

bulletin



March
2016

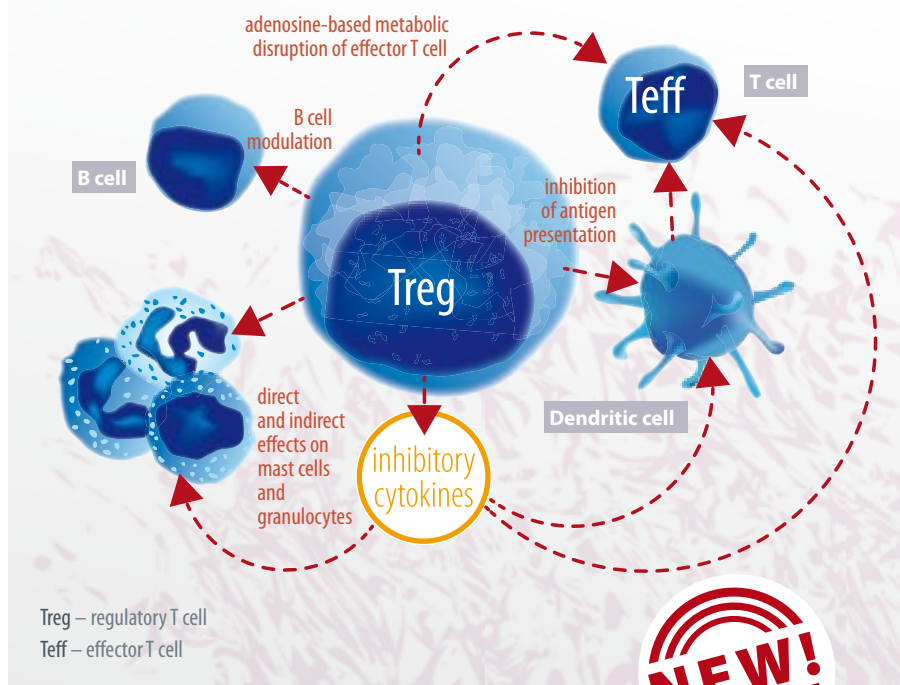
information magazine
for partners

EXBIO Praha, a.s. | www.exbio.cz

Brighter Future in Flow

Our Mission

EXBIO Praha strives to exceed the most demanding customer expectations in the field of analytical cytometry by providing a comprehensive portfolio of high quality products and services at affordable prices.



NEW!

KIT
EXBIO

Another New Product: TregFlowEx® Kit

Catalog No.	Product name	Reg. status	Quantity
ED7417	TregFlowEx® Kit	RUO	50 tests

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
status

RUO

Reagents provided:

- ED7417-1 **Fix and Lysing Solution** – 1 vial containing 10 ml of 10× concentrated reagent.
- ED7417-2 **Permeabilizing Solution** – 1 vial containing 25 ml of reagent, ready-to-use.
- ED7417-3 **Blocking Buffer** – 1 vial containing 2.5 ml of reagent, ready-to-use.
- ED7417-4 **CD4 FITC/CD25 PE** – 1 vial containing 0.50 ml of cell surface staining reagent, a mixture of mouse monoclonal antibody against CD4, FITC labelled (clone MEM-241, isotype IgG1) and antibody against CD25, R-PE labelled (clone MEM-181, isotype IgG1).
- ED7417-5 **FoxP3 APC** – 1 vial containing 0.25 ml of monoclonal antibody against FOXP3, APC labelled (clone 3G3, isotype IgG1).

more on page **2**

Flow
with us

**Flow
Cytometry
Kits**

check at
www.exbio.cz

Detection of Treg cells using Treg FlowEx® Kit

Regulatory T-cells (Treg cells), a subset of lymphocytes, have immune suppressive properties and play a critical role in the maintenance of self-tolerance. Their regulatory function leads to protection of tissues from collateral damage triggered by immune responses against microbes and allergens, they facilitate maternal tolerance to allogeneic fetus during pregnancy and maintain homeostasis with commensal microbiota. Tregs are usually characterized as CD4⁺ CD25^{high} T-cells expressing forkhead box transcription factor (FOXP3). Such phenotype is found in 5–12% of human peripheral blood CD4⁺ T-cells. Tregs can be further distinguished into the so called “natural” Tregs originating in thymus (tTreg cells) and the peripherally induced Tregs (pTreg cells). The discrimination between tTregs and pTregs is still disputable and several markers were proposed to be the tTreg unique features: the expression of Helios transcription factor and the expression of Neutropilin-1, a receptor for members of the vascular endothelial growth factor family.

The TregFlowEx® Kit is based on combined cell surface and intracellular detection of CD4, CD25 and FOXP3 proteins that are characteristic for the phenotype of regulatory T cells. Blood samples are initially stained with a mixture of anti-CD4 (fluorescein labelled) and anti-CD25 (R-phycoerythrin labelled) monoclonal antibodies which specifically bind to the antigenic determinants expressed on the surface of leukocytes. Specific staining of blood cells is followed by lysis of red blood cells. Afterwards, leukocytes are permeabilized and stained with monoclonal antibody against FOXP3 protein (allophycocyanine labelled). The fluorochromes attached to the monoclonal antibodies are excited by appropriate laser beam and emission of light from each fluorochrome on individual cells is collected and analyzed by a flow cytometer.

Related Products

Mouse monoclonal anti-FoxP3

Catalog No.	Clone	Reactivity	App.	Format	Quantity
11-601-C025	3G3 (IgG1)	Hu, Ms	FC, WB	purified	0.025 mg
11-601-C100				Alexa Fluor® 488	0.1 mg
A4-601-C025				PE	0.025 mg
A4-601-C100				APC	0.1 mg
1P-601-C025				Alexa Fluor® 647	0.025 mg
1P-601-C100					0.1 mg

Hamster monoclonal anti-Helios

Catalog No.	Clone	Reactivity	App.	Format	Quantity
11-771-C025	22F6 (IgG)	Hu, Ms	FC	purified	0.025 mg
11-771-C100				PE	0.1 mg
1P-771-T025					25 tests
1P-771-T100					100 tests

Fig. 1 Leukocyte scatter plot with the visualization of the threshold setting (A) and the lymphocyte gate (B).

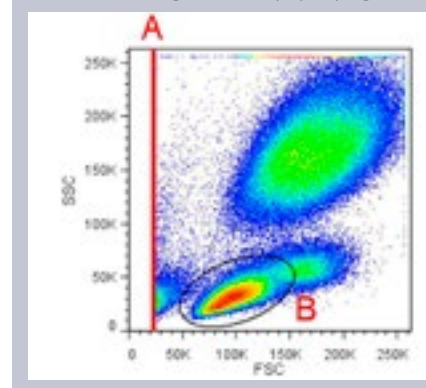


Fig. 2 Separation of singlets (C) from aggregates.

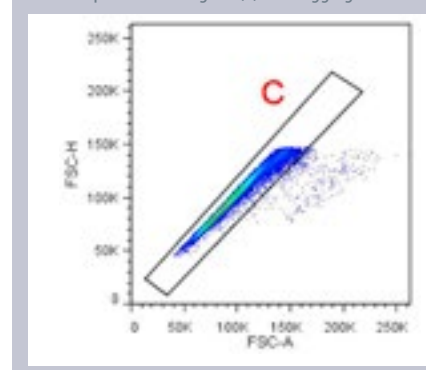


Fig. 3 Separation of CD4⁺ lymphocytes (D) from CD4⁺ monocytes and CD4 negative cells.

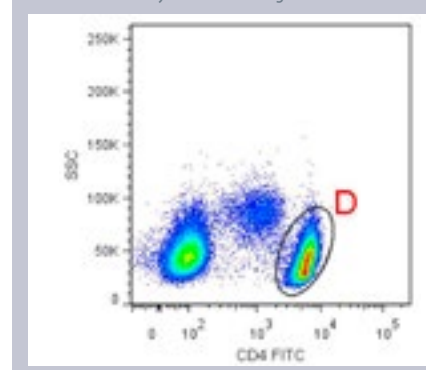
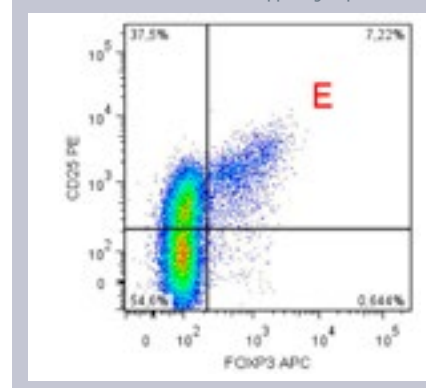


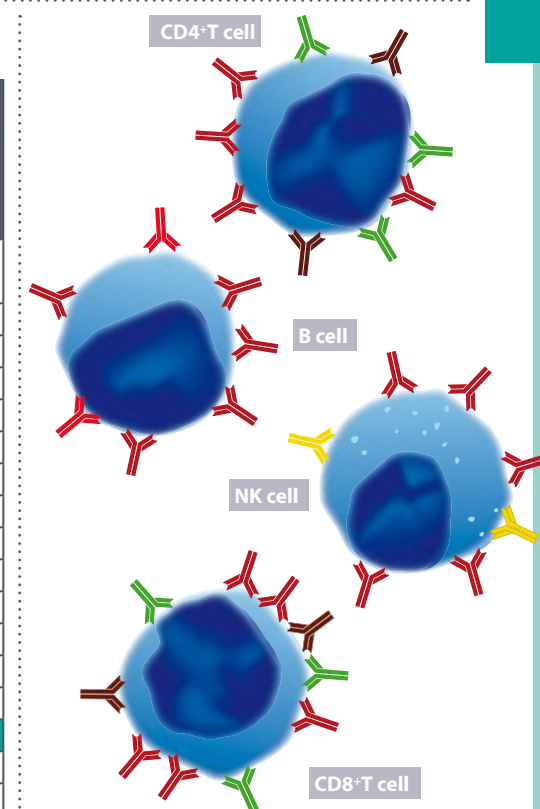
Fig. 4 CD4⁺ gated lymphocytes displayed according to their CD25 and FOXP3 expression. The Treg cells (CD25⁺ FoxP3⁺) are found in the upper right quadrant.



Multicolor Flow Cytometry

Human Blood Cells

Catal. No.	Markers	Fluorochromes	Detected Cell Populations						
			T	Th	Tc	B	NK	leukocytes	activ. T
ED7075	CD3 / CD4 / CD8 / CD19 / CD16+CD56 / CD45	FITC / PE-Cy™7 / APC-Cy™7 / APC / PE / PerCP-Cy™5.5							
ED7074	CD3 / CD19 / CD16+CD56 / CD45	FITC / APC / PE / PerCP							
ED7045	CD3 / CD4 / CD8 / CD45	FITC / APC / PE / PerCP							
ED7058	CD3 / CD4 / CD8	PE-Cy™5 / FITC / PE							
ED7059	CD3 / CD4 / CD8	PerCP / FITC / PE							
ED7076	CD3 / CD16+CD56 / CD45	FITC / PE / PerCP							
ED7077	CD3 / CD19 / CD45	FITC / PE / PerCP							
ED7047	CD3 / CD4 / CD45	FITC / PE / PerCP							
ED7048	CD3 / CD8 / CD45	FITC / PE / PerCP							
ED7050	CD3 / CD4	FITC / PE							
ED7051	CD3 / CD8	FITC / PE							
ED7052	CD4 / CD8	FITC / PE							
ED7053	CD3 / CD19	FITC / PE							
ED7054	CD3 / CD16+CD56	FITC / PE							
ED7055	CD3 / HLA-DR	FITC / PE							
ED7056	CD45 / CD14	FITC / PE							
ED7032	negative control	FITC / PE							



Lysing Solutions

EXCELLYSE I

Regulatory status: CE-IVD | Protocol: Lyse/wash, lyse/no wash

In case of lyse/no wash: • 2-step lysing procedure

- Excellent separation of lymphocytes and debris in SSC vs. FSC dot plot

Catalog no.	Product	Quantity
ED7064	EXCELLYSE I 30 ml	150–300 tests
ED7065	EXCELLYSE I 100 ml	500–1000 tests

EXCELLYSE Easy

Regulatory status: CE-IVD | Protocol: Lyse/wash, lyse/no wash

In case of lyse/no wash: • 1-step lysing procedure

- Suitable for lymphocyte gating via CD45 staining

Catalog no.	Product	Quantity
ED7070	EXCELLYSE Easy 30 ml	300–600 tests
ED7066	EXCELLYSE Easy 100 ml	1000–2000 tests

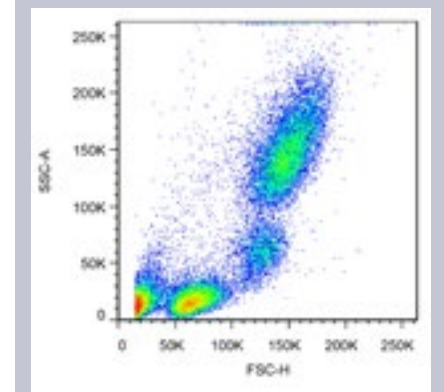
EXCELLYSE Live

Regulatory status: RUO | Protocol: Lyse/wash, lyse/no wash

Suitable for analysis of viable leukocytes

Catalog no.	Product	Quantity
ED7071	EXCELLYSE Live 30 ml	150 tests
ED7068	EXCELLYSE Live 100 ml	500 tests

Example of cell population separation in whole blood stained with CD45 PerCP and lysed using EXCELLYSE I (no wash protocol).



Regulatory status | CE | IVD

CE-IVD reagents are intended for *In Vitro* Diagnostic use in laboratories outside USA and Canada. These CE-IVD reagents are in conformity with the European *In Vitro* Diagnostic Medical Device Directive 98/79/EC.

Regulatory status | RUO

RUO reagents are intended for Research Use Only. Not for use in diagnostic or therapeutic procedures.



Newly Added Clones

With respect to our customers' needs we are continuously searching for valuable hybridomas to supplement our portfolio with high quality antibody clones, and we are also developing our own ones. Here we bring a table of clones that have been added to our portfolio since last issue. As your needs and requirements are of high importance for us, we are grateful for your feedback what other important clones you would like to see in our portfolio.

Regulatory
status

RUO

Catalog No.	Antigen	Clone	Host	Isotype	Reactivity	App.	Format	Quantity
11-696-C025 11-696-C100	FOLR2	EM-35	Ms	IgG1	Hu	FC, IP, WB ICC	purified purified	0.025 mg 0.1 mg
1P-696-C025 1P-696-C100							PE PE	0.025 mg 0.1 mg
11-800-C025 11-800-C100	Lysozyme	LZ598-10G9	Ms	IgG1	Hu	FC, ICC	purified purified	0.025 mg 0.1 mg
11-196-C100	FTL0187	polyclonal	Rb		<i>Francisella tularensis ssp. holarctica</i>	WB, ELISA	purified	0.1 mg
11-181-C100	FTT0571	polyclonal	Rb		<i>Francisella tularensis ssp. tularensis</i>	WB, ELISA	purified	0.1 mg
11-180-C100	PiIT	polyclonal	Rb		<i>Francisella tularensis ssp. tularensis</i>	WB, ELISA	purified	0.1 mg

You Can Meet Us:

- **WIRM X – 10th World Immune Regulation Meeting**
March 16–19, 2016 | Davos, Switzerland
- **ISLH 2016 – International Society for Laboratory Hematology**
May 12–14, 2016 | Milano, Italy
- **CYTO 2016 – 30th Congress of the International Society for Advancement of Cytometry**
June 11–15, 2016 | Seattle, USA
- **EAAI 2016**
June 11–16, 2016 | Wien, Austria
- **ESCCA 2016**
September 23 – October 7, 2016 | Edinburgh, Scotland

Abbreviations

APC	allophycocyanin
APC-Cy™7	allophycocyanin-Cyanine 7 tandem dye
FITC	fluorescein isothiocyanate
PE	R-phycoerythrin
PE-Cy™5	R-phycoerythrin-Cyanine 5 tandem dye
PE-Cy™7	R-phycoerythrin-Cyanine 7 tandem dye
PerCP	peridinin chlorophyll a protein
PerCP-Cy™5.5	peridinin chlorophyll a protein-Cyanine 5.5 tandem dye
RUO	research use only
CE-IVD	in conformity with the European <i>In Vitro</i> Diagnostic Medical Device Directive 98/79/EC

Quality statement

Our company has been assessed and certified as meeting the requirements of ISO 9001:2008, ISO 14001:2004, and ISO 13485: 2003 for the following activities:
Design, development and production of biotechnological products for research use.
Design, development and production of *in vitro* diagnostics for cell and protein analysis.

Alexa Fluor®, Pacific Blue™ and Pacific Orange™ are trademarks of Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation); DyLight™ is a trademark of Thermo Fisher Scientific; Cy™ is a trademark of GE Healthcare Limited.



Antibodies

EXBIO Praha, a.s.
Nad Safinou II 341
252 42 Vestec u Prahy
fax +420 261 090 660
orders@exbio.cz
technical@exbio.cz

www.exbio.cz

Brighter Future in Flow



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
OPERATIONAL PROGRAMME ENTERPRISE
AND INNOVATIONS FOR COMPETITIVENESS