Annexin V PE Assay Kit

Item No. 601420

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
601421	Annexin V PE Assay Reagent	1 vial/100 tests	4°C
600302	Cell-Based Assay Annexin V Binding Buffer (10X)	1 vial/50 ml	4°C
601361	DAPI Viability Dye	1 vial/100 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

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. A 96-well v-bottom plate or FACS tubes for staining and running samples, as appropriate for your flow cytometer.
2. Flow cytometer with 488 nm and 350 (or 405) nm lasers and filters at 585 nm and 450 nm.
3. PBS, pH 7.4

INTRODUCTION

About This Assay

Cayman's Annexin V PE Assay Kit employs a PE-conjugated annexin V as a probe for phosphatidylserine on the outer membrane of apoptotic cells. DAPI is used as a marker of cell membrane permeability seen in very late apoptotic or necrotic cells. 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 100 samples when using a 96-well plate format.

PRE-ASSAY PREPARATION

NOTE: Annexin V PE 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 1X Binding Buffer will be stable for one year at 4°C.

Annexin V PE/DAPI Staining Solution

Prepare sufficient Annexin V PE/DAPI Staining Solution to stain 100 samples by adding 100 µl of Annexin V PE Assay Reagent (Item No. 601421) and 100 µl of DAPI Viability Dye (Item No. 601361) to 10 ml of 1X Binding Buffer. For fewer samples, adjust volume as necessary. The Annexin V PE/DAPI Staining Solution will be stable for one hour at 4°C.

ASSAY PROTOCOL

Flow Cytometry

We recommend using only suspension cells for flow cytometric staining of annexin V. 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-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. Discard 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 100 µl of Annexin V PE/DAPI Staining Solution. Mix well to ensure separation of individual cells. Incubate the cells in the dark at room temperature for 10 minutes.
4. Centrifuge at 400 x g for five minutes and discard supernatant.
5. Add 200 µl of PBS, pH 7.4, and analyze immediately using 488 nm excitation and ~585 nm emission for PE and 350 (or 405) nm excitation and 450 nm emission for DAPI.

ANALYSIS

Performance Characteristics

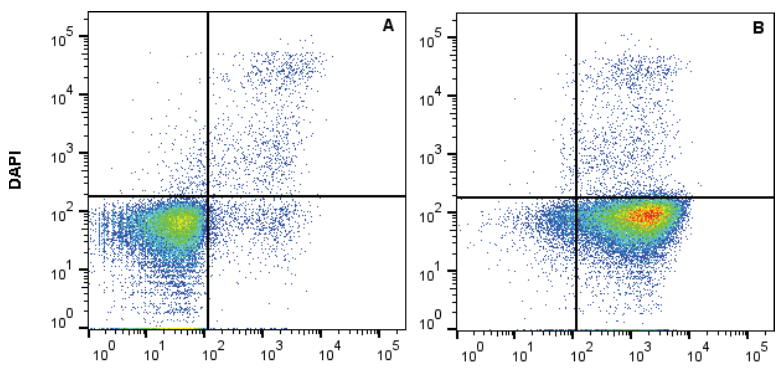

Figure 1. Ultraviolet light induces apoptosis in Jurkat cells. Jurkat cells were left untreated (*Panel A*) or stimulated with 200 mJ/cm^2 ultraviolet light (*Panel B*) and then incubated for four hours at 37°C. Cells were stained with Annexin V PE at a 1:200 dilution in 1X Binding Buffer for 15 minutes at room temperature in the dark. After centrifugation and removal of the supernatant, cells were resuspended in 1:100 DAPI in PBS. Data were collected on a MACSQuant® cytometer from Miltenyi and analyzed using FlowJo® software.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Strong staining for both annexin V PE and DAPI in all samples, including controls	A. Cells are not healthy B. Adherent cells may be compromised in processing	A. Start with healthy cells B. Try different cell line or different removal method
No signal for annexin V PE	A. Annexin V PE/DAPI Staining Solution not prepared properly B. No apoptosis induced	A. Check dilutions B. Vary treatment time or compound dosage
Cells lost during processing (adherent)	A. Annexin V staining protocol incompatible with your cells B. Cells progressed too far through apoptosis	A. Try different cell line B. Lower stimulus dosage or shorten incubation time

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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