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

Catalog number: 22838
Unit size: 100 Tests

Component	Storage	Amount
Component A: RPE-Annexin V	Refrigerate (2-8 °C), Minimize light exposure	1 vial
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °C)	50 mL
Component C: 100X Nuclear Red™ DCS	Refrigerate (2-8 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Annexin V may be conjugated to fluorochromes including PE. 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, PE Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation. PE 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 PE 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 (7-AAD negative, PE Annexin V positive). Viable cells with intact membranes exclude 7-AAD, whereas the membranes of dead and damaged cells are permeable to 7-AAD. For example, cells that are considered viable are both PE Annexin V and 7-AAD negative while cells that are in early apoptosis are PE Annexin V positive and 7-AAD negative, while cells that are in late apoptosis or already dead are both PE Annexin V and 7-AAD positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both PE Annexin V and 7-AAD. However, when apoptosis is measured over time, cells can be often tracked from PE Annexin V and 7-AAD negative (viable, or no measurable apoptosis), to PE Annexin V positive and 7-AAD negative (early apoptosis, membrane integrity is present) and finally to PE Annexin V and 7-AAD positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both PE Annexin V and 7-AAD positive, in of itself, reveals less information about the process by which the cells underwent their demise.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add RPE-Annexin V assay solution
3. Incubate at room temperature for 20 - 60 minutes
4. Analyze cells with a flow cytometer using 575/26 nm filter (PE channel)

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm or 561 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE 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. RPE-Annexin V stock solution (100X):
Add 200 µL PBS with 0.2% BSA into the vial of RPE-Annexin V (Component A) to make 100X RPE-Annexin V stock solution.

Note Store the reconstituted 100X RPE-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 RPE-Annexin V stock solution into the cells.
5. **Optional:** Add 2 µL of 100X Nuclear Red™ DCS (Component C) 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 RPE-Annexin V using a flow cytometer with 575/26 nm filter (PE channel). Measure the cell viability using 660/20 nm filter (APC channel) when Nuclear Red™ DCS is added into the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, RPE-Annexin V detects innate apoptosis in non-induced cells, which is typically 2- 6% of all cells. In apoptotic cells, RPE-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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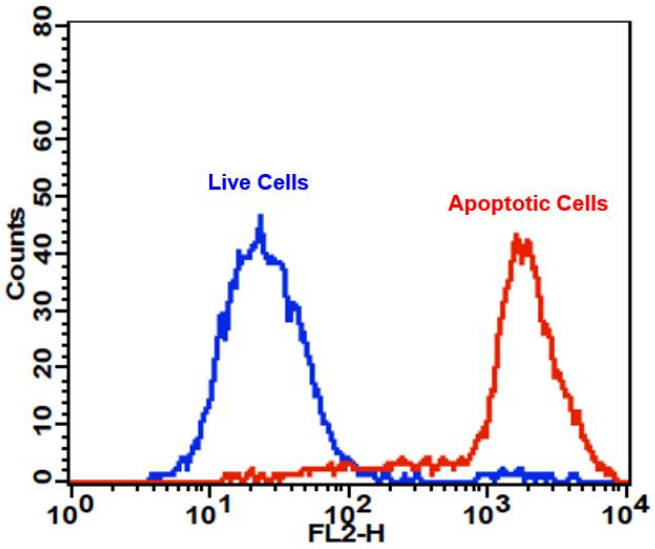

Figure 1. The detection of binding activity of RPE-Annexin V to phosphatidylserine in Jurkat cells with Cell Meter™ RPE-Annexin V Binding Apoptosis Assay Kit. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO2 incubator for 4-5 hours, and then dye loaded with RPE-Annexin V for 30 minutes. The fluorescence intensity of RPE-Annexin V was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL2 channel.

DISCLAIMER