

Annexin V-FineTest®647/DAPI Apoptosis Kit

Catalog No.: K021

Size: 20T/50T/100T

Kit components:

Reagents	20T	50T	100T
Annexin V-FineTest®647	100µL	250µL	500µL
1X Binding Buffer	10mL	25mL	50mL
DAPI	100µL	250µL	500µL

Storage:

2-8°C for 12 months. Annexin V-FineTest®647 and DAPI need to be stored away from light.

Shipping Conditions: ice pack

Description:

Annexin V-FineTest®647/DAPI apoptosis detection kit can be used to detect apoptosis in suspension cells and adherent cells.

Annexin V is a calcium-dependent phosphatidylserine binding protein with a high affinity for phosphatidylserine PS. Annexin V labelled FineTest®647 can bind to the membrane of early apoptotic cells by means of the PS exposed outside the cells. Apoptosis can be detected by flow cytometry or fluorescence microscopy.

Cell membrane integrity is lost due to late apoptosis or necrosis, and 4',6-Diamidino-2-Phenylindole dihydrochloride (4', 6-Diamidino-2-phenylindole, DAPI) can bind specifically to double-stranded DNA and produce strong fluorescence. Cells at different apoptotic stages can be distinguished.

Annexin V-FineTest®647/DAPI Assay Protocol:

A. Incubation of cells with Annexin V-FineTest®647

1. Induce apoptosis by desired method. Centrifuge at 300 g for 5 min, discard the supernatant, collect the cells, gently suspend the cells with PBS and count them.
2. Collect 1-5 x 10⁵ cells by centrifugation, and the supernatant was discarded. The the cells were washed with PBS once, the supernatant was abandoned after centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer.

4. Add 5 μ l of Annexin V-FineTest®647 and 5 μ l of DAPI.
 5. Incubate at room temperature for 15 min in the dark.
- Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Annexin V-FineTest®647 can be detected in APC channel while DAPI channel is preferred to Pacific Blue channel for DAPI detection.

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-FineTest®647 (A.3-5).

C. Detection by Fluorescence Microscopy

Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on glass slide and visualize cells.

Note:

1. It should be detected as soon as possible after staining. Too long a time may lead to an increase in the number of apoptotic or necrotic cells.
2. When detecting adherent cells, the suspension cells generated after inducing apoptosis should be collected and detected together with the adherent cells collected later.
3. Mechanical damage caused by digestive adherent cells should be avoided as much as possible. At the same time, the digestive juices of pancreatic enzymes should be as free of EDTA as possible, as EDTA affects Annexin V binding to phosphatidylserine.
4. If EDTA-containing pancreatic enzymes are used, the cells should be thoroughly washed after collection to ensure that EDTA is removed.
5. Fluorescent substances are prone to quenching, in the fluorescence observation, as far as possible to shorten the observation time, while in the operation and storage process also try to pay attention to the preservation of light.
6. For your safety and health, please wear a lab coat and disposable gloves.
7. This kit is for scientific research only.