Annexin-binding Buffer for Flow Cytometry

Catalog no. V13246

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	50 mL	5X	• 2–6˚C • DO NOT FREEZE	When stored as directed this product is stable for 1 year from the date of receipt.
Number of assays: Sufficient material is supplied for 500 assays based on the protocol below.				

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] The binding of annexin V to PS requires the presence of Ca^{++}. Under defined salt and calcium conditions, annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.[10] Refer to our website at www.invitrogen.com for a wide selection of products for apoptosis research.

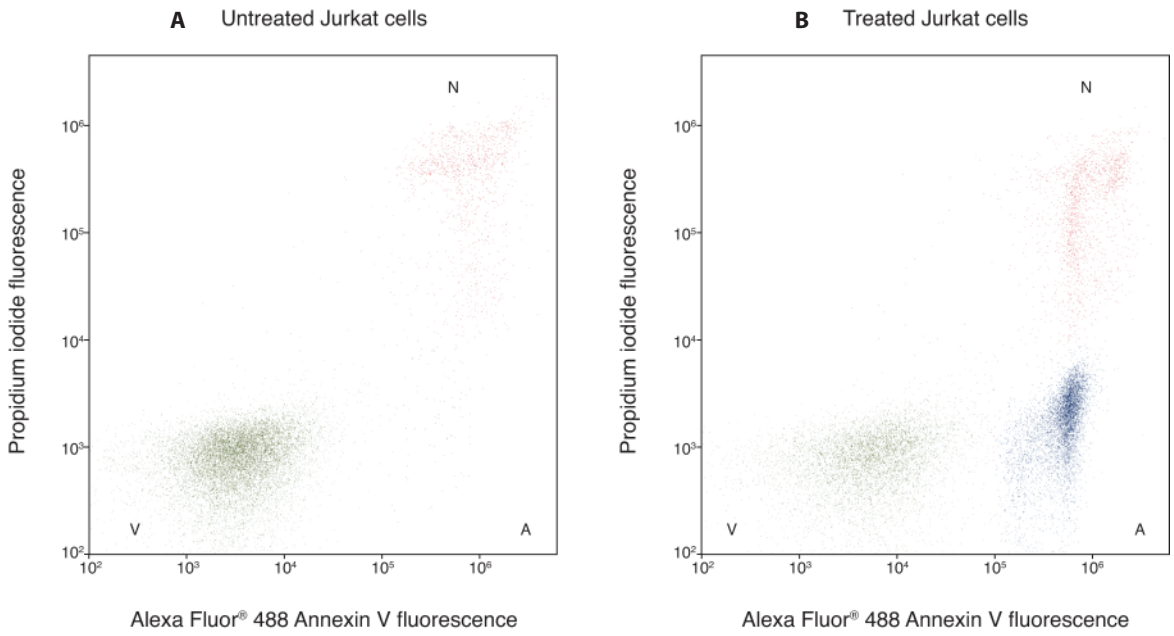

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (panel B) or untreated control (panel A). Alexa Fluor® 488 annexin V and Propidium Iodide dead cell stain were used with the annexin-binding buffer in this example. Cells were stained and analyzed by flow cytometry using 488 nm excitation on the Attune™ Acoustic Cytometer, 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 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

Deionized water

Preparing 1X Buffer

Dilute 5X Annexin-binding Buffer 1:4 with deionized water before use. Store the diluted buffer at 2–8˚C.

The final 1X concentration of Annexin-binding buffer is 100 mM HEPES, 140 mM NaCl, 25 mM $CaCl_2$, pH 7.4.

Experimental Protocol

This assay is optimized 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 using 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 to 4 mL deionized water.

4. Re-centrifuge the washed cells (from step 2), discard the supernatant, and resuspend the cells in 1X annexin-binding buffer.

5. 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 appropriate amount of annexin V conjugate and dead cell dye 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.

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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