



Annexin V

Annexin V-FITC
Annexin V-Biotin

130-093-060
130-092-773

Index

1. Description

1.1 Background and product applications

1.2 Reagent requirements

2. General protocol for immunofluorescent staining

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

4. References

1. Description

Product format 1 mL Annexin V:
Annexin V conjugated to fluorescein isothiocyanate (FITC) or to biotin.

All components are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests or up to 10^8 total cells.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

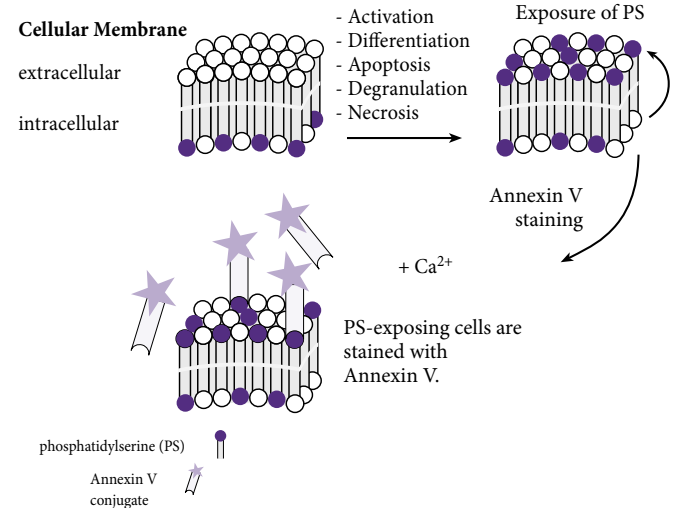
1.1 Background and product applications

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 platelets³, in certain cell anomalies such as sickle cell anemia⁴, in erythrocyte senescence⁵, upon degranulation of mast cells⁶, and in certain stages of B cell differentiation⁷. 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), can be directly detected through their staining with fluorochrome-conjugated Annexin V. Dead cells are stained with both Annexin V and PI, whereas viable cells cannot be stained with either.

Staining procedure



Product applications

- Studies on cell death (apoptosis 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).

1.2 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) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Annexin V-Biotin.
- (Optional) Propidium iodide (PI) 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.

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).

140-001-886-01

Miltenyi Biotec

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

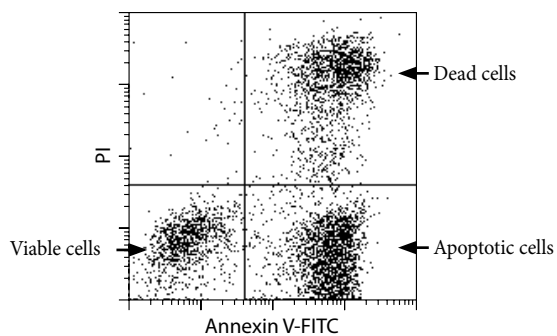


▲ When working with cell samples containing platelets (e.g. blood samples), wash samples carefully at a low centrifugation speed (200×g) in order to remove platelets. Use buffer containing the ion chelator EDTA for these washing steps. Finally, wash with Annexin V Binding Buffer to avoid chelation of Ca²⁺ ions. Activated platelets expose PS and therefore bind Annexin V.⁵

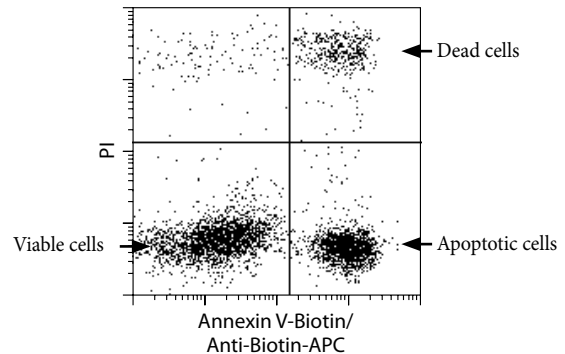
1. Wash 10⁶ cells in 1 mL of 1× Annexin V Binding Buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Repeat washing step.
3. Resuspend 10⁶ cells in 100 µL of 1× Annexin V Binding Buffer.
4. Add 10 µL of Annexin V fluorochrome or biotin conjugate.
5. Mix well 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 10⁶ 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 10⁶ cells, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), refrigerate for 10 minutes in the dark (4–8 °C), and continue as described in step 6.
9. Resuspend cell pellet in 500 µL of 1× Annexin V Binding Buffer per 10⁶ total cells.
10. (Optional) Add 1 µg/mL of PI solution immediately prior to analysis by flow cytometry or fluorescence microscopy.

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

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



(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

1. Koopman, G. *et al.* (1994) Annexin V for Flow Cytometric Detection of Phosphatidylserine expression on B Cells Undergoing Apoptosis. *Blood* 84: 1415–1420.
2. Martin, S.J. *et al.* (1995) Early Redistribution of Plasma Membrane Phosphatidylserine Is a General Feature of Apoptosis Regardless of the Initiating Stimulus: Inhibition by Overexpression of Bcl-2 and Abl. *J. Exp. Med.* 182: 1545–1556.
3. Thiagarajan, P. and Tait, J.F. (1990) Binding of annexin V/placental anticoagulant protein I to platelets. Evidence for phosphatidylserine exposure in the procoagulant response of activated platelets. *J. Biol. Chem.* 265: 17420–17423.
4. Kuypers, F.A. *et al.* (1996) Detection of Altered Membrane Phospholipid Asymmetry in Subpopulations of Human Red Blood Cells Using Fluorescently Labeled Annexin V. *Blood* 87: 1179–1187. [181]
5. Schroit, A.J. and Zwaal, R.F.A. (1991) Transbilayer movement of phospholipids in red cells and platelet membranes. *Acta Biochim. Biophys.* 1071: 313–329.
6. Demo, S.D. *et al.* (1999) Quantitative Measurement of Mast Cell Degranulation Using a Novel Flow Cytometric Annexin-V Binding Assay. *Cytometry* 36: 340–348.
7. Dillon, S.R. *et al.* (2001) Annexin V Binds to Positively Selected B Cells. *J. Immunol.* 166: 58–71.
8. Fadok, V.A. *et al.* (1992) Exposure of Phosphatidylserine on the Surface of Apoptotic Lymphocytes Triggers Specific Recognition and Removal by Macrophages. *J. Immunol.* 148: 2207–2216.
9. Fadok, V.A. *et al.* (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 405: 85–90.
10. Moss, S.E. *et al.* (1991) Diversity in the Annexin Family. In *Novel Calcium Binding Proteins*, Springer Verlag, 535–566.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

MACS is a registered trademark of Miltenyi Biotec GmbH.

© 2006 Miltenyi Biotec GmbH. Printed in Germany.