ab14159

Annexin V-PE-Cy5 Apoptosis Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Apoptosis in living cells (adherent and suspension)

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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Table of Contents

1. Overview 3

2. Protocol Summary 3

3. Components and Storage 4

4. Assay Protocol 5

5. Troubleshooting 7

1. Overview

Abcam's Annexin V-PE-Cy5 Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phospholipid phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a strong natural affinity for PS.

The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells, without the need for fixation. Results can be analyzed by flow cytometry or fluorescence microscopy.

2. Protocol Summary

Incubate Cells with Annexin V-PE-Cy5



Quantify Using Flow Cytometry

OR

Detect Using Fluorescence Microscopy

3. Components and Storage

A. Kit Components

Item	Quantity
Annexin V- PE-Cy5	500 µL
Binding Buffer II/1X Binding Buffer	50 mL

* Store kit at +4°C. All reagents are stable for one year under proper storage conditions.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Flow cytometer or fluorescence microscope
- Glass slides
- Orbital shaker

4. Assay Protocol

1. Incubation of cells with Annexin V-PE-Cy5:

a) Induce apoptosis by desired method.

b) Collect 1-5 x 10^5 cells by centrifugation.

c) Re-suspend cells in 500 µl of Binding Buffer II/1X Binding Buffer.

d) Add 5 µl of Annexin V-PE-Cy5

e) Incubate at room temperature for 5 min in the dark.

Proceed to step 2 or 3 below depending on method of analysis.

2. Quantification by Flow Cytometry:

Analyze Annexin V-PE-Cy5 binding by flow cytometry (Ex = 488 nm; Em = 670 nm).

For ***adherent cells***, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-PE (1.c-e).

3. Detection by Fluorescence Microscopy:

a) Place the cell suspension from Step 1.e on a glass slide. Cover the cells with a glass coverslip.

For analyzing ***adherent cells***, grow cells directly on a coverslip. Following incubation (1.e), invert coverslip on a glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization.

Note:

Cells must be incubated with Annexin V-PE *before* fixation since any cell membrane disruption can cause non-specific binding of Annexin V to PS on the inner surface of the cell membrane.

b) Observe the cells under a fluorescence microscope using the PE-Cy5 filter.

Cells that have bound Annexin V-PE-Cy5 will show bright purple staining in the plasma membrane.

5. Troubleshooting

Problem	Reason	Solution
High Background	Cell density is higher than recommended	Refer to datasheet and use the suggested cell number
	Increased volumes of components added	Use calibrated pipettes accurately
	Incubation of cell samples for extended periods	Refer to datasheets and incubate for exact times
	Use of extremely confluent cells	Perform assay when cells are at 80-95% confluency
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination

Problem	Reason	Solution
Lower signal levels	Washing cells with PBS before/after fixation (adherent cells)	Always use binding buffer for washing cells
	Cells did not initiate apoptosis	Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	Very few cells used for analysis	Refer to data sheet for appropriate cell number
	Incorrect setting of the equipment used to read samples	Refer to datasheet and use the recommended filter setting
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the components appropriately

Problem	Reason	Solution
Erratic results	Uneven number of cells seeded in the wells	Seed only healthy cells (correct passage number)
	Adherent cells dislodged at the time of experiment	Perform experiment gently and in duplicates or triplicates for each treatment
	Incorrect incubation times or temperatures	Refer to datasheet & verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Increased or random staining observed in adherent cells	Always stain cells with Annexin before fixation (makes cell membrane leaky)

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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