Product Information

CF®488A Annexin V and PI Apoptosis Kit

Catalog Number: 30061

Unit Size: 100 assays

Kit Contents

Component	Size
99902: 5X Annexin V Binding Buffer	3 x 15 mL
99946: CF®488A Annexin V	1 x 500 uL
99948: Propidium Iodide (PI)	1 x 20 uL

Storage and Handling

Storage at 4°C, protected from light. Do not freeze. Product is stable for at least 6 months from date of receipt when stored as recommended. PI binds nucleic acids, handle with universal laboratory safety precautions.

Spectral Properties

CF®488A Annexin V Ex/Em: 490/515 nm
PI Ex/Em: 530/622 nm (with DNA)

Product Description

The CF®488A and PI Apoptosis Kit provides a convenient method for quantifying apoptotic (green) and necrotic (red) cells within the same cell population by flow cytometry or fluorescence microscopy.

Fluorescent conjugates of Annexin V can be used to label apoptotic cells. The human anticoagulant Annexin V is a 35-36 kilodalton, Ca^{2+}-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. Our CF®488A dye is superior to fluorescein/FITC as it is brighter, not affected by pH, and has much better photostability.

Propidium iodide (PI) is a membrane-impermeant DNA-binding dye that is commonly used to selectively stain dead cells in a cell population. PI is excluded from live cells and early apoptotic cells, but stains necrotic and late apoptotic cells with compromised membrane integrity. PI can be excited by the 488, 532, or 546 nm laser lines, and emits red fluorescence.

Biotium also offers the CF®488A Annexin V and 7-AAD Apoptosis Kit, which works by the same assay principle, but features the far-red nucleic acid dye 7-AAD instead of PI (see related products).

General Assay Considerations

1. These protocols were optimized using Jurkat cells treated with staurosporine to induce apoptosis. Assay optimization may be required for use with other inducing agents or other cell types.
2. Annexin V requires calcium for binding; usually concentrations near 2.5 mM are used. Annexin V Binding Buffer contains calcium.
3. If you prefer not to wash your cells, staining can be performed in cell culture medium with serum instead of Annexin V Binding Buffer, but the concentration of Annexin V may require optimization.

Assay Protocols

Staining protocol for flow cytometry

1. Prepare a positive control by inducing apoptosis in your cells by the desired method. Include an untreated cell sample as a negative control. Also include samples for single-stained controls if compensation is required.
2. Dilute 5X Annexin V Binding Buffer 1:5 with distilled water. Prepare approximately 1 mL of 1X Binding Buffer for each sample to be stained.
3. Harvest cells after treatment by centrifugation and wash with PBS.
4. Centrifuge cells again, discard supernatant and resuspend cells at $5x10^6$ to 10^7 cells per mL in 1X Binding Buffer.
5. Aliquot cells into flow cytometry tubes at 100 uL/tube.
6. Prepare a working solution of PI by diluting 1:10 in 1X Binding Buffer.
7. Add 5 uL of CF®488A Annexin V and 1-2 uL of PI working solution to each tube. Also prepare single stained compensation controls.
8. Incubate at room temperature for 15-30 minutes in the dark. The incubation can be carried out on ice to arrest the apoptotic process if desired.
9. Add 400 uL 1X Binding Buffer to each tube and analyze the cells by flow cytometry within 30 minutes of staining.
10. Detect CF®488A Annexin V in the FITC channel, and PI in the PE-Texas Red® channel.

Staining protocol for fluorescence microscopy

Note: For cells in suspension, follow the staining protocol for flow cytometry.

1. Grow cells on coverslips or chamber slides.
1. Prepare a positive control by inducing apoptosis in cells by your desired method. Include an untreated cell sample as a negative control.
2. Wash cells with PBS.
3. Dilute 5X Annexin V Binding Buffer 1:5 with distilled water.
4. Prepare a working solution of PI by diluting 1:10 in 1X Binding Buffer.
5. Add 5-25 uL of CF®488A Annexin V and 1-2 uL of PI working solution for every 100 uL 1X Binding Buffer required for staining. Note: The optimal concentration may need to be determined empirically.
6. Add enough staining solution to completely cover the cells, and incubate at room temperature for 15-30 minutes in the dark. Incubation can be carried out on ice to arrest the apoptotic process if desired, but in that case staining time should be at least 30 min.
7. Wash cells with 1X Binding Buffer.
8. Mount coverslips onto slides with a drop of 1X Binding Buffer. For cells on chamber slides, add enough 1X Binding Buffer to completely cover cells.
9. Image using appropriate filters. CF®488A Annexin V can be imaged using FITC settings, while PI can be imaged using Cy®3 or Texas Red® settings.

Related Products

Catalog number	Product
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30065	Apoptosis and Necrosis Quantitation Kit Plus
99902	5X Annexin V Binding Buffer
29003-29085	Annexin V CF® Dye Conjugates
29006-29082	Annexin V Near IR CF® Dye Conjugates
29003R-29085R	Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized
30072	NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
70055	MitoView™ 633
32002-32013	Live-or-Dye™ Fixable Viability Staining Kits
32010	Live-or-Dye™ NucFix Red Staining Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay
30068	ViaFluor® SE 405 Cell Proliferation Kit
30086	ViaFluor® SE 488 Cell Proliferation Kit
30050	ViaFluor® SE CFSE Cell Proliferation Kit

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.