eBioscience™ Annexin V Apoptosis Detection Kit APC

Catalog Numbers 88-8007-74, 88-8007-72

Pub. No. MAN0025583 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in a calcium-dependent manner.

In early-stage apoptosis, the plasma membrane excludes viability dyes such as propidium iodide (PI), 7-AAD, or Fixable Viability Dyes such as eFluor™ 660/eFluor™ 780. Cells will stain with Annexin V but not a viability dye, thus distinguishing cells in early apoptosis. However, in late stage apoptosis, the cell membrane loses integrity, thereby allowing Annexin V to also access PS in the interior of the cell. A viability dye can be used to resolve these late-stage apoptotic and necrotic cells (Annexin V, viability dye-positive) from the early-stage apoptotic cells (Annexin V positive, viability dye-negative).

Note: The Annexin V Apoptosis Detection Kits are compatible with intracellular staining. Please refer to thermofisher.com/apoptosis for details.

Note: Fixable Viability Dye eFluor™ 450 is not recommended for use with Annexin V Apoptosis Detection Kits.

Contents and storage

Component	Cat. No. 88-8006-72 (50 tests)	Cat. No. 88-8006-74 (200 tests)	Storage
10X Binding Buffer	30 mL	100 mL	Store at 2 to 8°C
Annexin V eFluor™ 450	1 vial (0.25 mL)	1 vial (1 mL)	Store at 2 to 8°C Protect from light
7-AAD Viability Staining Solution	1 vial (0.5 mL)	2 vials (0.5 mL)	Store at 2 to 8°C Protect from light

Procedural guidelines

Due to the calcium dependence of the Annexin V:PS interaction, it is critical to avoid buffers containing EDTA or other calcium chelators during Annexin V experiments. Annexin V can only be used as a marker of apoptosis in cells where the plasma membrane is intact because destroying the integrity of the plasma membrane will allow non-specific binding of Annexin V to PS inside the cell.

Stain cells using Annexin V

1. Dilute 10X binding buffer to 1X using distilled water (e.g., 1 mL 10X binding buffer + 9 mL of dH_20).
2. Wash cells once in PBS, then once in 1X binding buffer.
3. Resuspend cells in 1X binding buffer at 1–5 x 10^6 cells/mL.
4. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.

5. Incubate for 10-15 minutes at room temperature.
6. Wash cells in 1X binding buffer, then resuspend in 200 µL of 1X binding buffer.
7. Add 5 µL of Propidium Iodide Staining Solution (Cat. No. 00-6990-50) or 7-AAD Viability Staining Solution (Cat. No. 00-6993-50).
8. Analyze by flow cytometry within 4 hours, then place at 2 to 8°C in the dark.

Stain cells using Annexin V and Fixable Viability Dyes

Required materials not supplied

- PBS without sodium azide
- Fixable Viability Dyes—see **thermofisher.com/livedead**
- Distilled water
- Flow Cytometry Staining Buffer (Cat. No. 00-4222-26)

Choose an appropriate viability stain that has an emission profile compatible with the Annexin V-conjugate to be used.

Note: Fixable Viability Dye eFluor™ 450 is not recommended for use with the Annexin V Apoptosis Detection Kits.

1. Follow the staining protocol for the chosen Fixable Viability Dye to stain late-apoptotic/dead cells. Go to **Best Protocols:Viability Staining for Flow Cytometry, Protocol C.**
2. After staining with Fixable Viability Dye, wash cells twice with a protein-containing buffer such as Flow Cytometry Staining Buffer (Cat. No. 00-4222-26).
3. Dilute 10X binding buffer to 1X using distilled water (e.g., 1 mL 10X binding buffer + 9 mL of dH_20).
4. Wash cells once with the 1X binding buffer.
5. Resuspend cells in 1X binding buffer at 1–5 x 10^6 cells/mL.
6. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
7. Incubate for 10-15 minutes at room temperature, protected from light.
8. Wash cells in 1X binding buffer and resuspend in 200 µL of 1X binding buffer.
9. Analyze by flow cytometry within 4 hours, storing at 2 to 8°C in the dark.

References

Andree HA, Reutelingsperger CP, Hauptmann R, Hemker HC, Hermens WT, Willems GM. Binding of vascular anticoagulant alpha (vAc alpha) to planar phospholipid bilayers. J Biol Chem. 1990; 265(9):4923-4928

Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 1994; 84(5):1415-1420

Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods. 1995; 184(1):39-51

Related products

For more information on other products for apoptosis research, visit **thermofisher.com/apoptosis**.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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Revision	Date	Description
A.0	24 March 2022	The content and format were updated.

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