Product Information

FITC-Annexin V and PI Apoptosis Kit

Catalog Number: F6012S, F6012M, F6012L

Product Size:10T, 50T, 100T

Contents:

Size / Component	F6012S (10T)	F6012M (50T)	F6012L (100T)
A. 1×Annexin V Binding buffer	10 mL	50 mL	50 mL×2
B. FITC-Annexin V	50 μL	250 μL	500 μL
C. PI	100 μL	500 μL	1 mL

Parameters

FITC-Annexin V: Abs/Em: 494 /518 nm

PI: Abs/Em: 535 /617 nm (with DNA)

Storage

Store at 4℃ and protect from light. Do not freeze. Expiration date marked on the outer packing.

Description

FITC-Annexin V and PI Apoptosis Kit provides a convenient method to make a distinction between 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. 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.

Propidium iodide (PI) is a membrane impermeant DNA-binding dye which 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.

Protocol

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. The test sample should contain untreated cell samples as a negative control; a group of samples should be single-stained to adjust compensation.

2. Collecting cells. Suspension cells: 300 g, 4℃ centrifugation. Adherent cells: After digestion with EDTA-free trypsin, the cells were collected by 300 g centrifugation at 4℃ for 5 min. The trypsin digestion time should not be too long to prevent false positives.

Note: After the cells are trypsinized, they are stained after

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recovering in the optimal culture conditions and medium for about 30 minutes to avoid false positives.

3. Wash the cells twice with pre-cooled PBS, centrifuge at 300 g for 5 min at 4°C, collect 1-5×10^5 cells and resuspend the cells in 100 µL 1× binding buffer.

4. Add 4-5 µL FITC-Annexin V and 5 µL PI to each tube.

Note: We recommend you set up two additional tubes, for each of the dyes alone (FITC-Annexin V and PI) as single stained compensation controls.

5. Incubate at room temperature for 10-15 minutes in the dark. To reduce the process of apoptosis, the process of Incubation can be operated on ice.

6. Add 400 µL 1× Binding Buffer or PBS to each tube and analyze by flow cytometry.

Staining protocol for fluorescence microscopy

For suspension cells, refer to protocol for flow cytometry .

1. Grow cells on coverslips or chamber slides.

2. Induce apoptosis. Including an untreated cell sample as a negative control.

3. Wash the cells with 1×PBS.

Note: Serum-containing medium can be used to directly replace Annexin V binding buffer, but the concentration of Annexin V may require optimization.

4. Add 5-25 µL of FITC-Annexin V and 5 µL of PI into every 100 µL Annexin Binding Buffer.

Note: The optimal concentration may need to be determined empirically.

5. 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.

6. Wash cells with 1× Binding Buffer.

7. Mount coverslips onto slides with a drop of 1× Binding Buffer. For cells on chamber slides, add enough 1× Binding Buffer to completely cover cells.

8. Use appropriate filters to image. FITC- Annexin V can be imaged with FITC filter, while PI can be imaged with Cy3 or Texas Red filter.

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