

# Product Information

## Annexin V Conjugates

Catalog number	Product	Unit Size	Abs/Ems
29013	Annexin V, Biotin Conjugate	0.5 mL	N/A
29001	Annexin V, FITC Conjugate	0.5 mL	495/519 nm
29002	Annexin V, Sulforhodamine 101 Conjugate	0.5 mL	583/603 nm
29012	Annexin V, CF@350 Conjugate	0.5 mL	347/448 nm
29009	Annexin V, CF@405M Conjugate	0.5 mL	408/452 nm
29083	Annexin V, CF@450 Conjugate	0.5 mL	450/538 nm
29005	Annexin V, CF@488A Conjugate	0.5 mL	490/515 nm
29004	Annexin V, CF@555 Conjugate	0.5 mL	555/565 nm
29010	Annexin V, CF@568 Conjugate	0.5 mL	562/583 nm
29011	Annexin V, CF@594 Conjugate	0.5 mL	593/614 nm
29008	Annexin V, CF@633 Conjugate	0.5 mL	630/650 nm
29014	Annexin V, CF@640R Conjugate	0.5 mL	642/662 nm
29003	Annexin V, CF@647 Conjugate	0.5 mL	650/665 nm
29069	Annexin V, CF@660R Conjugate	0.5 mL	663/682 nm

### Format

50 ug/mL in 10 mM Tris, 1 mM EDTA, 30 mM NaCl, 1 mg/mL bovine serum albumin, 0.1% sodium azide, pH 7.5.

### Spectral Properties

See product table above for absorption/emission maxima. Please visit [www.biotium.com](http://www.biotium.com) to view 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 kD, Ca<sup>2+</sup>-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, which can be detected by fluorescence microscopy or flow cytometry.

Biotium offers a variety of Annexin V conjugates including those labeled with our outstanding series of CF@ dyes. CF@ dyes are superior to Alexa Fluor® dyes and Cy@ dyes for protein labeling, with combined advantages in brightness, photostability, and solubility. We also offer azide-free, lyophilized Annexin V conjugated to our visible and near-infrared CF@ dyes, suitable for live cell and *in vivo* imaging. Please visit [www.biotium.com](http://www.biotium.com) to learn more.

## Staining Protocols

### Considerations for Annexin V staining

We recommend using our Annexin V Binding Buffer (Catalog no. 99902) with Annexin V conjugates. Staining can be performed in other buffers containing up to 2.5 mM calcium. We also have performed successful staining of cells in culture medium with serum at 37°C without a wash step. Different culture media vary in calcium concentration, which may require optimization for Annexin V binding. Note that buffers with phosphate or serum-free media may be harmful to cells if calcium is added. For long term cell staining, we recommend our azide-free lyophilized CF@ Dye Annexin V Conjugates (see Related Products).

The optimal staining concentration for each conjugate or staining buffer should be determined empirically. Typical staining concentrations range from 0.25 ug/mL to 2.5 ug/mL. Generally, a higher concentration of Annexin V is recommended for microscopy based assays and lower concentrations may be used for flow cytometry. 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<sub>2</sub> 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.

### Staining suspension cells for flow cytometry or fluorescence microscopy

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Wash cells with PBS once and resuspend cells at 2-3x10<sup>6</sup> cells/mL in 1X Annexin Binding Buffer or other calcium-containing buffer.
3. Aliquot 100 uL cells per tube.
4. Add Annexin V conjugate to tubes at a final concentration of 0.25-2.5 ug/mL. Note: the optimal staining concentration should be determined empirically.
5. Incubate at room temperature for 15 minutes, protected from light.
6. For flow cytometry analysis, add 400 uL binding buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.
7. For fluorescence microscopy analysis, wash cells with binding buffer and place cell suspension on a glass slide and coverslip or transfer to a dish or chamber slide for imaging.

### Staining of adherent cells for fluorescence microscopy

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Wash cells twice with 1X Annexin Binding Buffer or other calcium-containing buffer.
3. Prepare staining solution by diluting Annexin V conjugate in binding buffer to a final concentration of 0.25-2.5 ug/mL. Note: The optimal staining concentration should be determined empirically. Prepare enough staining solution to completely submerge cells.
4. Stain cells with the staining solution at room temperature for 15-30 minutes, protected from light.
5. Wash cells with 1X binding buffer 1-2 times.
6. Image cells in 1X binding buffer within 1 hour of staining.

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## Staining Protocols (continued)

### Adherent cells for flow cytometry

1. Induce apoptosis in cells by a desired method.
2. Wash cells with PBS twice and detach cells from cell culture plate or well by trypsin or cell dissociating buffer.
3. Pellet cells and discard supernatant. Resuspend cells at  $2-3 \times 10^6$  cells/mL in 1X Annexin Binding Buffer or other calcium-containing buffer.
4. Aliquot 100  $\mu$ L cells per tube.
5. Add Annexin V conjugate to tubes at a final concentration of 0.25-2.5  $\mu$ g/mL. Note: The optimal staining concentration should be determined empirically.
6. Incubate at room temperature for 15 minutes, protected from light.
7. Add 400  $\mu$ L of the binding buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.

## Related Products

Catalog number	Product
99902	5X Annexin V Binding Buffer
29009R-5ug	Annexin V, CF@405M Conjugate, Azide-Free and Lyophilized
29005R-5ug	Annexin V, CF@488A Conjugate, Azide-Free and Lyophilized
29010R-5ug	Annexin V, CF@568 Conjugate, Azide-Free and Lyophilized
29011R-5ug	Annexin V, CF@594 Conjugate, Azide-Free and Lyophilized
29014R-5ug	Annexin V, CF@640R Conjugate, Azide-Free and Lyophilized
29007	Annexin V, CF@680 Conjugate, Azide-Free and Lyophilized
29046	Annexin V, CF@770 Conjugate, Azide-Free and Lyophilized
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10407	NucView® 405 Caspase-3 Substrate, 1 mM in PBS
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
30067	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate & CF@594 Annexin V
30076	Dual Apoptosis Assay 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
40061	RedDot™2 Far-Red Nuclear Stain (for dead or fixed cells)
40083	NucSpot® 470 Green Nuclear Stain (for dead or fixed cells)
40017	Propidium Iodide, 1 mg/mL in water (dead cell stain)
40051	Ethidium Homodimer III 1 mM in DMSO (dead cell stain)
40084	7-AAD Solution, 1 mg/mL (dead cell stain)
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
30063	CF@488A TUNEL Assay Apoptosis Detection Kit
30064	CF@594 TUNEL Assay Apoptosis Detection Kit
30074	CF@640R TUNEL Assay Apoptosis Detection Kit
32010	Live-or-Dye NucFix™ Red Staining Kit
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits

Visit [www.biotium.com](http://www.biotium.com) to see our full selection of CF® dye labeled products, including azide-free Annexin V conjugates, near-infrared Annexin V conjugates, secondary antibodies, streptavidin and anti-biotin antibodies, antibody labeling kits, and other bioconjugates such as phalloidins, lectins, and  $\alpha$ -bungarotoxins.

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. Alexa Fluor is a registered trademark of Thermo Fisher Scientific. Cy Dye is a registered trademark of GE Healthcare.

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444

Email: [customerservice@lifetechindia.com](mailto:customerservice@lifetechindia.com) Website: [www.lifetechindia.com](http://www.lifetechindia.com)