For research use only

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Annexin V-Biotin Apoptosis Detection Kit

This kit is intended for research use only. Not for use in diagnostic procedure.

1. Introduction

The Annexin V-Biotin Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phospholipid phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can easily bind to Biotin-conjugated Annexin V, a protein that has a strong natural affinity for PS (1,2). Annexin V-Biotin can be detected in conjunction with conventional dye-staining using any streptavidine- or avidin-dye reagents, such as (strept)avidine-fluorescein, -peroxidase, -alkaline phosphatase (AP), and ß- gal, etc.

2. Kit Contents

Code No. BV-K109-2: 25assays BV-K109-3: 100assays BV-K109-4: 400assays

Materials	Quantity					
	25 assays		100 assays		400 assays	
Annexin V-Biotin	125µL	1 vial	500µL	1 vial	2mL	1 vial
1x Binding Buffer	12.5mL	1 vial	50mL	1 vial	100mL	2 vials
Propidium Iodide (PI)	125µL	1 vial	500µL	1 vial	2mL	1 vial

3. Annexin V-Biotin Assay Protocol

A. Incubation of cells with Annexin V-Biotin

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 200 µL of 1X Binding Buffer.
4. Add 5 µL of Annexin V-Biotin an 5 µL of propidium iodide (PI, optional.)
5. Incubate at room temperature for 5 min in the dark.
6. Wash the cells once in 200 µL of 1X Binding Buffer.
7. Fix cells with 2% formaldehyde for 15 min and wash cells once with PBS.
   Resuspend cells in 100 µL of PBS + 1 mg/mL BSA.
   Note: Cells must be incubated with Annexin V-Biotin before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.
8. Add 5 µg/mL of (strept)avidin-fluorescein or other -dye and incubate for 15min.
9. Collecting cells by centrifugation and wash once with PBS.

Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Analyze sample by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Biotin (A.3-5).

C. Detection by Fluorescence Microscopy

1. Place the cell suspension from Step A.9 on a glass slide. Cover the cells with a glass coverslip.
   For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.9), invert coverslip on glass slide and visualize cells.
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.
   Cells that have bound Annexin V-Biotin and stained with (strept)avidine-FITC will show green staining in the plasma membrane. Cell which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the sell surface (plasma membrane).

4. Storage and Stability

All kit components must be stored at 2~8°C.

All reagents are stable for one year after manufacturing when stored at 2~8°C.

5. References

1. Koopman,G., et al.(1994) Blood 84: 1415-1420.
2. Martin, S.J., et al. (1995) J.Exp.Med. 182: 1545-1556.

6. Related Products

1. Annexin V-FITC, -Cy3, and -EGFP Apoptosis Detection Kits
2. Annexin V-Biotin and Unlabeled Annexin V Reagents.
3. Caspase-1, -2, -3, -6, -8, -9 Fluorometric and Colorimetric Assay Kits.
4. Active Recombinant Human Caspase-3, and -9.
5. Ready-to-use Caspase Inhibitors and Sets.
6. Ready-to-use Caspase Substrates and Sets.
7. Quick Apoptosis DNA Ladder Detection Kit
8. Ready-to-use Apoptosis Inducers and Inducer Set.
9. Quick Cell Proliferation Assay Kit.