BD Pharmingen™ Technical Data Sheet

ANNEXIN V-FITC APOPTOSIS DETECTION KIT I

PRODUCT INFORMATION

Catalog Number: **556547** (Was: 6693KK)

Components:

51-65874X	Annexin V-FITC
Contents:	100 tests; buffered in 50 mM Tris (pH 8.0) with 80 mM NaCl, 0.2% BSA, 1 mM EDTA, and 0.09% sodium azide.
51-66211E	Propidium Iodide Staining Solution
Contents:	2.0 ml in PBS (pH 7.4)
51-66121E	Annexin V Binding Buffer, 10X Concentrate
Contents:	50 ml solution

BACKGROUND

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenence of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca^{2+} dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS (*reviewed in 1*). Annexin V may be conjugated to fluorochromes such as Propidium Iodide (PI). This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis.[2-5]

Since externalization of PS occurs in the earlier stages of apoptosis, Annexin V-FITC staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. Annexin V-FITC staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with Annexin V-FITC is typically used in conjunction with a vital dye such as Propidium Iodide (PI, Cat. No. 51-66211E) to allow the investigator to identify early apoptotic cells (Annexin V-FITC positive, PI negative).[2-5] For example, cells that are viable are Annexin V-FITC and PI negative; cells that are in early apoptosis are Annexin V-FITC positive and PI negative; and cells that are in late apoptosis or already dead are are both Annexin V-FITC and PI positive.[2-5] This assay does not distinguish, per se, between cells that have already undergone apoptotic death and those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both Annexin-FITC and PI. However, when apoptosis is measured over time, cells can be often tracked from Annexin V-FITC and PI negative (viable, or no measurable apoptosis), to Annexin V-FITC positive and PI negative (early apoptosis, membrane integrity is present) and finally to Annexin V-FITC and PI positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both Annexin V-FITC and PI positive, in of itself, reveals less information about the process by which the cells underwent their demise.

SPECIFICITY AND PREPARATION

Annexin V-FITC is a sensitive probe for identifying apoptotic cells.[2-5] It binds to negatively charged phospholipid surfaces (K_d of ~5 x 10^{-2})[6] with a higher specificity for phosphatidylserine (PS) than most other phospholipids. Defined calcium and salt concentrations are required for Annexin V-FITC binding as described in the Annexin V-FITC Staining Protocol. Purified recombinant Annexin V was conjugated to FITC under optimum conditions. Annexin V-FITC is routinely tested using primary cells or cell lines induced to undergo an apoptotic death.

USAGE AND STORAGE

Applications include flow cytometry (5 µl / test). See the Annexin V-FITC Staining Protocol for usage information. Store Annexin V-FITC at 4˚C.

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ANNEXIN V-FITC STAINING PROTOCOL

Annexin V-FITC is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment.

Annexin V is a Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for Annexin V-FITC and negative for PI are undergoing apoptosis. Cells that stain positive for both Annexin V-FITC and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both Annexin V-FITC and PI are alive and not undergoing measurable apoptosis.

Reagents

1. **Annexin V-FITC** (Cat. No. 51-65874X). Use 5 µl per test. Store at 4˚C.
2. **Propidium Iodide** (Cat. No. 51-66211E)**.** Use 5 µl per test. PI (Propidium Iodide) is a convenient, ready-to-use solution of the nucleic acid dye that can be used for the exclusion of nonviable cells in flow cytometric assays. PI fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter).[1,2] Store at 4˚C.
3. **10X Annexin V Binding Buffer.** (Cat. No. 51-66121E). 0.1 M Hepes / NaOH (pH 7.4) 1.4 M NaCl, 25 mM $CaCl_2$.[3] The solution was 0.2 µm sterile filtered. For a working solution (1X), dilute 1 part binding buffer to 9 parts distilled H_20. This will yield a working solution of 10 mM Hepes / NaOH (pH 7.4) 140 mM NaCl, 2.5 mM $CaCl_2$. Store the 10X concentrate and working solution at 2–8°C.

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1X binding buffer at a concentration of 1 x 10^6 cells / ml.
2. Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5 µl of Annexin V-FITC and 5 µl of PI.
4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5. Add 400 µl of 1X binding buffer to each tube. Analyze by flow cytometry within one hour.

NOTE: *Methods for utilizing Annexin V binding on adherent cells (i.e., monolayer) have been described by van Engeland et al[5] and Casciola-Rosen et al.[4] However, these methods are not performed as a routine quality control for the Annexin V-FITC Apoptosis Detection Kit I and Kit II.*

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SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

The following controls are used to set up compensation and quadrants:

1. Unstained cells.
2. Cells stained with Annexin V-FITC alone (no PI).
3. Cells stained with PI alone (no Annexin V-FITC).

Other Staining Controls

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with Annexin V-FITC and with both Annexin V-FITC and PI. It is important to note that the basal level of apoptosis and necrosis varies considerably within a population. Thus, even in the absence of induced apoptosis, most cell populations will contain at least a minor percentage of cells that are positive for apoptosis (Annexin V-FITC positive, PI negative or Annexin V-FITC and PI positive).

The untreated population is used to define the basal level of apoptotic and dead cells. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for PI as well as for Annexin V-FITC. Thus the assay does not distinguish between cells that have already undergone an apoptotic cell death and those that have died as a result of necrotic pathway, because in either case the dead cells will stain with both Annexin V-FITC and PI.

INDUCTION OF APOPTOSIS BY CAMPTOTHECIN

The following protocol is routinely used in house to test the Annexin V-FITC.

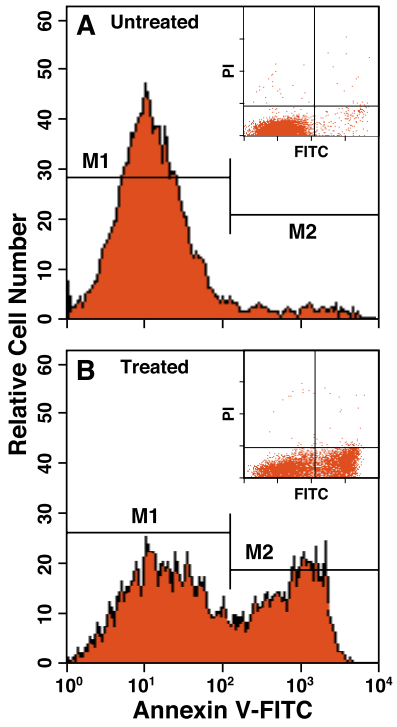
Materials

1. Prepare camptothecin stock solution (SIGMA Cat. No. C-9911): 1 mM in DMSO.
2. Jurkat T cells (ATCC TIB-152).

Procedure

1. Add camptothecin (final conc. 4-6 µM) to 1 x 10^6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the Annexin V-FITC Staining Protocol to measure apoptosis.

ANNEXIN V ASSAY ON JURKAT T CELLS



Annexin V-FITC: A tool for identifying cells that are undergoing apoptosis. Jurkat T cells were left untreated (A) or treated for 4 hr with 12 µM camptothecin (B). Cells were incubated with Annexin V-FITC in a buffer containing Propidium Iodide (PI, Cat. No. 51-66211E) and analyzed by flow cytometry. Untreated cells were primarily Annexin V-FITC and PI negative, indicating that they were viable and not undergoing apoptosis. After a 4 hr treatment (B), there were primarily two populations of cells: Cells that were viable and not undergoing apoptosis (Annexin V-FITC and PI negative) and cells undergoing apoptosis (Annexin V-FITC positive and PI negative). A minor population of cells were observed to be Annexin V-FITC and PI positive, indicating that they were in end stage apoptosis or already dead.

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