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DATA SHEET

Version: 03
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1. Identification

Product name

Annexin V-Biotin Apoptosis Detection Kit

100 assays

Cat. No: **CA013**

2. Description

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.

3. Composition

Item	Quantity
Labeled Annexin V-Biotin	500 µL
Binding Buffer (10X)	50 ml
Propidium iodide	1 mL

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

4. Storage specifications

All components should be stored at 4 °C.
Protect from light and do not freeze.

5. Applications

- Detect early/middle stages of Apoptosis.
- Differentiate apoptosis from necrosis.

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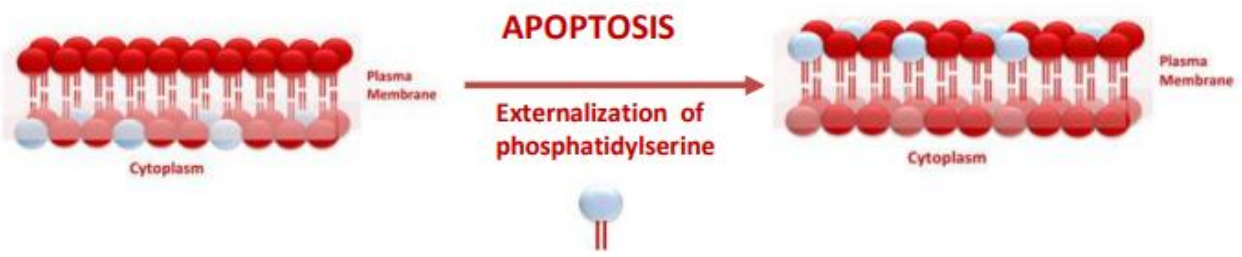
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6. Recommended Assay Procedure

Note: A fluorophore-conjugated streptavidin (e.g. Streptavidin-FITC, Streptavidin-PE or equivalent) is required for detection and is not included in this kit.

1. Dilute the Binding Buffer (10X) 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 1X Binding Buffer at a concentration of 1-5 x 10 6 cells/ml.
4. Transfer 500 µl of cell suspension in a 5 ml test tube.
5. Add 5 µl of labeled- Annexin V-Biotin.
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 the cells once with 1X Binding Buffer and resuspend in 100 µL of 1X Binding Buffer.
9. Add the recommended amount of a fluorophore-conjugated streptavidin according to the manufacturer's instructions and incubate for 15–30 minutes at room temperature in the dark.
10. Wash the cells once with 1X Binding Buffer and resuspend in 400 µL of 1X Binding Buffer.
11. Analyze by flow cytometry or fluorescence microscopy using the appropriate settings for the selected streptavidin conjugate.



7. Further information

Product Use Limitations

This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

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