

Product Information

Annexin V Conjugates

Cat. No.	Product Name	Unit Size
BGT-CHM-756	Annexin V, R-PE Conjugate	100 uL (20 tests)
		500 uL (100 tests)
BGT-CHM-757	Annexin V, APC Conjugate	100 uL (20 tests)
		500 uL (100 tests)

Storage and Handling

Store at 4°C, protected from light. Storage at -20°C is not recommended for R-PE/APC conjugates. Product is stable for at least 6 months from date of receipt when stored as recommended.

Product Description

Fluorescent conjugates of Annexin V can be used to label apoptotic cells. The human anticoagulant Annexin V is a 35-36 kD, Ca^{2+} -dependent phospholipid-binding protein with high affinity for phosphatidylserine (PS). In normal viable cells, PS is located on the inner leaflet of the cytoplasmic membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it is available for binding to fluorescently labeled Annexin V.

The fluorescent proteins R-phycoerythrin (R-PE) and allophycocyanin (APC) belong to the family of phycobiliproteins, which are derived from cyanobacteria and eukaryotic algae. R-PE is a 240 kDa protein with high extinction coefficient ($\sim 1,960,000 \text{ M}^{-1}\text{cm}^{-1}$) and high fluorescence quantum yield (~ 0.82). APC is a 105 kDa protein with high extinction coefficient ($240,000 \text{ M}^{-1}\text{cm}^{-1}$) and high quantum yield (0.68) in the far-red region. The APC in Annexin V-APC conjugates are chemically cross-linked. Annexin V R-PE and APC conjugates are designed for detection by flow cytometry.

Biotium offers a variety of Annexin V conjugates including those labeled with our outstanding series of CF® dyes for fluorescence microscopy or flow cytometry. Please visit www.biotium.com for details.

Staining Protocols

Staining can be performed in calcium-containing buffers such as HEPES-buffered saline containing 2.5 mM CaCl_2 . The optimal staining concentration for each conjugate should be determined empirically. The protocols provided below are intended to serve as general guidelines.

Note: Annexin V cannot be used to stain fixed cells or tissues. After staining with Annexin V and washing, cells can be fixed with 2% formaldehyde. Annexin V staining is calcium dependent, therefore 2.5 mM CaCl_2 should be included in all buffers used for washing and fixation. Annexin V binds to a phospholipid in the plasma membrane, therefore staining is not compatible with alcohol-based fixation or detergent permeabilization.

Suspension cells for flow cytometry

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Prepare 1X Annexin V Binding Buffer: HEPES-buffered saline containing 2.5 mM CaCl_2 . Wash cells with PBS once and resuspend cells at $2\text{-}3 \times 10^6$ cells/mL in 1X Binding Buffer.
3. Aliquot 100 uL cells per tube.
4. Add 5 uL Annexin V R-PE or APC conjugate to each tube and mix well.
5. Incubate at room temperature for 15 minutes, protected from light.
6. Add 400 uL 1X Binding Buffer to each tube. Analyze the cells by flow cytometry within 1 hour of staining.

Adherent cells for flow cytometry

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Prepare 1X Annexin V Binding Buffer: HEPES-buffered saline containing 2.5 mM CaCl_2 .
3. Wash cells with PBS twice and detach cells from cell culture plate or well by trypsin or cell dissociating buffer.
4. Pellet cells and discard supernatant. Resuspend cells at $2\text{-}3 \times 10^6$ cells/mL in 1X Binding Buffer.
5. Aliquot 100 uL cells per tube.
6. Add 5 uL Annexin V R-PE or APC conjugate to each tube and mix well.
7. Incubate at room temperature for 15 minutes, protected from light.
8. Add 400 uL 1X Binding Buffer to each tube. Analyze the cells by flow cytometry within 1 hour of staining.