**Annexin V-FITC/PI Apoptosis Detection Kit MD8831**

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**Technical literature is available at:** [**www.mesgenbio.com**](http://www.mesgenbio.com)**. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com**

**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**

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|  | **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 x 107 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 at2-8°C.

All reagent stored protecting from light.

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