Annexin V Apoptosis Detection Kit with 7-AAD, FITC

Apoptosis staining and detection kit

Catalog #100-0338 100 tests

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Product Description

The Annexin V Apoptosis Detection Kit includes Annexin V, FITC (Catalog #100-0333), Annexin V Binding Buffer (Catalog #100-0334), and 7-AAD (7-Aminoactinomycin D; Catalog #75001.1). The kit is used for the combined detection of early-stage cell apoptosis (using Annexin V) and late-stage cell apoptosis or necrosis (using both Annexin V and 7-AAD). Annexin V belongs to the annexin family of intracellular proteins that bind to membrane phospholipids in a calcium-dependent manner. This dye has a high affinity for phosphatidylserine (PS) that is present in the inner leaflet of the plasma membrane. During early-stage cell apoptosis, PS is translocated from the inner to the outer leaflet of the cell membrane, exposing it to the external environment, where it is detected by Annexin V. This process of PS translocation occurs prior to the loss of membrane integrity. As cells progress through apoptosis and towards necrosis, the cell membrane is compromised and consequently the 7-AAD viability dye passes into the cell. Thus, cells undergoing early apoptosis stain positive for Annexin V and negative for viability dyes, while apoptotic death or necrosis is characterized by positive staining for both Annexin V and 7-AAD.

Product Information

The following components are sold as a complete kit (Catalog #100-0338), and are also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Annexin V, FITC	100-0333	100 tests	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label. Protect from prolonged exposure to light.
7-AAD (7-Aminoactinomycin D)	75001.1	200 tests	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label. Protect from prolonged exposure to light.
Annexin V Binding Buffer	100-0334	50 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.

Applications

Verified: FC
Reported: FC

Abbreviations: CellSep: Cell separation; ChIP: Chromatin immunoprecipitation; FA: Functional assay; FACS: Fluorescence-activated cell sorting; FC: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence microscopy; IHC: Immunohistochemistry; IP: Immunoprecipitation; RIA: Radioimmunoassay; WB: Western blotting

Directions for Use

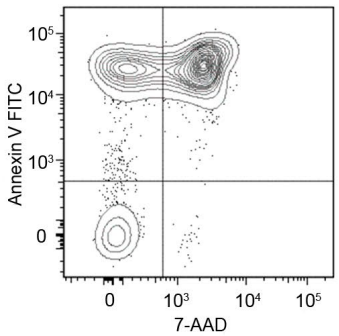
FLOW CYTOMETRY

1. Wash cells with 1 - 2 mL of phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) (e.g. Catalog #07905).
2. Centrifuge at 300 x *g* for 5 minutes at room temperature (15 - 25°C). Remove and discard supernatant.
3. Repeat steps 1 and 2 using PBS containing 2% FBS. To reduce background staining, 1 mL of Annexin V Binding Buffer (Catalog #100-0334) may be used instead.
4. Resuspend cells at a concentration of 1 - 10 x 10^6 cells/mL in Annexin V Binding Buffer.
5. Aliquot 100 µL of cell suspension to individual tubes for staining.
6. Add 5 µL of Annexin V, FITC to each tube.
7. Add 5 µL of 7-AAD (Catalog #75001.1) to each tube.
8. Gently vortex cells and incubate at room temperature for 15 minutes. Protect samples from light.

9. OPTIONAL: To reduce background staining, wash cells with 1 mL of Annexin V Binding Buffer. Centrifuge sample at 300 x *g* for 5 minutes at room temperature. Remove and discard supernatant.
10. Add 200 µL of Annexin V Binding Buffer to each tube.
11. Cells are now ready to be analyzed by flow cytometry.

NOTE: If washing cells with Annexin V Binding Buffer to reduce background staining, additional buffer may be required.

Data



Flow cytometry analysis of C57BL/6 mouse thymocytes incubated at 37°C with 1 µM dexamethasone overnight. Cells were harvested and labeled with Annexin V, FITC and 7-AAD.

References

1. Koopman G et al. (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood 84(5): 1415–20. (FC)