Product Information

Annexin V-APC and PI Apoptosis Kit

Catalog Number: A6030S, A6030M, A6030L

Product Size: 10T, 50T, 100T

Product content:

Components \ Size	A6030S（10T）	A6030M（50T）	A6030L(100T)
A: 1×Annexin V Binding buffer	10 mL	50 mL	50 mL×2
B: Annexin V-APC	50 μL	250 μL	500 μL
C: PI	100 μL	500 μL	1 mL

Storage

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

Spectral Characteristics

Annexin V-APC: Abs/Em: 650/660 nm

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

Description

Annexin V-APC and PI Apoptosis Kit provides a convenient method to make a distinction between cell apoptotic of early and late phase within the same cell population by flow cytometry or fluorescence microscopy.

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. Include an untreated cell sample as negative control. Also include samples for single-stained controls if compensation is required.

2. Collecting cells after treatment by centrifugation and wash with PBS.

Note: If you prefer not to wash 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. Centrifuge cells again, discard supernatant and resuspend cells for 5× 10^6 to 10^7 cellls per mL with 1× Binding Buffer.

4. Aliquot cells into flow cytometry tubes at 100 μL/tube.

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

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

6. Incubate at room temperature for 10-15 minutes in the dark. The incubation can be carried out on ice to arrest the apoptotic process if desired.

7. Add 400 μL 1× Binding Buffer to each tube and analyze by flow cytometry within 30 minutes. Use 633 nm excitation and measure Annexin V-APC fluorescence emission near 660 nm (FL4/RL1 channel) .Emission of PI is near 617 nm (FL3/BL3 channel).

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Staining protocol for fluorescence microscopy

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

1. Grow cells on coverslips or chamber slides.

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

3. Wash cells with PBS.

Note: If you prefer not to wash 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.

4. Add 5-25 μL of Annexin V-APC 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. Using appropriate filters to image.

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