ab14085 - Annexin V-FITC Apoptosis Detection Kit

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

View kit datasheet: www.abcam.com/ab14085

(use www.abcam.cn/ab14085 for China, or www.abcam.co.jp/ab14085 for Japan)

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.

Components and Storage

Item	Quantity
Annexin V-FITC II/Annexin V-FITC	500 µL
Binding Buffer II/1X Binding Buffer	50 mL
Propidium Iodide II/Propidium Iodide (PI)	500 µL

* Store kit at +4°C.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent Microscope
- Glass slides
- Orbital shaker

Assay Protocol

1. Incubation of cells with Annexin V-FITC:

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-FITC II/Annexin V-FITC 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-FITC 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 ***adherent cells***, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-FITC II/Annexin V-FITC (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.

b) For analyzing ***adherent cells***, grow cells directly on a coverslip. Following addition and incubation of dyes (1d and 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-FITC II/Annexin V-FITC 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.

c) Observe the cells under a fluorescence microscope using a dual filter set for FITC & Texas Red.

Note: Cells that have bound Annexin V-FITC II/Annexin V-FITC will show green staining in the plasma membrane. Cells that have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).

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
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
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)

FAQs

The protocol in the datasheet for flow cytometry doesn't mention a wash step after Annexin/PI addition. can I confirm that this is correct? I.e, 5min incubation then straight into flow cytometer.
Typically washing is not needed before analysing in the flow cytometer. However, if you wish to fix your cells, this can be done after incubating with Annexin V in binding buffer. The cells can then be washed in PBS and fixed in 2% PFA for 15 mins. Once your cells have been re-suspended in PBS, you can also add a preservative/stabiliser if you are planning on keeping your samples for a long time. For example, 1 mg/ml BSA or 1% sodium azide.

I am looking to investigate cell apoptosis using 96 well plates. I was wondering if your Annexin V-FITC Apoptosis Detection Kit could be adapted to a 96 well plate fluorescent plate reader format?
It is possible to adapt the assay to a plate reader format, however, the sensitivity can vary from sample to sample. For this particular assay, take 10^5 trypsinized cells into the plate wells, resuspend in 100 µl binding buffer, add 1µ Annexin V and PI respectively, incubate them in the dyes, give a gentle wash and then analyze. The cell number can vary from cell to cell and/or treatment protocol selected. Please take measures to prevent overcrowding in the wells and also aspirate solutions gently since the apoptotic cells tend to detach quickly.

Can you pre- label the cells and then follow apoptosis using this kit?
Unfortunately, the product has not yet been tested in this manner. We are unsure whether it might work and needs significant optimization with known controls and timepoints to determine if the kit is performing correctly in these conditions.

For this kit, where was the Annexin-V obtained from? Is it recombinant? Or purified? And from what species?
The annexin V is a recombinant protein expressed from E. coli.

Can the kit work on bacteria or yeast cells?
The kit has been standardized for mammalian cells only.

Will trypsinizing the cells removed the phosphatidylserine?
The trypsin used in this kit should in no way affect the PS on the cell membrane.

I see green colour with my control cells (no Annexin-FITC, only PI)?
As for the visualisation of PI under the FITC channel, this is going to depend on the excitation and emission filters/laser you are using. PI can be excited to about 50% efficiency in the 488nm range. If you have a broad pass, or long pass filter in your FITC set you will see the signal from the PI. (FITC Ex/Em is 495nm/519nm and Propidium iodide is 535nm/617nm).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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www.abcam.co.jp/contactus (Japan)