Store at 4°C

Annexin V-APC Apoptosis Analysis Kit

Catalog Number	Vial Size
AO2001-11A-G	25 tests
AO2001-11A-H	100 tests

天津三箭生物技术股份有限公司
Tianjin Sungene Biotech Co., Ltd.
精准 高效 稳定 Precision Efficient Stable

Market | 400-621-0003
marketing@sungenebiotech.com

Support | 022-66211636-8024
techsupport@sungenebiotech.com

Web | www.sungenebiotech.com

Important Note: Each Buffer should be diluted by using the same pH PBS as mentioned below.
This product is guaranteed up to one year from purchase.

Description

Annexin V (or Annexin A5) is a member of the annexin family of intracellular proteins that binds to phosphatidylserine (PS) in a calcium-dependent manner. PS is normally only found on the intracellular leaflet of the plasma membrane in healthy cells, but during early apoptosis, membrane asymmetry is lost and PS translocates to the external leaflet. Fluorochrome-labeled Annexin V can then be used to specifically target and identify apoptotic cells.

Annexin V Binding Buffer is recommended for use with Annexin V staining.

Products List

AO2001-11	Annexin V-APC	Keep as concentrated solution. Store at 4°C and protected from prolonged exposure to light. Do not freeze.
AO2003	7-AAD Viability Staining solution	Keep as concentrated solution. Store at 4°C and protected from prolonged exposure to light. Do not freeze.
AB2000	Annexin V Binding Buffer	Keep as concentrated solution. Store at 4°C as an undiluted liquid. For extended storage aliquot contents and freeze at -20°C
AO2004	Apoptosis Positive Control Solution	Keep as concentrated solution. Store at 4°C and protected from prolonged exposure to light. Do not freeze.

Suggested Staining Protocol

A.Parameters regulation

1. Harvest cell(1×10^6 -3×10^6 cells), then sepatate the cells in two parts. Wash cells with cold PBS, then centrifuge the cells and disgard the supernatant.
2. Suspend one part of cells in 200μL 1× binding buffer, store at 4℃ for use.
3. Suspend the other part of cells in 500μL Apoptosis Positive Control Solution, and incubate for 10 minutes in room temperature. Wash cells with more than 3.0 mL cold PBS, blot the supernatant, then suspend the cells in 200μL 1× binding buffer.
4. Mix the two parts cells together, then separate the cells in three tubes, and add 100 μL of cells in each tube.
5. The first tube is Blank Control, the second one adds 5 μL of Annexin V-APC, and the third one adds 5 μL 7-AAD solution.
6. Gently vortex each tube and incubate for 5 minutes in room temperature, protected from light.
7. Before analyzing by flow cytometry, using the blank control and single dye sample to regulate voltage and compensation, as shown in Figure Parameters regulation.

B.Sample detection

1. Dilute 3 mL 10× binding buffer with 27 mL distilled water for 10 tests.
2. Harvest cell，about 1×10^6 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 1×10^6 cells/mL.
5. Add 100 μL of cells (1×10^5 cells) to each labeled tube.
6. Add 5 μL of Annexin V-APC 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.

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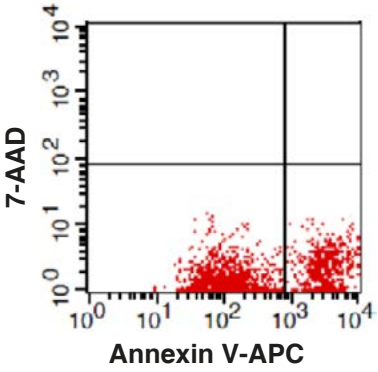
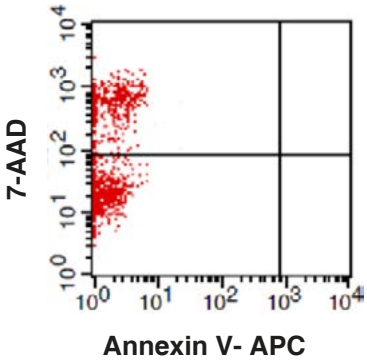
Illustration of Immunofluorescent Staining

Parameters regulation

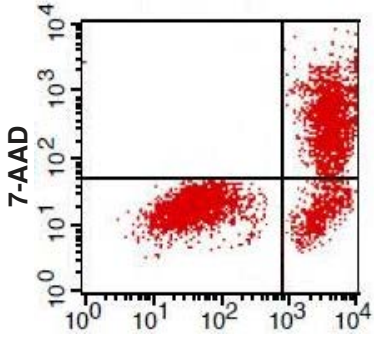


Annexin V- APC Single Staining Control



7-AAD Single Staining Control

Sample detection



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

References

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