PURPOSE
The ApoTarget™ Annexin-V FITC Apoptosis Kit is designed to detect apoptotic cells by flow cytometry or immunofluorescence.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

PRINCIPLE OF THE METHOD
Annexin-V is a 35-36 kDa, calcium-dependent, phospholipid binding protein with high affinity for phosphatidylserine. The $K_d$ for the binding of Annexin-V to phosphatidylserine (PS) has been estimated at $5 \times 10^{-10}$ M. The Annexin-V binding assay is based on the rapid and selective binding to phosphatidylserine found in the outer cell membrane beginning early in the process of apoptosis (programmed cell death). Viable cells maintain an asymmetric distribution of different phospholipids between the inner and outer leaflets of the plasma membrane. Choline-containing phospholipids such as phosphatidylethanolamine and sphingomyelin are primarily located on the outer leaflet of viable cells and aminophospholipids such as phosphatidylethanolamine and phosphatidylserine are found at the cytoplasmic (inner) face of viable cells. During apoptosis, the plasma membrane changes include a redistribution of phosphatidylserine from the cytoplasmic face to the outer leaflet making it available for Annexin-V binding.

The ApoTarget™ Annexin-V FITC Apoptosis Kit employs a fluorescein labeled Annexin-V (Annexin-V FITC) in concert with propidium iodide (PI) to detect the cells undergoing apoptosis. During the early stage of apoptosis, cells begin to display PS on cell surface membranes where it is readily detectable by staining the cells with Annexin-V FITC. As the plasma membrane becomes increasingly permeable during the later stages of apoptosis, PI can move across the cell membrane and bind to DNA. This provides a means of identifying the lost membrane integrity associated with necrosis or late stage of apoptosis. With the use of an Annexin-V-propidium iodide double staining regime, three populations of cells are distinguishable in two color flow cytometry (Figure, right): (1) Non-apoptotic cells: Annexin-V negative and PI negative; (2) Early apoptotic cells: Annexin-V positive and PI negative; (3) Necrotic cells or late apoptotic cells: Annexin-V positive and PI positive. Alternatively, the cell can be examined with a fluorescent microscope equipped with FITC and rhodamine filter sets.

REAGENTS PROVIDED
Note: Store kit at 2 to 8°C.
1. Annexin-V FITC Conjugate (0.1 mL or 1.5 mL): Recombinant Annexin-V conjugated with FITC in Tris buffered saline containing 1% BSA and 0.02% sodium azide, pH 7.4.
2. Propidium Iodide Buffer (0.5 mL or 3 mL); containing 50 µg/mL in PBS.
3. Annexin-V Binding Buffer (20 mL of 1X Annexin-V Binding Buffer or 50 mL of 10X Annexin-V Binding Buffer): 1X Annexin-V Binding Buffer or after dilution of 10X Annexin-V Binding Buffer contains 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl$_2$.

Caution: Sodium azide and propidium iodide are poisonous and hazardous substances. Handle with care and dispose of properly.

REAGENTS NOT PROVIDED
1. Fluorescence activated cell sorter with a laser using excitation at 488 nm (for detection by Flow cytometry).
2. PBS.
3. Calibrated adjustable precision pipettes, preferably with disposable plastic tips.
4. Tubes appropriate for holding cells during induction of apoptosis.
5. Microcentrifuge.
PROCEDURAL NOTES/LAB QUALITY CONTROL
1. When not in use, kit components should be stored refrigerated or frozen as indicated on vial or bottle label.
2. Cover or cap all reagents when not in use.
3. Do not mix or interchange different reagent lots from various kits.
4. Do not use reagents beyond the expiration date of the kit.

WARNINGS AND PRECAUTIONS
1. For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.
2. Never pipette by mouth.
3. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

ASSAY PROCEDURE
1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Dilute 10X Annexin-V binding Buffer 1:10 in distilled water.
3. Wash cells with PBS twice and resuspend cells at 2-3 x 10⁶ cells/mL in 1X Annexin-V Binding Buffer (containing 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂).
4. Aliquot cells at 100 µL/tube.
5. Add 5 µL of Annexin-V FITC and 10 µL of Propidium Iodide Buffer to each tube.
6. Incubate at room temperature for 15 minutes in the dark.
7. Add 400 µL 1X Annexin-V Binding Buffer to each tube.
8. Analyze the cells by flow cytometry within 1 hour of staining.

TYPICAL DATA
Jurkat cells were cultured with 2 µM camptothecin for 5 hours and stained by Annexin-V FITC. Apoptotic and non-apoptotic cells can be differentiated according to Annexin-V FITC binding (Figure, left). M1 represents non-apoptotic cells and M2 represents apoptotic cells. Staining with propidium iodide differentiates necrotic or late apoptotic cells. Propidium iodide stained cells are easily distinguished from early apoptotic population (Annexin-V positive and PI-negative) appearing in the lower right quadrant of the two color dot plot (Figure, right).
REFERENCES:


Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue Number</td>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>EUD</td>
<td>Research Use Only</td>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td></td>
<td>Use by</td>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>EC[REP]</td>
<td>European Community authorised representative</td>
<td></td>
</tr>
<tr>
<td>[-]</td>
<td>Without, does not contain</td>
<td>[+]</td>
<td>With, contains</td>
</tr>
<tr>
<td>®</td>
<td>Protect from light</td>
<td></td>
<td>Consult accompanying documents</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Directs the user to consult instructions for use (IFU), accompanying the product</td>
</tr>
</tbody>
</table>

Copyright © Invitrogen Corporation. 20 July 2010