Annexin V-PE Apoptosis Detection Kit

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.

1. Overview

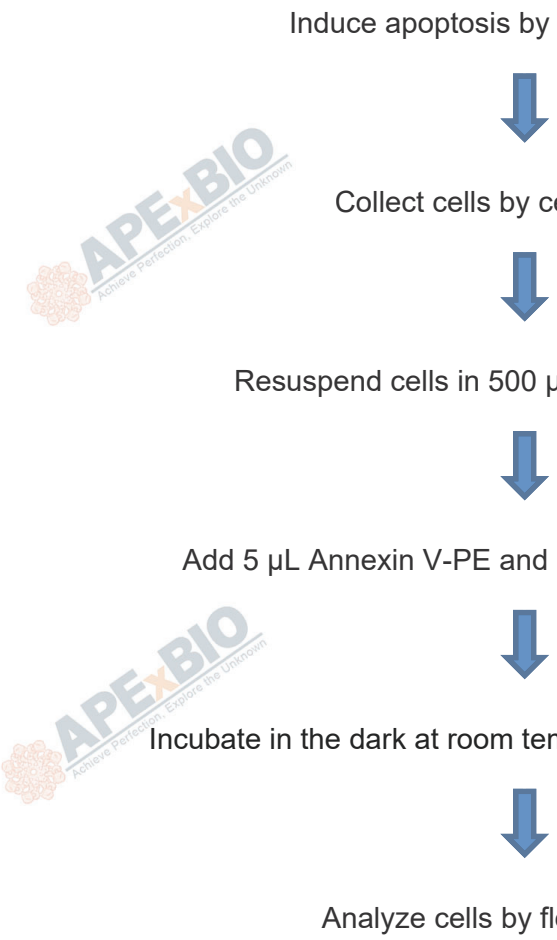
Annexin V-PE Apoptosis Staining / Detection Kit (with dead cell stain) is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phospholipid phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface.

Once on the cell surface, PS can easily be detected by staining with a fluorescent conjugate of Annexin V, a protein that has a strong natural affinity for PS. The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells, without the need of fixation.

This kit includes annexin V-PE, SYTOX green dye and binding buffer. The SYTOX green dye is impermeable to live cells and apoptotic cells but stains necrotic cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with annexin V-PE and SYTOX Green dye in the provided binding buffer, apoptotic cells show red fluorescence, dead cells show green fluorescence and live cells show little or no fluorescence.

These populations can easily be distinguished by Fluorescence microscopy using FITC and PE filters or by flow cytometry using the FL1 channel (Ex= 488 nm/Em= 530 nm) for SYTOX Green dye and FL2 channel for Annexin V-PE (Ex= 488 nm/Em= 578 nm).

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-PE	125 µL	4°C
SYTOX Green Dye	25 µL	4°C
Binding Buffer	12.5 mL	4°C

7. Materials Required, Not Supplied

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

– Flow cytometer with PE signal detector (Ex = 488 nm; Em = 530 nm)

– 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-PE:

10.1.1 Induce apoptosis by desired method. Concurrently incubate a control culture without induction.

∆ **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 105 cells by centrifugation.

10.1.3 Resuspend cells in 500 µL of 1X Binding Buffer.

10.1.4 Add 5 µl of Annexin V-PE and 1 µl of SYTOX Green dye.

∆ **Note:** Thaw the SYTOX Green dye in room temperature before use.

10.1.5 Incubate at room temperature for 5-10 min in the dark.

10.1.6 Analyze samples by flow cytometry using FL1 channel for SYTOX Green dye (Ex = 488 nm; Em = 530 nm) and FL2 channel for Annexin V-PE (Ex = 488 nm; Em = 578 nm).

Adherent cells

Gently trypsinize and wash cells once with serum-containing media.

– Proceed to step 10.1.4.

The cell population should separate into three groups:

– Live cells with only a low level of fluorescence;

– Apoptotic cells with moderate green fluorescence;

– Necrotic cells with high-intensity green fluorescence.

For research use only! Not to be used in humans.

For more details, please visit http://www.apexbt.com/or contact our technical team.

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