Annexin V Conjugates for Apoptosis Detection

Table 1. Spectral characteristics and storage information.

Material		Amount	Ex/Em (nm)*	Storage	Stability
Catalog no.	Annexin V conjugate				
A23202	Alexa Fluor® 350	500 µL	346/442	• 2–6˚C • Do not freeze • Protect from light	When stored as directed, the solutions should be stable for at least 6 months.
A35122	Pacific Blue™	500 µL	410/455		
A13199	Fluorescein	500 µL	494/518		
A13201	Alexa Fluor® 488	500 µL	495/519		
A13200	Oregon Green® 488	500 µL	496/524		
A35111	R-phycoerythrin	250 µL	496, 546, 565/578†		
A35108	Alexa Fluor® 555	500 µL	555/565		
A13202	Alexa Fluor® 568	500 µL	578/603		
A13203	Alexa Fluor® 594	500 µL	590/617		
A35110	Allophycocyanin	250 µL	650/660		
A23204	Alexa Fluor® 647	500 µL	650/665		
A35109	Alexa Fluor® 680	500 µL	679/702		
A13204	Biotin-X	500 µL	NA		

Number of reactions: A35110 and A35111 are supplied in a unit size of 250 µL, sufficient for 50 flow cytometry assays following the protocol outlined below. The remaining annexin V conjugates are supplied in a unit size of 500 µL, sufficient for 100 flow cytometry assays following the protocol outlined below.

* Approximate fluorescence excitation/emission maxima. † Multiple excitation peaks.

Introduction

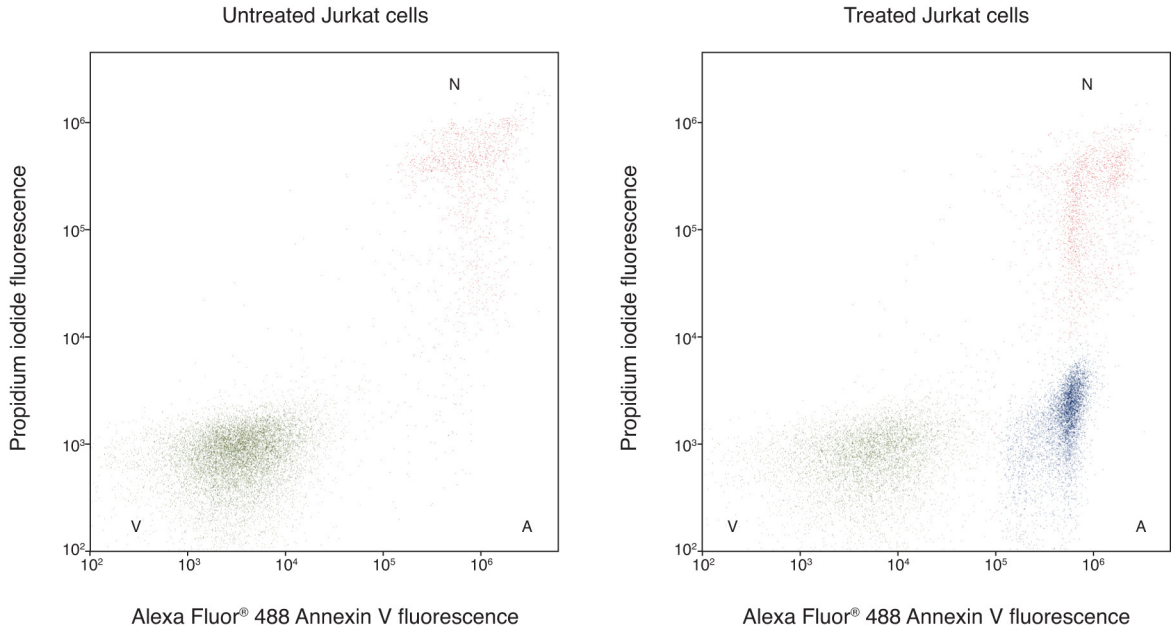
Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological 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. 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 kD 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 (Figure 1).[10]

Molecular Probes offers recombinant annexin V conjugated to some of our best and brightest fluorophores (Table 1). The Alexa Fluor® series of dyes have proven to make brighter and more photostable bioconjugates than other organic dyes with the same spectral characteristics. We also offer annexin V conjugated to fluorescein, Oregon Green® 488 dye, R-phycoerythrin (R-PE), allophycocyanin (APC), and Pacific Blue™ dye, as well as an annexin V biotin conjugate, which can be detected with fluorophore-labeled streptavidin. Molecular Probes carries streptavidin conjugated to a variety of fluorophores, including phycoerythrin, allophycocyanin, and our Alexa Fluor® dyes.

Annexin V conjugates bind to PS on apoptotic cell surfaces in the presence of Ca^{2+}, but can also pass through the compromised membranes of dead cells and bind to PS in the interior of the cell.[6] Therefore, we recommend using a cell-impermeant dead cell stain in combination with annexin V conjugate staining to distinguish dead cells from apoptotic cells.

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM of camptothecin for 4 hours (panel B) or untreated control (panel A). Cells were stained, then analyzed by flow cytometry using 488-nm excitation on the Attune® Acoustic Focusing Cytometer with 530/30 and 574/26 bandpass filters and collected by means of a standard 100 µL/minute collection rate. Note that the camptothecin-treated cells (panel B) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.



Before Starting

Fluorescence spectral characteristics

The excitation and emission maxima for the various conjugates are shown in Table 1.

Storage and handling

The fluorescent annexin V conjugates are in a solution containing 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, plus 0.1% bovine serum albumin (BSA). The biotin annexin V conjugate is in a solution of 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4. Upon receipt, store the labeled annexins at 2–6°C. The solutions should be stable for at least 6 months. DO NOT FREEZE. Protect the fluorescent conjugates from light.

Experimental Protocols

Staining cells with annexin V conjugates - flow cytometry

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

1.1 Prepare annexin-binding buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4.

1.2 Induce apoptosis in cells using the desired method. A negative control should be prepared by incubating cells in the absence of inducing agent.

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

1.4 Recentrifuge the washed cells (from step 1.3), discard the supernatants, then resuspend the cells in annexin-binding buffer. Determine the cell density and dilute in annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

1.5 Add 5 µL of the annexin V conjugate to each 100 µL of cell suspension. You may also wish to add an appropriate dead cell indicator, such as SYTOX® Blue, SYTOX® Green, or SYTOX® AADvanced™ dead cell stain.

1.6 Incubate the cells at room temperature for 15 minutes.

1.7 After the incubation period, add 400 µL of annexin-binding buffer, mix gently, then keep the samples on ice.

1.8 As soon as possible, analyze the stained cells by flow cytometry. Cells labeled with the biotin-X conjugate of annexin V will require a secondary detection agent, such as fluorophore-labeled streptavidin. The population should separate into at least two groups: live cells with only a low level of fluorescence and apoptotic cells with a substantially higher fluorescence intensity. If a dead cell stain is used, dead cells will be labeled with both the dead cell stain and with the annexin V conjugate (see Figure 1).

Tips and tricks for the Attune® Acoustic Focusing Cytometer

- This protocol is optimized for samples to be run without dilution at any collection rate.
- To analyze concentrated samples (that is, ≥1 × 10^6 cell/mL) at 200 µL/minute, 500 µL/minute, and/or 1000 µL/minute, dilute the samples in buffer that contains a cell-impermeant DNA dead cell stain, maintaining the appropriate final concentration for analysis.

Staining cells with annexin V conjugates - microscopy

The following protocol was developed using Jurkat cells treated with camptothecin to induce apoptosis and may be adapted for adherent cell lines.

2.1 Prepare annexin-binding buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4.

2.2 Induce apoptosis in cells using the desired method. A negative control should be prepared by incubating cells in the absence of inducing agent.

2.3 After the incubation period, wash the cells in cold phosphate-buffered saline (PBS).

2.4 Resuspend the cells in annexin-binding buffer. Determine the cell density and dilute the cells in annexin-binding buffer to ~1 × 10^6 cells/mL, preparing a sufficient volume for deposition on a slide.

2.5 Add 5–25 µL of the annexin V conjugate to each 100 µL of cell suspension. An appropriate dead cell indicator, such as propidium iodide or SYTOX® Green stain may be added at this point. If a dead cell stain or other fluorescent cell marker is used, we find that using the annexin V probe at the high end of the given concentration range tends to produce more satisfactory results.

2.6 Incubate the cells at room temperature for 15 minutes.

2.7 Wash the cells with annexin-binding buffer. Cells labeled with the biotin-X conjugate of annexin V will require a secondary detection agent, such as fluorophore-labeled streptavidin.

2.8 Mount the slides using the desired method, then observe the fluorescence using appropriate filters. The cells should separate into two groups: healthy cells should show only weak staining of the cellular membrane, while apoptotic cells should show a significantly higher degree of surface labeling.

References

1. Immunol Cell Biol 76, 1 (1998); **2.** Cytometry 27, 1 (1997); **3.** J Pharm 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
A23202	Annexin V, Alexa Fluor® 350 conjugate *100 assays*	500 µL
A13201	Annexin V, Alexa Fluor® 488 conjugate *100 assays*	500 µL
A35108	Annexin V, Alexa Fluor® 555 conjugate *100 assays*	500 µL
A13202	Annexin V, Alexa Fluor® 568 conjugate *100 assays*	500 µL
A13203	Annexin V, Alexa Fluor® 594 conjugate *100 assays*	500 µL
A23204	Annexin V, Alexa Fluor® 647 conjugate *100 assays*	500 µL
A35109	Annexin V, Alexa Fluor® 680 conjugate *100 assays*	500 µL
A35110	Annexin V, allophycocyanin conjugate (APC annexin V) *50 assays*	250 µL
A13204	Annexin V, biotin-X conjugate *100 assays*	500 µL
A13199	Annexin V, fluorescein conjugate (FITC annexin V) *100 assays*	500 µL
A13200	Annexin V, Oregon Green® 488 conjugate *100 assays*	500 µL
A35122	Annexin V, Pacific Blue™ conjugate *for flow cytometry* *100 assays*	500 µL
A35111	Annexin V, R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	250 µL
Related products		
S34860	SYTOX® Green dead cell stain *for flow cytometry* *30 µM* *1000 tests*	1 mL
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S10349	SYTOX® AADvanced™ dead cell stain *for 488-nm excitation* *for flow cytometry* *100 tests*	1 kit
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S34861	SYTOX® Orange dead cell stain *for flow cytometry* *250 µM* *1000 tests*	1 mL
S34862	SYTOX® dead cell stain sampler kit *for flow cytometry*	1 kit
V13246	Annexin-binding Buffer *5X concentrate* *for flow cytometry*	50 mL

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