ApoScreen® Annexin V Apoptosis Kit-PE

Description

ApoScreen® Annexin V Apoptosis Kit-PE (SB Cat. No. 10010-09) employs a R-phycoerythrin-conjugated Annexin V (Annexin V-PE) in concert with 7-Aminoactinomycin D (7-AAD) to evaluate subpopulations of cells undergoing apoptosis. During the early stages of apoptosis, cells begin to display phosphatidylserine (PS) on the outer cell membrane where it is readily detectable by staining the cells with Annexin V-PE. As the plasma membrane becomes increasingly permeable during the later stages of apoptosis, 7-AAD can readily move across the cell membrane and bind to cellular DNA, providing a means for identifying those cells that have lost membrane integrity through mechanisms including necrosis. When cells are double stained with Annexin V-PE and 7-AAD, three different cell populations may be observed - (i) live cells that do not stain with either Annexin V-PE or 7-AAD; (ii) necrotic cells that stain with both reagents; and (iii) apoptotic cells that stain with Annexin V-PE only. Analysis may be performed on any flow cytometer equipped with a single laser using excitation at 488 nm.

Applications

FC – Quality tested

Kit Components & Storage and Handling

- Annexin V-PE is supplied as 100 tests in 1.0 mL of PBS/NaN3 and a stabilizing agent. Store at 2-8°C.
- 7-Aminoactinomycin D (7-AAD) is supplied as 100 tests in 1.0 mL of PBS/NaN3. Store at 2-8°C.
- Annexin V Binding Buffer 10X is supplied as 7 x 1.5 mL. Store at 2-8°C.
- Protect PE conjugated and 7-AAD reagents from light. Reagents are stable for the period shown on the label if stored as directed.

Working Dilutions

<table>
<thead>
<tr>
<th>Flow Cytometry</th>
<th>Annexin V-PE</th>
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<tbody>
<tr>
<td>7-Aminoactinomycin D (7-AAD)</td>
<td>10 µL/10^6 cells</td>
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<tr>
<td>Annexin Binding Buffer 10X</td>
<td>Dilute 1 part 10X buffer with 9 parts dH2O just prior to use</td>
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For flow cytometry, the suggested use of these reagents is in a final volume of 100 µL of Annexin Binding Buffer 1X.

Other Applications

Since applications vary, you should determine the optimum working dilution for the product that is appropriate for your specific need.

Suggested Staining Protocol

- Wash cells twice in cold PBS
- Resuspend cells in cold 1X Annexin Binding Buffer to a concentration of 1 x 10^6 cells/mL
- Add 100 µL of cell suspension (10^6 cells) to 5 mL polystyrene round bottom tubes
- Add 10 µL of conjugated Annexin V-PE
- Gently vortex each tube and incubate for 15 minutes at 2-8°C and protected from light
- Without washing, add 380 µL of cold 1X Annexin Binding Buffer to each tube
- Add 10 µL of 7-Aminoactinomycin D (7-AAD)
- Analyze by flow cytometry immediately

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References


