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Annexin-V-FLUOS Staining Kit

Kit for the detection and quantification of apoptosis and differentiation from necrosis at single cell level, based on Annexin-V-labeling

Cat. No. 11 858 777 001 50 tests
Cat. No. 11 988 549 001 250 tests

Version 10
Content version: August 2016

Store at +2 to +8°C

1. Product overview

Kit contents

Vial/ Cap	Label	Content/Cat.No.		Use
		11 858 777 001	11 988 549 001	
1 green	Annexin-V-Fluorescein	110 µl	500 µl	Ready-to-use
2 red	Propidium iodide	150 µl	500 µl	• Ready-to-use • For the preparation of the Annexin-V-Fluorescein labeling solution
3 blue	Incubation buffer	50 ml HEPES buffer	4 × 50 ml HEPES buffer	• Ready-to-use • For the dilution of the Annexin-V-Fluorescein solution

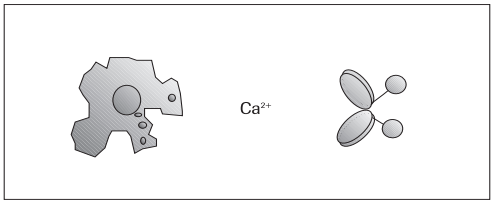
Introduction

In the early stages of apoptosis, changes occur at the cell surface (1, 2, 3). One of these plasma membrane alterations is the translocation of phosphatidylserine (PS) from the inner part of the plasma membrane to the outer layer, by which PS becomes exposed at the external surface of the cell (4). Fadok et al. showed that macrophages specifically recognize PS exposed on the surface of lymphocytes during the development of apoptosis (2). The recognition and phagocytosis of apoptotic cells and bodies protects organisms from the exposure to cellular compounds leading to inflammation, which mostly accompanies necrosis.

Assay principle

The analysis of phosphatidylserine on the outer leaflet of apoptotic cell-membranes is performed by using Annexin-V-Fluorescein and Proidium iodid (PI) for the differentiation from necrotic cells or labeling with a cell surface marker for cell characterization.
The procedure involves:

Stage	Description
1	Washing the cells in PBS.
2	Incubation of cells with Annexin-V-Fluorescein in a Hepes buffer containing PI or labeling reagent for cell surfaces (*e.g.*, CD-marker).
3	Analysis of the samples under a fluorescence microscope or on a flow cytometer.



Apoptotic cell with phosphatidylserine exposed on the outer leaflet of the membrane

Annexin-V-Fluos

Fig. 1: Test principle.

Application

Annexin V is a Ca^{2+} -dependent phospholipid-binding protein with high affinity for phosphatidylserine (4). This protein can hence be used as a sensitive probe for PS exposure upon the outer leaflet of the cell membrane and is therefore suited to detect apoptotic cells (4, 5, 6, 7) in cell populations but not on tissue sections. Since necrotic cells also expose PS according to the loss of membrane integrity, apoptotic cells have to be differentiated from these necrotic cells. The simultaneous application of a DNA stain which is used for dye exclusion tests allows the discrimination of necrotic cells from the Annexin V positively stained cell cluster. Any other secondary labeling should be possible, *e.g.*, membrane surface staining with a phycoerythrin or TRITC-labeled monoclonal antibody for further cellular characterization (8).

Sample material

- Cell lines
- Freshly isolated cells

Number of tests

For 50 tests (Cat. No. 11 858 777 001)
For 250 tests (Cat. No. 11 988 549 001)

Preparation

Recombinant Annexin- V is produced in *E. coli* (strain NB42). The GST-tagged protein is purified by standard purification protocols.

Fluorescence characteristics

Annexin-V-Fluorescein and propidium iodide show the following fluorescence characteristics:

	Fluorescein	Propidium Iodide
Excitation	488 nm	488–540 nm
Emission	518 nm	617 nm

Specificity

Annexin-V-Fluos binds in a Ca^{2+}-dependent manner to negatively charged phospholipid surfaces and shows high specificity to phosphatidylserine. Therefore, it stains apoptotic as well as necrotic cells. Propidium iodide stains DNA of leaky necrotic cells only.

Storage/Stability

Stable at +2 to +8°C until the expiration date printed on the label.

2. Procedures and required material

Additional solutions required

PBS*

Preparation of Annexin-V-FLUOS labeling solution

Predilute 20 µl Annexin-V-Fluos labeling reagent (vial 1) in 1 ml Incubation buffer (bottle 3) and add 20 µl Propidium iodide solution (vial 2).
Note: 1 ml is enough for 10 samples.

Staining of cell suspensions

In the following table please find the staining procedure for cell suspensions.

Step	Action
1	Wash 10^6 cells with PBS and centrifuge cells at 200 × *g* for 5 min
2	Resuspend the cell pellet in 100 µl of Annexin-V-FLUOS labeling solution. Incubate 10–15 min at 15–25°C.
3	Analyze by fluorescence microscopy or on a flow cytometer (see 3. Analysis).

* *available from Roche Diagnostics*

Staining of adherent cells

In the following table please find the staining procedure for adherent cells.

Step	Action
1	Remove chambers and silicon borders of cells grown on chamberslides.
2	Remove medium and cover slides with Annexin-V-FLUOS labeling solution (100 µl/chamber).
3	Put coverslips on slides and incubate for 10–15 min at 15–25°C.
4	Analyze by fluorescence microscopy or on a flow cytometer (see 3. Analysis). ***Note:*** We do not recommend to analyze adherent cells by flow cytometry, because trypsinization or scraping for monodispertion of the cells results in false positive staining and analysis of non-dispersed cell clusters.

3. Analysis

Fluorescence-microscopy

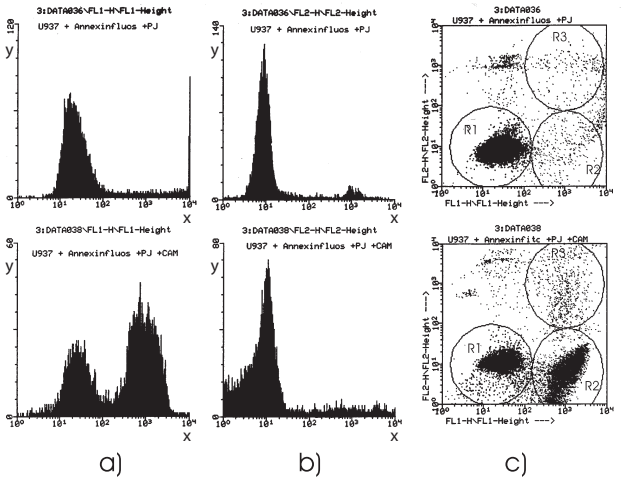
For evaluation by fluorescence microscopy use an excitation wavelength in the range of 450–500 nm (*e.g.*, 488 nm) and detection in the range of 515–565 nm (green).

Flowcytometry

Add 0.5 ml Incubation buffer (bottle 3) per 10^6 cells and analyze on a flow cytometer using 488 nm excitation and a 515 nm bandpass filter for fluorescein detection and a filter >600 nm for PI detection. Electronic compensation of the instrument is required to exclude overlapping of the two emission spectra. Typical histograms of apoptotic versus non-apoptotic and necrotic cells are shown in figure 2.

Figure 2

FACS analysis of apoptotic U937 cells after staining with Annexin-V-FLUOS and propidium iodide. Cells were then stained with the components of the Annexin-V-FLUOS Staining Kit and analyzed. Cultivation for 4 h in the presence (lower row) or absence (upper row) of 4 µg/ml camptothecin:
a) single parameter Annexin-V-FLUOS,
b) single parameter propidium iodide and
c) dual parameter (FL1 = Annexin-V-FLUOS; FL2 = propidium iodide); Cluster R1 =living cells, R2 = apoptotic cells and R3 = necrotic cells.



x-axis = increasing Annexin-V-fluorescence [relative light unit (rlu)]
y-axis = increasing Propidium Iodide (rlu)
Result: Flow cytometric analysis clearly differentiates normal (living) cells with low Annexin and low PI staining, apoptotic cells with high Annexin and low PI staining, and necrotic cells with high Annexin and high PI staining.

4. References

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Changes to previous version

Editorial changes

Trademarks

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