User Manual

Annexin V-FITC Apoptosis Detection Kit II

Fluorometric assay kit for measuring Annexin V in apoptotic cells

CBA059

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Product Overview

The Calbiochem® Annexin V-FITC Apoptosis Detection Kit II is a non-isotopic system that can be used to detect phosphatidylserine on the outer leaflet of the cell membrane of apoptotic cells using flow cytometry or fluorescence microscopy.

Apoptosis is a fundamental mode of cell death that serves as an important regulatory function during normal development, in tissue homeostasis, and in some disease processes. In normal viable cells phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. Upon induction of apoptosis, rapid alterations in the organization of phospholipids in most cell types occurs leading to exposure of PS on the cell surface. Recognition of PS by phagocytes *in vivo* results in the removal of cells programmed to die, thus apoptosis is not commonly associated with the local inflammatory response that accompanies necrosis. *In vitro* detection of externalized PS can be achieved through interaction with the anticoagulant annexin V. In the presence of calcium, rapid high affinity binding of annexin V to PS occurs. PS translocation to the cell surface precedes nuclear breakdown, DNA fragmentation, and the appearance of most apoptosis-associated molecules making annexin V binding a marker of early-stage apoptosis.

Materials Provided

There are sufficient reagents to label 20 samples with annexin V-biotin.

- Annexin V-FITC (Kit Component No. JA9201-100UL): 1 vial, 100 µL, recombinant, human annexin V, (expressed in *E. coli*, MW 35,800, > 98% pure by SDS-PAGE and HPLC) conjugated to FITC and supplied in 100 mM NaCl, 50 mM Tris-HCl, 1% BSA, 0.02% NaN_3, pH 7.4
- Binding Buffer (Kit Component No. JA9202-50ML): 1 vial, 50 mL, supplied as a 4X stock solution
- Propidium Iodide (Kit Component No. JA9203-EA): 1 vial, supplied at 20 µg/mL

Materials Required (Not supplied)

- 1X PBS
- Flow cytometer or fluorescence microscope

Storage and Stability

Upon arrival store the entire contents of the kit at 4 °C.

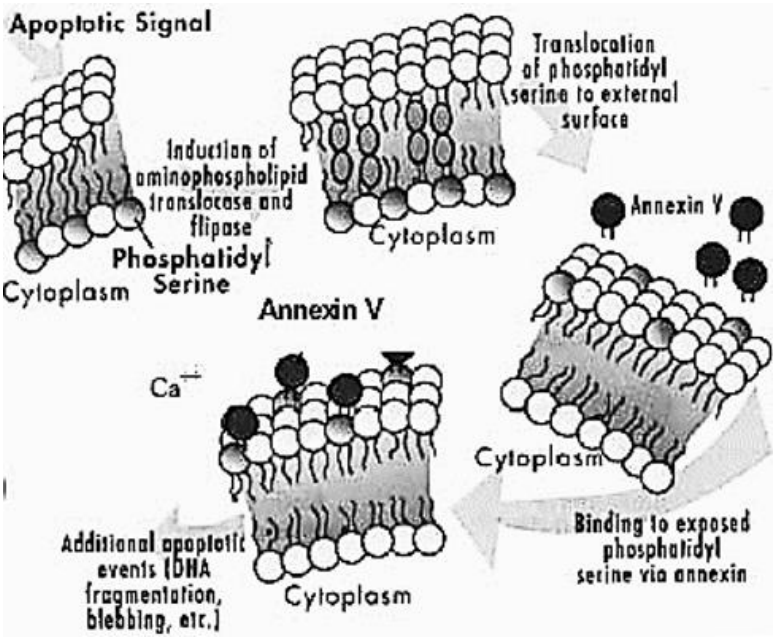
Protocol

1X Binding Buffer: Dilute the Binding Buffer 1:4 with dH_2O (e.g., 50 mL Binding Buffer + 150 mL dH_2O). This will yield a working concentration of 140 mM NaCl, 10 mM HEPES/NaOH, 2.5 mM $CaCl_2$.

1. Wash cells with 1X PBS and centrifuge at 1000 rpm to pellet the cells. Discard the supernatant and repeat for a total of 2-3 washes.
2. Resuspend the cell pellet in 1X Binding Buffer to a final concentration of 2-5 x 10^5 cells/mL.
3. Transfer 195 µL cell suspension to a clean tube and add 5 µL Annexin V-FITC. Mix and incubate for 10 min at room temperature.
4. Wash the cells with 1X Binding Buffer and centrifuge at 1000 rpm to pellet the cells. Discard the supernatant.
5. Resuspend the cell pellet in 190 µL 1X Binding Buffer and add 10 µL Propidium Iodide (final concentration = 1 µg/mL).
6. Analyze by flow cytometry or fluorescence microscopy.

Data Analysis

The Calbiochem® Annexin V-FITC Apoptosis Detection Kit II utilizes annexin V conjugated to FITC to bind to phosphatidylserine on the outer surface of cells undergoing apoptosis. Fluorescence is then detected by flow cytometry or fluorescence microscopy. Since membrane permeabilization is also observed in necrosis, necrotic cells will also bind Annexin V-FITC. Propidium iodide is included to help distinguish between viable, early apoptotic, and necrotic or late apoptotic cells. Necrotic cells will bind Annexin V-FITC and stain with propidium iodide while propidium iodide will be excluded from viable (FITC negative) and early apoptotic (FITC positive) cells. In the absence of phagocytosis final stages of apoptosis involve necrotic-like disintegration of the total cell, thus cells in late apoptosis will be labeled with both FITC and propidium iodide.



(*©1997 CN Biosciences Inc., all rights reserved*)

Figure 1. Annexin/Phosphatidyl Serine in Early Stages Apoptosis

References

1. Darzynkiewicz, Z., et al. 1997. *Cytometry* 27, 1.
2. Frey, T. 1997. *Cytometry* 28, 253.
3. Boersma, A.W.M., et al. 1996. *Cytometry* 24, 123.
4. White, E. 1996. *Genes and Devel.* 10, 1.
5. Martin, S.J., et al. 1995. *J. Exp. Med.* 182, 1545.
6. Koopman, G., et al. 1994. *Blood* 84, 1415.
7. Wyllie, A.H.,1993. *Br. J. Cancer* 67, 205.
8. Darzynkiewicz, Z., et al. 1992. *Cytometry* 13, 795.
9. Fadok, V.A., et al. 1992. *J. Immunology* 148, 2207.
10. Schmid, I., et al. 1992. *Cytometry* 13, 204.
11. Kerr, J.F.R., et al. 1972. *Cancer* 26, 239.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20769660 Ver 4.0

23236581 Ver 1.0, Rev 03JUL2025, UD