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Annexin V Conjugates for Apoptosis Detection

Table 1. Spectral Characteristics and Storage Information.

Catalog No.	Product Name	Unit Size	Ex/Em (nm)	Storage	Stability
A030	EGFP Annexin V	25 µg	475/509	• 2-6 °C, • Protect from light	The product is stable for 1 year when stored as directed.
A031	Biotin-X Annexin V	25 µg	NA		
A032	Andy Fluor™ 350 Annexin V	25 µg	350/450		
A033	FITC 488 Annexin V	25 µg	494/518		
A034	Andy Fluor™ 488 Annexin V	25 µg	495/520		
A035	Andy Fluor™ 555 Annexin V	25 µg	555/565		
A036	Andy Fluor™ 568 Annexin V	25 µg	575/602		
A037	Andy Fluor™ 594 Annexin V	25 µg	590/618		
A038	Andy Fluor™ 647 Annexin V	25 µg	650/665		
A039	Andy Fluor™ 680 Annexin V	25 µg	680/700		
A040	RPE Annexin V	25 µg	496, 546, 565/578		

Number of assays: 100 flow cytometry assays.

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. 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. The human anticoagulant, annexin V, is a 35–36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS. Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.

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. Therefore, we recommend using a cell-impermeant dead cell stain in combination with annexin V conjugate to distinguish dead cells from apoptotic cells.

Experimental Protocols

Apoptosis Analysis by Flow Cytometry

Note: The assay has been optimized using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types or other inducing agents.

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

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

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

1.4 Dissolve annexin V conjugate in 0.5 mL deionized water.

1.5 Re-centrifuge the washed cells (from step 1.2), discard the supernatant and 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.

1.6 Add 5 µL annexin V conjugate (from step 1.4) to each 100 µL of cell suspension. You may also wish to add an appropriate dead cell indicator, such as PI.

1.7 Incubate the cells at room temperature for 15 minutes in the dark.

1.8 After the incubation period, add 400 µL annexin-binding buffer, mix gently, and keep the samples on ice.

1.9 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 substantially higher fluorescence intensity.

Apoptosis Detection by Microscopy

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

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

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

2.4 Dissolve annexin V conjugate in 0.5 mL deionized water.

2.5 Re-centrifuge the washed cells (from step 2.2), discard the supernatant and 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.

2.6 Add 5-25 µL annexin V conjugate (from step 2.4) to each 100 µL of cell suspension. You may also wish to add an appropriate dead cell indicator, such as PI.

Note: *Higher concentrations of the solutions tend to produce better results for microscopy. The optimal concentration may need to be determined empirically.*

2.7 Incubate the cells at room temperature for 15-30 minutes in the dark.

2.8 Wash cells with 1X annexin-binding buffer. Cells labeled with the biotin-X conjugate of annexin V will require a secondary detection agent, such as fluorophore-labeled streptavidin.

2.9 Mount cells onto slides and observe 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.