Miltenyi Biotec

Annexin V

Contents

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. Description

This product is for research use only.

Components Annexin V conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-093-060	–
PE	130-108-077	130-108-112
Biotin	130-092-773	–

Capacity 1 mL: 100 tests or up to 10^8 total cells

300 µL: 30 tests or up to 3×10^7 total cells.

Product format Annexin V conjugates are 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.

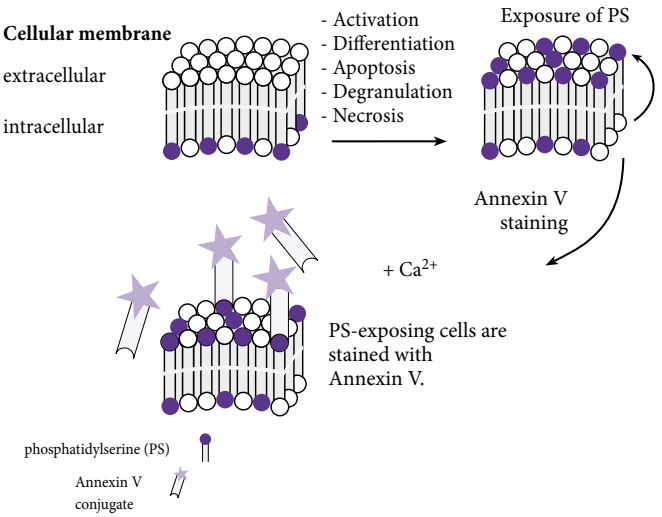
1.1 Background information

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 such as 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 36 kDa phospholipid-binding protein and has a high affinity to PS in the presence of physiological concentrations of calcium (Ca^{2+}).[10]

MACS® Annexin V fluorochrome and biotin conjugates have been developed for the detection and discrimination of apoptotic and dead cells. Apoptotic cells, which are otherwise undetectable by staining with propidium iodide (PI) or 4',6-diamidino-2-phenylindole (DAPI), can be directly detected through their staining with fluorochrome-conjugated Annexin V. Dead cells are stained with both Annexin V and PI or DAPI, whereas viable cells cannot be stained with either.

Staining procedure



1.2 Applications

- Studies on cell death (apoptotis and/or necrosis).
- Evaluation of MACS Separations with the Annexin V MicroBead Kit (# 130-090-201) and the Dead Cell Removal Kit (# 130-090-101).

1.3 Reagent requirements

- Buffer: Prepare 1× Annexin V Binding Buffer from the Annexin V Binding Buffer 20× Stock Solution (# 130-092-820):
  For 10^6 total cells, dilute 500 µL of the Annexin V Binding Buffer (20× Stock Solution) with 9.5 mL of sterile, distilled water. Alternatively, prepare 1× Annexin V Binding Buffer by diluting 25 mL of the 20× Stock Solution with 475 mL of sterile, distilled water. Store at 2–8 °C.
  ▲ **Note:** Handle under sterile conditions.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Annexin V-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233), DAPI, or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

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140-001-866.03

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for **up to** 10^6 nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

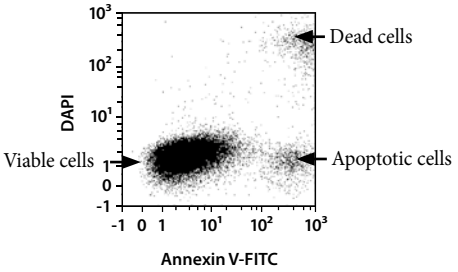
▲ When working with cell samples containing platelets (e.g. blood samples), wash samples carefully at a low centrifugation speed (200×g) in order to remove platelets. Use buffer containing the ion chelator EDTA for these washing steps. Finally, wash with Annexin V Binding Buffer to avoid chelation of Ca^{2+} ions. Activated platelets expose PS and therefore bind Annexin V.[5]

1. Wash 10^6 cells in 1 mL of 1× Annexin V Binding Buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Repeat washing step.
3. Resuspend 10^6 cells in 100 µL of 1× Annexin V Binding Buffer.
4. Add 10 µL of Annexin V conjugate.
5. Mix well and incubate for 15 minutes in the dark at room temperature.
   ▲ **Note:** Lower temperatures may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1 mL of 1× Annexin V Binding Buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) Repeat washing step.
8. (Optional) If Annexin V-Biotin was used, resuspend the cell pellet in 100 µL of 1× Annexin V Binding Buffer per 10^6 cells, add 10 µL of anti-biotin antibody, incubate for 10 minutes in the dark in the refrigerator (2–8 °C), and continue as described in step 6.
9. Resuspend cell pellet in 500 µL of 1× Annexin V Binding Buffer per 10^6 **total** cells.
10. (Optional) Add 1 µg/mL of Propidium Iodide Solution or 0.01 µg of DAPI immediately prior to analysis by flow cytometry or fluorescence microscopy.

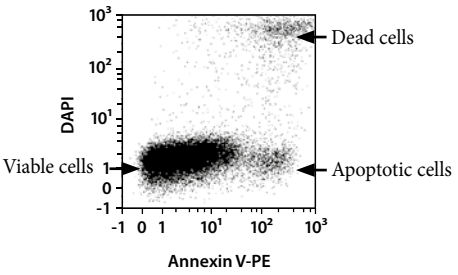
3. Examples of immunofluorescent staining with Annexin V fluorochromes and conjugates

Jurkat cells, cultured with staurosporine (50 nM) for 15 hours, were stained with Annexin V conjugated to FITC (A) or PE (B) followed by staining with DAPI and analyzed by flow cytometry using the MACSQuant® Analyzer.

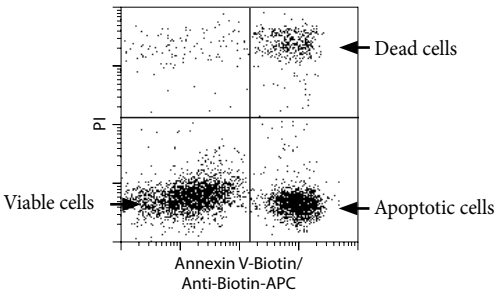
A)



B)



Jurkat cells were cultured with and without different concentrations of staurosporine (200 nM up to 2 µM) for different times (from 4 up to 20.5 hours). Cells were then pooled and stained with Annexin V-Biotin followed by Anti-Biotin-APC and PI and analyzed by flow cytometry.



4. References

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9. Fadok, V.A. *et al.* (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405: 85–90.
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Refer to **www.miltenyibiotec.com** for all data sheets and protocols.