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Annexin V Staining

Research Use Only

Introduction

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, as well as Fixable Viability Dyes (FVD) such as eFluor® 660, eFluor® 506 or eFluor® 780. These cells will stain with Annexin V but not with viability dyes, thus distinguishing cells in early apoptosis.
- 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 positive; viability dye positive) from the early-stage apoptotic cells (Annexin V positive; viability dye negative).

General Notes

- *Fixable Viability Dye (FVD) eFluor® 450 is not recommended for use with Annexin V Apoptosis Detection Kits.*
- *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. Destroying the integrity of the plasma membrane will allow binding of Annexin V to PS inside the cell.*

Protocol A: Annexin V staining

Materials

- 12 x 75 mm round-bottom tubes
- 1X PBS
- Annexin V Apoptosis Detection kit (cat. nos. 88-8103, 88-8007, 88-8006, 88-8005, 88-8102, or 88-8008)
- 10X Binding buffer
- Annexin conjugated to PE-Cyanine7, APC, eFluor® 450, FITC, PE or PerCP-eFluor® 710, respectively (see catalog numbers for detection kits)

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- Propidium Iodide Staining Solution (cat. no. 00-6990) or 7-AAD Viability Staining Solution (cat. no 00-6993)

  NOTE*: Cat. no. 88-8008 does not include a viability dye. We recommend using this format in combination with a Fixable Viability Dye (FVD) such as FVD eFluor® 660 (Cat. No. 65-0864), FVD eFluor® 506 (Cat. No. 65-0866), or FVD eFluor® 780 (Cat. No. 65-0865)*

Experimental Procedure

1. Prepare 1X binding buffer by mixing 1 part of 10X binding buffer with 9 parts of distilled water.
2. Harvest cells.
3. Wash cells once in 1X PBS, then once in 1X binding buffer.
4. Resuspend cells in 1X Binding Buffer at 1-5 x 10^6 cells/mL.
5. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
6. Incubate 10-15 minutes at room temperature. Protect from light.
7. Add 2 mL 1X binding buffer and centrifuge at 400-600 *x g* for 5 minutes at room temperature. Discard supernatant.
8. Resuspend cells in 200 µL of 1X binding buffer.
9. Add 5 µL of Propidium Iodide Staining Solution or 7-AAD Viability Staining Solution and incubate 5-15 minutes on ice or at room temperature.

   NOTE: Propidium iodide and 7-AAD must remain in the buffer during acquisition. Do not wash cells after the addition of propidium iodide or 7-AAD.

10. Analyze by flow cytometry.

    NOTE: Cells should be analyzed within 4 hours after the initial incubation period due to adverse effects on the viability of cells left in the presence of propidium iodide or 7-AAD for prolonged periods. Store at 2–8°C and protect from light until ready for analysis.

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Protocol B: Annexin V staining with Fixable Viability Dyes

Materials

- 12 x 75 mm round-bottom tubes
- 1X PBS
- Annexin V Apoptosis Detection kit (cat. nos. 88-8007, 88-8006, 88-8005, 88-8102, 88-8103, or 88-8008)
- 10X Binding buffer
- Annexin conjugated to APC, eFluor® 450, FITC, PE, PE-Cyanine7 or PerCP-eFluor® 710, respectively (see catalog numbers for detection kits)
- Flow Cytometry Staining Buffer (cat. no. 00-4222)
- PBS (azide- and serum/protein-free PBS)
- FVD eFluor® 660 (cat. no. 65-0864), FVD eFluor® 506 (cat. no. 65-0866) or FVD eFluor® 780 (cat. no. 65-0865)

NOTE: *FVD eFluor® 450 is not recommended for use with the Annexin V Apoptosis Detection Kits.*

Experimental Procedure

1. Prepare 1X binding buffer by mixing 1 part of 10X binding buffer with 9 parts of distilled water.
2. Wash cells twice in azide-free and serum/protein-free PBS.
3. Resuspend cells at 1-10 x 10^6 cells/mL in azide-free and serum/protein-free PBS.
4. Add 1 µL of FVD per 1 mL of cells and vortex immediately.
5. Incubate for 30 minutes at 2-8°C. Protect from light.
6. Wash cells twice with Flow Cytometry Staining Buffer or equivalent.
7. Wash cells once with 1X Binding Buffer.
8. Resuspend cells in 1X Binding Buffer at 1-5 x 10^6 cells/mL.
9. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
10. Incubate 10-15 minutes at room temperature. Protect from light.
11. Add 2 mL of 1X binding buffer and centrifuge at 400-600 *x g* for 5 minutes at room temperature. Discard supernatant.
12. Resuspend cells in 200 µL of 1X Binding Buffer.
13. Analyze by flow cytometry.

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Protocol C: Annexin V staining with surface and intracellular staining

Materials

- 12 x 75 mm round-bottom tubes
- 1X PBS (azide- and serum/protein-free PBS)
- Flow Cytometry Staining Buffer Set (cat. no. 00-4222)
- Foxp3/Transcription Factor Staining Buffer Set (Cat. No. 00-5523) or Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824)
- Annexin V Apoptosis Detection kit (cat. nos. 88-8103, 88-8007, 88-8006, 88-8005, 88-8102, or 88-8008)
- 10X Binding buffer
- Annexin conjugated to PE-Cyanine7, APC, eFluor® 450, FITC, PE or PerCP-eFluor® 710, respectively (see catalog numbers for detection kits)
- FVD eFluor® 660 (cat. no. 65-0864), FVD eFluor® 506 (cat. no. 65-0866) or FVD eFluor® 780 (cat. no. 65-0865)

> **NOTE**: *FVD eFluor® 450 is not recommended for use with the Annexin V Apoptosis Detection Kits.*

Experimental Procedure

1. Prepare 1X binding buffer by mixing 1 part of 10X binding buffer with 9 parts of distilled water.
2. Stain cell surface antigen(s). Refer to 'Staining Cell Surface Targets, Protocol A' as described in Best Protocols.
3. Wash cells twice in azide-free and serum/protein-free PBS.
4. Resuspend cells at 1-10 x 10^6 cells/mL in azide-free and serum/protein-free PBS.
5. Add 1 µL of Fixable Viability Dye per 1 mL of cells and vortex immediately.
6. Incubate for 30 minutes at 2-8°C. Protect from light.
7. Wash cells twice in Flow Cytometry Staining Buffer or equivalent.
8. Wash cells once with 1X binding buffer.
9. Resuspend cells in 1X binding buffer at 1-5 x 10^6 cells/mL.
10. Add 5 µL of fluorochrome-conjugated Annexin V to 100 µL of the cell suspension.
11. Incubate 10-15 minutes at room temperature. Protect from light.
12. Wash cells once with 1X binding buffer.
13. Stain intracellular antigen(s). Refer to 'Staining Intracellular Targets, Protocol A or B' as described in Best Protocols.
14. Analyze by flow cytometry.

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