Annexin V- iFluor™ 488 STAINING PROTOCOL

Cat. No. CTG-AP0004 1 Kit (100 tests)

Annexin V- iFluor™ 488 Apoptosis Detection Kit is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Annexin V is a calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for Annexin V- iFluor™ 488 and negative for PI are undergoing apoptosis. Cells that stain positive for both Annexin V- iFluor™ 488 and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both Annexin V- iFluor™ 488 and PI are alive and not undergoing measurable apoptosis.

Reagents Preparation

1. Annexin V- iFluor™ 488: Use 5 µl per test.
2. Propidium Iodide (PI) is a convenient, ready-to-use nucleic acid dye. Use 5 µl per test.
3. 10X Annexin V Binding Buffer: 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM $CaCl_2$. For a 1X working solution, dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.

Staining

1) Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/ml.
2) Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3) Add 5 µl of Annexin V- iFluor™ 488 and 5 µl PI.
4) Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5) Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

The following controls are used to set up compensation and quadrants:

- Unstained cells.
- Cells stained with Annexin V- iFluor™ 488 (no PI).
- Cells stained with PI (no Annexin V- iFluor™ 488).

References

1. Schmid I, Krall WJ, Uittenbogaart CH, Braun J, Giorgi JV. Cytometry. 1992; 13(2):204-208.
2. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. J Immunol Methods. 1995; 184(1):39-51.
3. Andree HA, Reutelingsperger CP, Hauptmann R, Hemker HC, Hermens WT, Willems GM. J Biol Chem. 1990; 265(9):4923-4928.
4. Casciola-Rosen L, Rosen A, Petri M, Schlissel M. Proc Natl Acad Sci U S A. 1996; 93(4):1624-1629.
5. Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Blood. 1995; 85(2):532-540.