Instructions

for use: 1. For suspended cells:

a. After performing apoptosis stimulation, centrifuge at 1000g for 5 minutes, discard the supernatant, collect the cells, gently resuspend the cells with PBS and count. Note: PBS resuspension can not be omitted, the process of PBS resuspension also plays a role in washing cells, which can ensure the binding of subsequent Annexin V-FITC.

b. Take 50,000-100,000 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, and gently resuspend the cells by adding 195 μl of Annexin V-FITC binding solution.

c. Add 5 μl of Annexin V-FITC and mix gently.

d. Add 10 μl of propidium iodide stain and mix gently.

e. Incubate at room temperature (20-25 ° C) for 10-20 minutes in the dark, then place in an ice bath. Aluminum foil can be used to protect from light. The cells can be resuspended 2-3 times during the incubation to improve the staining effect.

f. If used for flow cytometry, the machine detected immediately, Annexin V-FITC green fluorescence of propidium iodide (PI) is the red fluorescence of apoptotic cells detected by flow effect

results and verification Refer Figure 1 and Figure 2. For initial flow cytometry testing, it is recommended to select a suitable set of cells. Refer to Figure 2 for the three controls of unstained, PI single staining and Annexin V-FITC single staining. If used for fluorescence microscopy, centrifugation at 1000g for 5 minutes, collect the cells, gently resuspend the cells with 50-100 μl Annexin V-FITC binding solution, smear, and observe under a fluorescence microscope. Note: The cells should be tested as soon as possible after staining, and it is usually best to complete the test within 1 hour. For flow cytometry detection, if too many PI false positive cells are found when Annexin V-FITC is stained alone, and can not be improved by adjusting the relevant settings and parameters, Annexin V-FITC can be diluted with PBS 3- Test again after 10 times.

2. Post-digestion testing of adherent cells:

a. Aspirate the cell culture medium into a suitable centrifuge tube, wash the adherent cells once with PBS, and add the appropriate amount of trypsin cell digestive solution (which may contain EDTA) to digest the cells. Incubate the trypsin cell digest if you incubate the adherent cells by gently pipetting at room temperature. Avoid excessive digestion of trypsin. Note: For adherent cells, the trypsinization step is critical. If the trypsin digestion time is too short, the cells need to be blown hard to fall off, which may cause damage to the cell membrane, leading to false positives of cell necrosis; if the digestion time is too long, it may also cause cell membrane damage and false positive of cell necrosis, or even It affects the binding of phosphatidylserine to Annexin V-FITC on the cell membrane to interfere with the detection of apoptosis. At the same time, trypsin cell digestive juice should be as free of EDTA as possible, because EDTA may affect the binding of Annexin V to phosphatidylserine.

b. Add the cell culture medium collected in step 2a, gently puff the cells down, transfer to a centrifuge tube, centrifuge at 1000g for 5 minutes, discard the supernatant, collect the cells, gently resuspend the cells with PBS and count. Note: It is very important to add the cell culture solution in step 2a. On the one hand, it can collect cells that have been apoptotic or necrotic, and on the other hand, the serum in the cell culture solution can effectively inhibit or neutralize residual pancreatic enzyme. Residual trypsin digests and degrades the subsequent addition of Annexin V-FITC, resulting in staining failure.

c. Take 50,000-100,000 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, and gently resuspend the cells by adding 195 μl of Annexin V-FITC binding solution.

d. Add 5 μl of Annexin V-FITC and mix gently.

e. Add 10 μl of propidium iodide stain and mix gently.

f. Incubate at room temperature (20-25 ° C) for 10-20 minutes in the dark, then place in an ice bath. Aluminum foil can be used to protect from light. The cells can be resuspended 2-3 times during the incubation to improve the staining effect.

g. If used for flow cytometry, it can be detected immediately. Annexin V-FITC is green fluorescence, propidium iodide (PI) is red fluorescence, and the effect of flow detection and its test

Please refer to Figure 1 and Figure 2. If used for fluorescence microscopy, centrifugation at 1000g for 5 minutes, collect the cells, gently resuspend the cells with 50-100 μl Annexin V-FITC binding solution, smear, and observe under a fluorescence microscope. Note: The cells should be tested as soon as possible after staining, and it is usually best to complete the test

within 1 hour. For flow cytometry detection, if too many PI false positive cells are found when Annexin V-FITC is stained alone, and can not be improved by adjusting the relevant settings and parameters,
Annexin V-FITC can be diluted with PBS 3- Test again after 10 times.

3. In situ fluorescence detection of adherent cells:

Note: The advantage of this method is that it can observe apoptosis in situ, the disadvantage is that some of the apoptosis is not detected due to non-adherence.

a. **(Optional)** If conditions permit, culture the cells in 24-well, 48-well or 96-well plates. After the induction of apoptosis was completed, centrifugation was carried out for 5 minutes at 1000 g in a centrifuge capable of centrifuging the multiwell plate.

b. Aspirate the cell culture medium and wash it once with PBS. If conditions permit, centrifuge at 1000g for 5 minutes before PBS is aspirated.

c. Add 195 μl of Annexin V-FITC binding solution.

d. Add 5 μl of Annexin V-FITC and mix gently.

e. Add 10 μl of propidium iodide stain and mix gently.

f. Incubate at room temperature (20-25 ° C) for 10-20 minutes in the dark, then place in an ice bath. Aluminum foil can be used to protect from light. The cells can be resuspended 2-3 times during the incubation to improve the staining effect.

g. Immediately observed under a fluorescence microscope, Annexin V-FITC is green fluorescent, and propidium iodide (PI) is red fluorescent. Note: The cells should be tested as soon as possible after staining, and it is usually best to complete the test within 1 hour.

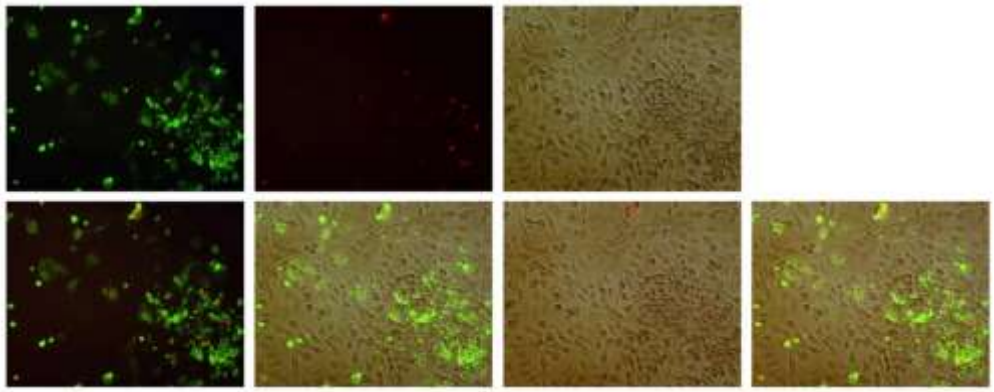

Figure 3. Effect of Annexin V-FITC and propidium iodide (PI) staining. The green fluorescence in the figure is Annexin V-FITC staining positive cells, and the red fluorescence is propidium iodide staining positive cells. Apoptotic cells stained only by green fluorescence, necrotic cells stained with green and red fluorescence, and normal cells not stained with fluorescence.