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

Catalog number: 22827
Unit size: 100 Tests

Component	Storage	Amount
Component A: Annexin V-iFluor™ 647 (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)	1 bottle (50 mL)

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 Cy5 filter set.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Annexin V-iFluor™ 647 assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells using flow cytometer with 660/20 nm filter (APC channel) or fluorescence microscope with Cy5 filter set

KEY PARAMETERS

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

Instrument:	Fluorescence microscope
Excitation:	Cy5 filter set
Emission:	Cy5 filter set
Recommended plate:	Black wall/clear bottom

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

Prepare and incubate cells with Annexin V-iFluor™ 647:

1. Treat cells with test compounds for a desired period of time (4 - 6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
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™ 647 (Component A) into the cells.
5. Incubate at room temperature for 30 to 60 minutes, protected from light.
6. Add 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope.
7. Monitor the fluorescence intensity using a flow cytometer with 660/20 nm filter (APC channel) or a fluorescence microscope with Cy5 filter set.

Analyze by using a flow cytometer:

1. Quantify Annexin V-iFluor™ 647 binding using a flow cytometer with 660/20 nm filter (APC channel).

   Note Annexin V binding 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.

Analyze by using a fluorescence microscope:

1. Pipette the cell suspension after incubation, rinse 1 - 2 times with Assay Buffer, and then resuspend the cells with assay buffer.
2. Add the cells on a glass slide that is covered with a glass cover-slip.

   Note For adherent cells, it is recommended to grow the cells directly on a cover-slip. After incubation with Annexin V-iFluor™ 647, rinse 1 - 2 times with assay buffer, and add assay buffer back to the cover-slip. Invert cover-slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after the incubation with Annexin V-iFluor™ 647 and visualized under a microscope.
3. Analyze the apoptotic cells with Annexin V-iFluor™ 647 under a fluorescence microscope using the Cy5 filter set. Measure the cell viability by using the TRITC channel when propidium iodide is added into the cells. The orange staining on the plasma membrane indicates the Annexin V-iFluor™ 647 binding to PS on cell surface.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, Annexin V-iFluor™ 647 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Annexin V-iFluor™ 647 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

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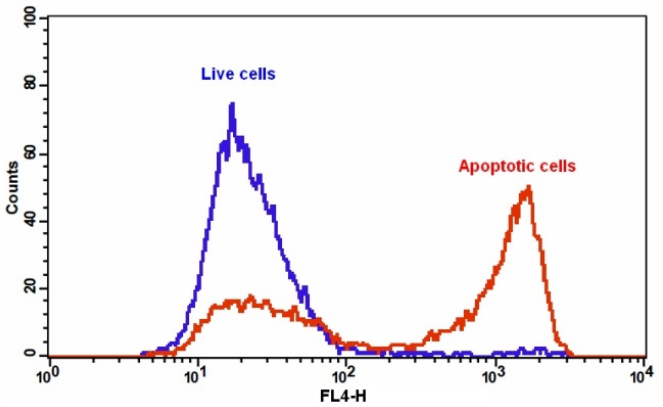

Figure 1. The detection of binding activity of Annexin V-iFluor™ 647 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO2 incubator for 4-5 hours, and then dye loaded with Annexin V-iFluor™ 647 for 30 minutes. The fluorescence intensity of Annexin V-iFluor™ 647 was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL4 channel.

DISCLAIMER

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