DATA SHEET

Product Information

Annexin V, Fluorescent Dye Conjugates

Catalog No.	Product Description	Unit Size
29001	Annexin V, FITC Conjugate	0.5 mL
29002	Annexin V, Sulforhodamine 101 Conjugate	0.5 mL
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

Concentration: 50 ug/mL, except for CF680 and CF750 (25 ug).

Form: Solution, except for CF680 Annexin V and CF750 Annexin V.
CF680 Annexin V and CF750 Annexin V: lyophilized solid. To reconstitute, add 0.5 mL of ultra-pure water to the vial to obtain a 50 ug/mL solution.

Spectral Properties

$\lambda_{abs}/\lambda_{em}$ (in pH 7.4 PBS buffer):

Product Description	Abs_{max} nm	Em_{max} nm
Annexin V, FITC Conjugate	495	519
Annexin V, Sulforhodamine 101 Conjugate	583	603
Annexin V, CF350 Conjugate	347	448
Annexin V, CF405M Conjugate	408	452
Annexin V, CF488A Conjugate	490	515
Annexin V, CF555 Conjugate	555	565
Annexin V, CF568 Conjugate	562	583
Annexin V, CF594 Conjugate	593	614
Annexin V, CF633 Conjugate	630	650
Annexin V, CF640R Conjugate	642	662
Annexin V, CF647 Conjugate	650	665
Annexin V, CF680 Conjugate	663	682
Annexin V, CF750 Conjugate	755	777

Please visit 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, 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 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 antibody labeling by having combined advantages in brightness, photostability, specificity and novel features ideal for in vivo imaging. Please visit www.biotium.com for details.

Staining Protocols

We recommend using our Annexin V Binding Buffer with Annexin V conjugates (Cat # 99902). HEPES-buffered saline containing 2.5 mM $CaCl_2$ 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 $CaCl_2$ 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

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 $CaCl_2$ can be used in place of 1X Binding Buffer.
3. Wash cells with PBS once and resuspend cells at 2-3x10^6 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 $CaCl_2$ can be used in place of 1X Binding Buffer.

Continued next page

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 $CaCl_2$ 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 2-3x10^6 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 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	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