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Not for use in diagnostic procedures.

Annexin-V-FLUOS

Version: 10
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Fluorescence-conjugated anticoagulant for the detection of phosphatidylserine on the outer leaflet of apoptotic cells

Cat. No. 11 828 681 001 500 µl
250 tests

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Annexin-V-FLUOS	Ready-to-use solution for labeling.	1 vial, 500 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	green	Annexin-V-FLUOS	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For staining and analyzing cells

See section, **Working Solution** *for preparation of solutions.*

- PBS
- Incubation buffer
- Propidium iodide solution
- Fluorescence microscope

1.4. Application

Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with high affinity for phosphatidylserine (PS). Therefore, this protein can 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 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, such as membrane surface staining with a phycoerythrin or TRITC-labeled monoclonal antibody for further cellular characterization.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Annexin-V-FLUOS can be used with:
- Cell lines
- Freshly isolated cells.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Content	Reconstitution/Preparation of Working Solution
1	Incubation buffer	Prepare a solution containing 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$. *Store at +2 to +8°C for 3 months.*
2	Propidium iodide solution	Prepare a 50 µg/ml stock solution.
3	Annexin-V-FLUOS labeling solution	Predilute 20 µl Annexin-V-FLUOS labeling reagent in 1 ml Incubation buffer and add 20 µl Propidium iodide solution. *1 ml is enough for 10 samples.*

2.2. Protocols

Staining of cell suspensions

See section, **Working Solution** *for additional information on preparing solutions.*

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

Staining of adherent cells

See section, **Working Solution** *for additional information on preparing solutions.*

1. Remove chambers and silicon borders of cells grown on chamber slides.
2. Remove medium and cover slides with Annexin-V-FLUOS labeling solution (100 µl/chamber).
3. Place coverslips on slides and incubate for 10 to 15 minutes at +15 to +25°C.
4. Analyze by fluorescence microscopy or on a flow cytometer.

Adherent cells are difficult to analyze by flow cytometry. This is not the preferred method because trypsinization or scraping for monodispertion of the cells results in false-positive staining and analysis of non-dispersed cell clusters.

Analysis by fluorescence microscopy

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

Analysis by flow cytometry

Add 0.4 to 0.8 ml Incubation buffer and analyze on a flow cytometer using 488 nm excitation and a 515 nm band-pass 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 1.

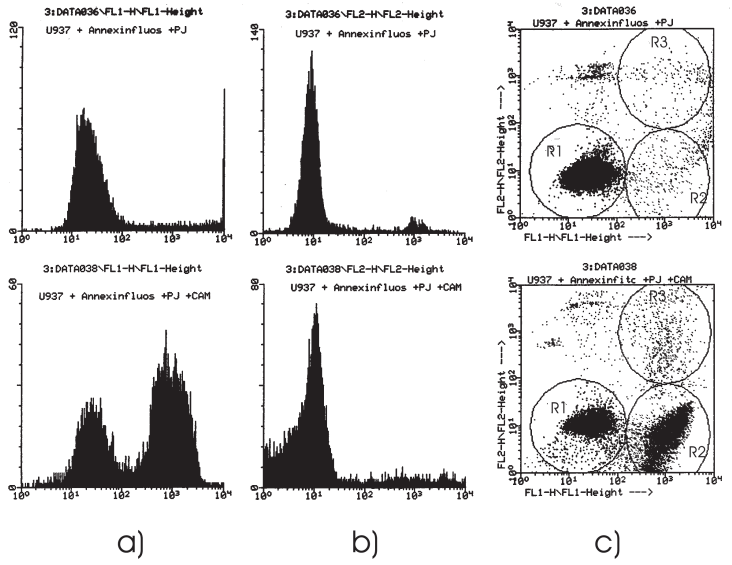

Fig. 1: FACS analysis of apoptotic U937 cells after staining with Annexin-V-FLUOS and propidium iodide. Cultivation for 4 hours in the presence (lower row) or absence (upper row) of 4 µg/ml camptothecin.
a) Single parameter Annexin-V-FLUOS.
b) Single parameter propidium iodide.
c) Dual parameter (FL1 = Annexin-V-FLUOS, FL2 = propidium iodide); Cluster R1 = living cells, R2 = apoptotic cells, and R3 = necrotic cells.

2.3. Parameters

Emission

Fluorescein: 518 nm
Propidium iodide: 617 nm

Excitation Maximum

Fluorescein: 488 nm
Propidium iodide: 488 to 540 nm

Specificity

Annexin-V binds in a Ca^{2+}-dependent manner to negatively charged phospholipid surfaces, and shows high affinity for phosphatidylserine. Therefore, it stains apoptotic and necrotic cells. Propidium iodide stains only the DNA of leaky necrotic cells and allows for a distinction between apoptotic and necrotic cells.

3. Additional Information on this Product

3.1. Test Principle

In the early stages of apoptosis, changes occur at the cell surface. One of these plasma membrane alterations is the translocation of 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. Scientists have showed that macrophages specifically recognize PS exposed on the surface of lymphocytes during the development of apoptosis. The recognition and phagocytosis of apoptotic cells and bodies protects organisms from the exposure to cellular compounds leading to inflammation, which mostly accompanies necrosis.

The analysis of phosphatidylserine on the outer leaflet of apoptotic cell membranes is performed by using Annexin-V-FLUOS and propidium iodide (PI) for differentiation from necrotic cells or labeling with a cell surface marker for cell characterization (Fig. 2). The following steps present an overview:

① Washing the cells in PBS.

② Incubation of cells with Annexin-V-FLUOS in a HEPES buffer containing PI or labeling reagent for cell surfaces, such as a CD marker.

③ Analysis of the samples under a fluorescence microscope or on a flow cytometer.

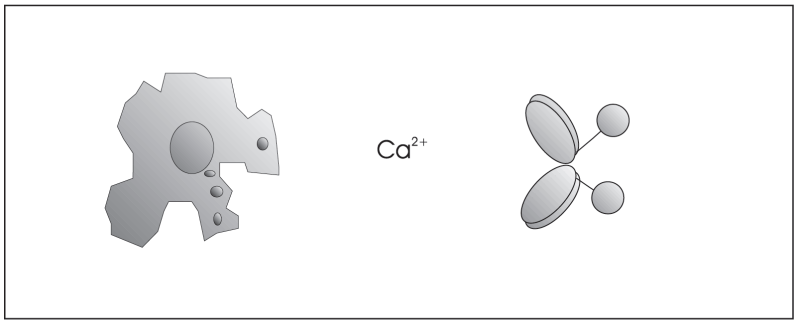

Fig. 2: Test principle

Preparation

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

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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