Biotium
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Product Information

CF™488A Annexin V and PI Apoptosis Kit

Catalog Number: 30061

Unit Size: 100 flow cytometry assays. Assay number may vary by application.

Kit Contents

Component	Size
99902: 5X Annexin V Binding Buffer	3 bottles (15 mL)
99946: CF™488A Annexin V	1 vial (500 uL)
99948: Propidium Iodide (PI)	1 vial (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. 7-AAD binds nucleic acids, handle with universal laboratory safety precautions.

Spectral Properties

CF™488A Annexin V: Abs/Em = 490/515 nm
PI: Abs/Em = 535/617 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 by 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 a similar mechanism, but features far-red fluorescent staining of necrotic cells with 7-AAD nucleic acid dye (see related products). PI and Annexin V conjugates are also available separately (visit www.biotium.com for more information).

References

1. Boersma A.W., et al. Quantification of apoptotic cells with fluorescein isothiocyanate-labeled annexin V in chinese hamster ovary cell cultures treated with cisplatin. Cytometry. 1996 Jun 1;24(2):123-30.
2. Homburg C.H., et al. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood. 1995 Jan 15;85(2):532-40.
3. Martin S.J., et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med. 1995 Nov 1;182(5):1545-56.
4. Vermes I., et al. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods. 1995 Jul 17;184(1):39-51.

Assay Protocols

These protocols were optimized using Jurkat cells treated with staurosporine to induce apoptosis. Additional assay optimization may be required for use with other inducing agents or other cell types.

Staining protocol for flow cytometry

1. Induce apoptosis in cells by your desired method. Include an untreated cell sample as a negative control. Also include samples for single-stained controls if compensation is required.
2. Harvest cells after treatment by centrifugation and wash with PBS.

   Note: If you prefer not to wash your cells, staining can be performed in cell culture medium with serum instead of Annexin Binding Buffer, but the concentration of Annexin V may require optimization.
3. Dilute 5X Annexin Binding Buffer 1:5 with distilled water. Prepare approximately 1 mL of 1X Annexin Binding Buffer for each sample to be stained.
4. Prepare a working solution of PI by diluting 1:10 with 1X Binding Buffer.
5. Centrifuge cells again, discard supernatant and resuspend cells at $5x10^6$ to 10^7 cellls per mL in 1X Binding Buffer. See note under step 2 above.
6. Aliquot cells into flow cytometry tubes at 100 uL/tube.
7. Add 5 uL of CF488A-Annexin V and 1-2 uL of PI working solution to each tube. Note: We recommend you set up two additional tubes, one for each of the dyes alone (CF488A-Annexin V and PI) to use as 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. Use 488 nm excitation and measure fluorescence emission near 530 nm (FITC channel) and 617 nm (PE or PI channel). If desired, staining can be confirmed by fluorescence microscopy using appropriate filters. Mount 5 uL of cell suspension on a slide with an 18 mm^2 coverslip for imaging.

Staining protocol for fluorescence microscopy

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

1. Grow cells on coverslips or chamber slides.
1. Induce apoptosis in cells by your desired method. Include an untreated cell sample as a negative control.
2. Wash cells with PBS.

   Note: If you prefer to not wash your cells, staining and imaging can be performed in cell culture medium with serum instead of Annexin Binding Buffer, but the concentration of Annexin V may require optimization.
3. Dilute 5X 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 CF488A-Annexin V and 1-2 uL of PI working solution for every 100 uL Annexin 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 staining time should be at least 30 min.
7. Wash cells with 1X Binding Buffer. See note under step 2 above.
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. See note under step 2 above.
9. Image using appropriate filters. CF488A Annexin V can be imaged using FITC settings, while PI can be imaged using Cy®3 or Texas Red® settings.

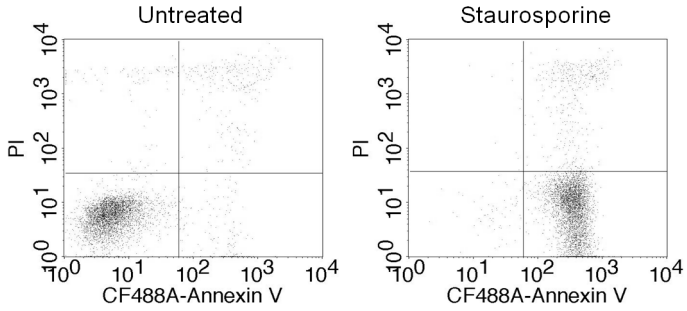

Figure 1: Jurkat cells were left untreated (left) or treated with 1 uM staurosporine (right) for 5 hours. Cells were then stained with the kit reagents and analyzed by flow cytometry using 488 nm argon laser excitation and 530/30 nm and 585/42 nm emission filters. The dot plot shows three distinct populations: viable cells which have low CF488A-Annexin V and low PI signal; apoptotic cells that have high CF488A-Annexinv V and low PI signal; and late stage apoptotic/secondary necrotic cells with compromised membranes exhibiting high CF488A-Annexin V and high PI signal. In some cases, a fourth population corresponding to damaged viable cells with low CF488A-Annexin V and high PI signal may be observed. The staurosporine treated cells exhibit a higher percentage of apoptotic cells compared to untreated cells.

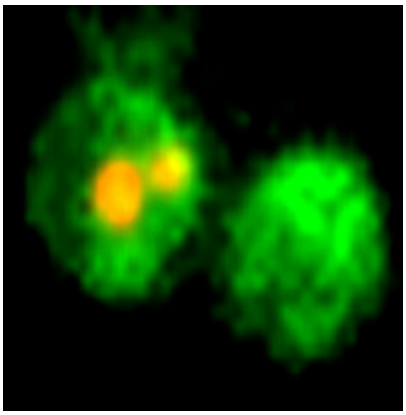

Figure 2: Jurkat cells were induced with 1 uM staurosporine for 5 hours and stained with CF488A-Annexin V and PI according to the protocol. Cells were placed on a glass slide and imaged by epifluorescence microscopy. Images were captured on a CCD camera. The cell on the right is representative of an apoptotic cell with only CF488A-Annexin V staining (green plasma membrane), while the cell on the left is a late stage apoptotic/secondary necrotic cell with both CF488A-Annexin V and PI staining (green membrane with red fragmented nucleus).

Related Products

Catalog number	Product
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits (choose from 8 fluorescent dye colors)
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30067	Dual Apoptosis Assay with NucView™ 488 Caspase-3 Substrate and CF™594 Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
10405	NucView™ 405 Caspase-3 Substrate
30026	Calcein AM Cell Viability Assay Kit
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay Kit
30068	ViaFluor™ 405-SE Cell Proliferation Kit
30050	CFDA SE Cell Proliferation Assay Kit
70055	MitoView™ 633
70052	MitoView™ Blue
30001	JC-1 Mitochondrial Membrane Potential Detection Kit
30019	MCB Glutathione Detection Kit

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly GelRed™ and GelGreen™ nucleic acid gel stains, EvaGreen® qPCR master mixes, fluorescent CF™dye antibody conjugates, Mix-n-Stain™ rapid antibody labeling kits, apoptosis detection reagents, and many more fluorescent probes and kits for cell biology research.