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Apoptosis Assay Kit
CF488A-Annexin V and 7-AAD (7-aminoactinomycin D)

Catalog Number: 30060 (100 assays)

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Description

Apoptosis and necrosis are two major processes by which cells die. Apoptosis is an active, genetically regulated disassembly of the cell from within. Disassembly creates changes in the phospholipid content of the cytoplasmic membrane outer leaflet. Phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell for phagocytic cell recognition. The human anticoagulant, annexin V, is a 35 kD Ca^{2+}-dependent phospholipid protein with a high affinity for PS. Annexin V labeled with CF488A ($\lambda_{abs}/\lambda_{em}$= 490/ 515 nm) can identify apoptotic cells in green by binding to PS exposed on the outer leaflet. Our CF488A dye is superior to fluorescein/FITC as it is not affected by pH and has much better photostability.

Necrosis normally results from a severe cellular insult. Both internal organelle and plasma membrane integrity are lost, resulting in spilling of cytosolic and organellar contents into the surrounding environment. Using a viability dye such as 7-AAD allows the distinction between early apoptotic, late apoptotic and necrotic cells. Moreover, 7-AAD emits in the far-red spectrum and can be well separated from CF488A and other green emitting dyes using the 488nm line of a flow cytometer. 7-AAD is impermeant to live cells or apoptotic cells, but stains necrotic cells with red fluorescence ($\lambda_{abs}/\lambda_{em}$ with DNA = 543/655 nm). 7-AAD is an alternative to propidium iodide (PI) or ethidium homodimer I used in other kits.

Kit Contents

CF488A-Annexin V, one vial, 500 uL in TE buffer containing 0.1% BSA and 0.1% NaN3, pH 7.5
7-AAD, one vial, 20 uL in DMSO
5X Binding Buffer, three bottles, 15 mL each

Caution: Sodium azide and 7-AAD are hazardous substances. Handle with care and dispose properly.

Storage Conditions

Store the kit at 4^{o}C and protected from light. Do not freeze!
The components of the kit should be stable for at least 6 months.

Experimental Protocols

The CF488A/7-AAD Apoptosis Assay Kit provides a convenient method for quantifying apoptotic (green) and necrotic (red) cells within the same cell population by flow cytometry or can be adapted for fluorescence microscopy. The assay has been optimized using Jurkat cells treated with staurosporine to induce apoptosis. Some additional optimization may be required for studies with other inducing agents or other cell types.

Flow Cytometry

1. Induce apoptosis in cells by a desired method. Untreated cells are also recommended as a negative control.
2. Harvest cells after treatment by centrifugation and wash cells with PBS.
3. Dilute 5X Binding Buffer 1:5 with distilled water. Make approximately 1 mL per sample to be stained.
4. Prepare a working solution of 7-AAD by diluting 1:10 with 1X Binding Buffer.

5. Centrifuge cells again, discard supernatant and resuspend cells at 5-10 x 10^6 cells /mL in 1X Binding Buffer.
6. Aliquot cells at 100 uL/tube.
7. Add 5 uL of CF488A-Annexin V and 1 - 2 uL of 7-AAD working solution to each tube. ***Note:*** *We recommend you set up two additional tubes, one for each of the staining dyes (CF488A-Annexin V and 7-AAD) as single stained controls if compensation is desired.*
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 the fluorescence emission using 530 nm and >670 nm (or close alternatives). The population should separate into distinctive populations. If desired, the staining can be confirmed by fluorescence microscopy using appropriate filters.

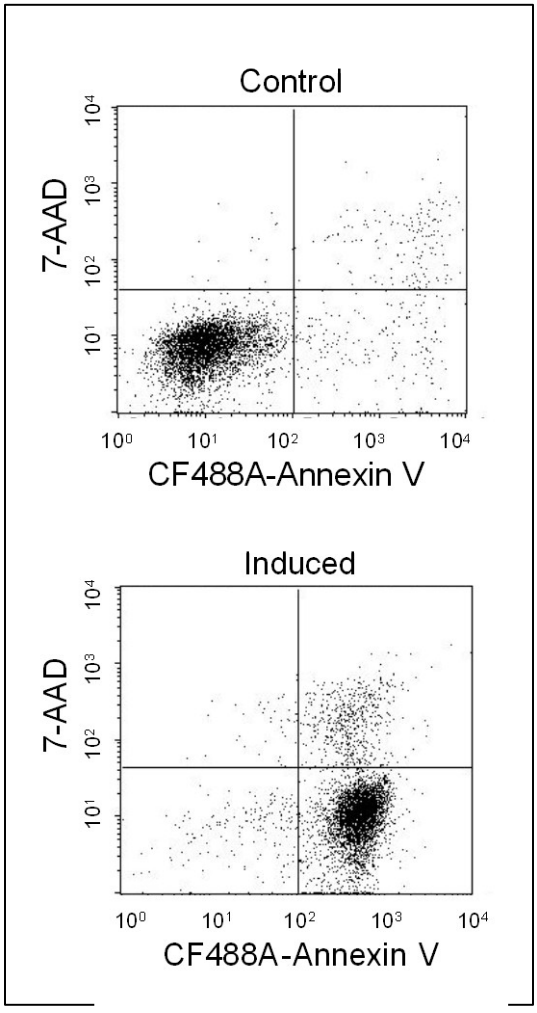

Figure 1: Jurkat cells were left untreated (Control) or treated (Induced) with 1 uM staurosporine for 5 hours. Cells were then stained with the kit reagents and analyzed by flow cytometry using 488 nm argon laser excitation and fluorescence was detected using 530/30 and 670 nm LP emission. The dot plot shows three distinct populations: a) the viable cells which have low CF488A-Annexin V and low 7-AAD signal, b) the apoptotic cells which have high CF488A-Annexinv V and low 7-AAD signal, and c) late stage apoptotic/secondary necrotic cells with compromised membranes exhibiting high CF488A-Annexin V and high 7-AAD signal. In some cases, a fourth population corresponding to damaged viable cells with low CF488A-Annexin V and high 7-AAD signal may be observed. The staurosporine treated cells (Induced) exhibit a higher percentage of apoptotic cells than that seen in the untreated (Control) cells.

Microscopy

1. Induce apoptosis in cells by a desired method. Untreated cells are also recommended as a negative control.
2. Harvest cells after treatment by centrifugation and wash cells with PBS.
3. Dilute 5X Binding Buffer 1:5 with distilled water. Make approximately 1 mL per sample to be stained.Dilute 5X Binding Buffer 1:5 with distilled water.
4. Prepare a working solution of 7-AAD by diluting 1:10 in 1X Binding Buffer.
5. Wash cells with PBS once and resuspend cells at 5-10 x 10^6 cells /mL in 1X Binding Buffer.
6. Aliquot cells at 100 uL/tube.
7. Add 5-25 uL of FITC-Annexin V and 1-2 uL of 7-AAD working solutions to each tube. ***Note:*** *Higher concentrations of the solutions tend to produce better results for microscopy. The optimal concentration may need to be determined empirically.*
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, but staining time should be at least 30 min.
9. Wash cells with 1X Binding Buffer by centrifugation.
10. Mount cells onto slides and observe using appropriate filters.

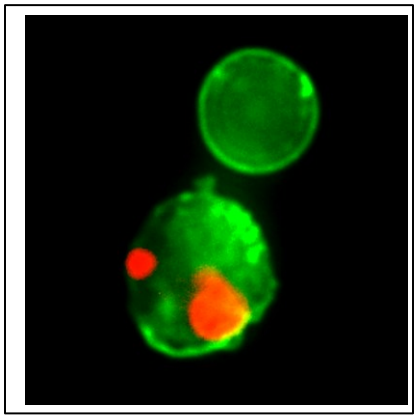

Figure 2: Jurkat cells were induced with 1 uM staurosporine for 5 hours and stained with CF488A-Annexin V and 7-AAD according to the protocol. Cells were placed on a glass slide and visualized on a mercury arc lamp microscope. Images were captured on a CCD camera. The top cell is a representative apoptotic cell with only CF488A-Annexin V staining (green plasma membrane), while the bottom cell is a late stage apoptotic/secondary necrotic cell with both CF488A-Annexin V and 7-AAD staining (green membrane with red fragmented nucleus).

References

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