Annexin V FITC Assay Kit

Item No. 600300

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600301	Cell-Based Annexin V FITC	1 vial/50 µl	4°C
600302	Cell-Based Assay Annexin V Binding Buffer (10X)	1 vial/50 ml	Room Temperature
10011234	Cell-Based Propidium Iodide Solution	1 vial/250 µl	4°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeat pipettor.
2. 6-, 12-, 24-, or 96-well plates for culturing cells.
3. Flow cytometer, fluorescence microscope, or plate reader equipped with laser or filters capable of detecting propidium iodide at excitation wavelengths between 520-570 nm and emission wavelengths between 570-610 nm and FITC at excitation/emission = 485/535 nm.
4. A plate centrifuge

INTRODUCTION

Background

Apoptosis is a programmed process of cell death in which a sequence of events leads to the elimination of cells. During an organism's life cycle, apoptosis plays a vital role in normal development and maintenance of tissue homeostasis by eliminating old, unnecessary, and unhealthy cells. Dysregulation of apoptosis results in pathological conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders, and cancer.[1] The ability to modulate cell fate (life or death) is thus recognized as an immense therapeutic potential in drug discovery. Research continues to focus on the elucidation and analysis of signaling pathways that control apoptosis.

One of the hallmarks of the early stages of apoptosis is that membrane phospholipids such as phosphatidylserine and phosphatidylethanolamine redistribute from the inner to outer leaflet of the membrane bilayer where they are exposed on the cell surface.[2,3] Externalization of phosphatidylserine residues to the outer plasma membrane leaflet allows their detection *via* their high-affinity for annexin V, a phospholipid binding protein. Apoptotic cells bound with fluorochrome-labeled annexin V can be visualized using fluorescence microscopy, flow cytometry, or a plate reader capable of fluorescence measurements. Compared to other apoptosis detection methods, such as TdT-mediated dUTP Nick-End Labeling (TUNEL) analysis, ISEL (*in situ* end labeling), and DNA laddering analysis for detection of fragmentation of DNA, Annexin-V analysis has advantages of being more sensitive and less time consuming.

About This Assay

Cayman's Annexin V FITC Assay Kit employs a FITC-conjugated annexin V as a probe for phosphatidylserine on the outer membrane of apoptotic cells. Propidium iodide is used as a marker of cell death. The reagents provided in the kit are sufficient to run 100 samples when using flow cytometry or 500 samples when using a 96-well plate format.

PRE-ASSAY PREPARATION

NOTE: Annexin V FITC is light sensitive. Do not expose to direct intense light.

Reagent Preparation

Assay Binding Buffer

Prepare a working binding buffer by diluting the Cell-Based Assay Annexin V Binding Buffer (10X) (Item No. 600302) 1:10 in distilled water (for example, add 10 ml of the Cell-Based Assay Annexin V Binding Buffer (10X) to 90 ml of distilled water). Mix well and keep at room temperature. The diluted Assay Binding Buffer will be stable for one year at room temperature.

Annexin V FITC/Propidium Iodide Staining Solution

Prepare an Annexin V FITC/Propidium Iodide Staining Solution by adding 10 µl of Cell-Based Annexin V FITC (Item No. 600301) and 10 µl of Cell-Based Propidium Iodide Solution (Item No. 10011234) to 5 ml of the diluted Assay Binding Buffer. Mix well. Prepare this staining solution immediately before adding to the samples. The Annexin V FITC/Propidium Iodide Staining Solution will be stable for one hour at 4°C. *NOTE: Protect from light.*

ASSAY PROTOCOL

NOTES

- Annexin V FITC and Propidium Iodide are light sensitive. All staining procedures must be performed without direct exposure to intense light. Incubations should be done in the dark.
- For all assay protocols described below, it is imperative that samples be analyzed immediately following completion of the staining.

Flow Cytometry

1. Culture cells in 6-, 12-, or 24-well plates at a density of 5 x 10^5 cells/ml in a CO_2 incubator overnight at 37°C. Treat the cells with experimental compounds or vehicle (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Collect the cells in a test tube and centrifuge at 400 x g for five minutes. Aspirate the supernatant.
3. Resuspend the cells with 2 ml of diluted Assay Binding Buffer. Mix well to ensure separation of individual cells.
4. Centrifuge the cells at 400 x g for five minutes and aspirate and discard the supernatant.
5. Resuspend the cells in 250 µl of Annexin V FITC/Propidium Iodide Staining Solution. Mix well to ensure separation of individual cells. Incubate the cells in the dark at room temperature for 10 minutes.
6. Centrifuge the cells at 400 x g for five minutes and aspirate and discard the supernatant.
7. Resuspend the cells in 2 ml of the diluted Assay Binding Buffer. Mix well to ensure separation of individual cells.
8. Centrifuge the cells at 400 x g for five minutes and aspirate and discard the supernatant.
9. Resuspend the cells in 0.5-1 ml of the diluted Assay Binding Buffer.

10. Analyze the cells with a flow cytometer. The cells must be analyzed immediately. Dead cells are stained by propidium iodide and are detectable in the FL2 channel of a flow cytometer. Early stage apoptotic cells recognized by Annexin V FITC are detectable in the FL1 channel.

Fluorescence Microscopy

A 6-, 12-, 24-, or 96-well culture plate can be used for this method. We recommend that the cell density be ≤1 x 10^6 cells/ml. Optimal conditions will depend on the cell type.

1. Culture cells in 6-, 12-, 24-, or 96-well plates at a density of 5 x 10^5 cells/ml in a CO_2 incubator overnight at 37°C. Treat the cells with experimental compounds or vehicle (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
3. Add 2 mL, 1 ml, 500 µl, or 100 µl of diluted Assay Binding Buffer to each well of a 6-, 12-, 24-, or 96-well plate, respectively.
4. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
5. Add 800 µl, 400 µl, 200 µl, or 50 µl of Annexin V FITC/Propidium Iodide Staining Solution to each well of the 6-, 12-, 24-, or 96-well plate respectively. Incubate for 10 minutes at room temperature in the dark.
6. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
7. Add 2 ml, 1 ml, 500 µl, or 100 µl of diluted Assay Binding Buffer to each well of a 6-, 12-, 24-, or 96-well plate respectively.
8. Repeat steps 6-7.
9. Examine the cells by fluorescence microscopy. Cells must be analyzed immediately. Dead cells are stained by propidium iodide and can be detected using a filter designed to detect rhodamine (excitation/emission = 540/570 nm) or Texas Red (excitation/emission = 590/610 nm). Early stage apoptotic cells stained by Annexin V FITC can be detected using a filter designed to detect fluorescein (excitation/emission = 485/535).

Plate Reader Fluorescence Detection

A 96-well **black** culture plate should be used for this method. We recommend that the cell density be 1 x 10^5-1 x 10^6 cells/well. Optimal conditions will depend on the cell type.

1. Culture cells in a 96-well black plate at a density of 1 x 10^5-5 x 10^5 cells/ml in a CO_2 incubator overnight at 37°C. Treat the cells with experimental compounds or vehicle (each sample should be run in duplicate or triplicate). Incubate the cells according to your normal protocol.
2. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
3. Add 100 µl of diluted Assay Binding Buffer to each well and centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
4. Incubate the cells in 50 µl of the Annexin V FITC/Propidium Iodide Staining Solution for 10 minutes at room temperature.
5. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate and discard supernatant.
6. Add 100 µl of diluted Assay Binding Buffer to each well. The cells are now ready for analysis with a plate reader equipped with appropriate fluorescence capabilities. Dead cells are stained by propidium iodide which display strong fluorescent intensity with excitation and emission at 560 nm and 595 nm, respectively. Early stage apoptotic cells stained by Annexin V FITC can be detected with excitation and emission at 485 nm and 535 nm, respectively.

ANALYSIS

Performance Characteristics

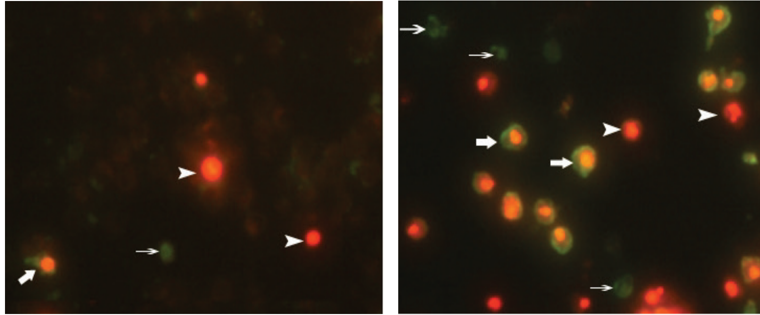

Figure 1. Staurosporine induces apoptosis in RAW 264.7 cells, as measured by an increase of Annexin V FITC positive cells. RAW 264.7 cells were plated at a density of 5 x 10^4 cells/well in a 96-well plate. The next day, cells were treated with vehicle (control) or 8 µg/ml of staurosporine (treatment) for five hours in a CO_2 incubator at 37°C. Cells were then processed for staining of dead cells and Annexin V FITC according to the protocol above. *Left Panel:* in control cells, there are a few propidium iodide positive dead cells (arrow heads) and few of these cells are from apoptosis (thick arrow, both propidium iodide and Annexin V FITC positive). A thin arrow indicates a cell at an early apoptotic stage, which is Annexin V FITC positive but propidium iodide negative. *Right Panel:* cells treated with 8 µg/ml of staurosporine, show an increase in both propidium iodide positive cells (arrowheads), Annexin V FITC positive cells (thin arrows), and cells which are both propidium iodide and Annexin V FITC positive (thick arrows), indicating that treatment of staurosporine in RAW 264.7 cells causes apoptosis.

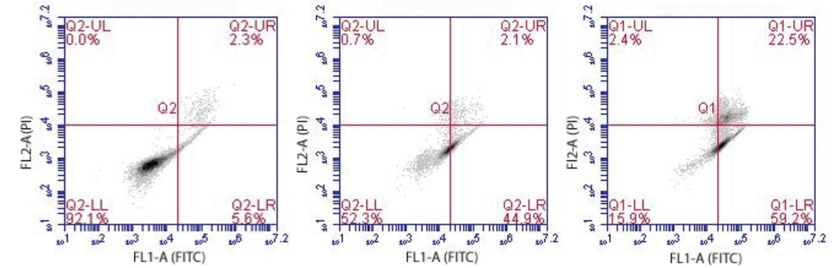

Figure 2. Staurosporine induces apoptosis in Jurkat cells as measured by flow cytometry. Jurkat cells were plated at a density of 1 x 10^6 cells/ml in a 6-well plate. The next day, cells were treated with vehicle (control), 0.5 µg/ml staurosporine for four hours, or 0.5 µg/ml staurosporine overnight in a CO_2 incubator at 37°C. Cells were then processed for staining of dead cells and apoptotic cells. A majority of control cells (left panel) were not stained with either Annexin V FITC or propidium iodide and appear in lower left quadrant. When cells were treated with 0.5 µg/ml staurosporine for four hours (middle panel), there is a significant increase in Annexin V FITC positive cells (lower right quadrant) whereas there is no change in cells that were both propidium iodide and Annexin V FITC positive (upper right quadrant). In contrast, cells treated with 0.5 µg/ml staurosporine overnight (right panel) had a significant increase in both Annexin V FITC positive cells (lower right quadrant) and cells that were both propidium iodide and Annexin V FITC positive (upper right quadrant). The results indicate that staurosporine causes Jurkat cell death through apoptosis and the effect can be detected as early as four hours after the treatment.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Strong staining for both Annexin V FITC and propidium iodide in all samples, including controls	Cells are not healthy	A. Use only healthy cells B. Keep staining time to 10 minutes (prolonged incubation time increases cell death)
High level of Annexin V FITC staining in all samples, including controls	Cells are damaged during harvesting or processing for staining	Process the sample gently; for example, disperse cells gently by pipetting cells up and down; Do not vortex the cells; Do not use scraping for adherent cells
No signal for Annexin V FITC	A. Annexin V FITC/Propidium Iodide Staining Solution not prepared properly B. Cells lost during processing	A. Use right amount of Annexin V FITC to prepare Annexin V FITC/Propidium Iodide Staining Solution B. Decrease treatment time or compound dosage

Reference

1. Schiller, M., Bekerdjian-Ding, I., Heyder, P., *et al.* Autoantigens are translocated into small apoptotic bodies during early stages of apoptosis. *Cell Death and Differentiation* **15**, 183-191 (2008).
2. van Engeland, M., Nieland, L.J.W., Ramaekers, F.C.S., *et al.* Annexin V-affinity assay: A review on an apoptosis detection system based on phosphatidylserine exposure. *Cytometry* **31**, 1-9 (1998).
3. Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* **35(4)**, 495-516 (2007).

Related Products

7-AAD Cell Viability Assay Kit - Item No. 10009856
7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit - Item No. 600120
Apoptotic Blebs Assay Kit - Item No. 10010750
Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Glutathione Cell-Based Detection Kit (Blue Fluorescence) - Item No. 600360
Glycolysis Cell-Based Assay Kit - Item No. 600450
JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172
LDH Cytotoxicity Assay Kit - Item No. 10008882
MTT Cell Proliferation Assay Kit - Item No. 10009365
Multi-Drug Resistance Assay Kit (Calcein AM) - Item No. 600370
Multi-Parameter Apoptosis Assay Kit - Item No. 600330
NAD^+/NADH Cell-Based Assay Kit - Item No. 600480
Neutrophil Elastase Activity Assay Kit - Item No. 600610
20S Proteasome Assay Kit - Item No. 10008041
WST-1 Cell Proliferation Assay Kit - Item No. 10008883
WST-8 Cell Proliferation Assay Kit - Item No. 10010199
XTT Cell Proliferation Assay Kit - Item No. 10010200

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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NOTES

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