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

Catalog number: 22826
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22826)
Component A: Annexin V-iFluor® 594 (100X stock solution)	Minimize light exposure, Freeze (< -15 °C)	1 vial (200 µL/vial)
Component B : Assay Buffer	Freeze (< -15 °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 Texas Red filter set.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds (200 µL/sample).
2. Add Annexin V-iFluor® 594 assay solution.
3. Incubate at room temperature for 30 - 60 minutes.
4. Analyze cells using a flow cytometer with a 610/20 nm filter (PE-Texas Red channel) or fluorescence microscope with a TRITC or Texas Red filter set.

CELL PREPARATION

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® 594:

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® 594 (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 a 610/20 nm filter (PE-Texas Red channel) or a fluorescence microscope with a TRITC or Texas Red filter set.

Analyze by using a flow cytometer:

1. Quantify Annexin V- iFluor® 594 binding using a flow cytometer with 610/20 nm filter (PE-Texas Red 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 Casciola-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® 594, rinse 1 - 2 times with Assay Buffer, and add Assay Buffer back to the cover-slip. Invert the cover-slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after incubation with Annexin V-iFluor® 594 and visualized under a microscope.
3. Analyze the apoptotic cells with Annexin V-iFluor® 594 under a fluorescence microscope using a TRITC or Texas Red filter set. The orange staining on the plasma membrane indicates the Annexin V-iFluor® 594 binding to PS on the cell surface.

EXAMPLE DATA ANALYSIS AND FIGURES

In live non-apoptotic cells, Annexin V-iFluor® 594 detects innate apoptosis in non-induced cells, which is typically 2 - 6% of all cells. In apoptotic cells, Annexin V-iFluor® 594 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulting in increased staining intensity.

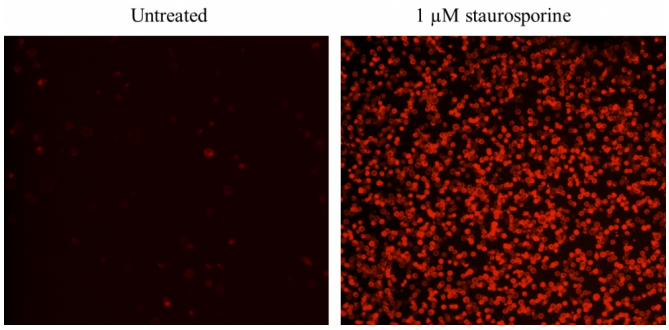

Figure 1. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with Cell Meter Annexin V Binding Apoptosis Assay Kit *Red Fluorescence*. (Left): Untreated control cells. (Right): Cells treated with 1 μM staurosporine for 5 hours.

DISCLAIMER

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