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. After opening the kit, store individual components as stated below

Item Number	Item	Quantity/Size	Storage
601281	Annexin V FITC Reagent	1 vial	4°C
600302	Cell-Based Assay Annexin V Binding Buffer (10X)	1 vial/50 ml	4°C
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) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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

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. For flow cytometry: a 96-well v-bottom plate or FACS tubes for staining and running samples, as appropriate for your flow cytometer; capability of exciting at 488 nm and detecting emission at 525 nm (FITC) and 650 nm (propidium iodide).
2. For fluorescence microscopy: cell culture plates (or chamber slides) appropriate for culturing and visualizing cells; filters designed for ex/em at 485/535 nm (FITC) and 535/617 nm (propidium iodide).

INTRODUCTION

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 (PI) is used as a marker of cell membrane permeability (see Figure 1 on page 7). This kit is valuable for researchers looking to assess the degree of apoptosis induced by experimental compounds, and can be useful in pharmacology, immunology, cancer or physiology research. The reagents provided in the kit are sufficient to run 500 samples when using a 96-well plate format.

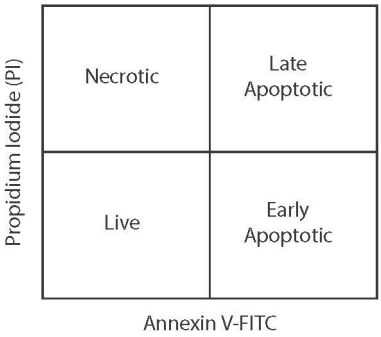

Figure 1. Illustration of expected staining of live and dead cell populations using Cayman's Annexin V FITC Assay Kit.

PRE-ASSAY PREPARATION

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

Reagent Preparation

Annexin V Binding Buffer

Prepare 1X Binding Buffer by diluting the Cell-Based Assay Annexin V Binding Buffer (10X) (Item No. 600302) 1:10 in distilled water. Mix well and keep at room temperature. The diluted 1X Binding Buffer will be stable for one year at 4°C.

Annexin V FITC/Propidium Iodide Staining Solution

Prepare sufficient Annexin V FITC/Propidium Iodide Staining Solution to stain up to 100 samples by adding 10 µl of Annexin V FITC Reagent (Item No. 601281) and 2.5 µl of Cell-Based Propidium Iodide Solution (Item No. 10011234) to 5 ml of 1X 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. It is important for the flow cytometry protocol to include a positive control and single stains for Annexin V-FITC and Propidium Iodide, for compensation purposes.

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

This protocol describes staining in a polypropylene 96-well v-bottom plate. Alternatively, FACS tubes can be used for staining by scaling up volumes approximately 5-10 fold.

1. Culture cells under assay conditions designed to induce/inhibit apoptosis according to your protocols.
2. Collect 1-5 x 10^5 cells into each well and centrifuge at 400 x g for five minutes. Flick out the supernatant. *Optional: Perform antibody staining of cell surface proteins as desired, wash once with 1X Binding Buffer and continue with the protocol.*
3. Resuspend the cells in 200 µl of 1X Binding Buffer. Mix well to ensure separation of individual cells.
4. Centrifuge the cells at 400 x g for five minutes and flick out the supernatant.
5. Resuspend the cells in 50 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. Add 150 ul of 1X Binding Buffer and analyze immediately using 488 nm excitation and ~525 nm emission for FITC and ~655-730 nm emission for PI.

Fluorescence Microscopy

This protocol is optimized for the use of a 96-well culture plate. Adjust volumes accordingly when using other culture vessels. We recommend that the cells not exceed ~80% confluence by the time of the assay. Optimal conditions will depend on the cell type. It is possible to use a fluorescent plate reader to measure total fluorescence using this protocol, but this is not recommended, as visualizing individual cells is important in evaluating the stage of apoptosis.

1. Culture cells in 96-well plates and treat the cells with experimental compounds or vehicle 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 1X Binding Buffer to each well.
4. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate supernatant.
5. Add 50 µl of Annexin V FITC/Propidium Iodide Staining Solution to each well. 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 supernatant.
7. Add 100 µl of 1X Binding Buffer to each well.
8. Examine the cells by fluorescence microscopy. Cells must be analyzed immediately. Dead cells are stained by propidium iodide, which has excitation and emission maxima at 535 nm and 617 nm, respectively. Early stage apoptotic cells stained by Annexin V FITC can be detected using a filter designed to detect fluorescein (excitation/emission = 485/535).

Performance Characteristics

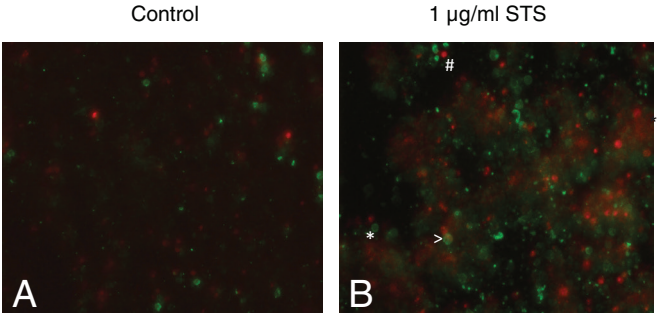

Figure 2. Staurosporine induces apoptosis in RAW 264.7 cells, as visualized by fluorescence microscopy. RAW 264.7 cells were plated at 1 x 10^5 cells/well and treated for 24 hours with 1 µg/ml staurosporine (STS) or vehicle (control). Cells were stained as described for fluorescence microscopy and visualized at 20X magnification. Annexin V single positive cells (*) are early apoptotic, double positive cells (>) are late apoptotic, and PI single positive cells (#) are necrotic.

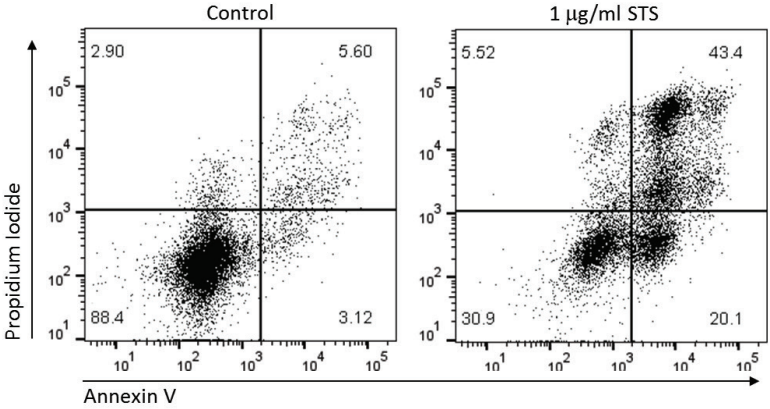

Figure 3. Staurosporine induces apoptosis in Jurkat cells as measured by flow cytometry. Jurkat cells were plated at a density of 0.5 x 10^6 cells/ml and treated with vehicle (control or 1 µg/ml staurosporine for 48 hours).

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

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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