Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*

Catalog number: 22828
Unit size: 100 Tests

Component	Storage	Amount
Component A: Annexin V-mFluor Violet™ 450 (100X stock solution)	Refrigerate (2-8 °C), Minimize light exposure	1 vial (200 µL)
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °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 Pacific Blue filter set.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Annexin V-mFluor Violet™ 450 assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells using flow cytometer with 450/40 nm filter (Pacific Blue channel) or fluorescence microscope with DAPI filter set

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm laser
Emission:	450/40 nm filter
Instrument specification(s):	Pacific Blue channel
Instrument:	Fluorescence microscope
Excitation:	DAPI filter set
Emission:	DAPI 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-mFluor Violet™ 450:

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-mFluor Violet™ 450 (Component A) into the cells.

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

6. Incubate at room temperature for 30 to 60 minutes, protected from light.

7. Add 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope.

8. Monitor the fluorescence intensity using a flow cytometer with Pacific Blue channel or a fluorescence microscope with DAPI filter set.

Analyze by using a flow cytometer:

1. Quantify Annexin V-mFluor Violet™ 450 binding using a flow cytometer with Pacific Blue channel. Measure the cell viability by using 610/20 nm filter (PE-Texas Red channel) when propidium iodide is added into the cells.

   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 V-mFluor Violet™ 450, 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 V-mFluor Violet™ 450 and visualized under a microscope.

3. Analyze the apoptotic cells with V-mFluor Violet™ 450 under a fluorescence microscope using DAPI filter set. Measure the cell viability by using the TRITC channel when propidium iodide is added into the cells. The blue staining on the plasma membrane indicates the V-mFluor Violet™ 450 binding to PS on cell surface.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, Annexin V-mFluor Violet™ 450 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Annexin V-mFluor Violet™ 450 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

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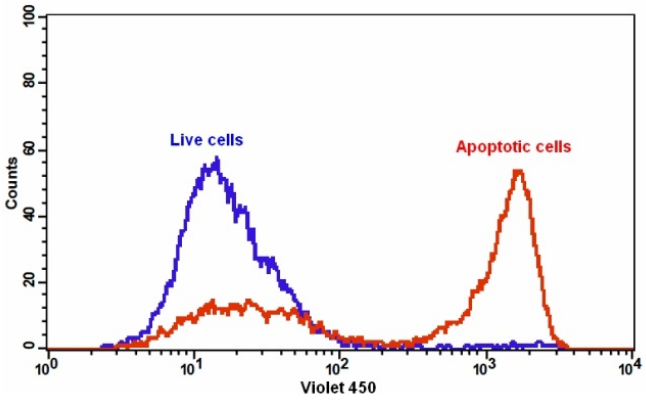

Figure 1. The detection of binding activity of Annexin V-mFluor™ Violet 450 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 µM staurosporine (Red) in a 37 °C, 5% CO2 incubator for 5 hours, and then dye loaded with Annexin V-mFluor™ Violet 450 for 30 minutes. The fluorescence intensity of Annexin V-mFluor™ Violet 450 was measured with a FACSCalibur (Becton Dickinson) flow cytometer using 405 nm laser at Ex/Em = 405/450 nm.

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