ApoFlowEx FITC Kit

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

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7044_TDS_v5_EN

Date of Issue: 20-12-2024

Symbols used in the product labeling

RUO	Research Use Only		Keep Dry Keep away from rain
	Manufacturer		Caution
	Consult instructions for use	CONC 10×	Concentrated solution (10x)
	Contains sufficient for <n> tests	CONTENTS	Contents
REF	Catalogue number		
LOT	Batch code		
	Use by date		
	Temperature limit		
	Keep away from sunlight		

Description

The product is For Research Use Only. Diagnostic or therapeutic applications are strictly forbidden.

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. This change can be detected using Annexin V, which binds specifically to PS, making it a reliable assay for identifying early apoptotic cells.

Reagent(s) provided

Contents

The product ApoFlowEx FITC Kit is sufficient for 100 tests and is provided with the following reagents:

Annexin V-FITC ED7044-1 (1 vial) containing 0.5 ml of Annexin-V labeled with fluorescein, diluted to optimum concentration in a stabilizing phosphate buffered saline (PBS) containing 15mM sodium azide.

Propidium Iodide ED7044-2 (1 vial) containing 0.5 ml of a 0.1 mg/ml solution of propidium iodide in deionized water.

Annexin V Binding Buffer ED7044-3 (1 bottle) containing 5 ml of a concentrated (10X), filtered (0.2 µm) solution composed of 0.1M HEPES/NaOH, pH 7.4, 1.4M NaCl, 25mM $CaCl_2$.

Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (reagent grade)

Phosphate buffered saline (1× PBS), pH 7.2 – 7.4

Equipment required

Automatic pipette with disposable tips (5 µl – 100 µl) for pipetting specimen and reagents

Centrifuge

Vortex mixer

Flow cytometer with laser excitation source (488 nm), detectors for scattered light, optical filters and emission detectors appropriate to collect signals from fluorochromes provided in Table 2.

Table 2 Spectral characteristic of fluorochromes use in the product

Flurochrome	Excitation [nm]	Emission [nm]
FITC	488	525
Propidium iodide	488	617

Storage and handling

Store at 2-8 °C.

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.

See Section Procedure (Preparation of reagent(s) provided) for information about the storage conditions and stability of working solutions (where applicable).

Warnings, precautions and limitations of use

GHS Hazard Classification

Consult Safety Data Sheet (SDS) available on the product page at www.exbio.cz for the full information on the risks posed by chemical substances and mixtures contained in the Product and how they should be handled and disposed.

Biological Hazard

Human biological samples and blood specimens and any materials coming into contact with them are always considered as infectious materials.

Use personal protective and safety equipment to avoid contact with skin, eyes and mucous membranes.

Follow all applicable laws, regulations and procedures for handling and disposing of infectious materials.

Evidence of deterioration

Normal appearance of the Annexin V Binding Buffer is a clear liquid. Do not use the reagents if you observe any change in appearance, for example turbidity or signs of precipitation.

Limitation of use

Do not use after the expiry date stated on the product labels.

Specimen

Use peripheral blood collected in specimen receptacle classified as a medical product with EDTA or heparin anticoagulant.

Process the blood specimen no later than 24 hours after collection. Blood specimen

in the collection tube must be stored at room temperature (20-25 °C). Do not refrigerate.

Procedure

Preparation of reagent(s) provided

Annexin V Binding Buffer

The reagent is 10X concentrated and must be diluted with deionized water prior use (1 volume of the concentrated solution and 9 volumes of deionized water).

Following the first opening, the reagent retains its performance characteristics until the expiry date when stored under the stated conditions in its original primary container.

The diluted solution (1X) is not intended for storage, always prepare fresh for each experiment.

Preparation of reagents required but not provided

1× PBS

Prepare 1× PBS and let the solution to cool to 2-8 °C by keeping it in a refrigerator.

Sample staining protocol

1. Harvest cells intended for analysis by centrifugation (different cells may need different centrifugation conditions). Discard the supernatant into a container with an appropriate disinfectant.

   Resuspend the cell pellet in cold PBS by gentle shaking or by up-and-down mixing with a pipette.

   Centrifuge the cells and discard supernatant.

2. Resuspend the cell pellet in diluted (1X) Annexin V Binding Buffer, count the cells and adjust the cell density to 2–5 x 10^5 cells / ml. Prepare a sufficient volume to use 100 µl of cell suspension per a test tube.

3. Add 5 µl of Annexin V - FITC and 5 µl of Propidium Iodide to each 100 µl of cell suspension. Vortex gently to mix the content.

4. Incubate for 15 minutes at room temperature in the dark.

5. Centrifuge the cells and resuspend the pellet in 100 µl of diluted (1X) Annexin V Binding Buffer (or in a suitable volume according to your preferences).

6. Acquire the stained sample immediately using flow cytometer.

Flow cytometry analysis

The flow cytometer selected for use with the product ApoFlowEx FITC Kit shall be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors according to the cytometer manufacturers instructions.

If not maintained properly the flow cytometer may produce false results.

Refer to the manufacturer's cytometer specifications for lasers and fluorescence detectors according to the excitation and emission characteristics of the fluorochromes in Section Equipment required.

Set voltages on the fluorescence detectors of interest prior to stained specimen analysis. Voltage on a PMT detector should be set high enough, so that minimum of negatively stained events interfere with 0th channel on the fluorescence axis. Also, PMT detector voltage should not exceed values at which positive events are pressed to the right axis.

Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

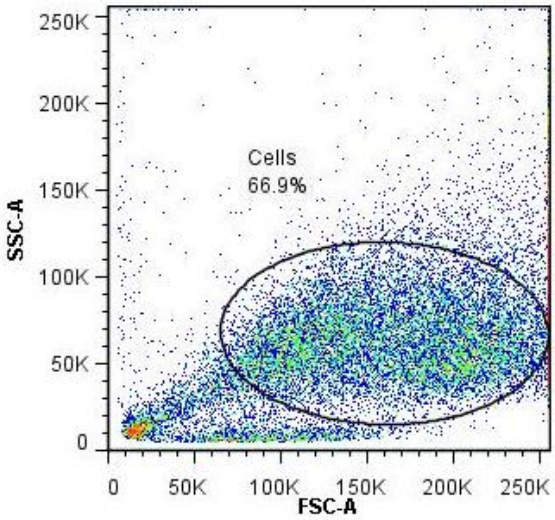
For measured data analysis, it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

To analyze a sufficient number of target events, acquire at least 5,000-10,000 cells per sample.

Data analysis

Visualize data in the side-scatter (SSC) versus forward-scatter (FSC) dot-plot. Set gate around the target cells (Figure 1).

Figure 1 Delineation of taget cells.



Plot the gated target cells in a propidium iodide (PE-Cy™5/PerCP/PerCP Cy™5.5 detector) versus Annexin-V (FITC detector). Set appropriate gates to identify the viable, apoptotic and necrotic cells (Figure 2A, 2B). Evaluate the percentage of apoptotic cells exhibiting high-intensity fluorescence in the FITC detector, as well as necrotic/late apoptotic cells exhibiting high-intensity fluorescence in both the FITC and propidium iodide detectors.

Figure 2A Apoptotic cells (camptothecin treated) in dot-plot propidium iodide vs Annexin V.

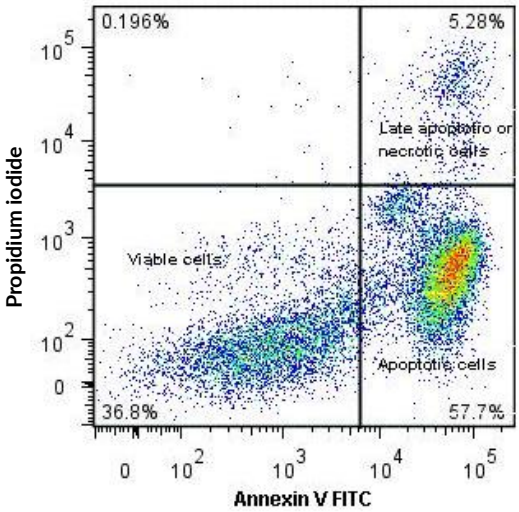
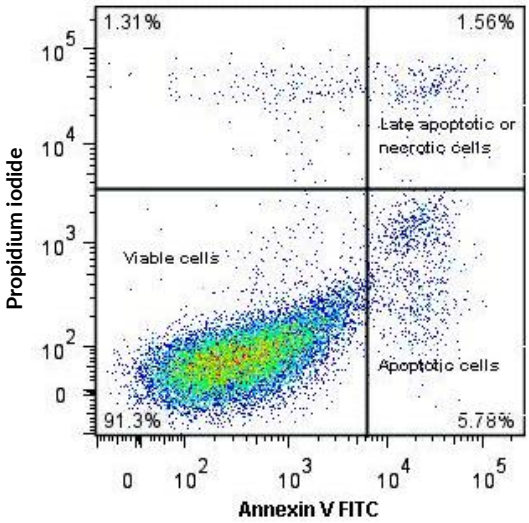
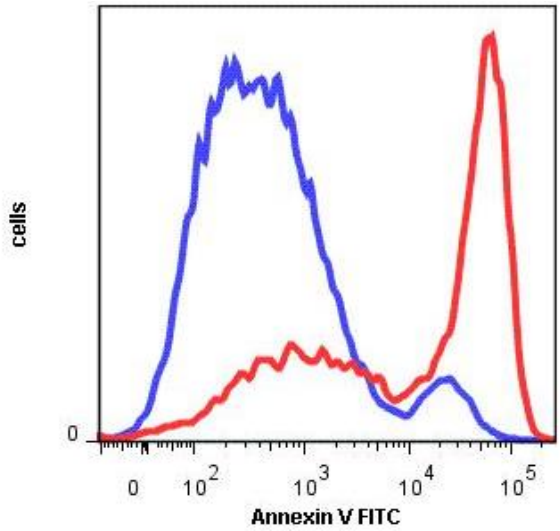


Figure 2B Negative control (untreated cells) in dot-plot propidium iodide vs Annexin V.



Apoptotic cells exhibit fluorescence of high intensity in detector for FITC (Figure 3).

Figure 3 Histogram overlay of fluorescence intensity in the FITC detector for Camptothecin-treated (Red) and untreated cells (Blue).



References

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2) 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.
3) 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.

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Revision History

Version 5, ED7044_TDS_v5

TDS layout has been redesigned for clarity and usability, with detailed sections on reagent preparation and flow cytometry analysis. Additional procedural refinements and expanded equipment details enhance the specificity and practicality of the instructions.

Manufacturer

EXBIO Praha, a.s.

Nad Safinou II 341

25250 Vestec

Czech Republic

Contact Information

info@exbio.cz

technical@exbio.cz

orders@exbio.cz

www.exbio.cz

NOTICE: Any serious incident that has occured in relation to the product shall be reported to the manufacturer and the local competent authority.