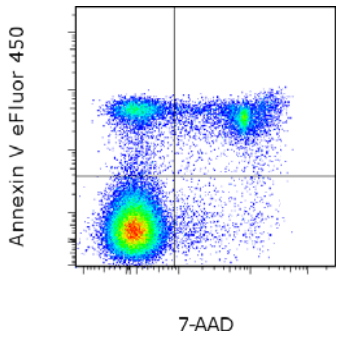
eBioscience™ Annexin V Apoptosis Detection Kit eFluor™ 450

Catalog Number: 88-8006

For Research Use Only. Not for use in diagnostic procedures.



Mouse thymocytes were prepared as a single cell suspension and incubated overnight at 37°C in medium. Cells were harvested and stained with Annexin V eFluor® 450 and 7-AAD Viability Staining Solution.

Product Information

Contents: eBioscience™ Annexin V Apoptosis Detection Kit eFluor™ 450

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Concentration: 5 uL/test

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer

Temperature Limitation: Store at 2-8°C. Do not freeze. Light sensitive material.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Contains sodium azide

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 or eFluor® 780. These 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).

Components

Cat. No. 88-8006-72:

10X Binding Buffer (cat. 00-0055): 30 mL, store at 2-8°C.
Annexin V eFluor® 450 (cat. 48-8006): 5 µL/test, 50 tests, store at 2-8°C. Protect from light.
7-AAD Viability Staining Solution (cat. 00-6993): 5 µL/test, 100 tests, store at 2-8°C. Protect from light.

Cat. No. 88-8006-74:

10X Binding Buffer (cat. 00-0055): 100 mL, store at 2-8°C.
Annexin V eFluor® 450 (cat. 48-8006): 5 µL/test, 200 tests, store at 2-8°C. Protect from light.
7-AAD Viability Staining Solution (cat. 00-6993): 5 µL/test, 2x100 tests, store at 2-8°C. Protect from light.

Applications Reported

The Annexin V Apoptosis Detection Kit eFluor® 450 has been reported for use in flow cytometric analysis.

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Applications Tested

The Annexin V Apoptosis Detection Kit eFluor® 450 has been pre-titrated and tested on mouse thymocytes cultured overnight in medium (to induce apoptosis) or on Jurkat cells treated with 10 µM Camptothecin for 4 hours. Both the Annexin V eFluor® 450 and the 7-AAD components can be used at 5 µL per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test.

Special Notes

The Annexin V Apoptosis Detection Kits are compatible with intracellular staining. Please refer to the Best Protocols: Annexin V Staining Protocol, Protocol C for details.

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

00-6990 eBioscience™ Propidium Iodide Staining Solution
65-0864 eBioscience™ Fixable Viability Dye eFluor™ 660
65-0865 eBioscience™ Fixable Viability Dye eFluor™ 780
88-8005 eBioscience™ Annexin V Apoptosis Detection Kit FITC
88-8007 eBioscience™ Annexin V Apoptosis Detection Kit APC
88-8008 eBioscience™ Annexin V Apoptosis Detection Kit PerCP-eFluor™ 710
88-8102 eBioscience™ Annexin V Apoptosis Detection Kit PE

Legal

Pat. No. EP 181 465 B2, EP 0509 026, USP 5,066,787

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Annexin V Staining Protocols

Protocol: Annexin V Staining

Note: 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.

Experimental Procedure

1. Dilute 10X Binding Buffer to 1X using distilled water (1 mL 10X Binding Buffer + 9 mL dH_20).
2. Wash cells once in PBS, then once in 1X Binding Buffer.
3. Resuspend cells in 1X Binding Buffer at 1-5x10^6/mL.
4. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
5. Incubate 10-15 minutes at room temperature.
6. Wash cells in 1X Binding Buffer and resuspend in 200 µL of 1X Binding Buffer.
7. Add 5 µL of Propidium Iodide Staining Solution (cat. 00-6990) or 7-AAD Viability Staining Solution (cat. 00-6993).
8. Analyze by flow cytometry within 4 hours, storing at 2-8°C in the dark

Protocol: Annexin V Staining with Fixable Viability Dyes

Note: 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.

Materials Needed

- PBS without sodium azide
- Fixable Viability Dyes
  - Fixable Viability Dye eFluor™ 455UV (Cat. No. 65-0868)
  - Fixable Viability Dye eFluor™ 450 (Cat. No. 65-0863)
  - Fixable Viability Dye eFluor™ 520 (Cat. No.65-0867)
  - Fixable Viability Dye eFluor™ 660 (Cat. No. 65-0864)
  - Fixable Viability Dye eFluor™ 780 (Cat. No. 65-0865)
- Distilled water
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)

Experimental Procedure

1. 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.
2. Follow the staining protocol for the chosen Fixable Viability Dye to stain late-apoptotic/dead cells.
   Refer to the Best Protocols webpage (Viability Staining Protocol C in the Resources tab of the home page).
3. After staining with Fixable Viability Dye, be sure to wash cells twice with a protein-containing buffer such as Flow Cytometry Staining Buffer (cat. 00-4222).
4. Dilute 10X Binding Buffer to 1X using distilled water (1 mL 10X Binding Buffer + 9 mL dH_20).
5. Wash cells once with the 1X Binding Buffer.
6. Resuspend cells in 1X Binding Buffer at 1-5x10^6/mL.
7. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
8. Incubate 10-15 minutes at room temperature, protected from light.
9. Wash cells in 1X Binding Buffer and resuspend in 200 µL of 1X Binding Buffer.
10. Analyze by flow cytometry within 4 hours, storing at 2-8°C in the dark.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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