Product Information

Annexin V-PE

Catalog Number: A6065S, A6065L

Product Size: 0.1 mL, 1 mL

Storage

Store at 4℃ and protect from light. Do not freeze. Expiration date marked on the outer packing.

Spectral Characteristics

Annexin V-PE: Ex/Em=488/578 nm

Description

Annexin V (membrane-linked protein-V) is a Ca^{2+}-dependent phospholipid-binding protein with a molecular weight of 35-36 KD that binds selectively to phosphatidylserine (PS). PS is mainly distributed on the inner side of the cell membrane, i.e., the side adjacent to the cytoplasm. In the early stages of apoptosis, different cell type exocytose phosphatidylserine to the cell surface and are exposed to the extracellular environment. At this point, the use of fluorescent protein PE-tagged Annexin V, Annexin V-PE, in combination with the outside-out PS allows flow cytometry to directly detect the outside-out PS, an important feature of apoptosis. Normal cells are not stained by Annexin V-PE, cells undergoing apoptosis or necrosis are stained by Annexin V-PE. Annexin V-PE can be used in combination with partially non-permeable nuclear dyes (7-AAD/PI) to differentiate cells in different periods of apoptosis.

Protocol

1. Induce cell apoptosis according to experimental requirements. The test sample should contain untreated cell samples as a negative control. In addition, set a group of samples for single dyeing for adjustment and compensation.

2. Collecting cells.

(1) For suspension cells:

a. After apoptosis stimulation, centrifuge at 1000 rpm for 5 min, discard supernatant, collect cells, resuspend cells gently with PBS and count.

Note: PBS resuspension should not be omitted, as the process of PBS resuspension also serves to wash the cells and ensure subsequent Annexin V-PE binding.

b. Take 5× 10^4-1× 10^5 resuspended cells, centrifuge at 1000 rpm for 5 min, discard the supernatant, and add 100 µL of 1× Annexin V binding buffer (optional final concentration of 2 mM Hepes/NaOH, pH 7.4, 28 mM NaCl, 0.5 mM $CaCl_2$ solution instead) and gently resuspend the cells.

c. Add 5 µL Annexin V-PE and mix gently.

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d. Add 10 μL 20 μg/mL 7-AAD or PI and mix gently.

e. Incubate at room temperature (20-25ºC) for 15 min protected from light. Aluminum foil may be used for light protection. Cells can be resuspended 2-3 times during incubation to improve staining.

(2) For adherent cells:

a. Aspirate the cell culture into a suitable centrifuge tube, wash the walled cells once with PBS, and digest the cells by adding the appropriate amount of trypsin cell digest (without EDTA). Incubate at room temperature until the cells can be blown off by gentle blowing, then aspirate the trypsin cell digest. Excessive digestion by trypsin needs to be avoided.

Note: For adherent cells, the trypsin digestion step is critical. If the trypsin digestion time is too short, the cells need to blow hard to fall off, which will easily cause damage to the cell membrane and lead to false positive cell necrosis; if the digestion time is too long, it will also easily cause damage to the cell membrane and lead to false positive cell necrosis, and even affect the binding of phosphatidylserine and Annexin V-PE on the cell membrane, thus interfering with the detection of apoptosis.

b. Add the cell culture solution collected in the previous step, gently blow down the cells, transfer to a centrifuge tube, centrifuge at 1000 rpm for 5 min, discard the supernatant, collect the cells, gently resuspend the cells with PBS and count them.

Note: It is very important to add the cell culture medium in the previous step, on the one hand to collect the cells that have undergone apoptosis or necrosis in suspension, and on the other hand the serum in the cell culture medium can effectively inhibit or neutralize the residual trypsin. Residual trypsin will digest and degrade the subsequently added Annexin V-PE, resulting in staining failure.

c. Take 5× 10^4-1× 10^5 resuspended cells, centrifuge at 1000 rpm for 5 min, discard the supernatant, and add 100 μL of 1× Annexin V binding buffer (optional final concentration of 2mM Hepes/NaOH, pH 7.4, 28mM NaCl, 0.5 mM $CaCl_2$ solution instead) and gently resuspend the cells.

d. Add 5 μL Annexin V-PE and mix gently.

e. Add 10 μL of 20 μg/mL 7-AAD or PI and mix gently.

f. Incubate at room temperature (20-25ºC) for 15 min protected from light. Aluminum foil may be used for light protection. Cells can be resuspended 2-3 times during incubation to improve staining.

3. Analysis of results:

(1) Flow cytometry assay:

a. After incubation, 400 μL of 1× Annexin V binding buffer can be added directly to resuspend the cells and immediately detected on the machine, Annexin V-PE was excited by 488 nm/566 nm laser and detected fluorescence emission spectra at 578 nm (BL2 (FL2)/YL1 channel) .

b. On the scatter plot of the bivariate flow cytometer, the lower left quadrant shows live cells as (Annexin V-PE-, 7-AAD/PI-); the lower right quadrant shows early apoptotic cells as (Annexin V-PE+, 7-AAD/PI-); the upper right quadrant shows necrotic and late apoptotic cells as (Annexin V-PE+, 7-AAD/PI+); and the upper left quadrant shows naked nuclei as (Annexin V-PE-, 7-AAD/PI+).

(2) Fluorescence microscopy assay:

a. Centrifuge at 1000 rpm for 5 min, collect the cells, and gently resuspend the cells with 400 μL 1× Annexin V binding buffer. Transfer the cells to a 96-well plate and settle for a few moments or perform cell smearing, then place under a fluorescence microscope for observation.

b. Annexin V-PE can be used with applicable PE channel filters.

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Notes

1. Please centrifuge the product instantaneously to the bottom of the tube before use for subsequent experiments.

2. To reduce the process of apoptosis, incubation can be done on ice, but the incubation time should be extended to at least 30 min.

3. Because apoptosis is a rapid process, it is recommended that samples be analyzed within 1 h of staining.

4. For adherent cells, digestion is a critical step. If there are floating cells during the induction of apoptosis in the adherent cells, collect the floating cells and the walled cells and combine them for staining. Be careful when handling walled cells and try to avoid artificial damage to the cells. If the digestion time of trypsin is too short, the cells need to be blown hard to fall off, which will easily cause damage to the cell membrane and excessive intake of PI; if the digestion time is too long, the cell membrane will also be easily damaged, and even affect the binding of phosphatidylserine and Annexin V-PE on the cell membrane. When digestion, trypsin is spread all over the bottom of the well plate, and then the trypsin is fully contacted with the cells when shaken gently, and then most of the trypsin is poured off, and the remaining small amount of trypsin is used to digest for a period of time, which can be terminated when the space between cells increases and the bottom of the bottle is flowery. Try not to use EDTA in the digestion solution, EDTA will affect the binding of Annexin V to PS.

5. After digestion with trypsin, it is recommended that the walled cells be stained after about 30 min of recovery in optimal culture conditions and medium to avoid false positives.

6. To avoid cell loss when washing cells, use a large Tip tip over a small Tip tip when aspirating.

7. The optimal concentration of the dye to be used is determined by the specific experimental requirements.

8. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

9. For your safety and health, please wear lab coats and disposable gloves.

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