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FITC Annexin V and PI Apoptosis Kit
Catalog Number: A026

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
FITC Annexin V (Component A)	**500 µL**	• **2-6 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
PI (Component B)	**200 µL**		
5X Annexin-binding buffer (Component C)	**50 mL**		

Number of assays: 100 flow cytometry assays.
Approximate fluorescence excitation/emission maxima, in nm: FITC: 494/518; PI: 535/617, bound to DNA.

Introduction

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry. In normal live cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment. The human anticoagulant, annexin V, is a 35–36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS. Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.

The FITC Annexin V and PI Apoptosis Kit provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to fluorescein to detect apoptotic cells, and a ready-to-use solution of the red-fluorescent propidium iodide (PI) nucleic acid binding dye to detect dead cells. After staining a cell population with FITC annexin V and PI in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show red and green fluorescence, and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometer with the 488 nm line of an argon-ion laser for excitation. Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis.

Materials Required but Not Provided

- Phosphate buffered saline (PBS)
- Inducing agent
- Deionized water

Experimental Protocols

Apoptosis Analysis by Flow Cytometry

Note: The assay has been optimized using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types or other inducing agents.

1.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

1.2 Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

1.3 Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component C) to 8 mL deionized water.

1.4 Re-centrifuge the washed cells (from step 1.2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

Determine the cell density and dilute in 1X annexin-binding buffer to ~1 × 10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

1.5 Add 5 µL FITC annexin V (Component A) and 1~2 µL PI (Component B) to each 100 µL of cell suspension.

1.6 Incubate the cells at room temperature for 15 minutes in the dark.

1.7 After the incubation period, add 400 µL 1X annexin-binding buffer, mix gently, and keep the samples on ice.

1.8 As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm and >575 nm (or equivalent) using 488 nm excitation.

Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein (FITC) and Texas Red dye.

Apoptosis Detection by Microscopy

2.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2.2 Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

2.3 Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component C) to 8 mL deionized water.

2.4 Re-centrifuge the washed cells (from step 2.2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

Determine the cell density and dilute in 1X annexin-binding buffer to ~1 × 10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

2.5 Add 5-25 µL FITC annexin V (Component A) and 1~2 µL PI (Component B) to each 100 µL of cell suspension.

Note: *Higher concentrations of the solutions tend to produce better results for microscopy. The optimal concentration may need to be determined empirically.*

2.6 Incubate the cells at room temperature for 15-30 minutes in the dark.

2.7 Wash cells with 1X annexin-binding buffer.

2.8 Mount cells onto slides and observe using appropriate filters.

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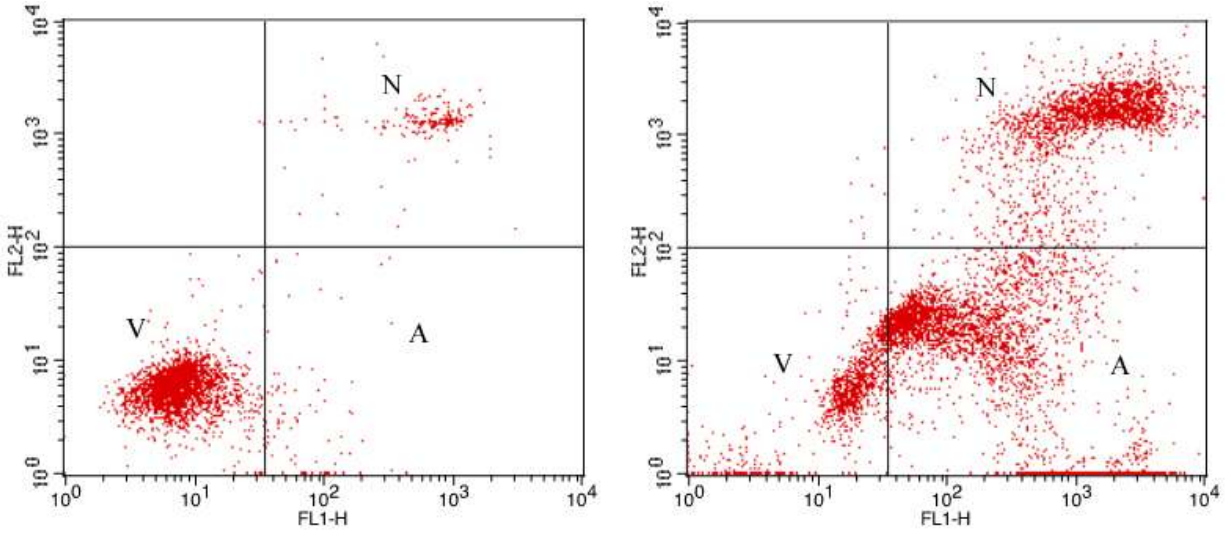

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (right panel) or untreated (as control, left panel). Cells were then treated with the reagents in the kit, followed by flow cytometric analysis. Note that the camptothecin-treated cells (right panel) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (left panel). A = apoptotic cells, V = viable cells, N = necrotic cells.