

Instructions for Use

Version: 1.0.1
Revision date: 7-Oct-25

Annexin V (APC/Cyanine 7) / 7-AAD Apoptosis Detection Kit

Catalog No.: abx092349

Size: 100 / 200 tests

Storage: Store all components at 2-8°C 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 (fluorescent, enzymatic, biotin or radioelement label), 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 7-AAD 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 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 as the APC/Cyanine 7 conjugated Annexin V bound to the outer cell membrane can be analyzed by flow cytometry or by fluorescence microscopy.

Kit components (100 tests)

1. Annexin V-APC/Cyanine 7
Reagent: 500 µl
2. Annexin V Binding Buffer (10X): 11 ml
3. 7-AAD Reagent (100 µg/ml): 500 µl

Kit components (200 tests)

1. Annexin V-APC/Cyanine 7
Reagent: 1 ml
2. Annexin V Binding Buffer (10X): 2 × 11 ml
3. 7-AAD Reagent (100 µg/ml): 1 ml

Materials Required But Not Provided

1. Flow cytometer or fluorescence microscope
2. Centrifuge and centrifuge tubes
3. Deionized water
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vortex mixer

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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 as per the experimental procedure. *If using adherent cells, use an appropriate detachment method to turn into a single-cell suspension.*
2. Collect the cell cultures and centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant.
3. Wash the cells in PBS (0.01 M, pH 7.4), then resuspend gently and count the cells.
4. Split the cell suspension into tubes with approximately $1 \sim 10 \times 10^5$ cells in each tube. Centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant.
5. Wash the cells again with PBS (0.01 M, pH 7.4) then discard the supernatant. Add 500 μ l of (1X) Annexin V Binding Buffer to resuspend the cells.
6. Add 5 μ l of Annexin V-APC/Cyanine 7 Reagent and 5 μ l of 7-AAD Reagent to each tube. Gently vortex the tubes then incubate at room temperature in the dark for 15 – 20 minutes.
7. Analyze the cells immediately using the correct channels or store on ice in the dark for up to 1 hour. Cells must be analyzed within 1 hour.

Two-Step Method

1. Induce apoptosis in the suspension/adherent cell sample as per the experimental procedure. *If using adherent cells, use an appropriate detachment method to turn into a single-cell suspension.*
2. Collect the cell cultures and centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant.
3. Wash the cells in PBS (0.01 M, pH 7.4), then resuspend gently and count the cells.
4. Split the cell suspension into tubes with approximately $1 \sim 10 \times 10^5$ cells in each tube. Centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant.
5. Wash the cells again with PBS (0.01 M, pH 7.4) then discard the supernatant. Add 100 μ l of (1X) Annexin V Binding Buffer to resuspend the cells.
6. Add 2.5 μ l of Annexin V-APC/Cyanine 7 Reagent and 2.5 μ l of 7-AAD Reagent to each tube. Gently vortex the tubes then incubate at room temperature in the dark for 15 – 20 minutes.
7. Add 400 μ l of (1X) Annexin V Binding Buffer to each tube and gently mix.
8. Analyze the cells immediately using the correct channels or store on ice in the dark for up to 1 hour. Cells must be analyzed within 1 hour.

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Dye	Channel
7-AAD	PerCP/Cy5.5
Annexin V-APC/Cyanine 7	APC/Cy7

Note:

- The One-Step Method has fewer steps whereas the Two-Step Method requires less volume of Annexin V-APC/Cyanine 7 and 7-AAD reagents.
- 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.