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

Catalog number: 22839
Unit size: 100 Tests

Component	Storage	Amount
Component A: Annexin V- FITC (100X stock solution)	Refrigerate (2-8 °C), Minimize light exposure	1 vial (200 µL/vial)
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °C)	50 mL
Component C: 100X Propidium Iodide	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Our Cell Meter™ FITC-Annexin V binding assay kit is designed to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed. This kit uses the green fluorescent FITC-Annexin V conjugate that specifically binds PS. The FITC-Annexin V conjugate has been demonstrated to selectively bind PS. This particular assay kit is optimized to monitor cell apoptosis using a flow cytometer with the FITC channel (green fluorescence).

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Annexin V-FITC assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells with a flow cytometer using FL1 channel (Ex/Em = 490/525 nm)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

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 2 - 5 × 10^5 cells/tube.

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

4. Add 2 µL of Annexin V-FITC (Component A) into the cells.

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

6. Incubate at room temperature for 30 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 Annexin V-FITC using a flow cytometer with FL1 channel (Ex/Em = 490/525 nm). Measure the cell viability using FL2 channel when propidium iodide is added into the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

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

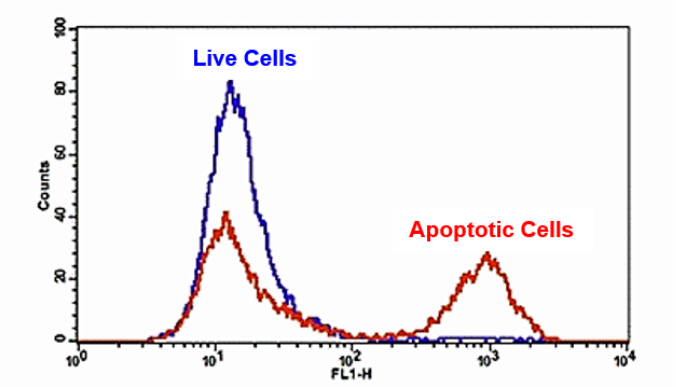

Figure 1.

The detection of binding activity of FITC-Annexin V to phosphatidylserine in Jurkat cells using Cell Meter™ FITC-Annexin V Binding Apoptosis Assay Kit. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37ºC, 5% CO2 incubator for 4-5 hours, and then dye loaded for 30 minutes. The fluorescence intensity of FITC-Annexin V was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using the FL1 channel.

DISCLAIMER