Version 1 Last updated 21 June 2018

ab236215 Annexin V-APC Assay Kit

For the study of apoptosis in suspension cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Annexin V-APC Assay Kit (ab236215) 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.

The reagents provided are sufficient to run 100 samples when using a 96-well format.

Culture cells to induce/inhibit apoptosis.

Collect 1-5 x 10^5 cells into each well and centrifuge. Discard supernatant.

Resuspend cells in Staining Solution and incubate in the dark at RT for 10 minutes.

Centrifuge. Discard supernatant.

Add PBS and analyze immediately using 633 nm excitation and ~70 nm emission for APC and 350 (or 405) nm excitation and 450 nm emission for DAPI.

2. Materials Supplied and Storage

Store kit at 4°C immediately in the dark on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Annexin V APC Assay Reagent	1 vial	4°C
Cell-Based Assay Annexin V Binding Buffer (10X)	1 vial	RT
DAPI Viability Dye	1 vial	4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Flow cytometer equipped with 633 nm and 350 (or 405) nm lasers and filters for ~700 nm and 450 nm
- 96-well v-bottom plate or FACS tubes
- PBS pH 7.4

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Cell-Based Assay Annexin V Binding Buffer (10X)

1. Prepare 1X Binding Buffer by diluting the Cell-Based Assay Annexin V Binding Buffer (10X) 1:10 in distilled water.
2. Mix well and keep at RT.
3. The diluted 1X Binding Buffer will be stable for 1 year at RT.

5.2 Annexin V APC Assay Reagent and DAPI Viability Dye

1. Prepare sufficient Annexin V APC/DAPI Staining Solution to stain 100 samples by adding 50 µl of Annexin V APC Assay Reagent and 100 µl of DAPI Viability Dye to 10 ml of 1X Binding Buffer.
2. For fewer samples adjust volume as necessary.
3. The Annexin V APC/DAPI Staining Solution will be stable for 1 hour at 4°C.

6. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.
- We recommend using only suspension cells for flow cytometric staining of Annexin V.
- The following describes staining in a 96-well v-bottom plate – FACS tubes can be used for staining by scaling up the volumes ~5-fold.

6.1 Suspension Cells:

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 5 minutes.
3. Discard the supernatant.
   Optional: Perform antibody staining of cell surface proteins as desired, wash once with 1X Binding Buffer and continue with the protocol.

7. Assay Procedure

— Assay all controls and samples in duplicate.

7.1 Flow Cytometry:

1. Resuspend the cells (Prepared in section 6) in 100 µl of Annexin V APC/DAPI Staining Solution (Prepared in section 5.2). Mix well to ensure separation of individual cells.
2. Incubate the cells in the dark at RT for 10 minutes.
3. Centrifuge at 400 x *g* for 5 minutes and discard supernatant.
4. 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.

8. FAQs / Troubleshooting

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

9. Typical Data

Data provided **for demonstration purposes only.**

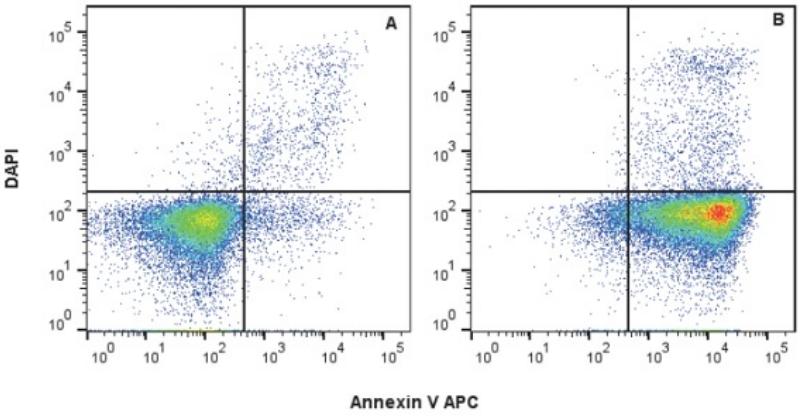

Figure 1. Ultraviolet light induces apoptosis in Jurkat cells.

Jurkat (Human T cell leukemia cell line from peripheral blood) cells were left untreated (panel A) or stimulated with 200 mJ/cm^2 ultraviolet light (panel B) and then incubated for 4 hours at 37°C. Cells were stained using Annexin V APC Assay Reagent at a 1:200 dilution in 1X Binding Buffer for 15 minutes at RT in the dark. After centrifugation and removal of the supernatant, cells were resuspended in 1:100 DAPI in PBS.

10. Notes

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