SYTOX® Dead Cell Stain Sampler Kit

for flow cytometry

Catalog no. S34862

Table 1 Contents and storage

Material	Amount	Concentration	Storage*	Stability
SYTOX® AADvanced™ dead cell stain (Component A)	50 µL	1 mM 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.
SYTOX® Blue dead cell stain (Component B)		1 mM in DMSO		
SYTOX® Green dead cell stain (Component C)		30 µM in DMSO		
SYTOX® Orange dead cell stain (Component D)		250 µM in DMSO		
SYTOX® Red dead cell stain (Component E)		5 µM in DMSO		
*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 50 tests per vial, based on the protocol below.				
Approximate fluorescence excitation and emission maxima (bound to DNA): SYTOX® AADvanced™ dead cell stain: 546/647 nm; SYTOX® Blue dead cell stain: 444/480 nm; SYTOX® Green dead cell stain 504/523 nm; SYTOX® Orange dead cell stain 547/570 nm; SYTOX® Red dead cell stain: 640/658 nm.				

Introduction

The SYTOX® series of dead cell stains are high-affinity nucleic acid stains that easily penetrate cells with compromised plasma membranes but will not penetrate healthy cell membranes. The SYTOX® dead cell stain sampler kit contains a selection of five different SYTOX® dead cell stains suitable for excitation using 405-nm, 488-nm, 532-nm, or 633/635-nm lasers. After a brief incubation with the appropriate SYTOX® dead cell stain, the nucleic acids of dead cells have a greater than 100-fold increase in fluorescence, making the SYTOX® dead cell stains simple and quantitative single-step dead-cell indicators. The emission of each SYTOX® dead cell stain allows dead cell discrimination with minimal compensation in the neighboring channels (Figure 1, page 2). Labeling of dead cells is achieved rapidly, within 5–20 minutes (dependent upon which SYTOX® dye is used).

Spectral Characteristics

The excitation and emission spectra of the SYTOX® dyes contained in the SYTOX® dead cell stain sampler kit are given in Table 2, page 3. These spectra were obtained in the presence of DNA; upon DNA binding, each SYTOX® stain exhibits a fluorescence enhancement of greater than 100-fold.

Figure 1 A mixture of heat-killed and live Jurkat cells were stained with (A) SYTOX® AADvanced™; (B) SYTOX® Blue; (C) SYTOX® Green; (D) SYTOX® Orange; or (E) SYTOX® Red. All stains were labeled according to the protocol described. Panels A–D: Cells were analyzed on the Attune® Acoustic Focusing Cytometer equipped with a 488-nm laser (for excitation of SYTOX® AADvanced, SYTOX® Green, and SYTOX® Orange) and a 405-nm laser (for excitation of SYTOX® Blue). Fluorescence emission was collected using a (A) 640 longpass filter; (B) 450/40 bandpass filter; (C) 530/30 bandpass filter; (D) 575/24 bandpass filter. (E) Cells were analyzed on a flow cytometer equipped with a 633-nm laser (for excitation of SYTOX® Red) and a 660/20 nm bandpass filter. Live cells are easily distinguished from the brighter dead cell population.

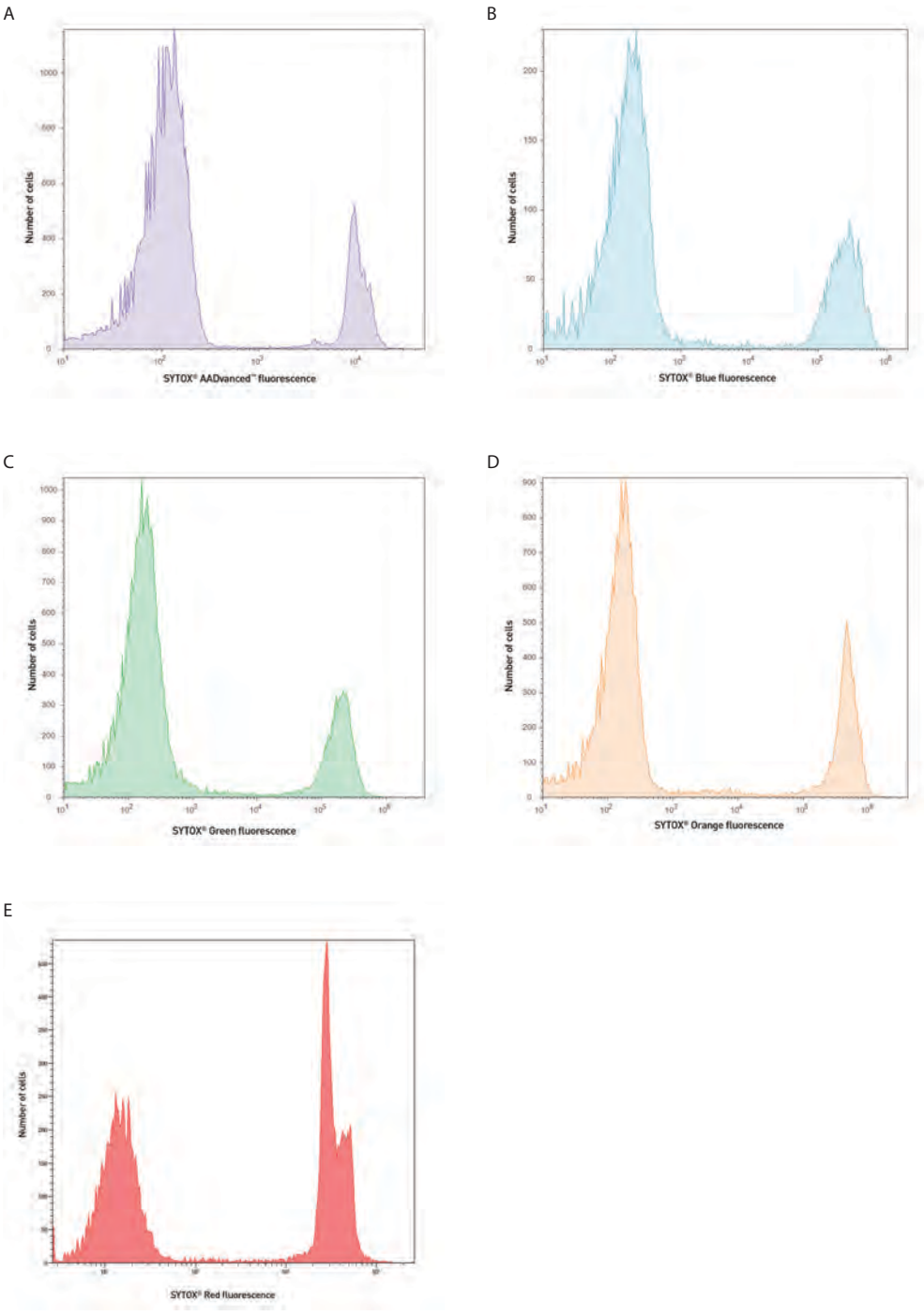

Table 2 Fluorescence excitation and emission maxima of SYTOX® dead cell stains

SYTOX® dead cell stain	Fluorescence excitation maximum (nm)	Fluorescence emission maximum (nm)	Suggested laser (nm)
SYTOX® AADvanced	546	647	488
SYTOX® Blue	444	480	405
SYTOX® Green	504	523	488
SYTOX® Orange	547	570	532 or 488
SYTOX® Red	640	658	633/635

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 these reagents. Because the SYTOX® dead cell stains bind to nucleic acids, treat them as potential mutagens and use with care. Handle the DMSO dye solutions 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 these reagents. 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 to bring the DMSO solution to the bottom. 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 and it is applicable for each SYTOX® dead cell stain included in the sampler kit. Growth medium, cell density, cell type variations, and other factors may influence staining. In initial experiments, test a range of stain concentrations to determine optimal stain concentration for the given cell type, buffer, and experimental conditions. If a SYTOX® dead cell stain is used in combination with other dyes for multicolor applications, see **Multicolor Staining** below.

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

The SYTOX® 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® dead cell stain solution in DMSO to each flow cytometry tube and mix well.

1.5 Incubate flow cytometry tubes for the time specified in Table 3 at room temperature or 2–6°C, protected from light.

1.6 Analyze samples without washing or fixing, using the suggested excitation and emission and the appropriate bandpass filter (or equivalent) as indicated in Table 3.

Table 3 Final concentration, incubation time, and cytometer configuration for SYTOX® dead cell stains

SYTOX® dead cell stain	Final concentration	Suggested incubation time (minutes)	Attune® channel; bandpass filter
SYTOX® AADvanced	1 µM	5	BL3; 640 longpass
SYTOX® Blue	1 µM	15	VL1; 450/40
SYTOX® Green	30 nM	20	BL1; 530/30
SYTOX® Orange*	250 nM	20	BL2; 575/24
SYTOX® Red	5 nM	15	NA; 660/20

* If excited by a 532-nm laser use at a final concentration of 4 nM.
NA = not applicable.

Multicolor Staining

The SYTOX® dead cell stain sampler kit allows for greater flexibility in multicolor cytometry. Each kit contains five different dead cell stains excitable by a 405-nm, 488-nm, 532-nm, or 633/635-nm laser. The SYTOX® dead cell stains have little spectral overlap with fluorophores excited by other laser lines and they can be easily combined with other dyes in multicolor cytometry. If a SYTOX® dead cell stain is used in combination with other dyes for multicolor applications, first apply the other dyes to the sample by following manufacturer's instructions, including the washes. Apply the SYTOX® dead cell 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.

Tips and Tricks for the Attune® Acoustic Focusing Cytometer

- SYTOX® dead cell stains may be analyzed at any collection rate using the Attune® Acoustic Focusing Cytometer.
- 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® dead cell stain at the appropriate final concentration (see Table 3, above) immediately prior to analysis. Failure to include the SYTOX® stain in the diluent may lead to inaccurate results.

References

1. Biotechnol Adv 28, 255 (2010); **2.** Methods Mol Biol 521, 449 (2009); **3.** Anal Chem 81, 5517 (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.** Leuk Res 29, 1029 (2005); **18.** J Microbiol Methods 64, 232 (2005); **19.** J Microbiol Methods 63, 276 (2005); **20.** Anal Chem 77, 554 (2005); **21.** Curr Protoc Cytom Chapter 7:Unit 7.23 (2004); **22.** Autoimmunity 37, 85 (2004); **23.** Cytometry A 60, 125 (2004); **24.** Cell Cycle 1, 132 (2002); **25.** Cytometry 48, 93 (2002); **26.** J Appl Microbiol 92, 866 (2002); **27.** Mutat Res 515, 3 (2002); **28.** Anal Biochem 286, 138 (2000); **29.** Photochem Photobiol 72, 28 (2000); **30.** Antimicrob Agents Chemother 44, 676 (2000); **31.** J Appl Environ Microbiol 63, 2421 (1997); **32.** 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
S34862	SYTOX® dead cell stain sampler kit *for flow cytometry*	1 kit
Related Products		
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S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
S34860	SYTOX® Green dead cell stain *for flow cytometry* *30 µM* *1,000 tests*	1 mL
S34861	SYTOX® Orange dead cell stain *for flow cytometry* *250 µM* *1,000 tests*	1 mL

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