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

Catalog number: 22825
Unit size: 100 Tests

Component	Storage	Amount
Component A: Annexin V-iFluor™ 555 (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 575/26 nm emission filter set (PE channel).

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Annexin V-iFluor™ 555 assay solution
3. Incubate at room temperature for 30 - 60 mintues
4. Analyze cells using flow cytometer with 575/26 nm filter (PE channel) or fluorescence microscope with Cy3 filter set

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm or 532 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE channel

Instrument:	Fluorescence microscope
Excitation:	Cy3 filter set
Emission:	Cy3 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™ 555:

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™ 555 (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 575/26 nm filter (PE channel) or a fluorescence microscope with Cy3 filter set.

Analyze by using a flow cytometer:

1. Quantify Annexin V- iFluor™ 555 binding using a flow cytometer with 575/26 nm filter (PE 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™ 555, 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™ 555 and visualized under a microscope.

3. Analyze the apoptotic cells with Annexin V-iFluor™ 555 under a fluorescence microscope using Cy3 filter set. Measure the cell viability by using the Cy5 channel when Nuclear Red™ DCS1 (AAT catalog #17552) is added into the cells. The orange staining on the plasma membrane indicates the Annexin V-iFluor™ 555 binding to PS on cell surface.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, Annexin V-iFluor™ 555 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Annexin V-iFluor™ 555 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

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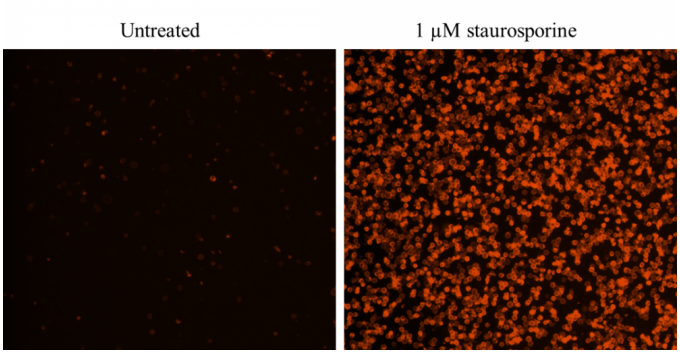

Figure 1. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with Cell Meter Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence*. (Left): Untreated control cells. (Right): Cells treated with 1 μM staurosporine for 5 hours.

DISCLAIMER