ab14143 – Annexin V/ANXA5-Cy3 Apoptosis Staining / Detection Reagent

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/ab14143

Assay Protocol

A) Incubation of cells with Annexin V-Cy3:

1. Induce apoptosis by desired methods.
2. Collect 1 x 105 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer.
4. Add 1 µl of Annexin V-Cy3. 5. Incubate at room temperature for 5 min in the dark.
5. Proceed to B or C below depending on method of analysis.

B) Quantification by Flow Cytometry:

1. Analyze cells by flow cytometry (Ex = 543 nm; Em = 570 nm) using FL2 channel. For adherent cells, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-Cy3 (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-Cy3 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 rhodamine filter. Cells that have bound Annexin V-Cy3 will show bright red staining on the plasma membrane.

Technical Support

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