Annexin V (APC) / PI Apoptosis Detection Kit

Catalog No.: abx090605

Size: 100 tests

Storage: Store all components at 2-8°C. Store the Annexin V-APC Reagent and PI Reagent in the dark.

Application: To detect the apoptosis of suspension and adherent cells.

Introduction

Annexin V is used as a probe to detect cells that have expressed phosphatidylserine (PS) on the cell surface, an event found in apoptosis as well as other forms of cell death. The Annexin V affinity assay typically uses a conjugate of annexin V and a fluorescent or enzymatic label, biotin or other tags, or a radioelement, in a suitable buffer (Annexin V binding to PS is Ca2+ dependent). The assay combines Annexin V staining of PS membrane events with the staining of the cell nucleus with PI to distinguish living cells from dead cells. Annexin V apoptosis detection is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner (cytoplasmic-facing) leaflet of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

Kit components

1. Annexin V-APC Reagent: 500 µl
2. Annexin V Binding Buffer (10X): 11 ml
3. PI Reagent (50 µg/ml): 500 µl

Materials Required But Not Provided

1. 96-well microplate, PCR plate, or equivalent
2. Fluorescence Microplate reader
3. Centrifuge
4. deionized water
5. PBS (0.01 M, pH 7.4)
6. Pipette and pipette tips
7. Vials/tubes

Protocol

A. Preparation of Reagents

- **(1X) Annexin V Binding Buffer:** Dilute (10X) Annexin V Binding Buffer 10-fold with deionized water to prepare (1X) Annexin V Binding Buffer. Prepare immediately before use.

B. Detection Procedure

One-Step Method

1. Induce apoptosis in the suspension/ adherent cell sample using conventional methods.
2. If using adherent cells, use an appropriate detachment method to turn into a single-cell suspension.
3. Collect the cell cultures and centrifuge at 300 × g for 5 minutes. Discard the supernatant.
4. Wash the cells in PBS (0.01 M, pH 7.4), then resuspend gently and count the cells.
5. Split the cell suspension into tubes with approximately 1 – 5 × 10^5 cells in each tube. Centrifuge at 300 × g for 5 minutes. Discard the supernatant.
6. Wash the cells again with PBS (0.01 M, pH 7.4) then discard the supernatant. Add 500 µl of (1X) Annexin V Binding Buffer to resuspend the cells.
7. Add 5 µl of Annexin V-APC Reagent and 5 µl of PI Reagent to each tube. Gently vortex the tubes then incubate at room temperature in the dark for 15 – 20 minutes.
8. Analyze the cells immediately or store on ice in the dark and analyze within 1 hour.

Two-Step Method

1. Induce apoptosis in the suspension/adherent cell sample using conventional methods.
2. If using adherent cells, use an appropriate detachment method to turn into a single-cell suspension.
3. Collect the cell cultures and centrifuge at 300 × g for 5 minutes. Discard the supernatant.
4. Wash the cells in PBS (0.01 M, pH 7.4), then resuspend gently and count the cells.
5. Split the cell suspension into tubes with approximately 1 – 5 × 10^5 cells in each tube. Centrifuge at 300 × g for 5 minutes. Discard the supernatant.
6. Wash the cells again with PBS (0.01 M, pH 7.4) then discard the supernatant. Add 100 µl of (1X) Annexin V Binding Buffer to resuspend the cells.
7. Add 2.5 µl of Annexin V-APC Reagent and 2.5 µl of PI Reagent to each tube. Gently vortex the tubes then incubate at room temperature in the dark for 15 – 20 minutes.
8. Add 400 µl of (1X) Annexin V Binding Buffer to each tube and gently mix.
9. Analyze the cells immediately or store on ice in the dark and analyze within 1 hour.

Note:

- The One-Step Method has fewer steps whereas the Two-Step Method requires less volume of Annexin V-APC and PI reagents.
- Annexin V-APC can be detected in the APC channel.
- It is recommended that the ECD channel should be used to detect PI rather than the PE channel. The PerCP/Cy5.5 channel can be used if autofluorescence is observed with the FITC channel.
- Adherent cells that have undergone apoptosis may lose their adhesion properties and become suspended. It is important to collect these along with the remaining adherent cells for a complete detection of apoptosis.
- Avoid mechanical damage of adherent cells when detaching.
- If using trypsin to detach adherent cells, if possible the solution should not contain EDTA as this will affect the binding of Annexin V to phosphatidylserine.
- If the trypsin solution contains EDTA, wash cells thoroughly after harvesting to remove the EDTA.
- Analyze cells as soon as possible. Avoid exposure to light.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.