Miltenyi Biotec

Annexin V-FITC Kit

Order no. 130-092-052

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1. Description

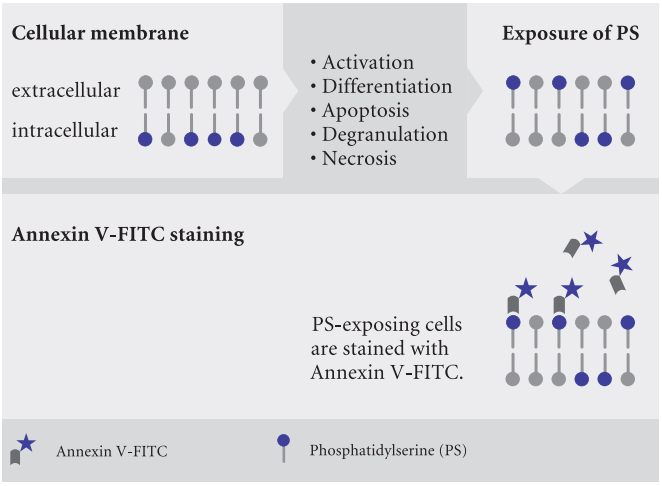
Components	**1 mL Annexin V-FITC**: Annexin V conjugated to FITC (fluorescein-isothiocyanate) **25 mL 20× Binding Buffer Stock Solution** **0.5 mL Propidium Iodide (100 µg/mL)**
Capacity	For 10^8 total cells, up to 100 stainings.
Product format	Annexin V-FITC is supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial label.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1.1 Principle of the staining procedure

The Annexin V-FITC Kit has been developed for detection and discrimination of apoptotic, necrotic, and dead cells. Apoptotic cells are stained positively for Annexin V-FITC that binds to phosphotidylserine (PS), but are negative for staining with propidium iodide (PI). Dead cells are stained positive for Annexin V-FITC and PI, whereas viable cells are negative for both Annexin V-FITC and PI.



1.2 Background information

In most normal, viable eukaryotic cells, the negatively charged phospholipid phosphatidylserine (PS) is located in the cytosolic leaflet of the plasma membrane lipid bilayer.[1] PS redistribution from the inner to the outer leaflet is an early and widespread event during apoptosis.[1,2] However, in necrosis, PS becomes accessible due to the disruption of membrane integrity.[2] Apart from necrosis and apoptosis, PS also becomes accessible in activated platelets[3], in certain cell anomalies like sickle cell anemia[4], in erythrocyte senescence[5], upon degranulation of mast cells[6] and in certain stages of B cell differentiation[7]. PS exposure also serves as a trigger for the recognition and removal of apoptotic cells by macrophages.[8,9]
Annexin V is a 35 kDa phospholipid-binding protein and a major cell membrane component of macrophages and other phagocytic cell types. Annexin V has a high affinity to PS in the presence of physiological concentrations of calcium (Ca^{2+}).[10]

1.3 Applications

- Studies on cell death (apoptotis and/or necrosis).
- Evaluation of separations with the Annexin V MicroBead Kit (# 130-090-201).

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1.4 Reagent and instrument requirements

- Buffer (1× Binding Buffer)
  Prepare 1× Binding Buffer from 20× Binding Buffer Stock Solution by diluting 250 µL of 20× Binding Buffer Stock Solution with 4.75 mL of sterile, distilled water. This volume is sufficient for 10^6 total cells. Alternatively, prepare 1× Binding Buffer Stock Solution by diluting 25 mL of 20× Binding Buffer with 475 mL of sterile, distilled water.
  Store at 4 °C.

  ▲ **Note:** Handle under sterile conditions.

2. General protocol for fluorescent staining

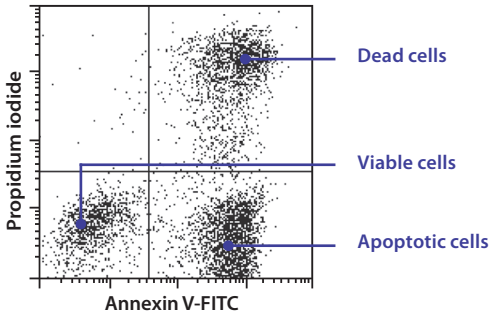
▲ Volumes for fluorescent labeling given below are for **up to** 10^6 total cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ When working with cell samples containing platelets, for example, blood samples, wash samples carefully at 200×g in order to remove platelets. Use buffer containing the ion chelator EDTA for these washing steps. Activated platelets expose PS and therefore bind Annexin V.[5]

1. Determine cell number.
2. Wash 10^6 cells in 1 mL of 1× Binding Buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. (Optional) Repeat washing step.
4. Resuspend cell pellet in 100 µL of 1× Binding Buffer per 10^6 cells.
5. Add 10 µL of Annexin V-FITC per 10^6 cells.
6. Mix well and incubate for 15 minutes in the dark at room temperature.
7. Wash cells by adding 1 mL of 1× Binding Buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. (Optional) Repeat washing step.
9. Resuspend cell pellet in 500 µL of 1× Binding Buffer per 10^6 cells.
10. Add 5 µL of PI solution immediately prior to analysis by flow cytometry or fluorescence microscopy.

3. Example of a fluorescent staining with Annexin V-FITC Kit

Jurkat cells cultured with staurosporine (50 nM) for 15 h, were stained with Annexin V-FITC and PI and analyzed by flow cytometry.



4. References

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