



Annexin V-PE-Cy5 Apoptosis Detection Kit

Catalog Number KA0718

100 assays

Version: 02

Intended for research use only

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Introduction

Background

The Annexin V-PE-Cy5 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 be easily 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 for fixation. Results can be analyzed by flow cytometry or fluorescence microscopy.

General Information

Materials Supplied

List of component

Component	Amount
Annexin V-PE-Cy5	500 µl
1X Binding Buffer	50 ml

Storage Instruction

Store kit at 4°C. All reagents are stable for one year under proper storage conditions.

Precautions for Use

FOR RESEARCH USE ONLY! Not to be used on humans

Assay Protocol

Assay Procedure

1. Incubation of cells with Annexin V-PE-Cy5

- A. Induce apoptosis by desired method.
 - B. Collect $1-5 \times 10^5$ cells by centrifugation.
 - C. Resuspend cells in 500 μ l of 1X Binding Buffer.
 - D. Add 5 μ l of Annexin V-PE-Cy5.
 - E. Incubate at room temperature for 5 min in the dark.
- Proceed to B or C below depending on method of analysis.

2. Quantification by Flow Cytometry

Analyze Annexin V-PE-Cy5 binding by flow cytometry (Ex = 488 nm; Em = 670 nm).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-PE-Cy5 (A. 3-5).

Data Analysis

Calculation of Results

- ✓ Detection by Fluorescence Microscopy
 - Place the cell suspension from the Step 1.E on a glass slide. Cover the cells with a glass coverslip.
 - For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (1.e), invert coverslip on glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-PE-Cy5 before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)
 - Observe the cells under a fluorescence microscope using the PE-Cy5 filter. Cells that have bound Annexin V-PE-Cy5 will show bright purple staining in the plasma membrane.