Fluorescent Annexin V Conjugates for Phosphatidylserine-Based Apoptosis Detection

Introduction

Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. Annexin V's preferential binding partner is phosphatidylserine (PS), which is usually kept on the inner-leaflet (the cytosolic side) of cell membranes. In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed. Our Annexin V conjugates are a set of tools for monitoring cellular functions, which are designed to monitor cell apoptosis through measuring the translocation of phosphatidylserine.

Annexin V conjugates bind to PS on apoptotic cell surfaces in the presence of Ca^{2+}, it also can pass through cell membranes of necrosis or dead cells and bind to PS in the interior of cells. Therefore, we recommend using a cell-impermeable nuclear stain in combination with annexin V conjugate to distinguish dead cells from apoptotic cells.

Spectral Properties

The excitation and emission maxima of the Annexin V conjugates are summarized in the following table.

Cat. #	Product Name	Amount	Ex/Em(nm)
20018	Annexin V-Biotin conjugate	100 tests (200 µL)	N/A
20030	Annexin V-FITC conjugate	100 tests (200 µL)	494/520
20031	Annexin V-TRITC conjugate	100 tests (200 µL)	541/568
20065	Annexin V-Cy3 conjugate	100 tests (200 µL)	556/574
20066	Annexin V-Cy5 conjugate	100 tests (200 µL)	651/660
20067	Annexin V-Cy5.5 conjugate	100 tests (200 µL)	681/705
20068	Annexin V-Cy7 conjugate	100 tests (200 µL)	750/778
20069	Annexin V-iFluor 633™ conjugate	100 tests (200 µL)	638/655
20070	Annexin V-iFluor 350™ conjugate	100 tests (200 µL)	347/443
20071	Annexin V-iFluor 488™ conjugate	100 tests (200 µL)	494/520
20072	Annexin V-iFluor 555™ conjugate	100 tests (200 µL)	552/567
20073	Annexin V-iFluor 594™ conjugate	100 tests (200 µL)	590/615
20074	Annexin V-iFluor 647™ conjugate	100 tests (200 µL)	651/660
20075	Annexin V-iFluor 680™ conjugate	100 tests (200 µL)	681/705
20076	Annexin V-iFluor 750™ conjugate	100 tests (200 µL)	750/778
20077	Annexin V-iFluor 700™ conjugate	100 tests (200 µL)	690/716
20080	Annexin V-mFluor Violet 450™ conjugate	100 tests (200 µL)	403/454
20081	Annexin V-mFluor Violet 510™ conjugate	100 tests (200 µL)	414/510
20082	Annexin V-mFluor Violet 540™ conjugate	100 tests (200 µL)	424/560
20085	Annexin V-mFluor™ Blue 570 conjugate	100 tests (200 µL)	553/570
20089	Annexin V-PacBlue conjugate	100 tests (200 µL)	410/455
20090	Annexin V-AF350 conjugate	100 tests (200 µL)	346/445
20092	Annexin V-AF488 conjugate	100 tests (200 µL)	494/517
20096	Annexin V-AF594 conjugate	100 tests (200 µL)	590/617

Storage and Handling Conditions

The fluorescent annexin V conjugates are in PBS buffer with 0.1% bovine serum albumin (BSA), pH = 7.4. The solutions should be stable for at least 6 months if store at -20 °C. Protect the fluorescent conjugates from light, and avoid freeze/thaw cycles.

Assay Protocol

Brief Summary

Prepare cells with test compounds (200 µL/sample) → Add Annexin V conjugate assay solution → Incubate at room temperature for 30-60 minutes → Analyze with a flow cytometer or a fluorescence microscope

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1. Prepare and incubate cells with Annexin V conjugates:

1.1 Prepare Annexin V-binding assay buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4.
1.2 Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
1.3 Centrifuge the cells to get 1-5×10^5 cells/tube.
1.4 Resuspend cells in 200 µL of Annexin V-binding assay buffer (from Step 1.1).
1.5 Add 2 µL of Annexin V conjugate into the cells.
Optional: Add a dead cell stain such as Propidium Iodide into the cells for necrosis cells.
1.6 Incubate at room temperature for 30 to 60 minutes, protected from light.
1.7 Add 300 µL of Annexin V-binding assay buffer (from Step 1.1) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope (see Step 1.8 below).
1.8 Monitor the fluorescence intensity by using a flow cytometer or a fluorescence microscope (See Step 2 or 3 below).

2. Analyze by using a flow cytometer:

Quantify Annexin V conjugates binding by using a flow cytometer with appropriated filters.
Note: Annexin V binding flow cytometric analysis on adherent cells is not routinely tested since specific membrane damage may occur during cell detachment or harvesting. However, methods for utilizing Annexin V for flow cytometry on adherent cell types have been previously reported by Casiola-Rosen et al. and van Engelend et al (see Refs 1 and 2).

3. Analyze by using a fluorescence microscope:

3.1 Pipette the cell suspension from Step 1.6, rinse 1-2 times with Annexin V-binding assay buffer (from Step 1.1), and then resuspend the cells with the Annexin V-binding assay buffer (from Step 1.1). Add the cells on a glass slide that is covered with a glass cover slip.
Note: For adherent cells, it is recommended to grow the cells directly on a cover slip. After incubation with Annexin V conjugate (Step 1.6), rinse 1-2 times with Annexin V-binding assay buffer (from Step 1.1), and add Annexin V-binding assay buffer (from Step 1.1) back to the cover slip. Invert cover slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after the incubation with Annexin V conjugate and visualized under a microscope.
3.2 Analyze the apoptotic cells with Annexin V conjugate under a fluorescence microscope with appropriated filters.

Data Analysis

The following figure is an example of detection of binding activity of Annexin V-mFluor Violet™ 450 with phosphatidylserine in Jurkat cell apoptosis using a flow cytometer. In live non-apoptotic cells, Annexin V-mFluor Violet™ 450 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Annexin V-mFluor Violet™ 450 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

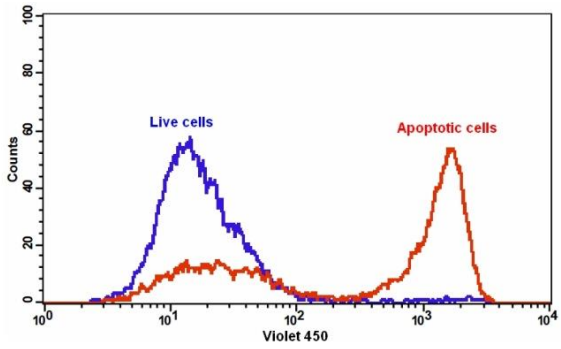

Figure 1. The detection of binding activity of Annexin V-mFluor Violet™ 450 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO_2 incubator for 5 hours, and then dye loaded with Annexin V-mFluor Violet™ 450 for 30 minutes. The fluorescence intensity of Annexin V-mFluor Violet™ 450 was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using violet laser at Ex/Em = 405/450 nm.

References:

1. Brad Larson, Peter Banks. Experimental Biology (2017)
2. Pascal Clerc, Pauline Jeanjean, Nicolas Halalli, Michel Gougeon, Bernard Pipy, Julian Carrey, Daniel Fourmy, Véronique Gigoux. Journal of Controlled Release (2017)
3. Hanshaw RG, Lakshmi C, Lambert TN, Johnson JR, Smith BD. Chembiochem, 6, 2214. (2005).