Reagent Preparation

1×Annexin V Binding Buffer： Dilute Annexin V Binding Buffer (10×) with deionized water to 1×Annexin V Binding Buffer before use.

Staining Procedure

One-step process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently followed by the cell counting.
2. Split the cell suspension into tubes, 1~5×10^5 cells for each, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and discard the supernatant. Add 500 µL of 1×Annexin V Binding Buffer to resuspend the cells.
3. Add 5 µL of Annexin V-APC and 5 µL of PI to each tube.
4. Gently vortex the cells and incubate at room temperature for 15~20 min in the dark.
5. Analyze the cells immediately with proper machine settings. Otherwise, place the cells on ice in the dark and analyze within 1h.

   Note: Annexin V-APC can be detected in APC channel while ECD channel is preferred to PE channel for PI detection; if the sample has the autofluorescence of the FITC channel, the PerCP/Cy5.5 channel is selected for PI detection.

Two-step process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently followed by the cell counting.
2. Split the cell suspension into tubes, 1~5×10^5 cells for each, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and discard the supernatant. Add 100 µL of 1×Annexin V Binding Buffer to resuspend the cells.
3. Add 2.5 µL of Annexin V-APC and 2.5 µL of PI to each tube.

   (Attributed to the higher resolution of two-step protocol, half the amount of the reagents can still guarantee a result of matched quality as in the one-step protocol. It's also recommended that users titrate the reagents for optimal performance in specific models.)
4. Gently vortex the cells and incubate at room temperature for 15~20 min in the dark.
5. Add 400 µL of 1×Annexin V Binding Buffer to the tube, and mix gently.
6. Analyze the cells immediately with proper machine settings. Otherwise, place the cells on ice in the dark and analyze within 1h

   Note: Annexin V-APC can be detected in APC channel while ECD channel is preferred to PE channel for PI detection; if the sample has the autofluorescence of the FITC channel, the PerCP/Cy5.5 channel is selected for PI detection.

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