Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit with Alexa® Fluor 488 annexin V and PI for Flow Cytometry

Catalog nos. V13241 and V13245

Table 1. Contents and storage information.

Material	Amount		Composition	Storage*	Stability
	V13241	V13245			
Alexa Fluor® 488 annexin V (Component A)	250 µL	5 × 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 1 year from the date of receipt.
Propidium iodide (PI, Component B)	100 µL	100 µL	1 mg/mL (1.5 mM) solution in deionized water		
5X annexin-binding buffer (Component C)	15 mL	50 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4		

*The Alexa Fluor® 488 annexin V and propidium iodide 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 (Cat. no. V13241) or 250 (Cat. no. V13245) flow cytometry assays based on a 100 µL assay volume.

Approximate fluorescence excitation/emission maxima: Alexa Fluor® 488 annexin V**:** 488/499 in nm; Propidium iodide: 535/617 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 live cells, phosphatidyl serine (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 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 Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit with Alexa® Fluor 488 annexin V and PI for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to one of our best and brightest fluorophores, the Alexa Fluor® 488 dye, to provide the maximum sensitivity. Alexa Fluor® 488 dye is an almost perfect spectral match to fluorescein (FITC), but it creates brighter and more

photostable conjugates.

In addition, the kit includes a ready-to-use solution of the red-fluorescent propidium iodide (PI) nucleic acid binding dye. PI is impermeant to live cells and apoptotic cells, but stains dead cells with red fluorescence, binding tightly to the nucleic acids in the cell. After staining a cell population with Alexa Fluor® 488 annexin V and PI in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show red and green fluorescence, and live cells show little or no fluorescence (Figure 1). These populations can easily be distinguished using a flow cytometer with the 488 nm line of an argon-ion laser for excitation.

We have optimized this assay 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. Because no single parameter defines apoptosis in all systems, we strongly suggest using 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.

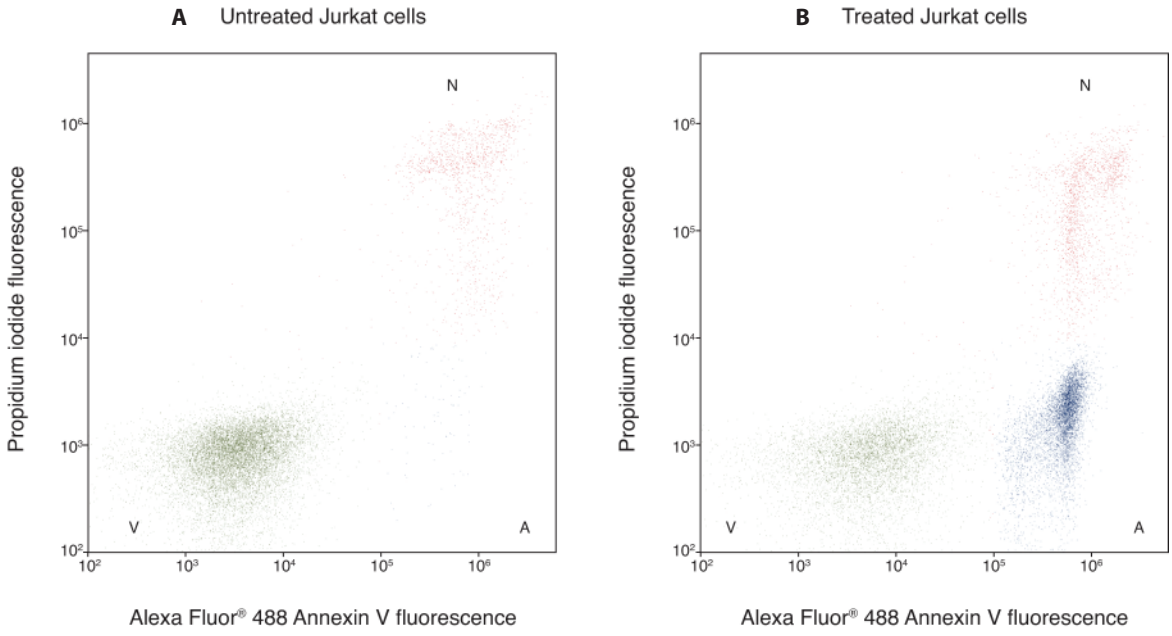

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (panel B) or untreated control (panel A). Cells were stained ,and analyzed by flow cytometry using 488 nm excitation on the Attune™ Acoustic Cytometer with 530/30 and 575/24 bandpass filters and collected by 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

Materials Required but Not Provided

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

Caution

Propidium iodide is a potential mutagen; use appropriate precautions when handling this reagent.

Experimental Protocol

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.

This assay is optimized using traditional hydrodynamic focusing flow cytometers as well as the Attune™ Acoustic Cytometer. Any collection rate (25 µL/min through 1,000 µL/min) may be used with the Attune™ Acoustic Cytometer.

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
2. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).
3. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 1 mL 5X annexin-binding buffer (Component C) to 4 mL deionized water.
4. Prepare a 100 µg/mL working solution of PI by diluting 5 µL of the 1 mg/mL PI stock solution (Component B) in 45 µL 1X annexin-binding buffer.

   Store the unused portion of this working solution for future experiments.
5. Re-centrifuge the washed cells (from step 2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

   Determine the cell density and dilute in 1X annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.
6. Add 5 µL Alexa Fluor® 488 annexin V (Component A) and 1 µL 100 µg/mL PI working solution (prepared in step 4) to each 100 µL of cell suspension.
7. Incubate the cells at room temperature for 15 minutes.
8. After the incubation period, add 400 µL 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 575 nm (or equivalent) using 488 nm excitation.

   Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein (FITC) and tetramethylrhodamine (TRITC) or Texas Red® dye.

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
V13241	Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit with Alexa® Fluor 488 annexin V and PI *for flow cytometry* *50 assays*	1 kit
V13245	Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit with Alexa® Fluor 488 annexin V and PI *for flow cytometry* *250 assays*	1 kit
Related Products		
V13240	Single Channel Annexin V/ Dead Cell Apoptosis Kit *Alexa Fluor® 488 annexin V/SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
V13246	Annexin-binding buffer *5X concentrate* *for flow cytometry*	50 mL
V35112	PE Annexin V/ Dead Cell Apoptosis Kit *with SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
V35113	APC Annexin V/Dead Cell Apoptosis Kit *with APC annexin V and SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
V35114	Metabolic Activity/Annexin V/Dead Cell Apoptosis Kit *with C12 resazurin, APC annexin V, and SYTOX® Green* *50 assays* *for flow cytometry*	1 kit
V35116	Mitochondrial Membrane Potential/Annexin V Apoptosis Kit *Alexa Fluor® 488 annexin V/MitoTracker® Red CMXRos* *50 assays* *for flow cytometry*	1 kit
V35136	Violet Annexin V/Dead Cell Apoptosis Kit *Pacific Blue™ annexin V/SYTOX® AADvanced™* *for flow cytometry* *50 assays*	1 kit
V23200	Vybrant® Apoptosis Assay Kit #6 *biotin-X annexin V/Alexa Fluor® 350 streptavidin/propidium iodide* *50 assays*	1 kit
A13201	annexin V, Alexa Fluor® 488 conjugate *100 assays*	100 assays
A23204	annexin V, Alexa Fluor® 647 conjugate *100 assays*	100 assays
A35110	annexin V, allophycocyanin conjugate (APC annexin V) *50 assays*	50 assays
A35111	annexin V, R-phycoerythrin conjugate (R-PE annexin V) *50 assays*	50 assays
A35122	annexin V, Pacific Blue™ conjugate *for flow cytometry* *100 assays*	100 assays
A35137	Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit *for flow cytometry* *100 assays*	1 kit

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