Annexin V-APC Apoptosis Detection Kit
(Annexin V-APC / PI)

Catalog# BAD1003

Size: 100T

Lot # Check on the product label

Introduction

Annexin A5 (or Annexin V) is a cellular protein in the annexin group. Annexin A5 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 A5 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 cell nucleus with PI or AAD-7 in living cells to distinguish from dead cells.

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner face 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

Components	100 tests	Storage Instruction
Annexin V-APC	500 µl	Store at 4°C in dark. Do not freeze.
Propidium iodide	500 µl	
Positive Control	5 ml	
Binding buffer (10×)	30 ml	Store at 4°C. For long term storage, aliquot and store at -20°C.

Staining Protocol

- **Positive Control**

1. Harvest fresh cells (about 1~3 x 10^6), then equally divide cells into two tubes. Wash cells with 4 ml of cold PBS, then centrifuge at 300 x g for 10 min to remove the

supernatant.

2. Suspend one tube of cells in 200 µl of 1 x Binding buffer and store at 4°C for use. (T1)
3. Suspend the rest cells in 500 µl of Positive Control provided by the kit, incubate at room temperature for 10 min. Wash cells with 3 ml of cold PBS, centrifuge at 300 x g for 10 min to remove the supernatant. Repeat this wash and centrifuge to remove the supernatant again. Then, suspend the precipitate / cells in 200 µl of 1x Binding buffer. (T2)
4. Mix the cells of T1 & T2 together, and label THREE new tubes with #A1, #A2 & #A3, then add 100 µl cells into each tube.
5. Take #A1 as the Blank Control, then add 5 µl of Annexin V-APC into #A2, and 5 µl of Propidium iodide into #A3.
6. Add 300~500 µl of PBS into #A1, #A2 & #A3, vortex gently and incubate at room temperature for 5 min in dark.
7. Before Sample detection, adjust the corresponding parameters of the machine (Voltage & Fluorescent compensation) according to the #A1, #A2 & #A3.

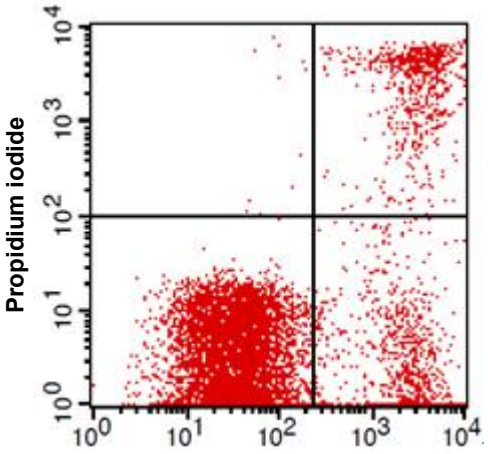
(Note: The Positive Control of the kit is for parameters adjustment use only, can NOT be used to process the cells for flow cytometry assay.)

- **Sample detection**

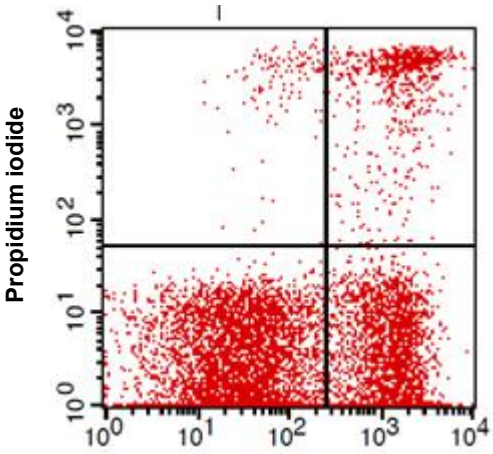
1. Dilute 3 ml of the Binding buffer (10x) to work solution (1x) with distilled water for 10 tests.
2. Harvest cell (about 1 x 10^5 cells per test), then wash once with 4 ml of cold PBS, centrifuge at 300 x g for 10 min to remove the supernatant.
3. Suspend cells in 100 µl of 1 x Binding buffer, centrifuge at 300 x g for 10 min, remove the Binding buffer from the cell pellet.
4. Resuspend cells in cold 1 x Binding buffer to a concentration of 1×10^6 cells/mL.
5. Add 100µL of cells (1×10^5) to each appropriate tube.
6. Add 5µL of Annexin V-APC to appropriate tubes.

7. Gently vortex each tube and incubate for 10 min at room temperature in dark.
8. Add 5μL of PI solution and incubate for 5 min at room temperature in dark.
9. Wash cells once in PBS and resuspend in PBS.
10. Analyze by flow cytometry within 1 hour.

Images of Immunofluorescent Staining



Annexin V-APC
Jurkat cell stainded with Annexin V-APC + PI



Annexin V-APC
Camptothecin treated Jurkat cell stained with Annexin V-APC + PI

Reference

1. Koopman G, Reutelingsperger CP, Kuijten GAM et al. (1994). "Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis". Blood 84 (5): 1415–20.
2. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C (1995). "A novel assay for apoptosis—flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V". J Immunol Methods 184 (1): 39–51.