ApoFlowEx FITC Kit

Cat.No. ED7044

Description

The ApoFlowEx FITC Kit is intended for identification of early apoptotic, necrotic and viable cells.
Apoptosis is a regulated cell death process characterized by morphological and biochemical features occurring at different stages. The translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer is one of many plasma membrane alterations during apoptosis. By this process PS becomes exposed on the external surface of the cell. Detection of such membrane changes by Annexin V binding to PS has been suggested as a suitable assay for early apoptotic cells.

Specification

Annexin V-FITC (Ready-to-Use) in stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.
Propidium Iodide (Ready-to-Use) solution in deionized water (0.1 mg/ml).
Annexin V Binding Buffer (10x concentrated) filter-sterilized solution (0.1M HEPES/NaOH, pH 7.4, 1.4M NaCl, 25mM CaCl2).

Reagents provided

ED7044-1 **Annexin V-FITC** – 1 x 0.5 ml, sufficient for 100 test.
ED7044-2 **Propidium Iodide**, 1 x 0.5 ml, sufficient for 100 test.
ED7044-3 **Annexin V Binding Buffer**, 1 x 5 ml.

Materials required but not provided

5ml test tubes (12 × 75 mm)
Ultrapure demineralized water
Phosphate buffered saline (PBS)

Storage and handling

Store the ApoFlowEx FITC Kit in dark at 2-8 °C. Do not freeze. Avoid prolonged exposure to light. Short-term exposure to room temperature should not affect the quality of the reagent. If the reagent will be stored under conditions other than those specified, the conditions must be verified by the user.

Warnings and precautions

- Intended for research use only.
- Do not use reagents after their expiration date.
- Avoid contamination of reagents.
- Avoid Annexin V – FITC (ED7044-1) prolonged exposure to light.
- Avoid Propidium Iodide (ED7044-2) prolonged exposure to light.
- Any non-performance of assay procedure may produce false results.
- Flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure the stable sensitivity of detectors.
- See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.

Application

Identification of early apoptotic, necrotic and viable cells using flow cytometry.

Reagent preparation

Annexin V Binding Buffer

Annexin V Binding Buffer is a 10x concentrated solution and must be diluted with deionized water prior use to prepare 1x Annexin V Binding Buffer. Use fresh solution for each experiment.

Required for handling

Automatic pipettes with disposable tips
Vortex mixer
Centrifuge with rotor suitable for test tubes
Flow cytometer - blue laser excitation at 488 nm, light emission at 525 nm (FITC) and 617 nm (Propidium iodide)

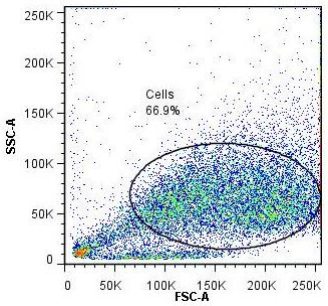
Procedure

1. Harvest cells intended for analysis by centrifugation (different cells may need different centrifugation conditions), discard supernatant. Resuspend cell pellet in cold PBS and wash cells by gentle shaking or by up-and-down mixing with a pipette tip. Re-centrifuge washed cells again and discard supernatant.
2. Resuspend cell pellet in **1x Annexin V Binding Buffer** and adjust cell density to 2 – 5 x 10^5 cells/ ml, preparing a sufficient volume of cell suspension (100 µl per assay).
3. Add **5 µl of Annexin V - FITC** and **5 µl of Propidium Iodide** to each 100 µl of cell suspension and mix gently.
4. Incubate for 15 minutes at room temperature in the dark.
5. Centrifuge cells and resuspend pellet in 100 µl of **1x Annexin V Binding Buffer** (or in an appropriate volume according to a method of sample acquisition)
6. Analyze the stained cells by flow cytometry as soon as possible.

Flow Cytometric Analysis

Analyze stained samples using flow cytometer. Acquire at least 5.000 – 10.000 cells per sample. Make compensation of fluorescent signals prior or after data acquisition if needed.
Visualize measured data from cells in the side-scatter (SSC) versus forward-scatter (FSC) dot-plot. Set the gate around cells as shown in figure 1.

Fig. 1 Delimitation of cells



Then bring the gated cells to dot-plot diagram (shown in figure 2) where the X-axis represents fluorescence intensity in FITC channel (FL1) and Y-axis represents PI channel (FL4). Set appropriate gates to calculate the percentage of viable, apoptotic and necrotic cells.

Fig. 2a Stained apoptotic cells (camptothecin treated)

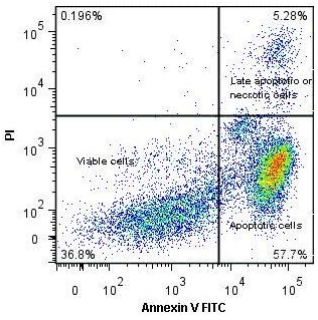
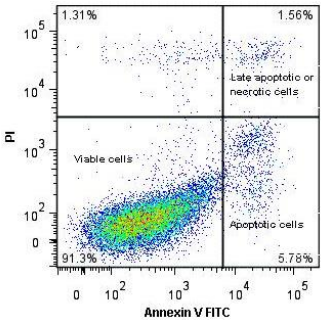
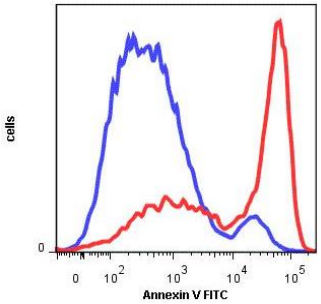


Fig. 2b Negative control – untreated and stained cells



Apoptotic cells exhibit high intensity of fluorescence in FITC channel (histogram figure 3).

Fig. 3 Histogram overlay of positive and negative cells (Blue – untreated cells, Red – Camptothecin treated cells)



Evaluate the percentage of apoptotic cells according to fig. 2. Apoptotic cells exhibit high intensity of fluorescence in FITC channel. Necrotic and late apoptotic cells exhibit high intensity of fluorescence in FITC and PI channels.

References

Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidyl-serine expression on B cells undergoing apoptosis. Blood. 1994; 84(5):1415-1420

Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods. 1995; 184(1):39-51

Zhang G, Gurtu V, Kain SR, Yan G. Early detection of apoptosis using a fluorescent conjugate of annexin V. Biotechniques. 1997 Sep;23(3):525-31.

Manufacturer

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Trademarks

n/a

Revision History

• Version 1, ED7044_TDS_v1
Initial Release
• Version 2, ED7044_TDS_v2
Annexin V buffer changed from "PBS with 0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA)" to "stabilizing phosphate buffered saline (PBS) solution".
• Version 3, ED7044_TDS_v3
Product description changed from "intended for differentiation of early apoptotic, necrotic and viable cells" to "intedned for identification of early apoptotic, necrotic and viable cells".
• Version 4, ED7044_TDS_v4
The company logo changed. IFU layout changed. Manufacturer postal code changed from 252 42 to 252 50.

Symbols

REF	Catalog number
LOT	Batch code
	Use-by date
	Temperature limits
	Consult instructions for use
	Keep away from sunlight
	Manufacturer
RUO	For Research use only. Not for use in diagnostic or therapeutic procedures.

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100 tests | Cat.No. **ED7044**

For Research use only.
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Technical Data Sheet

Version ED7044_TDS_v4_EN
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