Product Information

YF®647A-Annexin V and PI Apoptosis Kit

Catalog Number: Y6026

Product Size: 50 T, 100 T

Contents:

Component	50 T	100 T
A.1×Annexin V Binding buffer	50 mL	50 mL×2
B. YF®647A-Annexin V	250 μL	500 μL
C. PI	500 μL	1 mL

Storage

Store at 4℃ and protect from light. Do not freeze. When stored as directed, product is stable for at least 6 months.

Spectral Characteristics

YF®647A-Annexin V: Abs/Em: 650 /665 nm

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

Description

YF®647A-Annexin V and PI Apoptosis Kit provides a convenient method to make a distinction between apoptotic (far red) 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. 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.

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. 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

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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 4-5 μL YF®647A-Annexin V and 5 μL PI to each tube.

Note: We recommend you set up two additional tubes, for each of the dyes alone (YF®647A-Annexin V and 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 647 nm excitation and measure YF®647A-Annexin V fluorescence emission near 665 nm (APC channel) .Emission of PI is near 617 nm (PI channel).

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 to not wash 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.

4. Add 5-25 μL of YF®647A-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. Using appropriate filters to image. YF®647A-Annexin V can be imaged with APC filter, while PI can be imaged with Cy3 or Texas Red filter.

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