

# Product Information

## Annexin V Conjugates

Catalog no.	Conjugate	Unit Size	Ex/Em (nm)
29013	Annexin V, Biotin Conjugate	0.5 mL	N/A
29001	Annexin V, FITC Conjugate	0.5 mL	495/519
29012	Annexin V, CF@350 Conjugate	0.5 mL	347/448
29009	Annexin V, CF@405M Conjugate	0.5 mL	408/452
29083	Annexin V, CF@450 Conjugate	0.5 mL	450/538
29005	Annexin V, CF@488A Conjugate	0.5 mL	490/515
29004	Annexin V, CF@555 Conjugate	0.5 mL	555/565
29010	Annexin V, CF@568 Conjugate	0.5 mL	562/583
29085	Annexin V, CF@583R Conjugate	0.5 mL	586/609
29011	Annexin V, CF@594 Conjugate	0.5 mL	593/614
29008	Annexin V, CF@633 Conjugate	0.5 mL	630/650
29014	Annexin V, CF@640R Conjugate	0.5 mL	642/662
29003	Annexin V, CF@647 Conjugate	0.5 mL	650/665
29069	Annexin V, CF@660R Conjugate	0.5 mL	663/682
29007	Annexin V, CF@680 Conjugate, lyophilized solid	25 ug	681/698
29070	Annexin V, CF@680R Conjugate, lyophilized solid	25 ug	680/701
29082	Annexin V, CF@700 Conjugate, lyophilized solid	25 ug	699/737
29006	Annexin V, CF@750 Conjugate, lyophilized solid	25 ug	755/777
29046	Annexin V, CF@770 Conjugate, lyophilized solid	25 ug	770/797
29047	Annexin V, CF@790 Conjugate, lyophilized solid	25 ug	784/806
29078	Annexin V, CF@800 Conjugate, lyophilized solid	25 ug	797/816

### Form

Liquid 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.

Lyophilized solids: preservative-free lyophilized solid. To reconstitute, dissolve in 0.5 mL of PBS or other buffer of your choice to the vial to obtain a 50 ug/mL solution. The solution can be sterilized by 0.2 um filtration.

### 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 kilodalton, 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 by having combined advantages in brightness, photostability, specificity and novel features ideal for in vivo imaging. Please visit [www.biotium.com](http://www.biotium.com) for details.

### Staining Protocols

We recommend using our Annexin V Binding Buffer (Catalog no. 99902) with Annexin V conjugates. Staining can be performed in other calcium-containing buffers. We also have performed successful staining of cells in culture medium with serum at 37°C without a wash step. 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.

### Suspension cells for flow cytometry or fluorescence microscopy

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Dilute 5X Annexin V Binding Buffer (Catalog number 99902) 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl<sub>2</sub> can be used in place of 1X Binding Buffer.
3. Wash cells with PBS once and resuspend cells at 2-3x10<sup>6</sup> cells/mL in 1X Binding Buffer.
4. Aliquot 100 uL cells per tube.
5. 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.
6. Incubate at room temperature for 15 minutes, protected from light.
7. For flow cytometry analysis, add 400 uL 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 1 hour of staining.
8. For fluorescence microscopy analysis, wash cells with 1X Binding Buffer and place cell suspension on a glass slide and coverslip or transfer to a dish or chamber slide for imaging.

### Adherent cells for fluorescence microscopy

1. Induce apoptosis in cells by desired method. Include a control sample of untreated cells.
2. Dilute 5X Annexin V Binding Buffer (Catalog number 99902) 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl<sub>2</sub> can be used in place of 1X Binding Buffer.

### Staining Protocols (continued)

3. Wash cells twice with 1X Binding Buffer.
4. Prepare staining solution by diluting Annexin V conjugate in 1X 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.
5. Stain cells with the staining solution at room temperature for 15-30 minutes, protected from light.
6. Wash cells with 1X Binding Buffer 1-2 times.
7. Image cells in 1X Binding Buffer within 1 hour of staining.

### Adherent cells for flow cytometry

1. Induce apoptosis in cells by a desired method.
2. Dilute 5X Binding Buffer 1:5 in distilled water to obtain 1X Binding Buffer. HEPES-buffered saline containing 2.5 mM CaCl<sub>2</sub> can be used in place of 1X Binding Buffer.
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 2-3x10<sup>6</sup> cells/mL in 1X Binding Buffer.
5. Aliquot 100 uL cells per tube.
6. 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.
7. Incubate at room temperature for 15 minutes, protected from light.
8. Add 400 uL 1X 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
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
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
22020	10X Phosphate-Buffered Saline (PBS)

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 a-bungarotoxins are also available. Please visit the Biotium website at [www.biotium.com](http://www.biotium.com) for details.

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