NKFlowEx Kit

50 tests | Cat. No. **ED7078**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7078_TDS_v6_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 NKFlowEx Kit is intended for the detection of CD69 activation marker on Natural killer (NK) cells following activation with various stimuli in human heparinized whole blood, using flow cytometry.

Natural killer cells represent one of the three major types of lymphocytes (T cells, B cells and NK cells). NK cells play critical role in the innate immune system, providing a rapid response to protect the body from both tumors and pathogens at the site of infection. They recognize and kill infected or transformed cells, but do not usually harm normal cells.

Transmembrane signal transduction that regulates NK cell activation is generated by interactions between activating and inhibitory receptors on the NK cells and their corresponding ligands which are expressed on the surface of other cells. By recognizing changes or even absence of surface class I MHC molecules, NK cell can distinguish infected and/or stressed cells from normal cells and kill the target cells subsequently. In order to prevent NK cell activation and attack on normal cells, activating signals must be blocked by inhibitory signals. Generally, NK cell activation is regulated by balance between activating and inhibitory signals[1].

Five NK cell subtypes can be recognized in human peripheral blood based on the expression of surface markers CD16 (FcγRIIIa, the Fc receptor of immunoglobulin G) and CD56 (NCAM glycoprotein molecule with cell to cell adhesion role)[2], [3], [4]. In combination with other specific surface markers like CD45 (LCA – Leukocyte common antigen) and CD3 (TCR receptor complex component) it is possible to distinguish populations of NK cells from other lymphocytes in human peripheral blood sample.

When PBMC are stimulated with various stimuli like interleukin-2, interferon-alpha, mitogens (e.g. PWM, PMA), cells (e.g. K562, JAR) or specific antigens[5], [6] NK cells ($CD56^+$, $CD16^+$, $CD45^+$, $CD3^-$) express CD69 activation marker (C-type lectin-like receptor) on their surface. Analyzing the expression of the CD69 marker can determine whether a particular stimulus causes the activation of human natural killer cells.

The NK activation test detects the CD69 activation marker on the surface of NK cells following stimulation of human heparinized whole blood with a stimulation control (PWM) and/or other stimuli. A negative control sample, where no stimulating reagent is used, shows no CD69 marker expression on NK cells.

After 24–48 hours of incubation with stimulation reagents, the tested blood samples are stained with a cocktail of monoclonal antibodies labeled with different fluorochromes (anti-CD45 FITC, anti-CD16+CD56 PE, anti-CD3 PE Cy™5, and anti-

CD69 PE-Cy™7). These antibodies specifically bind to antigenic determinants expressed on the surface of leukocytes. The specific staining of blood cells is followed by a lysis of red blood cells. The fluorochromes attached to the monoclonal antibodies are excited via laser beam and subsequent emissions of light from the fluorochromes of each cell are collected and analyzed by a flow cytometer. The fluorescence intensity differences enable the separation of cell subsets based on the expression of analyzed antigens.

Reagent(s) provided

Contents

The product NKFlowEx Kit is sufficient for 50 tests and is provided with the following reagents:

Staining Reagent ED7078-1 (1 vial) containing 0.5 ml a premixed combination of fluorochrome-labeled monoclonal antibodies (see Table 1), diluted at optimum concentrations in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.

Stumulation Control ED7078-2 (2 vials) containing lyophilized pokeweed mitogen (PWM), one vial is intended for stimulation of 13 blood samples.

Lysing Solution ED7078-3 (1 bottle) containing 10 ml of a 10X concentrated lysing solution with a fixative**.**

Antibody reagents specifications

Table 1 Description of the NKFlowEx Kit antibody conjugates in the Staining Reagent

Antigen	Flurochrome	Clone	Isotype
CD45	FITC	MEM-28	IgG1
CD16	PE	3G8	IgG1 kappa
CD56	PE	LT56	IgG2a
CD3	PE-Cy™5	UCHT1	IgG1
CD69	PE-Cy™7	FN50	IgG1

Materials required but not provided

Round bottom test tubes (12 x 75 mm)

Deionized water (Reagent-grade)

Culture medium suitable for cultivation of blood samples (serum free X-VIVO 10, Lonza, cat. no. BE04-380Q recommended)

96 well tissue culture test plates

Equipment required

Automatic pipette with disposable tips (5 µl – 1 ml) for pipetting specimen and reagents

Cell/tissue culture incubator (5% CO_2, 37 °C)

Laminar flow tissue culture cabinet

Racks for the test tubes

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
PE	488	576
PE-Cy™5	488	664
PE-Cy™7	488	780

Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

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

WARNING: Lysing Solution (ED7078-3) contains formaldehyde (CAS No. 50-00-0), methanol (CAS No. 67-56-1) and 2,2'-oxybisethanol (CAS No. 111-46-6) in concentrations classified as hazardous.

Label elements	Signal word
	**Danger**
H-phrases	H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H371: May cause damage to organs. H373: May cause damage to the kidneys through prolonged or repeated exposure if swallowed. H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled.
P-phrases	P201: Obtain special instructions before use. P260: Do not breathe vapours. P264: Wash hands and exposed parts of the body thoroughly after handling. P280: Wear protective gloves/eye protection/face protection. P301+P312: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P302+P352: IF ON SKIN: Wash with plenty of water and soap. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P308+P313: IF exposed or concerned: Get medical advice/ attention. P314: Get medical advice/attention if you feel unwell.

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 Lysing Solution is a clear liquid. Do not use the reagent if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the lyophilized Stimulation Control is a white powder. Do not use the reagent if you observe any change in appearance, for example liquefaction or a change in powder color.

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 heparin anticoagulant.

Process the blood specimen no later than 6 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

Stimulation Control

Aseptically reconstitute lyophilized Stimulation Control using 150 µl of the recommended culture medium (X-VIVO 10). Aliquot the unused reagent in appropriate volumes, freeze and store for later use at ≤ 20 °C. Avoid repeated freeze/thaw cycles.

Lysing Solution

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 lysing solution (1X) is stable for 1 month when stored in a liquid dispenser or closed container at room temperature.

Sample stimulation protocol

Work in a laminar flow tissue culture cabinet. Prepare standard tissue culture test plate, culture medium, Stimulation Control and the evaluated stimulant of your choice.

1. Add into the wells of the tissue culture plate:
    - **negative control sample:** 100 µl of culture medium + 100 µl of blood sample,
    - **positive control sample:** 10 µl of Stimulation Control + 90 µl of culture medium + 100 of µl blood sample,
    - **stimulated sample:** 10 µl of the evaluated **stimulant of your choice** + 90 µl of culture medium + 100 of µl blood sample.
2. Incubate for 24 – 48 h at 37 °C and 5 % CO_2 atmosphere in the cell/tissue culture incubator.

Sample staining protocol

1. Remove the tissue culture plate with the samples from the the cell/tissue culture incubator and place it securely at the working bench.
2. Prepare round bottom test tubes (12 x 75 mm), the rack for the test tubes, vortex mixer, the Staining Reagent and the diluted (1X) Lysing solution (450 µl per reaction).
3. Label the test tubes according to the specimen ID and according to the reaction type (negative control, positive control, the evaluated stimulant of your choice).
4. Pipette 10 µl of Staining Reagent into into the bottom of the test tubes (12 x 75 mm).
5. Pipette 50 µl of the sample from tissue culture plate well into the corresponding test tube.
6. Vortex and incubate the test tubes for 20 minutes at room temperature in the dark.
7. Add 500 µl of diluted (1X) lysing solution per test tube.
8. Vortex and incubate the test tubes for 10 minutes at room temperature in the dark.

Acquire the stained samples immediately using flow cytometer. If the satined sample will not be acquired immediately, store at 2-8 °C in the dark and analyze within 24 hours.

Flow cytometry analysis

The flow cytometer selected for use with the product NKFlowEx 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 end of the axis scale.

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™).

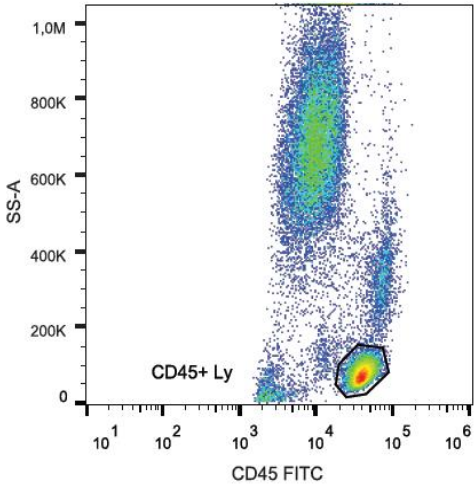
To analyze a sufficient number of NK cells (~2,000), acquire at least 10,000–20,000 leukocytes (CD45+ events) per sample.

Single-color reagents needed to determine the compensation matrix are available from EXBIO Praha, a.s..

Data analysis

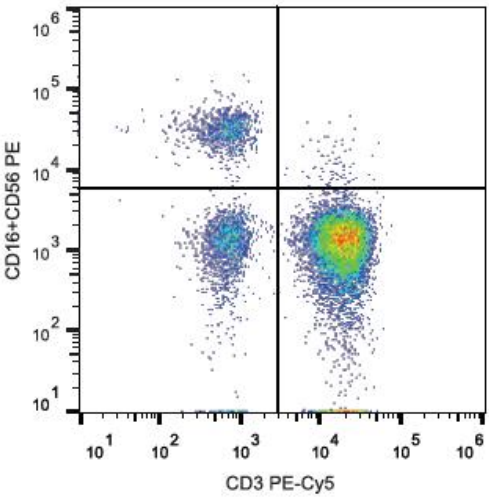
Visualize compensated data in the side-scatter (SSC) versus CD45 (FITC) plot. Set the gate for CD45+ lymphocyte population as shown in Figure 1.

Figure 1 Delineation of CD45+ lymphocyte population.
(data acquired on BD FACSCanto™)



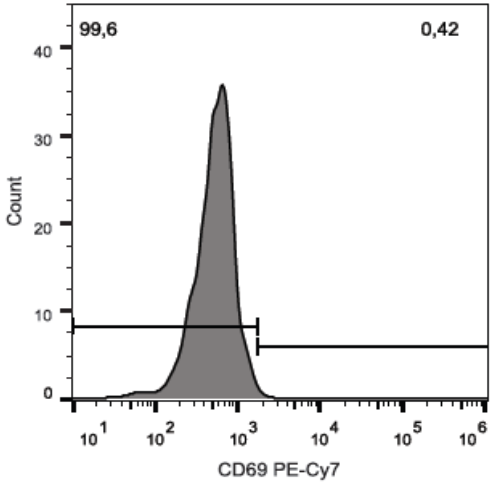
Plot the gated CD45+ lymphocytes in a CD16+CD56 (PE) versus CD3 (PE-Cy™5) plot as shown in Figure 2. Set appropriate gates to identify the natural killer (NK) lymphocytes (CD16+CD56+, CD3-) and T lymphocytes (CD3+).

Figure 2 CD45+ lymphocytes in dot-plot CD16+CD56 (PE) vs. CD3 (PE-Cy™5). (data acquired on BD FACSCanto™)



Visualize the NK cells (CD16+CD56+, CD3-) as a histogram where the X-axis represents fluorescence intensity in PE-Cy™7 detector. Use the negative control to set the discrimination gate between CD69 negative (resting) and CD69 positive events (activated).

Figure 3 Delineation of resting (CD69-) and activated (CD69+) NK lymphocytes (CD16+CD56+, CD3-) with the negative control.
(data acquired on BD FACSCanto™)



Copy the CD69+ vs CD69- delineating gate from negative control to the data from positive control and from the evaluated stimulant of your choice. Calculate the percentage of CD69+ (activated) NK lymphocytes in the positive control (Figure 4) and in the evaluated stimulant (examples of throphoblast and semen in Figure 5 and Figure 6).

Figure 4 NK lymphocytes from a sample activated by Stimulation Control.

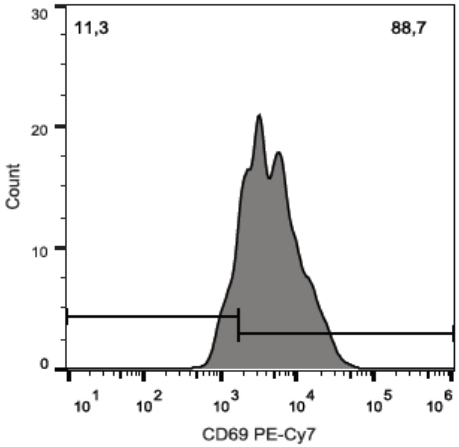


Figure 5 NK lymphocytes from a sample activated by trophoblast cells (JAR cells).

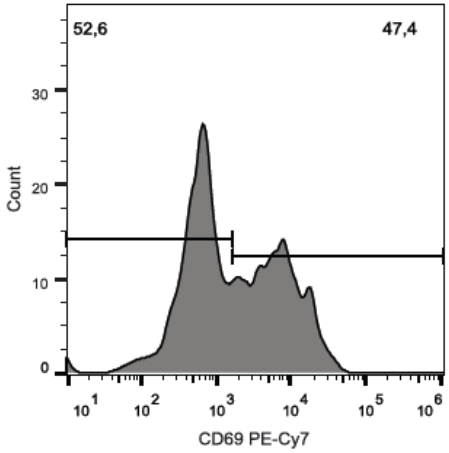
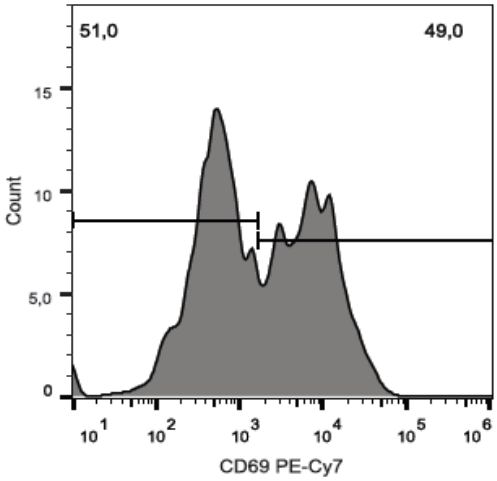


Figure 6 NK lymphocytes from a sample activated by human semen components.



References

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2) Caligiuri M. Human natural killer cells. Blood 112, 461-469 (2008).
3) Poli A. et al. CD56bright natural killer (NK) cells: an important NK cell subset. Immunology 126, 458-465 (2009).
4) Béziat V. et al. CD56brightCD16+ NK Cells: A Functional Intermediate Stage of NK Cell Differentiation. J. Immunol. 186, 6753-6761 (2001).
5) Werfel T. et al. Rapid expression of the CD69 antigen on T cells and natural killer cells upon antigenic stimulation of peripheral blood mononuclear cell suspensions. Allergy 52, 465-469 (1997).
6) Dons'koi B. V. et al. Measurement of NK activity in whole blood by the CD69 up-regulation after co-incubation with K562, comparison with NK cytotoxicity assays and CD107a degranulation assay. J. Immunol. Methods 372, 187-195 (2011).

Use of Third Party Trademarks

Revision History

Version 6, ED7078_TDS_v6

TDS layout has been changed. More detailed information is provided, especially in the procedure for sample stimulation, staining and flow cytometry analysis. This version includes enhanced safety warnings, particularly regarding the Lysing Solution, and provides additional clarification on data analysis and flow cytometer setup.

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