APC Annexin V/Dead Cell Apoptosis Kit with APC annexin V and SYTOX® Green for Flow Cytometry

Catalog no. V35113

Table 1. Contents and storage information.

Material	Amount	Composition	Storage*	Stability
Allophycocyanin (APC) annexin V (Component A)	250 µL	Solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, 0.1% bovine serum albumin (BSA)	• 2–6°C • Protect from light • DO NOT FREEZE COMPONENT A	When stored as directed this kit is stable for 6 months.
SYTOX® Green Stain (Component B)	100 µL	10 µM solution in DMSO		
5X annexin-binding buffer (Component C)	15 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4		

*For long-term storage, store the vial of SYTOX® Green stain at ≤–20°C. The SYTOX® Green stain and APC annexin V are light sensitive and may be handled in normal room light, but avoid prolonged exposure to light.

Number of assays: Sufficient material is supplied for 50 flow cytometry assays based on a 100 µL assay volume.

Approximate fluorescence excitation/emission maxima: APC annexin V: 650/660 in nm; SYTOX® Green 488**:** 503/554 in nm, bound to DNA.

Introduction

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.[1-5] In normal viable cells, phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment.[6] In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages.[7,8] The human vascular anticoagulant, annexin V, is a 35–36 kilodalton Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS.[9] Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.[10]

The APC Annexin V/Dead Cell Apoptosis Kit with APC annexin V and SYTOX® Green for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to allophycocyanin (APC). APC is an extremely fluorescent phycobiliprotein, which can be easily excited with a helium-neon (HeNe) laser at 633 nm on a standard flow cytometer and has an emission maximum at approximately 660 nm. In addition to APC annexin V, the kit includes a solution of SYTOX® Green nucleic acid stain. The SYTOX® Green dye is impermeant to live cells and apoptotic cells, but stains

dead cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with APC annexin V and SYTOX® Green stain, apoptotic cells show red fluorescence and very little green fluorescence, dead cells show a higher level of green and red fluorescence, and live cells show little or no fluorescence (Figure 1). These populations can easily be distinguished using a flow cytometer with both the 488 nm line of an argon-ion laser and the 633 nm line of a HeNe laser for excitation.

The assay has been optimized using Jurkat cells, a human T-cell leukemia clone, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types. Since no single parameter defines apoptosis in all systems, we recommend to use a combination of different measurements for reliable detection of apoptosis. Refer to our website at probes.invitrogen.com for a wide selection of products for apoptosis research.

Before Starting

Materials Required but Not Provided

- Samples (appropriate sample concentrations range from 2×10^5 to 1×10^6 cells/mL)
- Inducing agent
- 2 mM hydrogen peroxide
- Phosphate buffered saline (PBS)
- Deionized water

Caution

No data are available addressing the mutagenicity or toxicity of SYTOX® Green stain. Because this reagent binds to nucleic acids, treat it as a potential mutagen and handle with appropriate care. Handle the DMSO stock solution with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of stains in compliance with all pertaining local regulations.

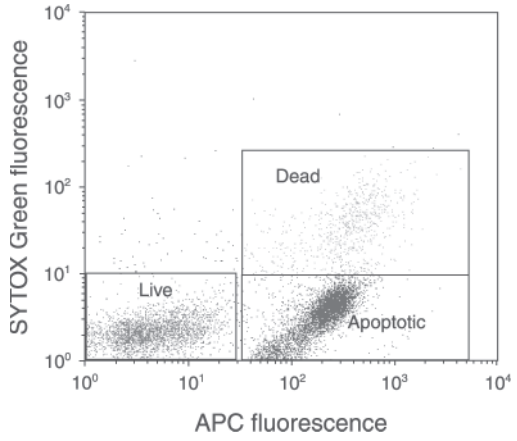

Figure 1. Jurkat cells (T-cell leukemia, human) were treated with 10 µM camptothecin for 4 hours at 37°C, 5% CO_2. The cells were incubated with the reagents in the kit, and then analyzed by flow cytometry. The SYTOX® Green fluorescence versus APC (allophycocyanin) fluorescence dot plot shows resolution of live, apoptotic, and dead cell populations.

Experimental Protocol

The assay has been optimized using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

1. Induce apoptosis in cells using the desired method. Prepare negative control by incubating cells in the absence of inducing agent. Prepare a positive control for necrosis by incubating cells with 2 mM hydrogen peroxide for 4 hours at 37°C.

2. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component C) to 8 mL deionized water.

3. Prior to opening, warm the vial of SYTOX® Green stain to room temperature. Prepare a 1 M working solution of SYTOX® Green stain.

   For example, dilute 5 µL of the 10 µM SYTOX® Green stain stock solution (Component B) in 45 µL of 1X annexin-binding buffer. The SYTOX® Green stain concentration may have to be adjusted for individual cell types.

   Store the unused portion of this working solution at ≤–20°C for up to 1 month. Reseal this vial tightly before returning to storage.

4. Harvest the cells following apoptosis induction and wash in 1X annexin -binding buffer.

5. Pellet the washed cells (from step 4) by centrifugation, discard the supernatant, and resuspend the cells at a concentration of ~1×10^6 cells/mL in 1X annexin-binding buffer.

6. Add 5 µL APC annexin V (Component A) and 1 µL of the 1 µM SYTOX® Green stain working solution (prepared in step 3) to each 100 µL cell suspension.

7. Incubate the cells at 37°C in an atmosphere of 5% CO_2 for 15 minutes.

8. After the incubation period, add 400 µL of the 1X annexin-binding buffer, mix gently, and keep the samples on ice.

9. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm and 660 nm. The population should separate into three groups: live cells with only a low level of green and red fluorescence, apoptotic cells with a high level of red fluorescence and little green fluorescence, and dead cells with a high level of green and red fluorescence. Confirm the flow cytometry results by viewing the cells with a fluorescence microscope, using filters appropriate for fluorescein (FITC) and allophycocyanin (APC).

References

1. Immunol Cell Biol 76, 1 (1998); **2.** Cytometry 27, 1 (1997); **3.** J Pharmacol Toxicol Methods 37, 215 (1997); **4.** FASEB J 9, 1277 (1995); **5.** Am J Pathol 146, 3 (1995); **6.** Cytometry 31, 1 (1998); **7.** J Immunol 148, 2207 (1992); **8.** J Immunol 151, 4274 (1993); **9.** J Biol Chem 265, 4923 (1990); **10.** Blood 84, 1415 (1994).

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

Cat. no.	Product Name	Unit Size
V35113	APC Annexin V/Dead Cell Apoptosis Kit with APC annexin V and SYTOX® Green *for flow cytometry* *50 assays*	1 kit

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.