ab14085

Annexin V-FITC

Apoptosis Detection Kit

Instructions for Use

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.
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1. Overview

Abcam’s Annexin V-FITC Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane 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 high affinity for PS.

The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy. The kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining.
2. Protocol Summary

Incubate Cells with Annexin V-FITC

\[\downarrow\]

Quantify Using Flow Cytometry

OR

Detect Using Fluorescence Microscopy
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin V-FITC</td>
<td>500 µL</td>
</tr>
<tr>
<td>1X Binding Buffer</td>
<td>50 mL</td>
</tr>
<tr>
<td>Propidium Iodide (PI)</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

* Store kit at +4°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent Microscope
- Glass slides
- Orbital shaker
4. 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 1X Binding Buffer.
   
d) Add 5 μl of Annexin V-FITC and 5 μl of 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 (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 *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 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).
## 5. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Background</strong></td>
<td>Cell density is higher than recommended</td>
<td>Refer to datasheet and use the suggested cell number</td>
</tr>
<tr>
<td></td>
<td>Increased volumes of components added</td>
<td>Use calibrated pipettes accurately</td>
</tr>
<tr>
<td></td>
<td>Incubation of cell samples for extended periods</td>
<td>Refer to datasheets and incubate for exact times</td>
</tr>
<tr>
<td></td>
<td>Use of extremely confluent cells</td>
<td>Perform assay when cells are at 80-95% confluency</td>
</tr>
<tr>
<td></td>
<td>Contaminated cells</td>
<td>Check for bacteria/ yeast/ mycoplasma contamination</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Lower signal levels</strong></td>
<td>Washing cells with PBS before/after fixation (adherent cells)</td>
<td>Always use binding buffer for washing cells</td>
</tr>
<tr>
<td></td>
<td>Cells did not initiate apoptosis</td>
<td>Determine the time-point for initiation of apoptosis after induction (time-course experiment)</td>
</tr>
<tr>
<td></td>
<td>Very few cells used for analysis</td>
<td>Refer to data sheet for appropriate cell number</td>
</tr>
<tr>
<td></td>
<td>Incorrect setting of the equipment used to read samples</td>
<td>Refer to datasheet and use the recommended filter setting</td>
</tr>
<tr>
<td></td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Always check the expiry date and store the components appropriately</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Erratic results</td>
<td>Uneven number of cells seeded in the wells</td>
<td>Seed only healthy cells (correct passage number)</td>
</tr>
<tr>
<td></td>
<td>Adherent cells dislodged at the time of experiment</td>
<td>Perform experiment gently and in duplicates or triplicates for each treatment</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to datasheet &amp; verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td></td>
<td>Increased or random staining observed in adherent cells</td>
<td>Always stain cells with Annexin before fixation (makes cell membrane leaky)</td>
</tr>
</tbody>
</table>
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