

Product Information

Annexin V Conjugates

See [product page](#) for a full list of product names and catalog numbers.

Concentration

CF® Dye, Biotin, and FITC conjugates are supplied at 50 µg/mL in 10 mM Tris pH 7.5, 1 mM EDTA, 30 mM NaCl, 1 mg/mL BSA, <0.1% sodium azide.

R-PE and APC conjugates are supplied in PBS, 5 mg/mL BSA, 0.05% sodium azide (use 5 µL per test).

Spectral Properties

See [product page](#) for dye spectral properties including Ex/Em maxima. Please use the [Spectra Viewer](#) on our website to view and download individual dye spectra.

Storage and Handling

Store at 4°C and protect from light. DO NOT FREEZE. 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 kilodalton, Ca²⁺-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 actively translocated from the inner to the outer leaflet of the plasma membrane. Once exposed on the cell surface, PS can bind fluorescently labeled Annexin V conjugates for detection by fluorescence microscopy or flow cytometry.

Biotium offers a wide selection of Annexin V conjugates of CF® Dyes and other labels. Our outstanding series of CF® Dyes offers superior brightness, photostability, and signal-to-noise when compared to other commercially available fluorescent dyes.

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 (~1,960,000 M⁻¹cm⁻¹) and high fluorescence quantum yield (~0.82). APC is a 105 kDa protein with high extinction coefficient (240,000 M⁻¹cm⁻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.

Considerations for Staining

- We strongly recommend using our Annexin V Binding Buffer (Cat. No. 99902) with Annexin V conjugates. Staining can be performed in other calcium-containing buffers.
- Staining can be performed in culture medium with serum at 37°C without a wash step for confocal microscopy. Staining in culture medium generally results in broader fluorescence peaks in flow cytometry compared to staining in 1X Binding Buffer. For long-term live cell staining, we recommend our Azide-Free Lyophilized Annexin V Conjugates.
- The optimal staining concentration for each conjugate should be determined empirically. Typical recommended staining concentrations are 0.25 µg/mL to 2.5 µg/mL. Generally, a higher concentration of Annexin V is recommended for microscopy based assays and lower concentrations may be used for flow cytometry.
- Annexin V detection of apoptotic cells relies on the integrity of the plasma membrane and cannot be used in fixed cells or tissues.
- After staining with Annexin V and washing, cells can be fixed with 2% formaldehyde. All buffers used for washing and fixation should contain 2.5 mM CaCl₂. Staining with Annexin V is not compatible with alcohol-based fixation or detergent permeabilization.
- We recommend including the following control samples:
 1. Untreated cells, stained with Annexin V
 2. Cells induced to undergo apoptosis by a validated method, stained with Annexin V
 3. Unstained, untreated cells
 4. Unstained, apoptosis-induced cells
- The protocols provided below are intended to serve as general guidelines and may be modified for different experimental systems.

Staining suspension cells for flow cytometry or fluorescence microscopy

Note: R-PE is not recommended for fluorescence microscopy due to poor photostability.

1. Dilute 5X Annexin V Binding Buffer (Cat. No. 99902) 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl₂ can be used in place of 1X Binding Buffer.
2. Wash cells with PBS once and resuspend cells at 2-3x10⁶ cells/mL in 1X Binding Buffer.
3. Aliquot 100 µL cells per tube.

- Add Annexin V conjugate to tubes at a final concentration of 0.25-2.5 ug/mL. For R-PE or APC conjugates, use 5 uL per tube.
Note: The optimal staining concentration should be determined empirically.
- Incubate the mixture at room temperature for 15 minutes, protected from light.
- For flow cytometry analysis, add 400 uL of 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.
- For imaging, transfer the stained cells to a suitable plate, or place a drop of the suspension on a glass slide and coverslip.

Staining adherent cells for fluorescence microscopy

Note: R-PE is not recommended for fluorescence microscopy due to poor photostability.

- Dilute 5X Annexin V Binding Buffer (Cat. No. 99902) 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl₂ can be used in place of 1X Binding Buffer.
- Wash cells twice with 1X Binding Buffer.
- Prepare staining solution by diluting Annexin V conjugate in 1X Binding Buffer to a final concentration of 0.25-2.5 ug/mL. Prepare enough staining solution to completely submerge the cells.
Note: The optimal staining concentration should be determined empirically.
- Stain cells with the staining solution at room temperature for 15-30 minutes, protected from light.
- Wash cells with 1X Binding Buffer 1-2 times.
- Image cells in 1X Binding Buffer within 1 hour of staining.

Staining adherent cells for flow cytometry

- Dilute 5X Binding Buffer 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl₂ can be used in place of 1X Binding Buffer.
- Wash cells with PBS twice and detach cells from cell culture plate or well by trypsin or cell dissociating buffer.
- Pellet cells and discard supernatant. Resuspend cells at 2-3x10⁶ cells/mL in 1X Binding Buffer.
- Aliquot 100 uL cells per tube.
- Add Annexin V conjugate to tubes at a final concentration of 0.25-2.5 ug/mL. For R-PE or APC conjugates, we recommend using 5 uL per tube.
Note: The optimal staining concentration should be determined empirically.
- Incubate the mixture at room temperature for 15 minutes, protected from light.
- Add 400 uL of 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.

Related Products

| Cat. No. | Product |
|-----------------------------|--|
| 99902 | 5X Annexin V Binding Buffer |
| 29088-29089 | Recombinant Annexin V (Lyophilized) |
| 29004R-5ug... 29085R-5ug | Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized |
| 10405 | NucView® 405 Caspase-3 Substrate, 1 mM in DMSO |
| 10402 | NucView® 488 Caspase-3 Substrate, 1 mM in DMSO |
| 10403 | NucView® 488 Caspase-3 Substrate, 1 mM in PBS |
| 10406 | NucView® 530 Caspase-3 Substrate, 1 mM in DMSO |
| 30029 | NucView® 488 Caspase-3 Substrate Assay Kit for Live Cells |
| 30067 | Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®594 Annexin V |
| 30076 | Dual Apoptosis Assay Kit with NucView® 488 Caspase-3 Substrate & CF®640R Annexin V |
| 30062 | NucView® 488 and MitoView™ 633 Apoptosis Kit |
| 30072 | NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit |
| 30065 | Apoptosis & Necrosis Quantitation Kit Plus |
| 30066 | Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus |
| 30060 | CF®488A Annexin V and 7-AAD Apoptosis Kit |
| 30061 | CF®488A Annexin V and PI Apoptosis Kit |
| 30001 | JC-1 Mitochondrial Membrane Detection Kit |
| 70076 | Aquaphile™ JC-1 |
| 30063 | CF®488A TUNEL Assay Apoptosis Detection Kit |
| 30064 | CF®594 TUNEL Assay Apoptosis Detection Kit |
| 30074 | CF®640R TUNEL Assay Apoptosis Detection Kit |
| 80027 | PathoGreen™ Histofluorescent Stain |
| 32010 | Live-or-Dye NucFix™ Red Staining Kit |
| 32002-32009 | Live-or-Dye™ Fixable Viability Staining Kits |
| 10226 | Ac-DEVD-R110 |
| 30008 | Caspase-3 DEVD-R110 Fluorometric and Colorimetric Assay Kit |
| 30009 | Caspase-3 DEVD-R110 Fluorometric-HTS Assay Kit |

A full selection of CF® Dye labeled products including secondary antibodies, streptavidin and anti-biotin antibodies, antibody labeling kits, and other bioconjugates such as phalloidins, lectins, and α-bungarotoxins are also available. Please visit the Biotium website at www.biotium.com for details.

Biotium products are for research use only, and are not intended for food, drug, or household use. CF Dye technology is covered by U.S. and international patents.