Technical Data Sheet

PE Annexin V Apoptosis Detection Kit I

Product Information

Material Number:	**559763**
Component:	**51-66121E**
Description:	10X Annexin V Binding Buffer
Size:	50 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing no preservative.
Component:	**51-68981E**
Description:	7-AAD
Size:	2.0 ml (1 ea)
Vol. per Test:	5 µl
Storage Buffer:	Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.
Component:	**51-65875X**
Description:	PE Annexin V
Size:	0.5 ml (1 ea)
Vol. per Test:	5 µl
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

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 Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including Phycoerythrin (PE). 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. Since externalization of PS occurs in the earlier stages of apoptosis, PE Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

PE Annexin V 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 PE Annexin V is typically used in conjunction with a vital dye such as 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (7-AAD negative, PE Annexin V positive). Viable cells with intact membranes exclude 7-AAD, wheras the membranes of dead and damaged cells are permeable to 7-AAD. For example, cells that are considered viable are PE Annexin V and 7-AAD negative; cells that are in early apoptosis are PE Annexin V positive and 7-AAD negative; and cells that are in late apoptosis or already dead are are both PE Annexin V and 7-AAD positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both PE Annexin V and 7-AAD. However, when apoptosis is measured over time, cells can be often tracked from PE Annexin V and 7-AAD negative (viable, or no measurable apoptosis), to PE Annexin V positive and 7-AAD negative (early apoptosis, membrane integrity is present) and finally to PE Annexin V and 7-AAD 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 PE Annexin V and 7-AAD positive, in of itself, reveals less information about the process by which the cells underwent their demise.

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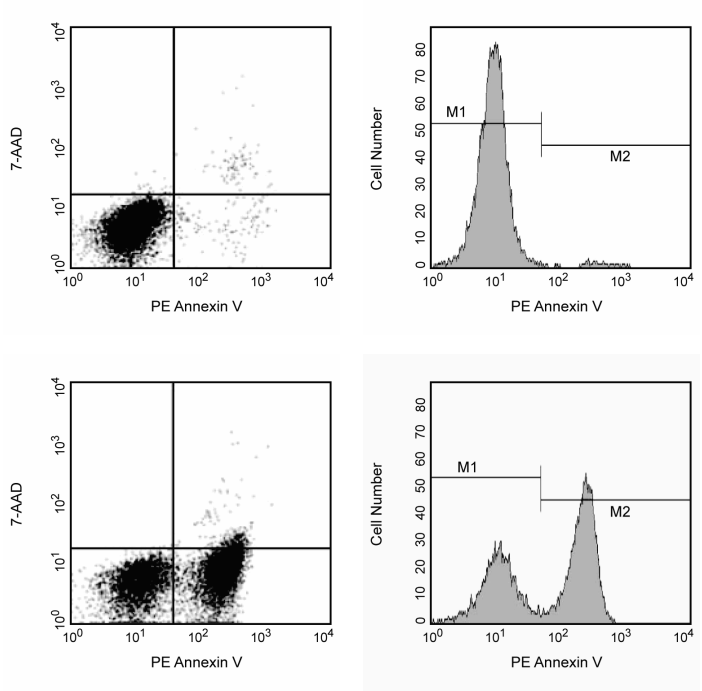

Flow Cytometric Analysis of PE Annexin V staining. *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were left untreated (top panels) or treated for 4 hours with 4 µM Camptothecin (bottom panels). Cells were incubated with PE Annexin V in a buffer containing 7-Amino-Actinomycin (7-AAD) and analyzed by flow cytometry. Untreated cells were primarily PE Annexin V and 7-AAD negative, indicating that they were viable and not undergoing apoptosis. After a 4 hour treatment (bottom panels), there were primarily two populations of cells: Cells that were viable and not undergoing apoptosis (PE Annexin V and 7-AAD negative); cells undergoing apoptosis (PE Annexin V positive and 7-AAD negative). A minor population of cells were observed to be PE Annexin V and 7-AAD positive, indicating that they were in end stage apoptosis or already dead.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

PE Annexin V is a sensitive probe for identifying apoptotic cells, binding to negatively charged phospholipid surfaces (Kd of ~5 x 10^-2) with a higher affinity for phosphatidylserine (PS) than most other phospholipids. PE Annexin V binding is calcium dependent and defined calcium and salt concentrations are required for optimal staining as described in the PE Annexin V Staining Protocol. **Investigators should note that PE Annexin V flow cytometric analysis on adherent cell types (e.g HeLa, NIH 3T3, etc.) is not routinely tested as specific membrane damage may occur during cell detachment or harvesting. Methods for utilizing Annexin V for flow cytometry on adherent cell types, however, have been previously reported (Casiola-Rosen et al. and van Engelend et al.).**

INDUCTION OF APOPTOSIS BY CAMPTOTHECIN

The following protocol is provided as an illustration on how PE Annexin V may be used on a cell line (Jurkat).

Materials

1. Prepare Camptothecin stock solution (Sigma-Aldrich 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 PE Annexin V Staining Protocol to measure apoptosis.

PE ANNEXIN V STAINING PROTOCOL

PE Annexin V 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 calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. 7-Amino-Actinomycin (7-AAD) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude 7-AAD, whereas the membranes of dead and damaged cells are permeable to 7-AAD. Cells that stain positive for PE Annexin V and negative for 7-AAD are undergoing apoptosis. Cells that stain positive for both PE Annexin V and 7-AAD are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both PE Annexin V and 7-AAD are alive and not undergoing measurable apoptosis.

Reagents

1. PE Annexin V (component no. 51-65875X): Use 5 µl per test.
2. 7-Amino-Actinomycin (7-AAD) (component no. 51-68981E) is a convenient, ready-to-use nucleic acid dye. Use 5 µl per test.
3. 10X Annexin V Binding Buffer (component no. 51-66121E): 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM CaCl2. For a 1X working solution, dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.

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 PE Annexin V and 5 µl 7-AAD.
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 1 hr.

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 PE Annexin V (no 7-AAD).
3. Cells stained with 7-AAD (no PE Annexin V).

Other Staining Controls:

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with PE Annexin V and/or PE Annexin V and 7-AAD. 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 a minor percentage of cells that are positive for apoptosis (PE Annexin V positive, 7-AAD negative or PE Annexin V positive, 7-AAD 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 7-AAD as well as for PE Annexin V. 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 PE Annexin V and 7-AAD.

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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