SYTOX® AADvanced™ Dead Cell Stain Kits

Catalog nos. S10274, S10349

Table 1. Contents and storage information.

Material	Amount		Storage	Stability
	S10274	S10349		
SYTOX® AADvanced™ dead cell stain (Component A)	5 vials	1 vial	• ≤–20˚C • Desiccate • Protect from light	When stored as directed, the kits are stable for at least 1 year.
Dimethylsulfoxide (DMSO, Component B)	0.5 mL	0.2 mL		
Number of assays: Sufficient material is supplied for 500 assays (Cat. no. S10274) or 100 assays (Cat. no. S10349) based on the protocol below.				
Approximate fluorescence excitation/emission maxima: SYTOX® AADvanced™ dead cell stain**:** 546/647 in nm, bound to DNA.				

Introduction

SYTOX® AADvanced™ dead cell stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes but will not cross healthy cell membranes. After brief incubation with SYTOX® AADvanced™ dead cell stain, the nucleic acid of dead cells fluoresce bright red-orange when excited with 488 nm blue laser light. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX® AADvanced™ dead cell stain a simple and quantitative single-step dead-cell indicator. The emission of SYTOX® AADvanced™ dead cell stain easily allows dead cell discrimination with the common 488 nm laser, with minimal compensation in the green, orange, and near-infrared channels (Figure 1). Labeling of dead cells is achieved very rapidly, within 5 minutes (Figure 2). SYTOX® AADvanced™ dead cell stain may also be used for DNA content cell cycle analysis with the addition of RNase A with fixed cells (Figure 3).

Spectral Characteristics

The fluorescence excitation and emission spectra of the SYTOX® AADvanced™ dead cell stain are shown in Figure 1 and were obtained from samples of the dye bound to DNA. The SYTOX® AADvanced™ dead cell stain exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX® AADvanced™ dead cell stain/DNA complex has fluorescence excitation and emission maxima of 546 nm and 647 nm, respectively.

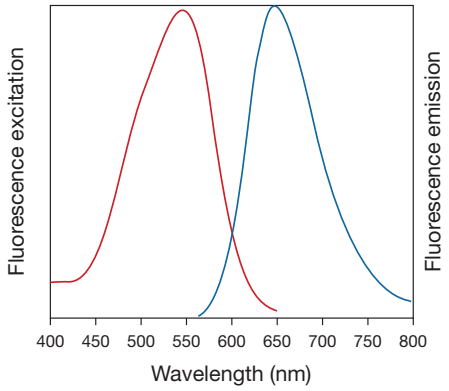

Figure 1. Fluorescence excitation and emission spectra of the SYTOX® AADvanced™ dead cell stain bound to DNA.

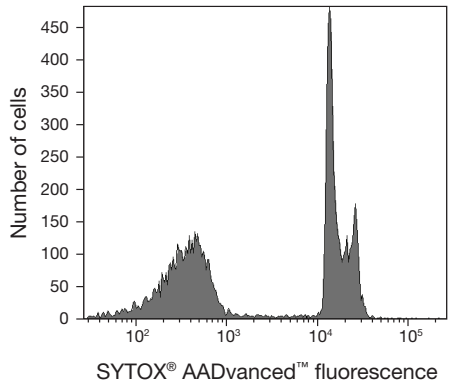

Figure 2. A mixture of heat-killed and untreated Jurkat cells were stained with 1 µM SYTOX® AADvanced™ dead cell stain for 5 minutes. Cells were analyzed on a flow cytometer equipped with a 488 nm laser and a 695/40 nm bandpass filter. Live cells are easily distinguished from the dead cell population.

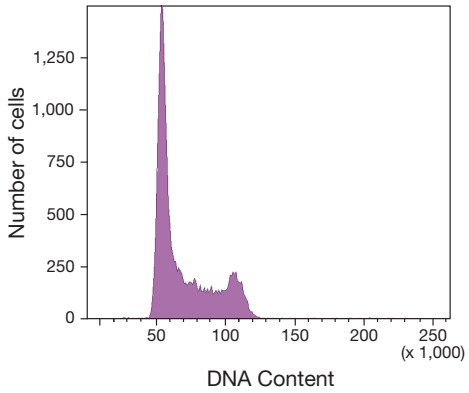

Figure 3. HL-60 human promyeloblastic leukemia cells were alcohol fixed and then suspended in 0.1% Triton® X-100/PBS/1% BSA. Cells were stained with 1 µM SYTOX® AADvanced™ dead cell stain with the addition of RNase A for 30 minutes at room temperature. Cells were analyzed on a flow cytometer equipped with a 488 nm laser and a 695/40 nm bandpass filter.

Before Starting

Materials Required but Not Provided

- Cells and culture medium
- Appropriate cell culture buffer
- Flow cytometry tubes
- RNase A (20 mg/mL, Invitrogen Cat. no. 12091) for DNA content cell cycle analysis

Caution

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

Experimental Protocol for Dead Cell Discrimination

The following procedure was developed using the Jurkat T-cell leukemia 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, test a range of stain concentrations to determine optimal stain concentration for the given cell type, buffer, and experimental conditions.

If SYTOX® AADvanced™ dead cell stain is used in combination with other dyes for multicolor applications, apply other dyes to the sample first following manufacturers instructions, including washes. Apply the SYTOX® AADvanced™ dead cell stain as the last stain to the sample, and do not wash or fix samples prior to flow cytometric analysis.

SYTOX® AADvanced™ Dead Cell Staining Procedure

1.1 Remove the vial containing the SYTOX® AADvanced™ dead cell stain (Component A) and the vial of DMSO (Component B) from the freezer, and allow the contents to equilibrate to room temperature.

1.2 Add 100 µL DMSO to one vial of SYTOX® AADvanced™ dead cell stain and mix well to prepare a 1 mM SYTOX® AADvanced™ dead cell stain solution in DMSO.

The SYTOX® AADvanced™ 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.

1.3 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.

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

1.5 Add 1 µL of SYTOX® AADvanced™ dead cell stain solution in DMSO to each flow cytometry tube and mix well. The final labeling concentration of stain is 1 µM.

1.6 Incubate flow cytometry tubes for a minimum of 5 minutes at room temperature or 2–6°C, **protected from light**.

1.7 Analyze samples without washing or fixing, using 488 nm excitation and emission collected in a 695/40 bandpass or equivalent. Alternative laser light sources such as 532 nm and 561 nm are also compatible with SYTOX® AADvanced™ dead cell stain.

Experimental Protocol for DNA Content Cell Cycle on Fixed Cells

The following procedure was developed using the Jurkat T-cell leukemia cell line, but can be adapted for any cell type. Fixative, 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 and experimental conditions. Since SYTOX® AADvanced™ dead cell stain also labels RNA, treat cells with RNase A to ensure specific DNA signal.

If SYTOX® AADvanced™ dead cell stain is used in combination with other dyes for multicolor applications, apply other dyes to the sample first following manufacturers instructions,

including washes. Apply the SYTOX® AADvanced™ dead cell stain as the last stain to the sample, and do not wash or fix samples prior to flow cytometric analysis.

SYTOX® AADvanced™ Stain for Cell Cycle Analysis on Fixed Cells

2.1 Remove the vial containing the SYTOX® AADvanced™ dead cell stain (Component A) and the vial of DMSO (Component B) from the freezer and allow the contents to equilibrate to room temperature.

2.2 Add 100 µL DMSO to one vial of SYTOX® AADvanced™ dead cell stain and mix well to prepare a 1 mM SYTOX® AADvanced dead cell stain solution in DMSO.

The SYTOX® AADvanced™ 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.

2.3 Harvest the cell sample(s).

2.4 Fix cells, or fix and permeabilize cells. Remove the fixative from cells before proceeding with cell staining.

2.5 Using an appropriate buffer, adjust the cell concentration of the sample(s) to be 1×10^6 cells/mL.

2.6 Prepare flow cytometry samples each containing 1 mL of cell suspension.

2.7 Add 5 µL RNase A (20 mg/ml) to each sample.

2.8 Add 1 µL of SYTOX® AADvanced™ dead cell stain solution in DMSO to each flow cytometry sample and mix well. The final labeling concentration of the stain is 1 µM.

2.9 Incubate flow cytometry tubes for 30 minutes at room temperature or 2–6°C, **protected from light**.

2.10 Analyze samples without washing, using 488 nm excitation and emission collected in a 695/40 bandpass or equivalent. Alternative laser light sources such as 532 nm and 561 nm are also compatible with SYTOX® AADvanced™ dead cell stain.

Multicolor Staining

SYTOX® AADvanced™ dead cell stain has little spectral overlap with fluorophores excited by other laser lines, and can be easily combined with other dyes excitable by the 488 nm laser. SYTOX® AADvanced™ dead cell stain may be easily combined with many annexin-V conjugates to distinguish live, apoptotic, and dead cells (Figure 4).

If SYTOX® AADvanced™ dead cell stain is used in combination with other dyes for multicolor applications, apply the other dyes to the sample first following manufacturers instructions, including washes. Apply the SYTOX® AADvanced™ stain as the last stain to the sample, and do not wash or fix samples prior to flow cytometric analysis.

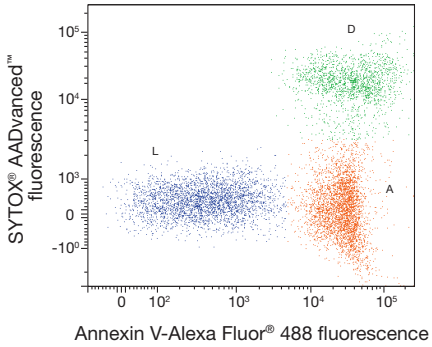

Figure 4. Staining pattern of Jurkat cells (human T-cell leukemia), treated with 10 µM camptothecin for 4 hours. Cells were suspended in annexin-binding buffer and then treated with Alexa Fluor® 488 annexin V conjugate for 30 minutes and SYTOX® AADvanced™ dead cell stain for 5 minutes, followed by flow cytometric analysis. Three cell types are identified: live (L), apoptotic (A), and dead (D). SYTOX® AADvanced™ fluorescence was collected in 695/40 bandpass and Alexa Fluor® 488 fluorescence was collected in 530/30 bandpass (both using the 488 nm blue laser).

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

Cat. no.	Product Name	Unit Size
S10274	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *500 tests*	1 kit
S10349	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *100 tests*	1 kit
Related Products		
A13199	annexin V, fluorescein conjugate (FITC annexin V) *100 assays*	500 µL
A13201	annexin V, Alexa Fluor® 488 conjugate *100 assays*	500 µL
A23204	annexin V, Alexa Fluor® 647 conjugate *100 assays*	500 µL
A35110	annexin V, allophycocyanin conjugate (APC annexin V) *50 assays*	250 µL
A35111	annexin V, R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	250 µL
A35122	annexin V, Pacific Blue™ conjugate *for flow cytometry* *100 assays*	500 µL
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
12091-039	RNase A (20 mg/ml)	25 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

For country-specific contact information, visit www.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.