Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*

Catalog number: 22824
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22824)
Component A: Annexin V-iFluor® 488 (100X stock solution)	Freeze (< -15 °C), Minimize light exposure	1 vial (200 µL/vial)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component C: 100X Propidium Iodide	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. This particular 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 a fluorescent Annexin V that specifically binds PS. Annexin V conjugates have 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-iFluor® 488 stock solution.
3. Incubate at room temperature for 30 - 60 minutes.
4. Analyze cells using a flow cytometer with a 530/30 nm filter (FITC channel).

Important Note

Before starting the experiment, thaw the vial of 100X Propidium Iodide (Component C) at room temperature.

KEY PARAMETERS

Flow cytometer

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

CELL PREPARATION

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 camptothecin) 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 Annexin V-iFluor® 488 (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-iFluor® 488 using a flow cytometer with a 530/30 nm filter (FITC channel). Measure the cell viability using the 610/20 nm filter (PE-Texas Red channel) after adding propidium iodide to the cells.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, Annexin V-iFluor® 488 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Annexin V-iFluor® 488 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulting in increased staining intensity.

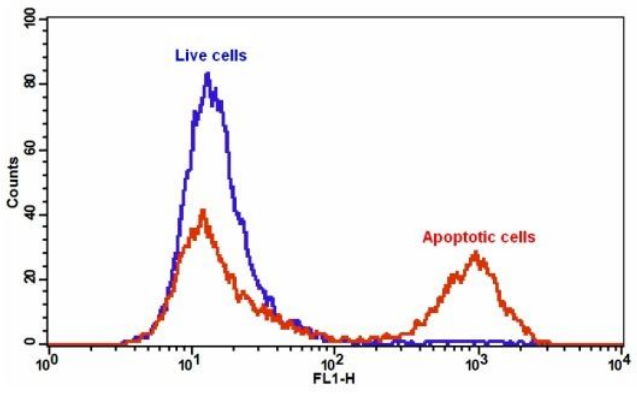

Figure 1. The detection of binding activity of Annexin V-iFluor® 488 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37 °C, 5% CO2 incubator for 4-5 hours, and then dye loaded with Annexin V-iFluor® 488 for 30

minutes. The fluorescence intensity of Annexin V-iFluor® 488 was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL1 channel.

DISCLAIMER

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