# Annexin V APC Assay Kit

Item No. 601410

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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 |
|---|---|---|---|
| 601411 | Annexin V APC 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. A flow cytometer equipped with 633 nm and 350 (or 405) nm lasers and filters for around 700 nm and 450 nm.
3. PBS, pH 7.4

# INTRODUCTION

## About This Assay

Cayman's Annexin V APC Assay Kit employs an APC-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 APC 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 APC/DAPI Staining Solution

Prepare sufficient Annexin V APC/DAPI Staining Solution to stain 100 samples by adding 50 µl of Annexin V APC Assay Reagent (Item No. 601411) 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 APC/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 APC/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 633 nm excitation and ~700 nm emission for APC and 350 (or 405) nm excitation and 450 nm emission for DAPI.



# ANALYSIS

## Performance Characteristics

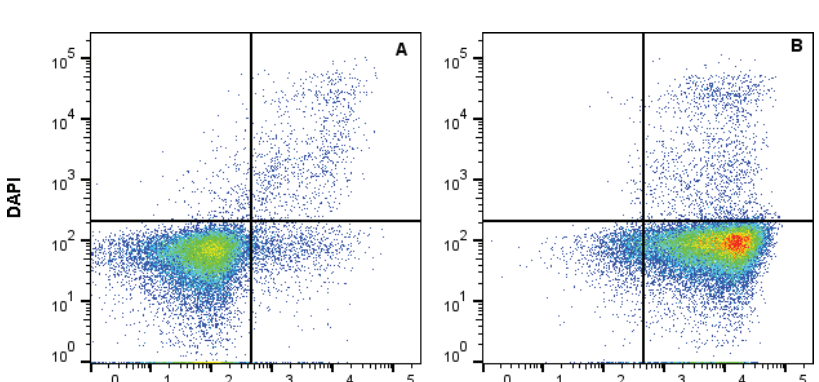


**Figure 1. Ultraviolet light induces apoptosis in Jurkat cells.** Jurkat cells were left untreated (*Panel A*) or stimulated with 200 mJ/cm$^2$ ultraviolet light (*Panel B*) and then incubated for four hours at 37°C. Cells were stained with Annexin V APC at a 1:200 dilution in 1X Binding Buffer for 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 APC and DAPI in all samples, including controls | A. Cells are not healthy<br>B. Adherent cells may be compromised in processing | A. Start with healthy cells<br>B. Try different cell line or different removal method |
| No signal for annexin V APC | A. Annexin V APC/DAPI Staining Solution not prepared properly<br>B. No apoptosis induced | A. Check dilutions<br>B. Vary treatment time or compound dosage |
| Cells lost during processing (adherent) | A. Annexin V staining protocol incompatible with your cells<br>B. Cells progressed too far through apoptosis | A. Try different cell line<br>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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