T-cell BlastoFlowEx Kit

100 tests | Cat. No. **ED7642**

RUO

Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet (EN)

Version: ED7642_TDS_v8_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.

T-cell BlastoFlowEx Kit is designed to measure the proliferative response of T-lymphocytes in activated samples of whole human blood.

The kit utilizes anti-CD3 / anti-Ki67 antibody cocktail to detect the proliferating lymphocytes.

Reagent(s) provided

Contents

The product T-cell BlastoFlowEx Kit is sufficient for 100 tests, is provided with the following reagents:

Fix and Lysing Solution ED7642-1 (1 bottle) containing 25 ml of concentrated (10X) solution of fixation-lysing reagent.

Permeabilizing Solution ED7642-2 (1 bottle) containing 10 ml of concentrated (10X) solution of permeabilizing reagent.

EDTA ED7642-3 (1 vial) containing 2.5 ml of ready to use buffered solution of divalent ions chelating reagent.

CD3 APC/Ki-67 PE ED7642-4 (2 vials) each containing 2.5 ml a premixed combination of fluorochrome-labeled monoclonal antibodies, diluted at optimum concentrations in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.

Antibody reagents specifications

Table 1 Description of the T-cell BlastoFlowEx Kit antibody conjugates

Antigen	Flurochrome	Clone	Isotype
CD3	APC	MEM-57	IgG2a
Ki-67	PE	Ki-67	IgG1

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 (25 µl – 2 ml) for pipetting specimen and reagents

Cylinders and beakers to dilute the reagents

Vortex mixer

Centrifuge

Waste container with disinfectant to collect the supernatants after cell centrifugations

Flow cytometer with two laser excitation sources (488 nm and ~635 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]
PE	488	575
APC	630 – 640	660

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: Fix and Lysing solution (ED7642-1) 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	H302: Harmful if swallowed. 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.
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. P308+P313: IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/attention if you feel unwell.

WARNING: Permeabilizing solution (ED7642-2) 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	H302: Harmful if swallowed. 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.
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. P308+P313: IF exposed or concerned: Get medical advice/attention. P314: Get medical advice/attention if you feel unwell.

WARNING: EDTA (ED7642-3) contains ethylenediaminetetraacetic acid-Na_2-salt-$2H_2O$ (CAS No. 6381-92-6) in concentrations classified as hazardous.

Label elements	**Signal word**
	**Warning**
H-phrases	H373: May cause damage to organs through prolonged or repeated exposure.
P-phrases	P260: Do not breathe vapours.

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 Fix and Lysing Solution, Permeabilizing Solution and EDTA 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 heparin anticoagulant. Anticoagulants EDTA and citrate negatively affect the stimulation response.

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

Measurement the proliferative response of T-lymphocytes.

Whole blood stimulation in tubes

NOTE: The kit was optimized for use with whole blood cultures stimulated inside tubes (12 x 75 mm). The stimulation tubes containing dried mitogens are available from EXBIO: Cat.No. ED7634 (PHA), Cat.No. ED7635 (PWM), Cat.No. ED7636 (Con A), Cat.No. ED7637 (Stimulation Negative Control), Cat. No. ED7673 (CD3/CD28).

1. Place a mitogen containing tube inside a laminar flow cabinet. Remove the cap, do not discard it, keep it inside the cabinet.
2. Add 0.5 ml of culture medium to the tube.
3. Add 50 µl of blood (heparin anticoagulated).
4. Cap the tube and vortex gently to mix the content.
5. Ensure the cap ventilation position and place the capped tubes in a cell incubator for 3 days (37 °C with 5% CO_2).

Preparation of reagent(s) provided

Fix and 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.

Prepare **2 ml** of the diluted solution for each staining reaction.

Permeabilization 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).

Prepare **0.5 ml** of the diluted solution for each staining reaction.

Detection of Ki67 expressing T-lymphocytes

1. Remove the tubes from the incubator. Remove and discard the caps. Add 25 µl of EDTA solution into each tube and mix.
2. Incubate for 10 minutes at 37 °C.
3. Add 2 ml of PBS, mix. Centrifuge the cells for 5 minutes at 400× g. Decant supernatant.

4. Shake the tubes a little to disturb the pellet. Add 2 ml of the diluted Fix and Lysing Solution, mix. Incubate for 10 minutes at room temperature.
5. Centrifuge the cells for 5 minutes at 400× g. Decant supernatant.
6. Add 0.5 ml of the diluted Permeabilizing Solution, mix. Incubate for 10 minutes at room temperature.
7. Add 2 ml of PBS. Centrifuge the cells for 5 minutes at 400× g. Decant supernatant.
8. Add 50 µl of CD3 APC / Ki-67 PE antibody cocktail, mix. Incubate for at least 30 minutes at room temperature in the dark.
9. Add 2 ml of PBS. Centrifuge the cells for 5 minutes at 400× g. Decant supernatant.
10. Resuspend the cells in 0.1-0.3 ml of 1% formaldehyde in PBS or Fix and Lysing solution diluted in PBS (mix 1 part of Fixation Buffer with 9 parts of PBS). Store the processed samples at 2-8 °C in the dark until analysis.

Flow cytometry analysis

The flow cytometer selected for use with the product T-cell BlastoFlowEx 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™).

Set the voltage on **light scatter detectors**, forward light scatter and side (perpendicular) light scatter so that the events of interest are on scale (consider that stimulated and unstimulated samples differ in their properties).

Set the threshold on **forward light scatter** so that only cells of interest are recorded and most of the debris excluded. Do not set threshold on APC fluorescence, in our experience this setting causes distortion of results due to the inappropriate fluorescence signal/background processing.

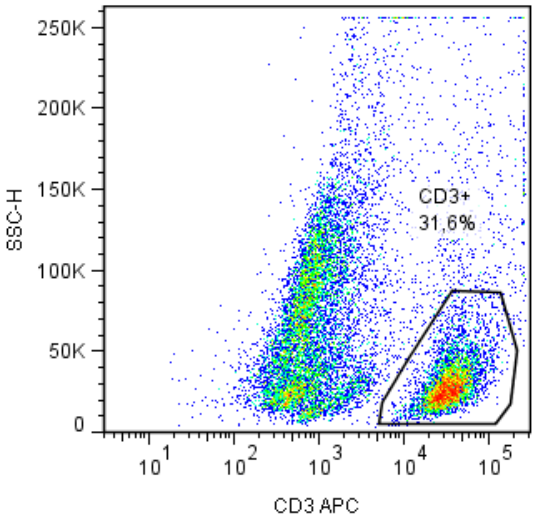
Set voltage on **fluorescence detectors** so that all events of interest are on scale.

Adjust the forward scatter area scaling factor (BD instruments) to enhance singlets vs doublets discrimination. When this factor is set properly the FSC peak area and the FSC peak height will have the same values and the singlet events will form a diagonal line (45 degrees, passing through zero).

Data analysis

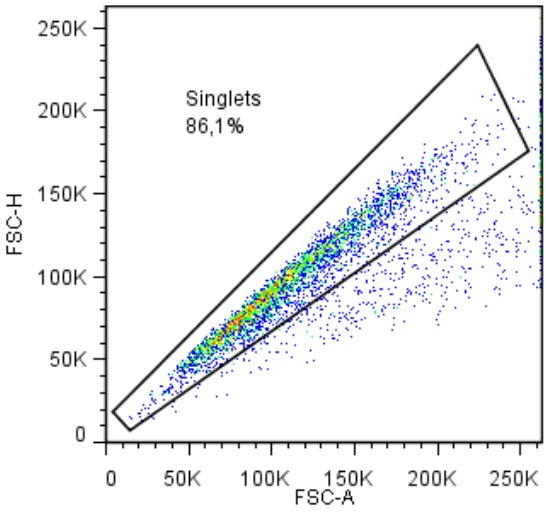
Plot the ungated events as side scatter vs CD3 APC fluorescence. Draw a gate around CD3+ events (Figure 1). Acquire at least 3,000 of CD3+ lymphocytes per sample.

Figure 1 Gate for CD3+ cells (PHA stimulation).



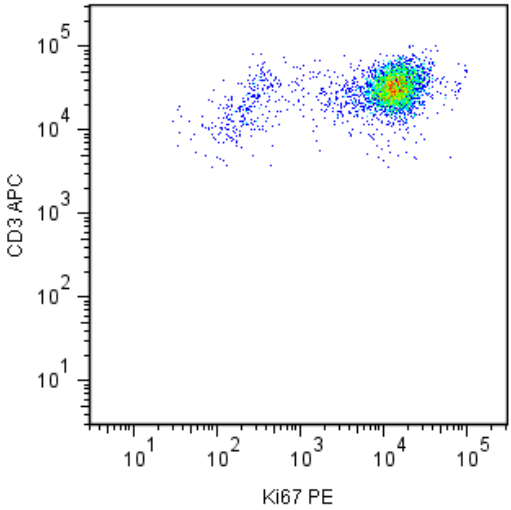
Plot the events from CD3+ gate as light forward scatter signal peak height vs light forward scatter signal peak area. Draw a diagonal gate around singlets (Figure 2).

Figure 2 Gates for Singlets (PHA stimulation).



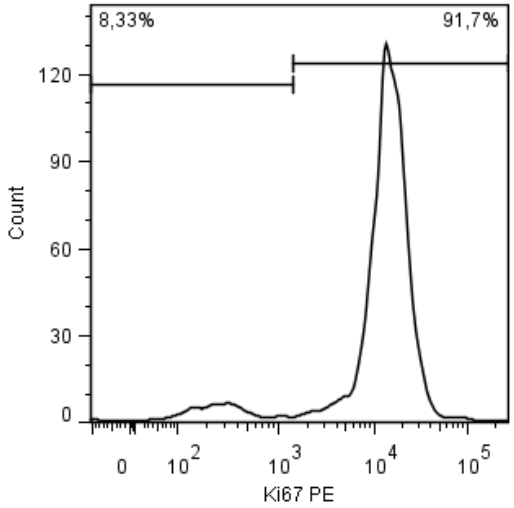
Check the distribution of Ki-67 signal by displaying the CD3+/singlets as a dot plot with CD3 APC signal on the Y axis vs Ki-67 PE signal on the X axis. Look for the best discrimination value between the negative and positive population (dashed line) (Figure 3).

Figure 3 Ki-67 fluorescence (PHA stimulation).



Display the CD3+/singlets in histogram of PE fluorescence. Draw the discrimination line between the negative and positive peaks. Apply to all tubes and evaluate the Frequency of CD3+Ki-67+ percentage (Figure 4).

Figure 4 Frequency quantitation (PHA stimulation).



References

N/A

Use of Third Party Trademarks

FlowJo™ is registered trademark of Becton, Dickinson and Company, VenturiOne® is a registered trademark of Applied Cytometry, Infinicyt™ is a registered trademark of Cytognos S.L..

Revision History

Version 8, ED7642_TDS_v8

TDS layout has been changed.

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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.