SYTOX® Red Dead Cell Stain

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
SYTOX® Red dead cell stain	1 mL	5 µM solution in DMSO	• ≤–20°C * • Store vial upright • Desiccate • 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.

Approximate fluorescence excitation and emission maxima: 640/658 nm, bound to DNA

Introduction

SYTOX® Red 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® Red stain, the nucleic acids of dead cells fluoresce bright red when excited with 633 or 635 nm red laser light. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® Red stain a simple and quantitative single-step dead-cell indicator for use with red laser–equipped flow cytometers (Figure 1). Using 633 or 635 nm excitation, SYTOX® Red dead cell stain is distinct from other dead cell probes like 7-AAD and PI, which are excited using 488 nm. The emission of SYTOX® Red stain is limited to one channel with minimal spectral overlap. This effectively frees all the channels of the 488 nm laser line.

Spectral Characteristics

The fluorescence excitation and emission spectra of the SYTOX® Red stain are shown in Figure 1. These spectra were obtained from samples of the dye bound to DNA. The SYTOX® Red stain exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX® Red stain/DNA complex has fluorescence excitation and emission maxima of 640 nm and 658 nm, respectively.

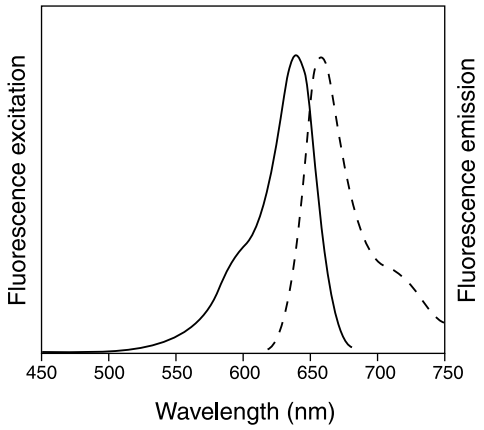

Figure 1. Fluorescence excitation and emission spectra of the SYTOX® Red stain bound to DNA.

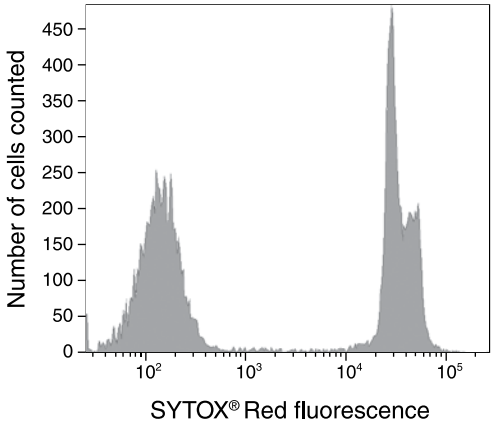

Figure 2. A mixture of heat-killed and untreated Jurkat cells were stained with 5 nM SYTOX® Red stain for 15 minutes. Cells were analyzed on a flow cytometer equipped with a 633 nm laser and a 660/20 nm bandpass filter. Live cells are easily distinguished from the dead cell population

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, cell type variations, 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.

If SYTOX® Red 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® Red stain should be the last stain applied to the sample, and samples should not be washed prior to flow cytometric analysis.

Caution

No data are available addressing the mutagenicity or toxicity of this reagent. However, SYTOX® Red 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.

1.1 Remove the vial containing the SYTOX® Red 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® Red stain (Component A) to each flow cytometry tube. The final concentration of dye will be 5 nM.

1.5 Incubate flow cytometry tubes for a minimum of 15 minutes at room temperature or 2–6°C, protected from light.

1.6 Analyze samples without washing or fixing, using 633 nm or 635 nm excitation and emission monitored at ~660 nm.

Multicolor Staining

SYTOX® Red dead cell stain has little spectral overlap with fluorophores excited by other laser lines, and may be easily combined with other dyes. SYTOX® Red dead cell stain may be easily combined with many annexin-V conjugates to distinguish live, apoptotic, and dead cells (Figure 3).

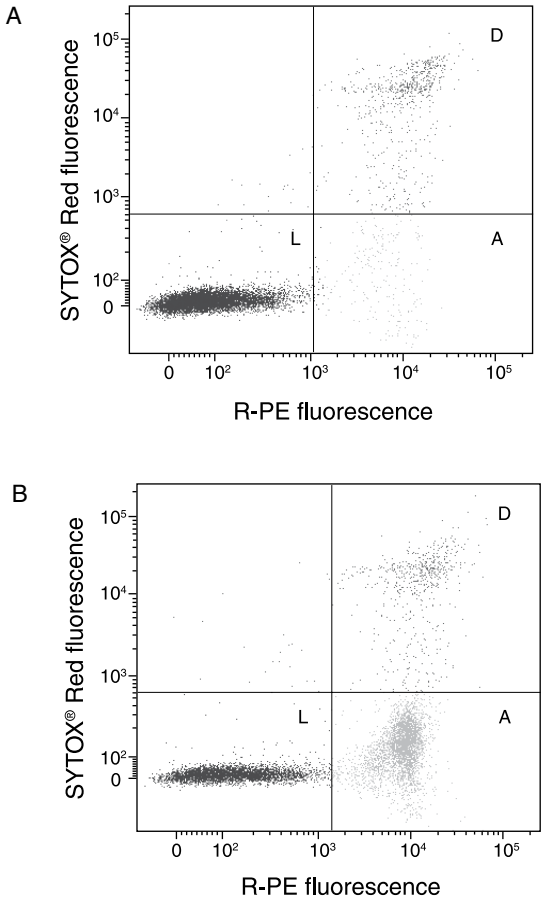

Figure 3. Staining pattern of jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for 4 hours (panel B) or untreated (as control, panel A). Cells were suspended in annexin-binding buffer and then treated with R-phycoerythrin (R-PE) annexin V conjugate and SYTOX® Red dead cell stain for 30 minutes, followed by flow cytometric analysis. Three cell types are identified: live (L), apoptotic (A), and dead (D). SYTOX® Red fluorescence was collected in 660/20 bandpass (633 nm red laser) and R-PE fluorescence was collected in 585/42 bandpass (488 nm blue laser).

Product List **Current prices may be obtained from our website 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
S7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL

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

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