ab14153

Annexin V-EGFP Apoptosis Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of apoptosis in various samples

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.

Version 3a, Last updated 5 June 2023

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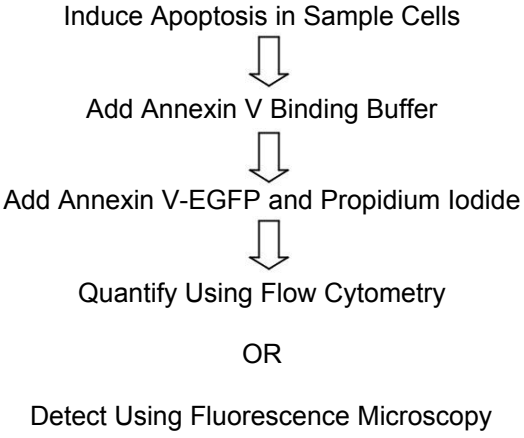
1. Overview

Abcam's Annexin V-EGFP 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 an enhanced green fluorescent protein (EGFP) fusion 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. Detection can be analyzed by flow cytometry or by fluorescence microscopy with a FITC filter. EGFP is brighter and more photo-stable than other fluorescent reagents. The kit can differentiate apoptosis vs. necrosis when performing both Annexin V-EGFP and PI staining.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Annexin V-EGFP	500 µL
Binding Buffer II/1X Binding Buffer	50 mL
Propidium Iodide II/Propidium Iodide	500 µL

* Store at +4°C. Do not freeze. Stable for one year under proper storage conditions.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Flow Cytometer or Fluorescence Microscope
- Glass slides and coverslips

4. Assay Protocol

1. Incubation of cells with Annexin V-EGFP:

a) Induce apoptosis by desired methods.

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-EGFP and 5 µl of Propidium Iodide II/Propidium Iodide (PI 50µg/ml, optional.)

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-EGFP binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For analyzing ***adherent cells***, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (Step 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 glass slide and visualize cells. The cells can also be washed with Binding Buffer II/1X Annexin V Binding Buffer and fixed in 2% formaldehyde before visualization.

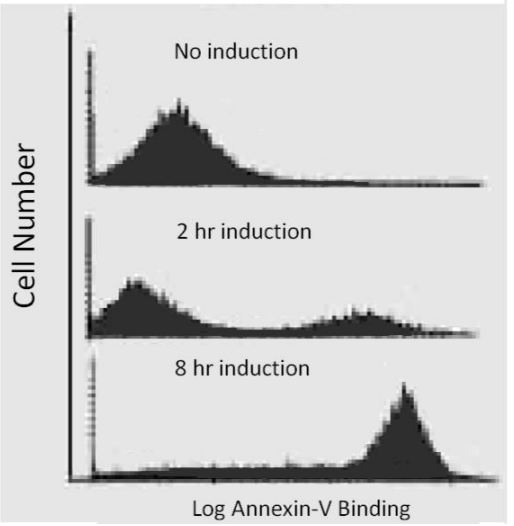
Note:

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

b) Observe the cells under a fluorescence microscope using a dual filter set for FITC and rhodamine.

Cells which have bound Annexin V-EGFP will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (EGFP) on the cell surface (plasma membrane).

5. Data Analysis



Detection of Apoptosis with Annexin V-EGFP. Apoptosis was induced in Jurkat T Cells by camptothecin (2 μM) for various times as indicated. Cells were collected and incubated with Annexin V-EGFP for 5 minutes according to the kit protocol. Results were analyzed by flow cytometry.

6. Troubleshooting

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)
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

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

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

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

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