Violet Annexin V/Dead Cell Apoptosis Kit with Pacific Blue™ Annexin V/SYTOX® AADvanced™ for Flow Cytometry

Catalog no. A35136

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Pacific Blue™ annexin V (Component A)	250 µL solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, with 0.1% bovine serum albumin (BSA)	• 2–6°C • Protect from light • Do not freeze	When stored as directed, this kit is stable for at least 6 months.
SYTOX® AADvanced™ Dead Cell Stain (Component B)	1 vial	• 2–6°C • Protect from light	
Dimethylsulfoxide (DMSO), anhydrous (Component C)	100 µL	• 2–6°C	
5X annexin binding buffer (Component D)	15 mL		
Number of assays: Sufficient material is supplied for 50 assays, based on the protocol below.			
Approximate fluorescence excitation/emission maxima: Pacific Blue™ annexin V conjugate**:** 415/455 in nm; SYTOX® AADvanced™ Dead Cell Stain: 546/647 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, or accidental cell death, 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. In apoptotic cells, however, 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 kDa 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 Violet Annexin V/Dead Cell Apoptosis Kit with Pacific Blue™ annexin V/SYTOX® AADvanced™ provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to the violet-excitable fluorophore, Pacific Blue™ dye, to provide the maximum sensitivity. Because Pacific Blue™ dye absorbs maximally at 415 nm with fluorescence emission 455 nm, it is a good choice for violet diode laser excitation in flow cytometry. The kit includes the red fluorescent dye, SYTOX® AADvanced™ Dead Cell Stain,

for identifying necrotic cells based on membrane integrity. After staining a cell population with Pacific Blue™ annexin V and SYTOX® AADvanced™ stains in the supplied binding buffer, apoptotic cells show bright violet fluorescence, dead cells show red fluorescence, and live cells show dim violet fluorescence (Figure 1). Because there is very little spectral overlap between the two dyes, very little or no compensation is required. We have optimized this assay using Jurkat cells, a human T-cell leukemia line, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Molecular Probes offers a wide selection of products for apoptosis research; for more information, refer to www.invitrogen.com.

Spectral Characteristics

The fluorescence excitation and emission spectra of the Pacific Blue™ annexin V conjugate (Component A) has fluorescence excitation/emission maxima of 415 nm and 455 nm, respectively.

The fluorescence excitation and emission spectra of the SYTOX® AADvanced™ Dead Cell Stain (Component B) 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.

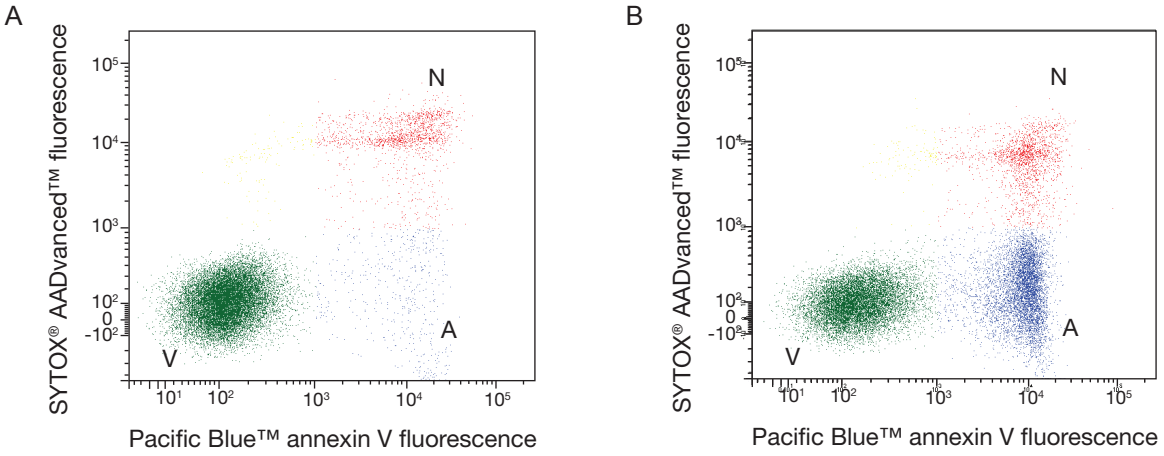

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (panel B) or untreated control (panel A). Cells were treated with the reagents in the kit and analyzed by flow cytometry using 405 nm and 488 nm excitation. Note that the camptothecin-treated cells have a higher percentage of apoptotic cells (panel B) than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.

Before Starting

Materials Required but Not Provided

- Flow cytometry tubes
- Cells and culture medium
- Deionized water

Caution

The hazards posed by these stains have not been fully investigated. SYTOX® AADvanced™ dye (Component B) is known to bind to nucleic acids; treat each dye as a potential mutagen and use it with appropriate care. Handle the DMSO (dimethyl sulfoxide) dye solution with particular caution, because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye and face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Preparing Reagents

1.1 Remove the kit from the refrigerator, and allow the contents to equilibrate to room temperature.

1.2 Prepare 1X annexin binding buffer. For example, for ~10 assays, add 1 mL of 5X annexin binding buffer (Component D) to 4 mL of deionized water.

1.3 Prepare 500 µM SYTOX® AADvanced™ Dead Cell Stain working solution by adding 200 µL DMSO (Component C) to one vial of SYTOX® AADvanced™ Dead Cell Stain (Component B). Mix the solution well. When stored at 2–6°C, the SYTOX® AADvanced™ Dead Cell Stain working solution may be subjected to many freeze-thaw cycles without reagent degradation.

Assay Guidelines

- We have optimized this assay using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.
- Make sure that all tubes contain the same number of cells for a given experiment. Tube-to-tube variation in cell number leads to significant differences in staining, and such variation can affect results.
- We recommend phosphate-buffered saline (PBS) for suspending cells during staining.
- Do **not** use glass containers or tubes.

Experimental Protocols

2.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of apoptosis inducing agent.

2.2 Harvest the cells after the incubation period and wash them in cold phosphate-buffered saline (PBS).

2.3 Discard the supernatant and resuspend the cells in 1X annexin binding buffer (prepared in step 1.2) at ~1 × 10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

2.4 Add 5 µL of Pacific Blue™ annexin V (Component A) and 1 µL of 500 µM SYTOX® AADvanced™ Dead Cell Stain working solution (prepared in step 1.3) to each 100 µL of cell

suspension.

2.5 Incubate the cells at room temperature for 30 minutes, **protected from light**.

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

2.7 As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission using a 450 nm bandpass or equivalent with 405 nm excitation (Pacific Blue™ dye) and a 670 bandpass or equivalent with 488 nm excitation (SYTOX® AADvanced™).

The sample can contain three populations: live cells showing a low level of violet and red fluorescence, apoptotic cells showing a high level of violet fluorescence and no red fluorescence, and necrotic cells showing a high intensity red and violet fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope using the appropriate filters.

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
A35136	Violet Annexin V/Dead Cell Apoptosis Kit with Pacific Blue™ annexin V/SYTOX® AADvanced™ *for flow cytometry* *50 assays*	1 kit
Related Products		
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	R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	250 µL
A35116	annexin V, Pacific Blue™ conjugate *for flow cytometry* *100 assays*	500 µL
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
S34857	SYTOX® Blue dead cell stain *for flow cytometry* *1000 assays* *1 mM solution in DMSO*	1 mL
S34859	SYTOX® Red dead cell stain *for 633 or 635 nm excitation* *5 µM solution in DMSO*	1 mL
V35112	PE Annexin V/Dead Cell Apoptosis Kit with SYTOX® Green *for flow cytometry* *50 assays*	1 kit
V35113	APC Annexin V/Dead Cell Apoptosis Kit with APC annexin V and SYTOX® Green *for flow cytometry* *50 assays*	1 kit
V35114	Metabolic Activity/Annexin V/Dead Cell Apoptosis Kit with C_{12} Reaszurin, APC annexin V, and SYTOX® Green *for flow cytometry* *50 assays*	1 kit
V35116	Mitochondrial Membrane Potential/Annexin V Apoptosis Kit with MitoTracker® Red and Alexa® Fluor 488 annexin V *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

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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.

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