full spectrum cell analysis
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Annexin V kit for detection of early apoptotic events

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Introduction

Annexins are a family of calcium-dependent phospholipid-binding proteins abundant in the eukaryotic kingdom. Annexin V preferentially binds to phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet or cytosol-facing part of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution in the phospholipid bilayer and is translocated to the extracellular membrane leaflet where it identifies cells as targets for phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin-V in a Ca^{2+} -dependent manner.

In early stage apoptosis, the plasma membrane excludes viability dyes such as propidium iodide and 7-AAD, so these cells will only stain with Annexin-V. Thus, Annexin-V binding serves as an early marker of apoptosis. However, in late stage apoptosis, the integrity of the cell membrane is lost allowing Annexin-V to access PS in the interior of the cell. A viability dye such as 7-AAD or propidium iodide (PI) can be used to resolve these late stage apoptotic and necrotic cells (Annexin-V and 7-AAD or PI double positive) from the early stage apoptotic cells (Annexin-V positive, 7-AAD or PI negative).

Useful websites

Protocol Online (http://www.protocol-online.org/prot/Cell_Biology/Apoptosis/index.html)
Protocol Online is a database of research protocols contributed by researchers worldwide.

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Materials

- Annexin-V FITC Apoptosis Detection Kit (eBioscience Cat. No. 88-8005), Annexin-V APC Apoptosis Detection Kit (eBioscience Cat. No. 88-8007) or Annexin-V eFluor® 450 Apoptosis Detection Kit (eBioscience Cat. No. 88-8006)
- Propidium Iodide Staining Solution (eBioscience Cat. No. 00-6990)
- PBS

Buffers

- Prepare 1X Binding Buffer by diluting 10X stock in distilled water

Experimental Procedure

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 staining. If you intend to fix your cells, incubate them with Annexin-V prior to fixation to avoid access of Annexin-V to the

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inner leaflet of the plasma membrane. Thoroughly wash the cells with Binding Buffer to remove unbound Annexin-V before fixation.

1. Prepare cells for assay according to your experimental protocol.
2. Centrifuge samples at 300-400 x g at 4°C for 5 minutes, discard supernatant.
3. Resuspend cell pellet in 2 ml PBS.
4. Centrifuge samples at 300-400 x g at 4°C for 5 minutes, discard supernatant.
5. Resuspend cell pellet in 2 ml 1X Binding Buffer.
6. Centrifuge samples at 300-400 x g at 4°C for 5 minutes, discard supernatant.
7. Resuspend cells in 1X Binding Buffer at a concentration of 1-5 x 10^6/ml.
8. Gently mix 100 ul of the cell suspension with 5 µl of fluorochrome-conjugated Annexin-V.
9. Incubate for 10-15 minutes at room temperature in the dark.
10. Wash cells once with 2 ml 1X Binding Buffer followed by centrifugation at 300-400 x g for 5 minutes, discard supernatant.
11. Resuspend in 200 µl of 1X Binding Buffer.
12. Add 5 µl of Propidium Iodide Staining Solution to each sample
13. Analyze with a flow cytometer using the PE detector to collect the PI signal. It is recommended that analysis be performed immediately after staining as apoptosis is a swift and dynamic process.
14. Recommended controls include cells stained with Annexin-V alone or PI alone in order to optimize cytometer settings.

References

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