Product Information

Annexin V-PE and RedNucleus II Apoptosis Kit

Catalog Number: A6079

Product Size: 50 T, 100 T

Contents:

Component	50 T	100 T
A.1×Annexin V Binding buffer	50 mL	50 mL×2
B. Annexin V-PE	250 μL	500 μL
C. RedNucleus II	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.

Store Annexin V-PE at 50mM Tris, 100mM NaCl, 1% BSA, 0.02% NaN_3, pH7.4 solution.

Spectral Characteristics

Annexin V-PE: Abs/Em: 488 /578 nm

RedNucleus II: Abs/Em: 635/695 nm (with DNA)

Description

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.

RedNucleus II provided in this kit is a far red fluorescence dye, belonging to anthraquinone compounds, which can not penetrate the intact cell membrane of living cells and early apoptotic cells, and is impermeable, but can rapidly stain nucleus/dsDNA in dead and permeable cells. Rednucleus II is an ideal substitute of propidium iodide (PI) and 7-AAD. It can be used in multicolor analysis with FITC, PE and purple fluorescent dyes because it is not excited by ultraviolet and overlapped with PE/PE homologues without compensation controls. When combined with Annexin V-PE, RedNucleus II was excluded from living cells and early apoptotic cells, while late apoptotic cells and dead cells were stained with Annexin V-PE and RedNucleus II at the same time.

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

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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-PE and 5 μL RedNucleus II to each tube.

Optional: You can set up two additional tubes, for each of the dyes alone (Annexin V-PE and RedNucleus II) 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 488 nm excitation and measure Annexin V-PE fluorescence emission near 578 nm (FL2/BL2 channel) .Emission of RedNucleus II is near 695 nm (FL4/RL1 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 Annexin V-PE and 5 μL of RedNucleus II 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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