invitrogen USER GUIDE

Annexin V Conjugates for Apoptosis Detection

Catalog Numbers A23202, A13199 , A13200, A13201, A13202, A13203, A13204, A23204, A35108, A35109, A35110, A35111, A35122

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiological conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet, marking cells as targets for phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorophore-labeled annexin V in a calcium-dependent manner.

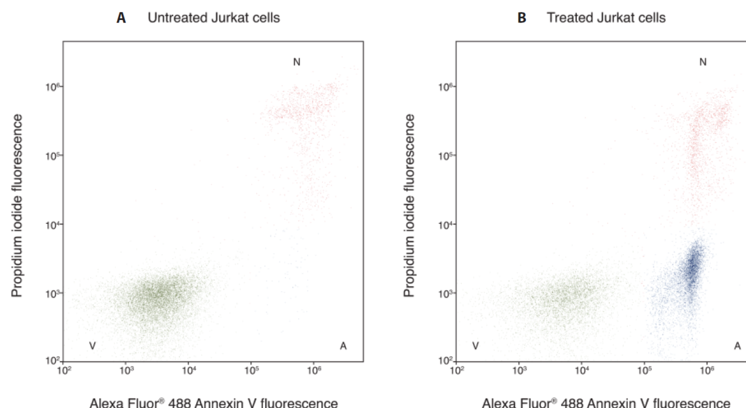

Figure 1 Jurkat cells (human T-cell lymphocytes) untreated control (panel A) or treated with 10 µM camptothecin for 4 hours (panel B).

Cells were stained, then analyzed by flow cytometry using 488-nm excitation on the Attune™ Acoustic Focusing Cytometer with 530/30-nm and 574/26-nm 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.

We offer recombinant Annexin V conjugated to a numerous Alexa Fluor™ fluorophores, for brighter and more photostable bioconjugates compared to other organic dyes with the similar 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 (available at **thermofisher.com**).

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 an Annexin V Conjugate staining to distinguish dead cells from apoptotic cells.

Contents and storage

Annexin V conjugate	Cat. No.	Amount[1]	Ex/Em (nm)[2]	Storage[3]
Alexa Fluor™ 350[4]	A23202	500 µL	346/442	2 to 6°C Do not freeze. Protect from light.
Pacific Blue™	A35122	500 µL	410/455	
Fluorescein (FITC)[4]	A13199	500 µL	494/518	
Alexa Fluor™ 488[4]	A13201	500 µL	495/519	
R-phycoerythrin (R-PE)[4]	A35111	250 µL	496, 546, 565/578[5]	
Alexa Fluor™ 555[4]	A35108	500 µL	555/565	
Alexa Fluor™ 568[4]	A13202	500 µL	578/603	
Alexa Fluor™ 594[4]	A13203	500 µL	590/617	
Allophycocyanin (APC)[4]	A35110	250 µL	650/660	
Alexa Fluor™ 647[4]	A23204	500 µL	650/665	
Alexa Fluor™ 680[4]	A35109	500 µL	679/702	
Biotin-X[6]	A13204	500 µL	NA	

[1] 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.
[2] Approximate fluorescence excitation/emission maxima.
[3] When stored as directed, the solutions are stable for at least 6 months.
[4] The fluorescent annexin V conjugates are supplied in a solution containing 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, plus 0.1% bovine serum albumin (BSA).
[5] Multiple excitation peaks.
[6] The biotin annexin V conjugate is supplied in a solution of 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4.

Stain cells with Annexin V Conjugates for microscopy

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

1. Prepare annexin-binding buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4.
2. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of an inducing agent.
3. After the incubation period, wash the cells in cold phosphate-buffered saline (PBS).
4. Resuspend the cells in annexin-binding buffer.
5. Count the cells, then adjust the cell density to $\sim 1 \times 10^6$ cells/mL with annexin-binding buffer.
6. Transfer a 100-µL aliquot of each cell suspension to a new tube, then add 5–25 µL of the Annexin V Conjugate to each tube.

   Note: An appropriate dead cell indicator, such as propidium iodide or SYTOX™ Green stain can be added at this point. If a dead cell stain or other fluorescent cell marker is used, we recommend using the annexin V probe at the high end of the given concentration range.
7. Incubate for 15 minutes at room temperature
8. Wash the cells with annexin-binding buffer.

   Note: Cells labeled with the Biotin-X conjugate of annexin V will require a secondary detection agent, such as fluorophore-labeled streptavidin.
9. Mount the slides using the desired method, then observe the fluorescence using appropriate filters.

   Note: 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.

Stain cells with Annexin V Conjugates for flow cytometry

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

1. Prepare annexin-binding buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4.
2. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of an inducing agent.
3. Harvest the cells after the incubation period, then wash in cold phosphate-buffered saline (PBS).
4. Centrifuge the washed cells (from step 3) again, discard the supernatants, then resuspend the cells in annexin-binding buffer.
5. Count the cells, then adjust the cell density to $\sim 1 \times 10^6$ cells/mL with annexin-binding buffer.
6. Transfer a 100-µL aliquot of each cell suspension to a new tube, then add 5 µL of the Annexin V Conjugate to each tube.

   Note: An appropriate dead cell indicator, such as SYTOX™ Blue, SYTOX™ Green, or SYTOX™ AADvanced™ Dead Cell Stain, can be added at this point.
7. Incubate for 15 minutes at room temperature.
8. After the incubation period, add 400 µL of annexin-binding buffer, mix gently, then place the samples on ice.
9. Immediately, 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.

   Note: 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).

Related products

For more information on other products for apoptosis research, visit **thermofisher.com/apoptosis**.

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Revision	Date	Description
A.0	3 May 2022	The content and document layout were updated. This document supercedes Rev 2.0, revision date August 2011.

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