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MESGEN[™]
INNOVATION BIOTECHNOLOGY

Description

Annexins are a family of calcium-dependent phospholipid-binding proteins, which bind to phosphatidylserine (PS) to identify apoptotic cells. In healthy cells, PS is predominantly located along the cytosolic side of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution in the phospholipid bilayer and translocates to the extracellular membrane, which is detectable with fluorescently labeled Annexin V. In early stages of apoptosis, the plasma membrane excludes viability dyes such as propidium iodide (PI), therefore cells which display only Annexin V staining (PI negative) are in early stages of apoptosis. During late-stage apoptosis, loss of cell membrane integrity allows Annexin V binding to cytosolic PS, as well as cell uptake of PI. Annexin V staining, paired with PI is widely used to identify apoptotic stages by flow cytometry.

Principles of the Test

Annexin V exhibits anti-phospholipase activity and binds to phosphatidylserine. FITC labelling allows simple direct detection by FACS analysis. Counterstaining by propidium iodide allows the discrimination of apoptotic cells.

Kit Components

	20T	50T	100T
Annexin V-FITC	100µl	250µl	500µl
1×Binding Buffer	8ml	20ml	40ml
Propidium iodide (PI)	200µl	500µl	1000µl

Staining Procedure

1. Wash cells twice with cold PBS (MesGen Cat.No.MG3150), and then resuspend cells in 400µl 1×Binding Buffer at a concentration of $0.25-1.0 \times 10^7$ cells/ml.
2. Add 5 µl of Annexin V-FITC. Gently vortex the cells and incubate for 15 min at room temperature (25°C) in the dark.
4. Add 10 µl of Propidium Iodide Solution and incubate for 5 min at 4°C in the dark.
6. Analyze by flow cytometry with proper machine settings.

Caution

Propidium Iodide Solution is toxigenic and mutagenic; handle with care

Storage

Annexin V-FITC store at -20°C.

1×Binding Buffer and Propidium iodide (PI) store at 2-8°C.

All reagent stored protecting from light.

For Research Use Only. Not For Use In Diagnostic Procedures.