Technical Data Sheet

Annexin V-FITC Fluorescence Microscopy Kit

Product Information

<table>
<thead>
<tr>
<th>Component</th>
<th>51-8074KC</th>
<th>Description</th>
<th>Annexin V-FITC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size:</td>
<td>5.0 ml (1 ea)</td>
<td>Storage Buffer:</td>
<td>Aqueous buffered solution containing BSA and ≤0.09% sodium azide.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>51-66121E</th>
<th>Description</th>
<th>10X Annexin V Binding Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size:</td>
<td>50 ml (1 ea)</td>
<td>Storage Buffer:</td>
<td>Aqueous buffered solution containing no preservative.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>51-6635KC</th>
<th>Description</th>
<th>10X PBS Buffer</th>
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</thead>
<tbody>
<tr>
<td>Size:</td>
<td>50 ml (1 ea)</td>
<td>Storage Buffer:</td>
<td>Aqueous buffered solution containing no preservative.</td>
</tr>
</tbody>
</table>

Description

The loss of plasma asymmetry is a key feature of apoptosis. In normal cells, membrane phospholipids are distributed asymmetrically between the inner and outer leaflets of the plasma membrane. For example, phosphatidylserine (PS), an aminophospholipid, is normally present in the inner leaflet of the plasma membrane. Early in apoptosis, before the loss of membrane integrity, PS translocates from the inner to the outer leaflet of the plasma membrane, exposing it to the external cellular environment at the surface of the plasma membrane. Annexin V is a 35-36 kDa, calcium-dependent, phospholipid-binding protein with a high affinity for PS and is used to indirectly monitor PS translocation. The Annexin V assay is perhaps the most widely used assay today, facilitated by the observation that PS translocation appears to be a universal apoptotic phenomenon. It has been detected in mammalian, insect, and plant cells under the action of most, if not all, triggers of apoptosis.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The Annexin V was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

For a working solution (1X), dilute 1 part either 10X Annexin V Binding Buffer or 10X PBS Buffer to 9 parts distilled H2O.

Application Notes

Application

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<tr>
<th>Fluorescence microscopy</th>
<th>Routinely Tested</th>
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Recommended Assay Procedure:

Annexin V-FITC binds to negatively charged phospholipid surfaces (Kd of ~5x10^-2) with a higher specificity for PS than most other phospholipids. Defined calcium and salt concentrations are required for Annexin V-FITC binding.

Annexin V-FITC Microscopy Staining Protocol

1. Wash cells twice with 1X PBS.
2. Wash cells once with 1X Annexin V Binding Buffer.
3. Stain cells with Annexin V-FITC diluted 1:10 in 1X Annexin V Binding Buffer for 15 min at RT. We typically use 2 ml total volume per Labtek II chamber slide (i.e., 200 µl Annexin V-FITC and 1.8 ml 1X Binding Buffer. For example, for a 4 well chambered slide, use 500 µl Annexin V-FITC solution/well.
4. Wash cells once with 1X Binding Buffer.
5. Add additional Binding Buffer and observe under the microscope. The amount of Binding Buffer added should be sufficient to ensure that the slide does not dry out during microscopic observation.
Fluorescence Microscopy Analysis of Annexin V-FITC Staining. HeLa cells were left untreated [top left panel] or induced to undergo apoptosis with camptothecin (8µM camptothecin, 24 h) [top middle panel], or staurosporine (1µM, 4 h) [top right panel] and stained with Annexin V-FITC according to the protocol provided. Bottom left panel, bottom middle panel, and bottom right panel represent phase contrast correlates of top left panel, top middle panel, and top bottom panel, respectively. The data shows that untreated cells were negative for Annexin V-FITC staining, whereas positive cells were seen following either camptothecin or staurosporine treatment. The data also shows that more positive cells were seen in the staurosporine- than the camptothecin-treated populations. The staurosporine treatment was also associated with dramatic morphological changes. The amount of Annexin V-FITC positive cells and the cell morphology can vary according to the cell type, model system, treatment type, or time after apoptosis induction.

Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References