 1 mL. Upon receipt, this vial should be stored upright, frozen at ≤–20°C, desiccated, and protected from light. Before refreezing, the vial should be sealed 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.

Caution: No data are available addressing the mutagenicity or toxicity of this reagent. However, SYTOX® Blue stain binds to nucleic acids and 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. Please dispose of the reagents in compliance with all pertaining local regulations.

Experimental Protocol

The following procedure was developed using Jurkat cells (a humanT-cell line) but can be adapted for any cell type. Growth medium, cell density, the presence of other cell types and other factors may influence staining. In initial experiments, it may be best to try a range of dye concentrations to determine the one that yields optimal staining for the given cell type and experimental conditions (suggested starting range: 625 nM to 10 µM).

If SYTOX® Blue stain is used in combination with other dyes for multicolor applications, we recommend that the other stain(s) is applied to the sample first, following all manufacturer's instructions, including washes. SYTOX® Blue stain should be the last stain applied to the sample, and samples should not be washed prior to flow cytometric analysis.

1.1 Remove the vial containing the SYTOX® Blue stain from the freezer and allow the contents to equilibrate to room temperature.

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® Blue stain (Component A) to each flow cytometry tube. The final concentration of dye will be 1 µM.

1.5 Incubate flow cytometry tubes for at least 5 minutes at room temperature, protected from light. Do not allow the staining reaction to proceed for longer than 30 minutes.

1.6 Analyze samples without washing or fixing with either a 440/40 nm or a 530/30 nm bandpass filter.

Multicolor Staining

SYTOX® Blue dead cell stain has little spectral overlap with fluorophores excited by other (nonviolet) lasers. Although Pacific Blue™ dye and SYTOX® Blue dead cell stain have considerable spectral overlap, they may be combined without using compensation when SYTOX® Blue dead cell stain is read using the 525/50 channel as a "dump channel" to exclude dead cells from an analysis (Figure 3).

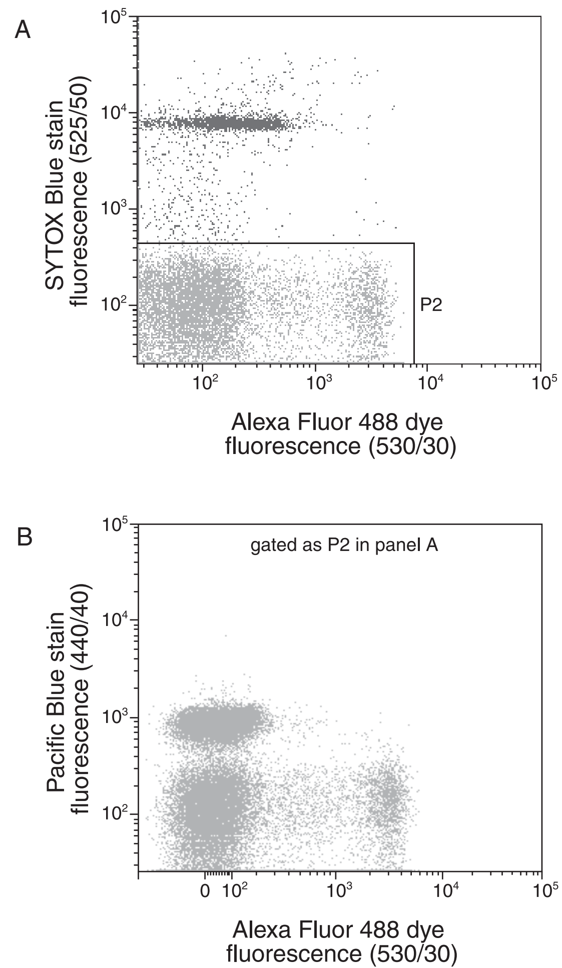

Figure 3. An aliquot of human peripheral blood mononuclear cells was heat treated (10 minutes at 67˚C) and mixed with untreated cells. Cells were stained for 30 minutes with an Alexa Fluor® 488 dye–labeled antibody against CD8 and a Pacific Blue™ dye–labeled antibody against CD4 (CD4 antibody was labeled using Zenon® technology). Cells were washed, resuspended in 1 mL buffer, and stained with SYTOX® Blue dead cell stain for 5 minutes. Cells were analyzed on a flow cytometer equipped with an argon-ion 488 nm laser and a 407 nm violet diode laser. Emission was collected in 530/30 nm (argon-ion laser) and 440/40 nm and 525/50 nm bandpass filters (violet diode laser). SYTOX® Blue stain–negative cells were gated using the 525/50 channel (Figure 3A) and CD4/CD8 phenotype of gated cells was displayed (Figure 3B).

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

Cat #	Product Name	Unit Size
S34857	SYTOX® Blue Dead Cell Stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL

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.

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