Annexin V-Biotin/PI Apoptosis Kit

Introduction

Annexin V is a cellular protein, which plays important roles in the inhibition of the activity of phospholipase A1 and blood coagulation by competing for phosphatidylserine (PS) binding sites with prothrombin. Annexin V has a high affinity to PS and is used as a probe to detect cells that have expressed PS on the cell surface. After initiating apoptosis, cells translocate phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface soon, which can be easily detected by Annexin V.

Annexin V-Biotin/PI Apoptosis Kit uses Annexin-Biotin to bind the PS, then bind biotin by fluorescently labeled streptavidin such as FITC-labeled streptavidin or PE-labeled streptavidin, and finally easily detect PS on the cell surface after initiating apoptosis. The one-step staining procedure needs only 10-20 minutes. The result can be analyzed by fluorescence microscopy or by flow cytometry. The Annexin V-Biotin/PI Apoptosis Kit can differentiate apoptosis vs necrosis when performing both Annexin V-Biotin and PI staining.

Components and Storage

Components	20 Assays	100 Assays	200 Assays	Storage
Annexin V-Biotin	100 μL	500 μL	1 mL	4°C
10X Binding Buffer	1 mL	5 mL	10 mL	4°C
PI	100 μL	500 μL	1 mL	4°C away from light
Shipping: Blue ice	**Shelf life: 6 months**			

Protocol

1. Induction of apoptosis by the desired method.
2. Collect cells.
    1) For suspension cells: 300×g centrifugation for 5 min, and the supernatant of the medium is discarded.
    2) For adherent cells: Try to use EDTA-Free trypsin to digest cells, 300×g centrifugation for 5 min, and the supernatant is discarded.
3. Wash cells with pre-chilled PBS and collect 1-5×10^5 cells.
4. Resuspend cells in 200 μL of pre-prepared 1X Binding Buffer.

*Note: Dilute 1 mL 10X Binding Buffer per 9 mL ddH_2O to make the 1X Binding Buffer.

5. Add 5 μL of Annexin V-Biotin and 5 μL of PI. Incubate at room temperature for 5 min in the dark.
6. After incubation, wash the cells in 200 μL of 1X Binding Buffer. Then centrifuge to remove the buffer.
7. Fix cells with 4% formaldehyde in PBS for 15 min. then wash cells with PBS once. Resuspend cells in 100 μL PBS (containing 1 mg/mL BSA).
8. Add 5 μg/mL of fluorescein-streptavidin and incubate for 15 min.

*Note: Fluorescently labeled streptavidin needs to be prepared on your own, such as Streptavidin-FITC (Cat. No. K1081).

9. Collect cells by centrifugation and resuspend in PBS.
10. Proceed to flow cytometry or fluorescence microscopy as soon as possible. If testing is not possible immediately, place the sample on ice and test within 1 hour.
    1) Flow Cytometry: The detection channel of Annexin V-Biotin needs to be determined based on streptavidin-labeled fluorescein. If use Streptavidin-FITC, Streptavidin-FITC is green fluorescent (Ex/Em: 490/525 nm); PI is red fluorescent (Ex/Em: 535/615 nm).
    2) Fluorescence Microscopy: Add 30-50 μL of the cell suspension from step 9 dropwise to a glass slide, cover the cells with a coverslip, and observe under fluorescence microscopy.

*Note: In general, microscopy-based analysis recommends an appropriate increase in the concentration of Annexin V compared to the dosage used for flow cytometry.

Note

1. Since the Annexin V method determines the occurrence and stage of apoptosis by detecting changes in the cell membrane, it is not suggested to fix or permeabilize the membrane with fixatives or permeabilizers that disrupt the integrity of the cell membrane before staining with Annexin V-Biotin and PI.
2. During the whole operation, the action should be as gentle as possible, and the cells should not be pipetted forcefully to avoid mechanical damage to the cells.
3. If the sample is derived from blood, be sure to remove platelets from the blood. Because platelets contain PS, they can bind to Annexin V, which can interfere with the results. Platelets can be washed using a buffer containing EDTA and centrifugation at 1400 rpm (200×g).
4. Please centrifuge the reagent briefly before opening the cap, and throw the liquid on the inner wall of the cap to the bottom of the tube to avoid the liquid spilling when the cap is opened.
5. Annexin V-Biotin and PI are photosensitizing substances, please take care to protect from light when handling. PI is a known mutagen. So be careful when using it.
6. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the

same time, the digestion fluid of trypsin should be EDTA-free as much as possible, because EDTA will affect the binding of Annexin V to PS. If EDTA-containing trypsin is used, the cells should be washed thoroughly after collection to ensure that the EDTA is cleanly removed.

7. For your safety and health, please wear lab coats and gloves during the experiment.

8. For research use only. Not to be used in clinical diagnostic or clinical trials.

Troubleshooting

Problems	Cause	Solution
High Background	▪ Cell density is higher than recommended ▪ Increased volumes of components added ▪ Incubation of cell samples for extended periods ▪ Use of extremely confluent cells ▪ Contaminated cells	▪ Refer to datasheet and use the suggested cell number ▪ Use calibrated pipettes accurately ▪ Refer to datasheets and incubate for exact times ▪ Perform assay when cells are at 80-95% confluency ▪ Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	▪ Washing cells with PBS before/after fixation (adherent cells) ▪ Cells did not initiate apoptosis ▪ Very few cells used for analysis ▪ Incorrect setting of the equipment used to read samples ▪ Use of expired kit or improperly stored reagents	▪ Always use binding buffer for washing cells ▪ Determine the time-point for initiation of apoptosis after induction (time-course experiment) ▪ Refer to data sheet for appropriate cell number ▪ Refer to datasheet and use the recommended filter setting ▪ Always check the expiry date and store the components appropriately
Erratic results	▪ Uneven number of cells seeded in the wells ▪ Adherent cells dislodged at the time of experiment ▪ Incorrect incubation times or temperatures ▪ Incorrect volumes used ▪ Increased or random staining observed in adherent cells	▪ Seed only healthy cells (correct passage number) ▪ Perform experiment gently and in duplicates or triplicates for each treatment ▪ Refer to datasheet & verify correct incubation times and temperatures ▪ Use calibrated pipettes and aliquot correctly ▪ Always stain cells with Annexin before fixation (makes cell membrane leaky)

***Note:** The most probable cause is listed under each section. Causes may overlap with other sections.

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