

Product Information

Annexin V, Fluorescent Protein Conjugates

Volume per assay: 5 uL

Technical Summary

Cat. No.	Unit Size	Fluorescent protein	Abs _{max} (nm)	Em _{max} (nm)
29045-100uL	100uL	R-PE	496,546, 565	578
29045-500uL	500uL	R-PE	496,546, 565	578
29057-100uL	100uL	APC	633, 640	660
29057-500uL	500uL	APC	633, 640	660

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

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 translocated from the inner to the outer leaflet of the plasma membrane, where it can be detected by a fluorescently labeled annexin V. We recommend using our Annexin V Binding Buffer (Cat # 99902) with these conjugates.

The red fluorescent protein 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 AnnexinV-APC conjugates are chemically cross-linked.

Staining Protocols

We recommend using our Annexin V Binding Buffer (Cat # 99902) with Annexin V conjugates. HEPES-buffered saline containing 2.5 mM CaCl₂ can be used in place of 1X Binding Buffer. The optimal staining concentration for each conjugate 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 are recommended for microscopy based assays and lower concentrations may be used for flow cytometry. Protocols are provided below as general guidelines. Note: Annexin V cannot be used to stain fixed cells or tissues. After staining with Annexin V, cells may be fixed with 2% formaldehyde. Annexin V staining is calcium dependent, therefore 2.5 mM CaCl₂ 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

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 CaCl₂ can be used in place of 1X Binding Buffer.
3. Wash cells with PBS once and resuspend cells at 2-3x10⁶ cells /mL in 1X

Binding Buffer.

4. Aliquot 100 uL cells per tube.
5. Add 5 uL R-PE or APC Annexin V conjugate to each tube and mix gently.
6. Incubate at room temperature for 15 minutes, protected from light.
7. 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.
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 CaCl₂ can be used in place of 1X Binding Buffer.
3. Wash cells twice with 1X Binding Buffer.
4. Prepare staining solution by diluting R-PE or APC Annexin V conjugate 1:20 in 1X binding buffer. 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 desired method. Include a control sample of untreated cells.
2. 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.
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 2-3x10⁶ cells /mL in 1X Binding Buffer.
5. Aliquot 100 uL cells per tube.
6. Add 5 uL R-PE or APC Annexin V conjugate to each tube and mix gently.
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.

Other Related Products

You also may be interested in the following related products:

Annexin V, CF™ Dye Conjugates

Catalog No.	Product Description	Unit Size
29012	Annexin V, CF350 Conjugate	0.5 mL
29009	Annexin V, CF405M Conjugate	0.5 mL
29005	Annexin V, CF488A Conjugate	0.5 mL
29004	Annexin V, CF555 Conjugate	0.5 mL
29010	Annexin V, CF568 Conjugate	0.5 mL
29011	Annexin V, CF594 Conjugate	0.5 mL
29008	Annexin V, CF633 Conjugate	0.5 mL
29014	Annexin V, CF640R Conjugate	0.5 mL
29003	Annexin V, CF647 Conjugate	0.5 mL
29007	Annexin V, CF680 Conjugate	25 ug
29006	Annexin V, CF750 Conjugate	25 ug
29047	Annexin V, CF790 Conjugate	25 ug

Related Products

Catalog No.	Product Description	Unit Size
99902	5X Annexin Binding Buffer	15 mL
30065	Apoptosis & Necrosis Quantitation Kit Plus	50 assays
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit	50 assays
30061	CF™488A Annexin V and PI Apoptosis Kit	50 assays
30029	NucView™488 Caspase-3 Assay Kit for Live Cells	50 assays
30067	NucView™488 Caspase-3 Substrate and CF™594 Annexin V Dual Apoptosis Assay Kit	50 assays
29013	Annexin V, Biotin Conjugate, 50 ug/mL	0.5 mL
30062	NucView™488 and MitoView™633 Apoptosis Kit	50 assays
30001	JC-1 Mitochondrial Membrane Detection Kit	100 assays
30063	CF™488A TUNEL Assay Apoptosis Detection Kit	50 reactions
30064	CF™594 TUNEL Assay Apoptosis Detection Kit	50 reactions

Other R-PE Bioconjugates

Catalog No.	Product Description	Unit Size
29044-200uL	Streptavidin, R-PE Conjugate	200 uL
29044-1mL	Streptavidin, R-PE Conjugate	1 mL
20357-200uL	R-PE goat anti-mouse IgG (H+L), F(ab')2 fragment	200 uL
20357-500uL	R-PE goat anti-mouse IgG (H+L), F(ab')2 fragment	500 uL
20358-200uL	R-PE goat anti-rabbit IgG (H+L), F(ab')2 fragment	200 uL
20358-500uL	R-PE goat anti-rabbit IgG (H+L), F(ab')2 fragment	500 uL
20355-200uL	R-PE goat anti-human IgG (H+L), highly cross-adsorbed	200 uL
20355-1mL	R-PE goat anti-human IgG(H+L), highly cross-adsorbed	1 mL
20352-200uL	R-PE Goat anti-mouse IgG (H+L)	200 uL
20352-1mL	R-PE Goat anti-mouse IgG (H+L)	1 mL
20353-200uL	R-PE Goat anti-rabbit IgG (H+L)	200 uL
20353-1mL	R-PE Goat anti-rabbit IgG (H+L)	1 mL
20354-200uL	R-PE goat anti-rat IgG (H+L), highly cross-adsorbed	200 uL
20354-1mL	R-PE goat anti-rat IgG (H+L), highly cross-adsorbed	1 mL

Biotium offers a wide selection of fluorescent dye labeled products including secondary antibodies, antibody labeling kits, and other bioconjugates, featuring our proprietary line of exceptionally bright and photostable CF™ dyes. Please visit our website at www.biotium.com for details.

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Other APC Bioconjugates

Catalog No.	Product Description	Unit Size
20411-100uL	Goat Anti-Mouse IgG (H+L)	100uL
20411-500uL	Goat Anti-Mouse IgG (H+L)	500uL
20412-100uL	Goat Anti-Rabbit IgG (H+L)	100uL
20412-500uL	Goat Anti-Rabbit IgG (H+L)	500uL
20413-100uL	Goat Anti-Rat IgG (H+L), highly cross-adsorbed Min X Hu, Bv, Hs and Rb	100uL
20413-500uL	Goat Anti-Rat IgG (H+L), highly cross-adsorbed Min X Hu, Bv, Hs and Rb	500uL
20414-100uL	F(ab')2 fragment of Goat anti-Mouse IgG (H+L)	100uL
20414-500uL	F(ab')2 fragment of Goat anti-Mouse IgG (H+L)	500uL
20415-100uL	F(ab')2 fragment Goat Anti-Rabbit IgG (H+L)	100uL
20415-500uL	F(ab')2 fragment Goat Anti-Rabbit IgG (H+L)	500uL
29048-200uL	Streptavidin	200uL
29048-1mL	Streptavidin	1mL

Bv: bovine; Ch: chicken; Gt: goat; GP: Guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat