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ab273267
Annexin V-Biotin Assay Kit

View Kit datasheet: https://www.abcam.com/ab273267
(use https://www.abcam.cn/ab273267 for china, or
https://www.abcam.co.jp/ab273267 for Japan)

For the detection of apoptosis based on the translocation of phosphatidylserine from the inner face of the cell membrane to the cell surface.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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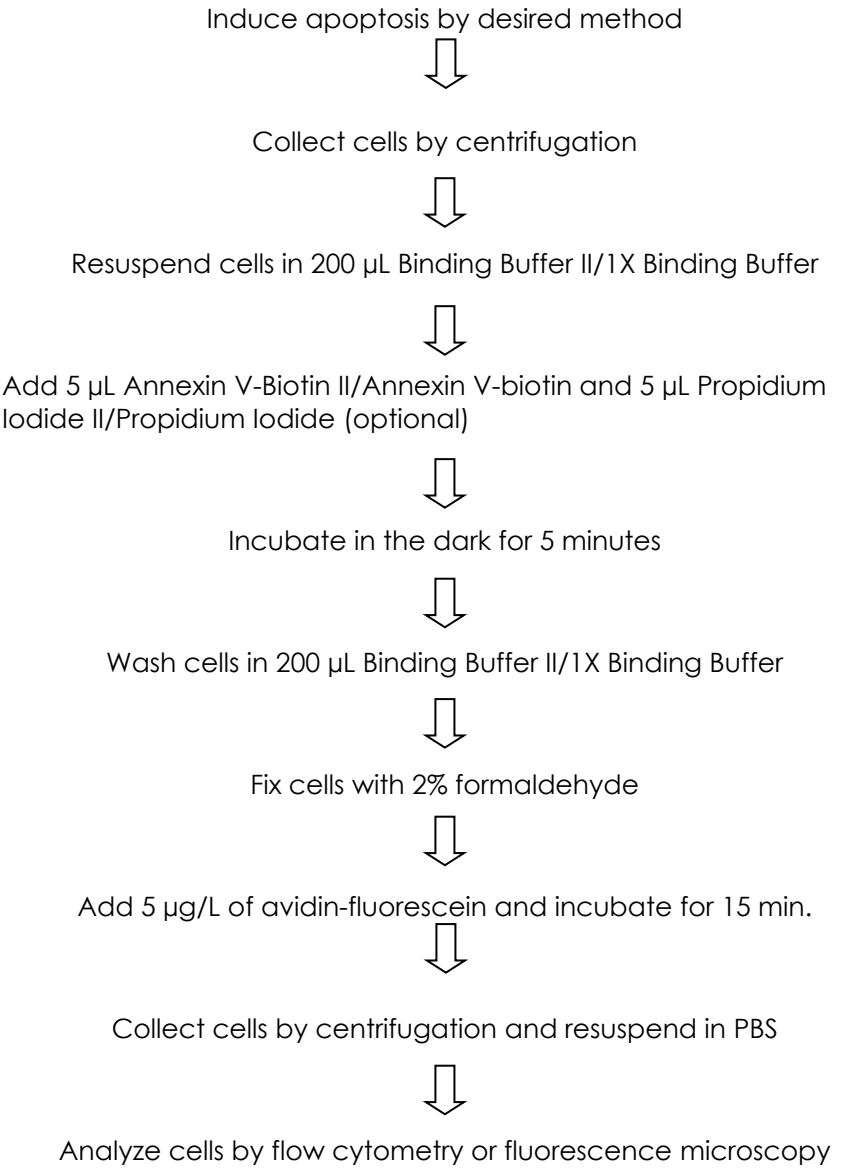
1. Overview

Annexin V-Biotin Assay Kit (ab273267) 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. Annexin V-Biotin can be detected in conjunction with conventional dye-staining using any streptavidin- or avidin-dye reagents, such as (strept)avidin-fluorescein, -peroxidase, -alkaline phosphatase (AP), and -β -gal, etc

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Annexin V-Biotin II/Annexin V-biotin	125 µL	4°C
Binding Buffer II/1X Binding Buffer	12.5 mL	4°C
Propidium Iodide II/Propidium Iodide	125 µL	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- PBS
- PBS + 1 mg/mL BSA
- Avidin-fluorescein
- Flow cytometer with FITC and phycoerythrin (for Propidium Iodide II/Propidium Iodide) signal detector (Ex = 488 nm; Em = 530 nm)
- Fluorescence microscope with a dual filter set for FITC & rhodamine
- Cell line of choice
- Reagents for induction of apoptosis

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Hot plate/dry heat block or microplate incubator

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Reagents are supplied ready to use.

10. Assay Protocol

10.1 Incubation with Annexin V-Biotin II/Annexin V-biotin:

10.1.1 Induce apoptosis by desired method.

Δ Note: Typically, perform assays when cells are at 80-95% confluency.

Δ Note: If necessary, perform a time-course experiment to determine the time-point for initiation of apoptosis after induction.

10.1.2 Collect 1-5 x 10^5 cells by centrifugation.

10.1.3 Resuspend cells in 200 µL of Binding Buffer II/1X Binding Buffer

10.1.4 Add 5 µl of Annexin V-Biotin II/Annexin V-Biotin and 5 µl of Propidium Iodide II/Propidium Iodide (PI, optional).

10.1.5 Incubate at room temperature for 5 min in the dark.

10.1.6 Wash the cells once in 200 µL of Binding Buffer II/1X Binding Buffer. Centrifuge to remove the buffer.

10.1.7 Fix cells with 2% formaldehyde in PBS for 15 min and wash cells once with PBS. Resuspend cells in 100 µL of PBS + 1 mg/mL BSA.

Δ Note: Cells must be incubated with Annexin V-Biotin II/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.

10.1.8 Add 5 µg/mL of avidin-fluorescein (not provided) and incubate for 15 min.

10.1.9 Collecting cells by centrifugation and resuspend in PBS.

10.1.10 Proceed to Flow cytometry or Fluorescence Microscopy below depending on method of analysis.

10.2 Flow Cytometry:

10.2.1 Analyze samples 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).

10.2.2 For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Biotin II/Annexin V-Biotin (Steps 10.3 – 1-.5, above).

10.3 Fluorescence Microscopy:

10.3.1 Place the cell suspension from Step 10.1.9 on a glass slide. Cover the cells with a glass coverslip. For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (10.1.9), invert coverslip on glass slide and visualize cells.

10.3.2 Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.

10.3.3 Cells that have bound Annexin V-Biotin II/Annexin V-Biotin and stained with (strept)avidin-FITC will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).

11. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)