ab14082 – Annexin V-FITC Apoptosis Detection Reagent (500X)

For the detection of apoptosis through fluorescence microscopy or flow cytometry.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab14082

Materials Supplied

Item	Quantity	Storage Condition
Annexin V-FITC Apoptosis Detection Reagent (500X)	1 vial	4 °C

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

Materials Required, Not Supplied

- 1X Annexin V Binding Buffer (ab14084)
- Propidium Iodide (ab14083)
- 2% Paraformaldehyde
- Microcentrifuge
- Pipettes and pipette tips
- Flow Cytometer or Fluorescence Microscope
- Glass slides and coverslips

Assay Protocol

A) Incubation of cells with Annexin V-FITC:

1. Induce apoptosis by desired methods.
2. Collect 1 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer (ab14084).
4. Add 1 µl of Annexin V-FITC and 1 µl Propidium Iodide (ab14083).
5. Incubate at room temperature for 5 min in the dark.
6. Proceed to B or C below depending on method of analysis.

B) Quantification by Flow Cytometry:

1. Analyze cells by flow cytometry (Ex. = 488 nm; Em. = 530 nm) using FL1 channel for detecting Annexin V-FITC staining and FL2 channel for detecting PI staining.
2. For adherent cells, gently trypsinize and wash cells with serum-containing medium before incubation with Annexin V-FITC (A.3-5).

C) Detection by Fluorescence Microscopy:

1. Place the cell suspension from Step A.5 on a glass slide, and cover with a glass coverslip. For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed with 1X Annexin V Binding Buffer and fixed in 2% formaldehyde before visualization
   ∆ **Note:** Cells must be incubated with Annexin V-FITC before fixation because any cell membrane disruption can cause nonspecific binding of annexin V to PS on the inner surface of the cell membrane.
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC and rhodamine, or separate filters.

∆ **Note:** Cells that have bound Annexin V-FITC will show green staining on the plasma membrane. Cells that have lost membrane integrity will show red PI staining throughout the nuclei and a halo of green staining (FITC) on the plasma membrane.

Test Results:

- Jurkat cells (treated with 2 µM camptothecin for 6 hours) were collected for annexin V assay according to the kit instructions. Results show 40-60% apoptotic cells as analyzed by flow cytometry.

Technical Support

For all technical or commercial enquiries please go to:

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