

# exbio

## TregFlowEx Kit

50 tests | Cat. No. **ED7417**

**RUO**

Not for use in diagnostic or therapeutic procedures.

## Technical Data Sheet (EN)

Version: ED7417\_TDS\_v5\_EN

Date of Issue: 10-02-2026

Symbols used in the product labeling

 <b>RUO</b>	Research Use Only		Keep Dry Keep away from rain
	Manufacturer		Caution
	Consult instructions for use		Concentrated solution (10x)
	Contains sufficient for <n> tests		Contents
	Catalogue number		
	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 TregFlowEx Kit is designed for the detection of regulatory T-cells (CD4+CD25+FOXP3+ cells) in human peripheral blood or human umbilical cord blood using flow cytometry.

Regulatory T-cells (Treg cells) are a subset of lymphocytes that have immune suppressive properties and play a critical role in the maintenance of self-tolerance. Their regulatory actions protect tissues from collateral damage triggered by immune responses against microbes and allergens, facilitate maternal tolerance to allogeneic fetus during pregnancy and maintain homeostasis with commensal microbiota<sup>[1]</sup>.

Tregs are usually characterized as CD4+CD25<sup>high</sup> CD127<sup>low</sup> T-cells expressing forkhead box transcription factor (FOXP3). Such phenotype is found in 5-12% of human peripheral blood CD4+ T-cells<sup>[2]</sup>. Tregs can be further distinguished into the so-called "natural" Tregs originating in thymus (tTreg cells) and the peripherally induced Tregs (pTreg cells)<sup>[3]</sup>. The discrimination between tTregs and pTregs is still disputable, however several proteins had been proposed to be the tTreg defining markers: the transcription factor Helios and the receptor for proteins of the vascular endothelial growth factor family Neutropilin-1 <sup>[4]</sup>.

## Reagent(s) provided

### Contents

The product TregFlowEx Kit is sufficient for 50 tests, is provided with the following reagents:

**Fix and Lysing Solution ED7417-1** (1 bottle) containing 10 ml of concentrated (10×) solution containing formaldehyde as fixative.

**Permeabilizing Solution ED7417-2** (1 bottle) containing 25 ml of ready to use solution.

**Blocking Buffer ED7417-3** (1 bottle) containing 2.5 ml of ready to use solution containing 15mM sodium azide.

**CD4 FITC/CD25 PE ED7417-4** (1 vial) containing 0.5 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.

**FOXP3 APC ED7417-5** (1 vial) containing 0.25 ml fluorochrome-labeled monoclonal antibody, diluted at optimum concentrations in a stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.

## Antibody reagents specifications

**Table 1** Description of the TregFlowEx Kit antibody conjugates

Antigen	Fluorochrome	Clone	Isotype
CD4	FITC	MEM-241	IgG1
CD25	PE	MEM-181	IgG1
FOXP3	APC	3G3	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

Phosphate buffered saline (PBS) with 1% formaldehyde

## Equipment required

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

Liquid dispenser or pipette with disposable tips (1 - 2 ml) for dispensing phosphate buffered saline

Refrigerated centrifuge

Refrigerator (2-8 °C)

Vortex mixer

Centrifuge

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

Fluorochrome	Excitation [nm]	Emission [nm]
FITC	488	525
PE	488	576
APC	630 – 640	660

Liquid waste container with appropriate disinfectant

Vacuum connected Pasteur pipettes to aspirate supernatant during cell washes (optional)

## Storage and handling

Store at 2-8 °C.

Avoid prolonged exposure to light.

Reagents were specially formulated to perform at low temperatures. It is important to adhere to the low temperature requirements throughout the procedure otherwise the fluorescence signals, the total number of cells and the number of the identified target cells will decrease considerably.

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 (ED7417-1)** 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
	<b>Danger</b>
	
<b>H-phrases</b>	H302: Harmful if swallowed. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. H331: Toxic if inhaled. 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. EUH071: Corrosive to the respiratory tract.
<b>P-phrases</b>	P201: Obtain special instructions before use. P260: Do not breathe vapours. P280: Wear protective gloves/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention.

P403+P233: Store in a well-ventilated place. Keep container tightly closed.
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Consult Safety Data Sheet (SDS) available on the product page at [www.exbio.cz](http://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, Blocking Buffer, CD4 FITC / CD25 PE and FOXP3 APC is a clear liquid. Do not use the reagents if you observe any change in appearance, for example turbidity or signs of precipitation.

Normal appearance of the Permeabilisation buffer is a light yellow to yellow-brown liquid usually with a precipitate if stored at 2-8 °C. The precipitate will dissolve completely if the solution is brought to room temperature (or warmed to 37 °C if necessary).

### **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.

The kit is not suitable for processing Ficoll purified cell suspensions (PBMCs).

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**

##### 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.

The diluted lysing solution (1X) is stable for 1 month when stored in a liquid dispenser or closed container at 2-8 °C.

Cool the diluted reagent to 2-8 °C prior to use.

#### Permeabilization Solution

The reagent contains the detergent Sodium dodecyl sulphate (SDS) that precipitates if the solution is stored at low temperatures. If necessary, dissolve the SDS precipitate by warming the vial to 37 °C. Afterwards store the Permeabilizing Solution at room temperature (15-25 °C).

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.

Bring the reagent to room temperature prior to use.

#### Blocking Buffer

Cool the reagent to 2-8 °C prior to use.

#### PBS

Prepare PBS according to recipe below (other recipes may apply).

Dissolve:

8.0 g of Sodium Chloride (NaCl)

0.2 g of Potassium Chloride (KCl)

2.0 g of Potassium Phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>)

1.42 g of Sodium Phosphate dibasic dihydrate (Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O) in 800 ml of deionized H<sub>2</sub>O.

Adjust the pH to 7.4 with HCl.

Add deionized H<sub>2</sub>O to 1 liter.

Sterilize by autoclaving for 20 minutes at 15 psi or by filter sterilization.

Cool the diluted reagent to 2-8°C prior to use.

#### 1% formaldehyde in PBS

Prepare 1% formaldehyde in PBS by mixing 1 part of methanol stabilized 37% formaldehyde solution from Sigma-Aldrich (Cat. No. F1635) and 36 parts of PBS. Store at 2-8 °C. The solution is stable for 1 month.

Cool the diluted reagent to 2-8 °C prior to use.

**CAUTION:** The concentrated formaldehyde solution is classified as acutely toxic substance. Refer to the safety precautions provided by your formaldehyde supplier.

## **Surface staining protocol**

1. For each specimen, label a 12 × 75 mm round bottom test tube with the appropriate sample identification.
2. Pipette 10 µl of CD4 FITC/CD25 PE reagent into the bottom of the 12 x 75 mm test tube.
3. Pipette 100 µl of blood specimen to the bottom of the test tube.
4. Vortex and incubate for 10 minutes in the refrigerator in the dark.
5. (optional wash) Wash cells by adding 2 ml of cold PBS and centrifuge at 300x g for 5 minutes at 4 °C.
6. Aspirate supernatant and proceed to the intracellular staining.

## **Intracellular staining protocol**

1. Add 1 ml of the diluted cold Fix and Lysing Solution.
2. Vortex to resuspend the cell pellet and incubate for 10 minutes in the refrigerator in the dark.
3. Add 0.5 ml of Permeabilizing Solution.
4. Vortex and incubate for 10 minutes in the refrigerator in the dark.
5. Centrifuge for 5 minutes at 400x g at 4 °C.
6. Decant supernatant.
7. Pipette 50 µl of cold Blocking Buffer into the bottom of the tube.
8. Pipette 50 µl of cold PBS into the bottom of the tube.
9. Vortex and incubate for 5 minutes in the refrigerator in the dark.
10. Pipette 5 µl of FOXP3 APC into the bottom of the tube.
11. Vortex and incubate for 30 minutes in the refrigerator in the dark.
12. Wash the cells twice: add 2 ml of cold PBS, centrifugate at 400x g for 5 minutes at 4 °C, decant supernatant.
13. Resuspend the cells in 300 µl of 1% formaldehyde in PBS.
14. Acquire the stained sample using flow cytometer. If the stained sample will not be acquired immediately, store at 2-8 °C in the dark and analyze within 4 hours.

## **Flow cytometry analysis**

The flow cytometer selected for use with the product TregFlowEx 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.

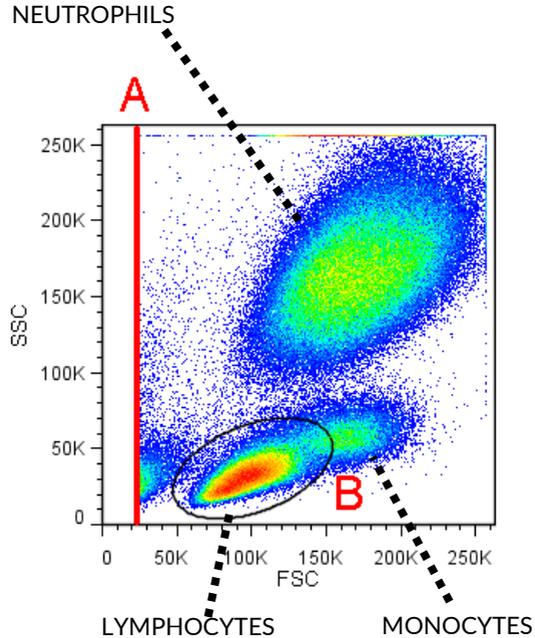
Compensate fluorescence signals between detectors prior to or after data acquisition. Data may be incorrectly interpreted if fluorescence signals are compensated improperly or if gates are positioned inaccurately.

For measured data analysis (10 000 – 30 000 CD4+ lymphocytes per sample), it is possible to use cytometer software developed by the manufacturer, or software dedicated for offline cytometry data analysis (for example FlowJo™, VenturiOne®, Infinicyt™).

## Data analysis

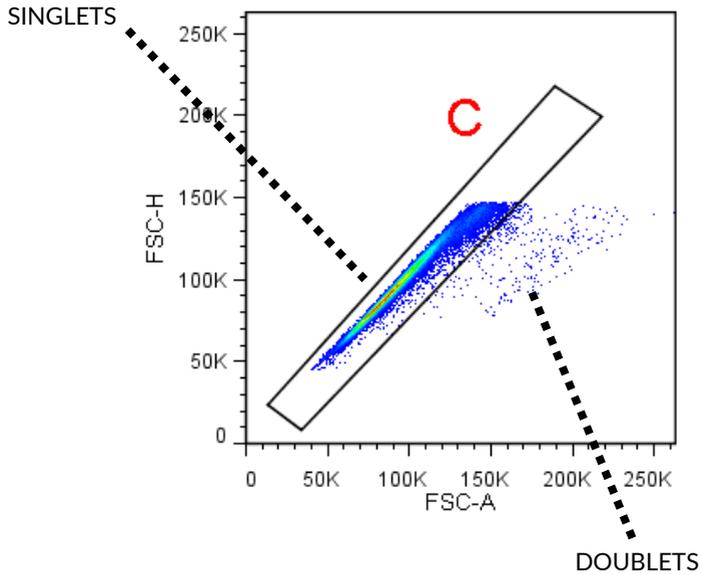
Visualize compensated data in a side-scatter (SSC) versus forward-scatter (FSC) plot. Set the gate for lymphocytes as shown in Figure 1.

**Figure 1** Two-dimensional density dot-plot showing clusters of peripheral blood leukocytes of TregFlowEx Kit processed sample analyzed on BD FACSCanto™ II cytometer: threshold setting (A) and the target cell population gate (B).



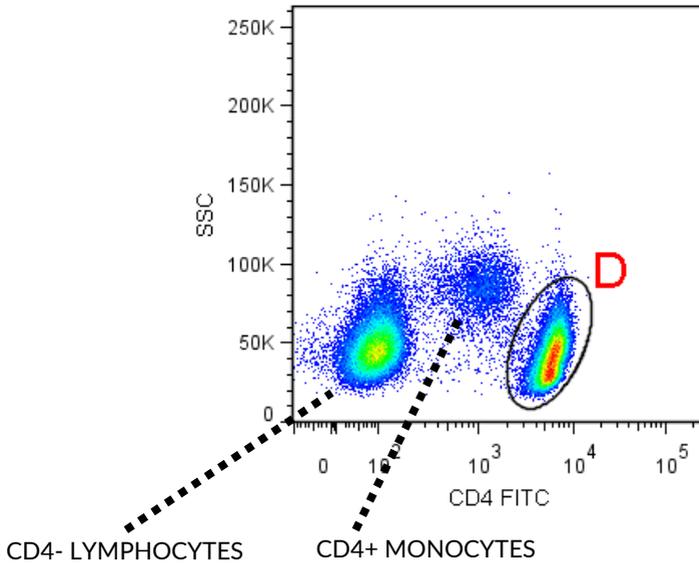
Plot the gated lymphocytes in forward-scatter height (FSC-H) vs forward-scatter area (FSC-A) plot. Separate the singlets and doublets with using a diagonal gate (C).

**Figure 2** Separation of singlets and doublets



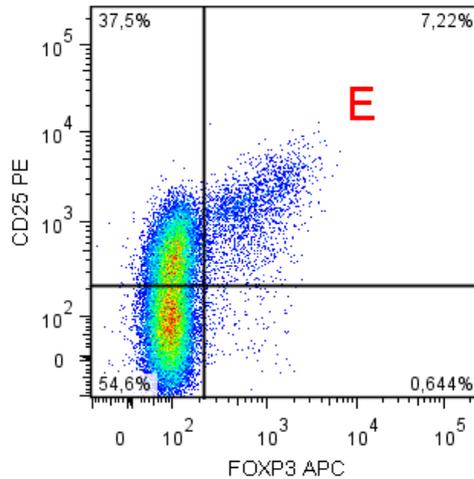
Plot the gated lymphocyte singlets in side-scatter (SSC) vs fluorescence intensity in FITC detector (CD4 FITC) plot. Create a gate around CD4+ lymphocytes (D).

**Figure 3** Separation of CD4+ lymphocytes (D) from CD4+ monocytes and CD4 negative lymphocytes



Plot the gated CD4+ lymphocytes in CD25 PE vs FOXP3 APC. Set appropriate gate and calculate the percentage of Treg cells (CD4+CD25+FOXP3+; region E in the Figure 4). Use appropriate controls to set the discrimination lines correctly.

**Figure 4** CD4+ lymphocytes displayed in a dot-plot CD25 PE vs. FOXP3 APC. Treg cells (CD4+CD25+FOXP3+) are found in the upper right quadrant.



## References

- 1) Sakaguchi S, Miyara M, Constantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* (2010) 10:490–500.
- 2) Churlaud G, Pitoiset F, Jebbawi F, Lorenzon R, Bellier B, Rosenzweig M, Klatzmann D. Human and Mouse CD8(+)/CD25(+)/FOXP3(+) Regulatory T Cells at Steady State and during Interleukin-2 Therapy. *Front Immunol*. 2015 Apr 15;6:171.
- 3) Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, Jiang S, Kuchroo VK, Mathis D, Roncarolo MG, Rudensky A, Sakaguchi S, Shevach EM, Vignali DA, Ziegler SF. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol*. 2013 Apr;14(4):307-8.
- 4) Lin X, Chen M, Liu Y, Guo Z, He X, Brand D, Zheng SG. Advances in distinguishing natural from induced Foxp3(+) regulatory T cells. *Int J Clin Exp Pathol*. 2013;6(2):116-23.

## Use of Third Party Trademarks

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## Revision History

Version 5, ED7417\_TDS\_v5

Change in hazard classification for component ED7417-1 Fix and Lysing Solution.

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