

PRODUCT INFORMATION

Annexin V-Biotin Apoptosis Detection Kit

Cat. No. 39902

Product Description:

General Annexins are a family of calcium-dependent phospholipid-binding proteins, which bind to phosphatidylserine (PS). Externalization of phosphatidylserine residues on the outer plasma membrane of apoptotic cells allows detection via Annexin V. Once the apoptotic cells are bound with labelled Annexin V, they can be visualized with fluorescent microscopy or cytometry. Since loss of membrane integrity is a pathognomonic feature of necrotic cell death, necrotic cells will stain with specific membrane-impermeant nucleic acid dyes such as propidium iodide, the membrane integrity of apoptotic cells can be demonstrated by the exclusion of these dyes.

Content The kit is sufficient for 100 reactions.

Application

- Detect early/middle stages of apoptosis
- Differentiation of apoptosis from necrosis

Storage Store at +2 °C to + 8 °C. Protect from light. **Do not freeze.**

Assay Procedure

1. Dilute the 10X Annexin V Binding Buffer with dH₂O to 1X and keep on ice.
2. Collect cells of interest by centrifugation. Wash cells with ice-cold culture medium or PBS.
3. Resuspend cells in Annexin V Binding Buffer at a concentration of 1-5 x 10⁶ cells/ml.
4. Transfer 500 µl of cell suspension in a 5 ml test tube.
5. Add 5 µl of labeled- Annexin V.
6. Add 10 µl of Propidium Iodide Solution.
7. Gently vortex the cells and incubate for 15 min at room temperature (25°C) in the dark.
8. Wash cells in 1X Binding Buffer and resuspend in 400 µl of 1X Binding Buffer.
9. Analyze by flow cytometry with proper machine settings.

Caution:

Propidium iodide is a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper wear proper protective clothing and gloves when handling the solution.