Cell Meter™ APC-Annexin V Binding Apoptosis Assay Kit *Optimized for Flow Cytometry*

Catalog number: 22837
Unit size: 100 Tests

Component	Storage	Amount
Component A: APC-Annexin V conjugate	Refrigerate (2-8 °C), Minimize light exposure	1 vial
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °C)	1 bottle (50 mL)
Component C: 100X Propidium Iodide	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Annexin V may be conjugated to fluorochromes including APC. This format retains its high affinity for phosphatidylserine (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, APC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. APC 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 APC 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.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add APC-Annexin V assay solution
3. Incubate at room temperature for 20 - 60 minutes
4. Analyze cells using flow cytometer with 660/20 nm filter (APC channel)

Important Thaw 100X Propidium Iodide (Component C) at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC channel

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. APC-Annexin V stock solution (100X):
Add 200 µL PBS with 0.2% BSA into the vial of APC-Annexin V conjugate (Component A) and mix well to make 100X APC-Annexin V stock solution.

Note Store the reconstituted 100X APC-Annexin V stock solution at 4 °C. Do Not Freeze.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

   Note Annexin V flow cytometric analysis on adherent cells is not routinely tested since specific membrane damage may occur during cell detachment or harvesting. However, methods for utilizing Annexin V for flow cytometry on adherent cell types have been previously reported by Casiola-Rosen et al. and van Engelend et al.

2. Centrifuge the cells to get 1-5 × 10^5 cells/tube.

3. Resuspend cells in 200 µL of Assay Buffer (Component B).

4. Add 2 µL of 100X APC-Annexin V stock solution into the cells.

5. **Optional:** Add 2 µL of 100X Propidium Iodide (Component C) into the cells for necrosis cells.

6. Incubate at room temperature for 20 to 60 minutes, protected from light.

7. **Optional:** Add 200 to 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer.

8. Monitor the fluorescence intensity of APC-Annexin V using a flow cytometer with 660/20 nm filter (APC channel). Measure the cell viability using 610/20 nm filter (PE-Texas Red channel) when propidium iodide is added into the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, APC-Annexin V detects innate apoptosis in non-induced cells, which is typically 2- 6% of all cells. In apoptotic cells, APC-Annexin V binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, therefore resulted in increased staining intensity.

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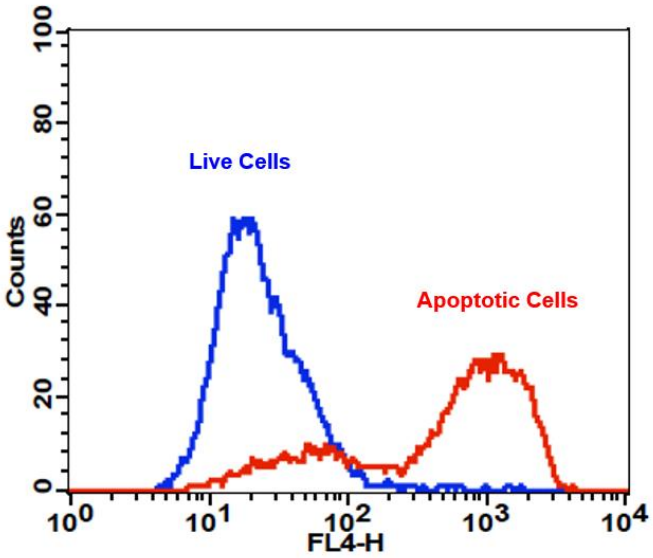

Figure 1. The detection of binding activity of APC-Annexin V to phosphatidylserine in Jurkat cells with Cell Meter™ APC-Annexin V Binding Apoptosis Assay Kit. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO2 incubator for ~4 hours, and then dye loaded with APC-Annexin V for 30 minutes. The fluorescence intensity of APC-Annexin V was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL4 channel.