ab14147

Annexin V-Cy5

Apoptosis Detection Reagent

Instructions for Use

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

This product is for research use only and is not intended for diagnostic use.
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1. Overview

Annexin V-Cy5 is a bright fluorescent reagent for detecting the early stages of apoptosis. During apoptosis, phosphatidylserine (PS) is translocated from the cytoplasmic face of the plasma membrane to the cell surface.

Annexin V has a strong, Ca$^{2+}$-dependent affinity for PS and therefore serves as a probe for detecting apoptosis. Cy5 fluorescent dye produces an intense signal in the far-red region of the spectrum and therefore it is very useful for multiple labeling of cells with green and red colored fluorescent probes. Cy5 yields fluorescence with a $\lambda_{\text{max}}$ emission of 670 nm.

2. Protocol Summary

Induce Apoptosis in Sample Cells

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Add Annexin V Binding Buffer (ab14084)

↓

Add Annexin V-Cy5

↓

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-Cy5 Apoptosis Detection Reagent</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

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

B. Additional Materials Required

- 1X Annexin V Binding Buffer (ab14084)
- Microcentrifuge
- Pipettes and pipette tips
- Flow Cytometer or Fluorescence Microscope
- Glass slides and coverslips
4. Assay Protocol

1. Incubation of cells with Annexin V-Cy5:
   a) Induce apoptosis by desired methods.
   b) Collect $1 \times 10^5$ cells by centrifugation.
   c) Re-suspend cells in 500 µl of 1X Annexin V Binding Buffer
   d) Add 1 µl of Annexin V-Cy5.
   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-Cy5 binding by flow cytometry (Ex = 649 nm; Em = 670 nm) using Helium-Neon Laser.

   For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Cy5 (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 and fixed in 2% paraformaldehyde before visualization.

Note:
Cells must be incubated with Annexin V-Cy5 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.

b) Observe the cells under a fluorescence microscope using a Cy5 filter or a FITC/Cy3/Cy5 triple band filter set (Chroma Technology) if you perform triple labeling with these dyes, or detect cells using CCD camera.

Cells that have bound Annexin V-Cy5 will show bright red-blue staining on the plasma membrane.
# 5. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Background</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>
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<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>
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<tr>
<td>---------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Lower signal levels</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>
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<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>
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<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>
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<td>------------------------------</td>
<td>----------------------------------------------------------</td>
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<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>Adherent cells dislodged at</td>
<td>Perform experiment gently and in duplicates or triplicates for each treatment</td>
<td></td>
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<tr>
<td>the time of experiment</td>
<td></td>
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<tr>
<td>Incorrect incubation times</td>
<td>Refer to datasheet &amp; verify correct incubation times and</td>
<td></td>
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<tr>
<td>or temperatures</td>
<td>temperatures</td>
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<tr>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<tr>
<td>Increased or random staining</td>
<td>Always stain cells with Annexin before fixation (makes cell membrane leaky)</td>
<td></td>
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<tr>
<td>observed in adherent cells</td>
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