Annexin V, PE

A cellular protein for detection of apoptotic cells

Catalog #100-0330	25 tests	5 µL/test
#100-0331	100 tests	5 µL/test

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Product Description

Annexin V is a member of the annexin family of proteins that bind to membrane phospholipids in the presence of calcium. This dye has 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 plasma membrane, exposing it to the external environment. Annexin V, a characteristic marker for early cell apoptosis, detects the translocation of PS to the external environment. Annexin V is used along with viability dyes such as 7-AAD (7-Aminoactinomycin D; Catalog #75001) or Propidium Iodide (Catalog #75002). The process of PS translocation occurs prior to the loss of membrane integrity. Therefore, as cells progress through apoptosis and towards necrosis, the cell membrane is compromised and consequently, viability dyes pass 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 the viability dye.

Properties

Storage: Product stable at 2 - 8°C when stored undiluted. Do not freeze. Protect product from prolonged exposure to light.
Shelf Life: Stable until expiry date (EXP) on label.
Conjugate: PE (Phycoerythrin)

Applications

Verified: FC
Reported: FC

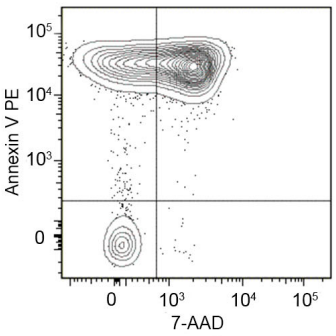
Handling / Directions for Use

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 sample 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, PE to each tube.
7. Add 5 µL of 7-AAD (7-Aminoactinomycin D; Catalog #75001) to each tube.
8. Gently vortex cells and incubate at room temperature (15 - 25°C) 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, PE and 7-AAD (7-Aminoactinomycin D; Catalog #75001).

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.