PE Annexin V Apoptosis Detection Kit

User Manual

KT40001

For research use only. Not intended for diagnostic testing.

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PE Annexin−V Apoptosis Detection Kit

Product Information
Material Number
Component:
Description: PE Annexin V
Storage Buffer: PBS pH 7.2, ≤0.09% sodium azide

Component:
Description: 7-AAD
Storage Buffer: PBS pH 7.2, ≤0.09% sodium azide

Component:
Description: 10X Annexin V Binding Buffer
Storage Buffer: PBS pH 7.2, ≤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 Ca_2+ 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 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 propidium iodide (PI) or 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 Annexin-PE 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.

Preparation and Storage

Keep as concentrated solution. Store at 4°C and protected from prolonged exposure to light.

Do not freeze.

Application

Notes

Application

Flow cytometry Routinely Tested

Other Applications (For the relevant formats): Immunohistochemistry

Induction of apotosis by camptothecin

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

Reagents

1. PE Annexin V : Use 5 µl per test.
2. 7-AAD is a convenient, ready-to-use nucleic acid dye. Use 5 µl per test.
3. 10X Annexin V Binding Buffer: dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.

Suggested Staining Protocol

1. Dilute 3 mL 10× binding buffer with 27 mL distilled water for 10 tests.
2. Harvest cell（about 1 ×106 cells per test） then wash with cold PBS.
3. Suspend cells in 1 mL 1× Binding Buffer, 300×g centrifugation for 10 minutes, then remove the Binding Buffer from the cell pellet.
4. Resuspend cells in 1 mL 1× Binding Buffer , adjust cell concentration to 1×106 cells/mL.
5. Add 100 µL of cells (1×105 cells) to each labeled tube.
6. Add 5 µL of Annexin V-PE to appropriate tubes.
7. Gently vortex each tube and incubate for 10 minutes in room temperature, protected from light.
8. Add 5 µL 7-AAD solution incubation for 5min in room temperature, protected from light.
9. Add PBS to 500µL and vortex gently.
10. Analyze by flow cytometry in 1 hour.

Suggest control for setting up flow cytomtry

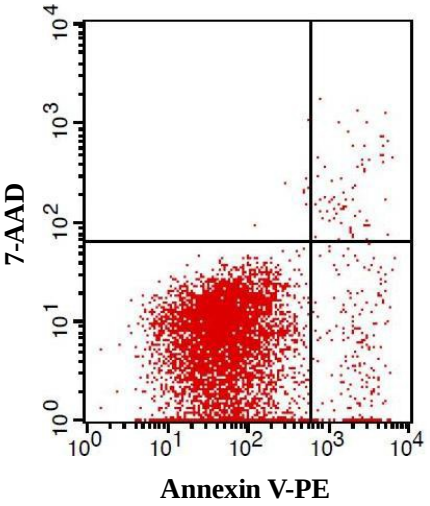
The following controls are used to set up compensation quadrants:

1. Unstained cells.
2. Cells stained with PE Annexin V (no 7-AAD).
3. Cells stained with 7-AAD (no PE Annexin V).

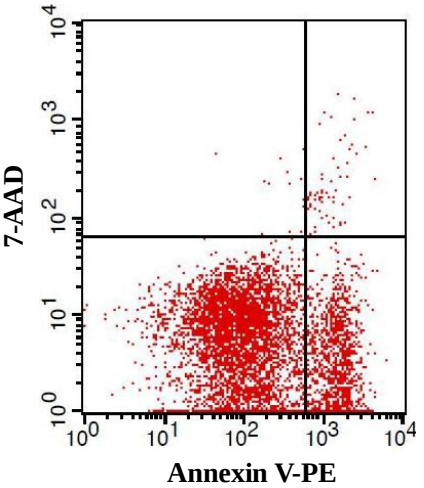
Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.

Illustration of Immunofluorescent Staining



Jurkat Cell stained with Annexin V-PE and 7-AAD



Camptothecin treated Jurkat Cell stained with Annexin V-PE and 7-AAD

Flow Cytometric Analysis of PE −Annexin V staining. Jurkat cells were left untreated or treated for campotothecin . 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 treatment, there were primarily two populations of cells: Cells that were viable and not undergoing apoptosis (PE−Annexin V and 7-AAD negative) and 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.

References

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