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Antibodies





EXBIO Praha

Dedicated to Superior Cytometry Reagents

Mission

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

EXBIO Praha is a dynamic privately-owned European biotechnology company focused on design, development, manufacture and sale of monoclonal antibodies and other reagents for research and clinical applications.

Who we are

EXBIO Praha was established at the beginning of 1999 and soon became a trusted and reliable OEM supplier to all leading antibody vendors. Our flow cytometry antibodies have become widely recognized reagents among life science researchers and diagnostic laboratories. In addition to OEM supplies, we distribute our products worldwide under the EXBIO trademark through a network of experienced local distributors.

Our portfolio

Flow cytometry validated antibodies for a broad range of research applications (immunology, hematology, cancer research, etc.) available in many colors and formulations (suitable for 8-color immunophenotyping).

Pre-mixed antibody conjugate cocktails for in vitro diagnostic (CE IVD) applications (immunophenotyping of white blood cells).

Flow cytometry assays (FlowEx® Kits) for comprehensive diagnosis and analysis of patient health status: **FagoFlowEx® Kit (CE-IVD)** and **IngoFlowEx® Kit (RUO)** for examination of **phagocytosis**, **BasoFlowEx® Kit (CE-IVD)** for analysis of **allergy specificity**, **NKFlowEx® Kit (RUO)** for analysis of **NK cell activation**, **CD34 QuantiFlowEx® Kit (CE-IVD)** for quantification of **CD34+ stem cells**, **TregFlowEx® Kit (RUO)** for detection of **regulatory T cells**, **SpermFlowEx® Kit (RUO)** for evaluation of human **semen quality**, **ApoFlowEx® FITC Kit (RUO)** for detection of **apoptosis**, and **BrdUFlowEx® FITC Kit (RUO)** and **CellCycleFlowEx® Kit (RUO)** for analysis of **proliferation**.

Other monoclonal antibodies for applications such as Western blotting, immunohistochemistry, immunoprecipitation, ELISA or functional *in vivo* studies. Antibodies to cytokeratins, betaIII-tubulin, sHLA-G and IgE belong to our best sellers.

sHLA-G ELISA kit for determination of soluble forms of Human Leukocyte Antigen-G (sHLA-G) in amniotic fluid, cell culture supernatant, plasma and serum.

EXBIO products are offered under the following brands:



Antibodies

Monoclonal and polyclonal antibodies and kits for research applications.



Diagnostics

Reagents and kits for flow cytometry-based *in vitro* diagnostic applications:

- Immunophenotyping
- Examination of allergy
- Examination of phagocytosis

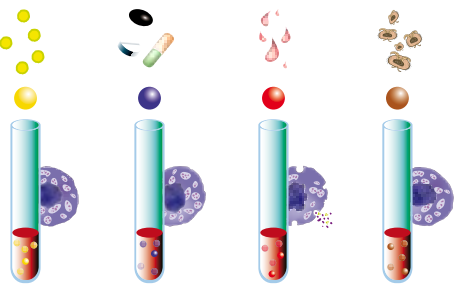
Basophil Activation Test – BAT

Allergic hypersensitivity – a challenge to modern diagnostics

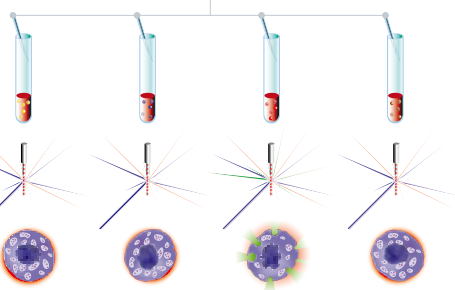
A rapid increase of patients with allergic hypersensitivity over the last decades has become a serious social and economical burden. According to WHO, e.g. the current numbers of asthmatic patients and of patients with food allergy are around 300 million and 250 million, respectively. Not only the worldwide increase of allergic patients, but also the increase of particular allergens occurs. To face this problem the reliable diagnostics is crucial.

Advantages of the Basophil Activation Test (BAT) in diagnosing allergies

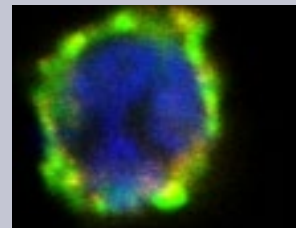
When diagnosing allergies, the crucial task is determining the causal allergen(s). The commonly used method of skin prick tests (SPT) is not optimal due to its low specificity and the risk of further aggravating the condition. More suitable are *in vitro* approaches, such as the determination of specific IgE in the serum (sIgE) or the analysis of basophil activation markers. In most cases, the specificity of sIgE detection is sufficient, but the sensitivity is usually only about 75%, and in cases of some allergens (mainly food and drug) both sensitivity and specificity of sIgE detection are substantially lower. **Basophil Activation Test (BAT)**, however, is known not only for very high specificity, but also for high sensitivity, even in cases of many problematic antigens. This test is based on the detection of basophil activation markers using flow cytometry. The **BasoFlowEx® Kit** is based on the principles of the BAT. Its use is **simple and suitable for every laboratory with basic equipment and with a flow cytometer**, which uses blue laser excitation (488 nm) and detection channels for FITC and PE.



BasoFlowEx® Kit
anti-CD203c-PE
anti-CD63-FITC



Surface staining of activated human basophil by FITC-conjugated anti-CD63 (clone MEM-259 – green) and PE-conjugated anti-CD203c (clone NP4D6 – red); merged signal yellow. DNA visualized by DAPI (blue).



Baso FlowEx® Kit

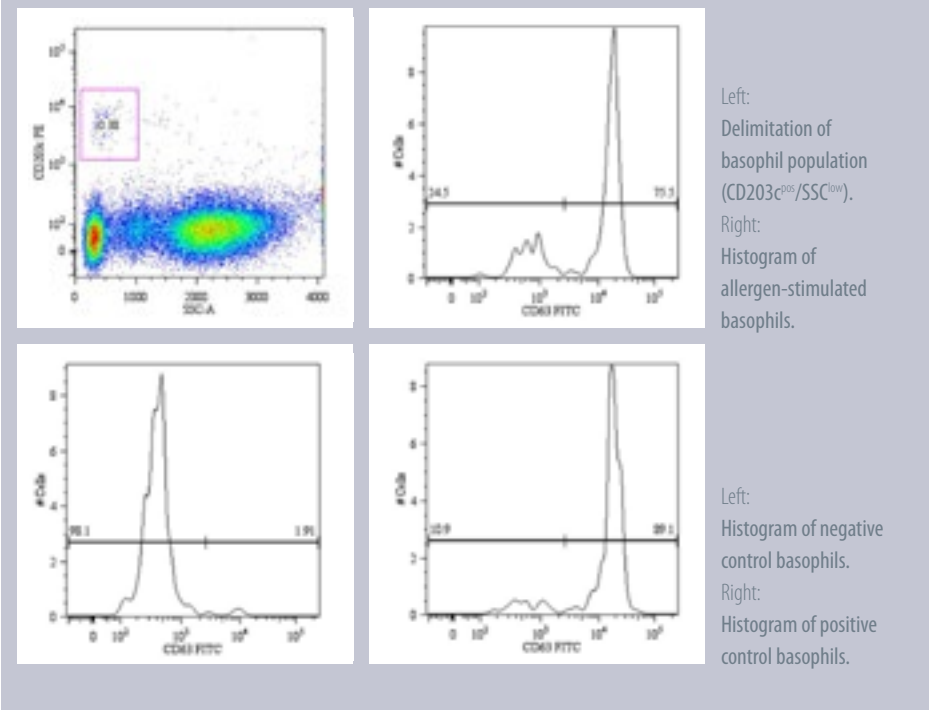
Catalog No.: **ED7043** Regulatory status: **CE-IVD** Quantity: **100 tests**

Reagents provided:

ED7043-1	Stimulation Buffer	• 5 lyophilized vials • 1 vial is intended for stimulation of 20 tubes
ED7043-2	Stimulation Control	• 2 lyophilized vials • 1 vial is intended for stimulation of 25 positive controls
ED7043-3	Staining Reagent	1 vial containing 2 ml of premixed antibody cocktail: anti-CD63, FITC labeled + anti-CD203c, PE labeled
ED7043-4	Lysing Solution	30 ml

The BasoFlowEx® Kit is intended for flow cytometry examination of IgE-mediated allergic reactions via the analysis of CD63 antigen surface exposure on basophils in human heparinized whole blood upon allergen stimulation. Commercially available allergens (e.g. used for skin prick tests) can be used for stimulation.

Example Data



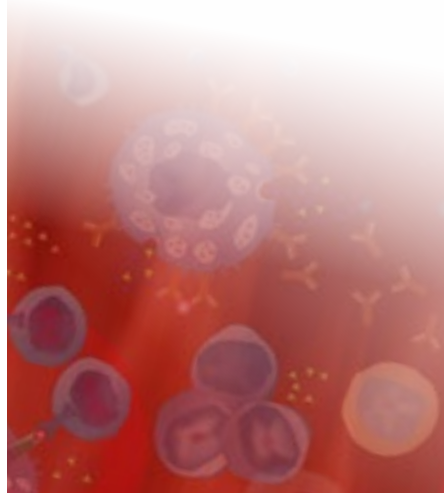
Allergens and their use in BAT

The measure of basophil response to a particular allergen exposure is usually expressed as % of CD63⁺ basophils. The response is triggered by antigen-dependent cross-linking of specific IgE bond to the high-affinity IgE receptor (FcεRI) on the surface of basophils. Amount of IgE-FcεRI receptor complexes on the basophil surface directly depends on the free IgE concentration in the blood. Hence the signal obtained out of the basophil activation test (BAT) reflects functional activation of basophil in relation to the sensitivity to particular activating allergen and its concentration in the test tube. Whereas CD63 externalization after challenge with one high allergen dose gives an information about cell reactivity, allergen titration reveals allergen threshold, which is a measure of allergen sensitivity.

Allergens representing different areas of allergy like inhalant allergy, food allergy, hymenoptera venom allergy, drug allergy or chronic urticaria can be used in BAT for diagnostic evaluation of a patient allergy profile together with typically performed tests such as skin prick testing and determination of specific IgEs. Whole allergen extracts as well as recombinant molecules of dominant allergens are commercially available from different sources and regions. Thus it can happen that the values of basophil reactivity reached in BAT with different compounds of the same allergen type may even vary based on geographical origin of the reagent. Therefore it is very important to standardize the use of allergen compounds of particular origin with BAT diagnostic products like **BasoFlowEx® Kit**.

Various allergens differ in the optimal concentration which provokes the maximum basophil activation, and also in individual variations of the dose-response curves. If the activation by allergen takes place over a broad concentration range and with low number of individual variations, it is much easier to choose the allergen concentration to be used in BAT, than in case that the effective concentration range is narrow and shows great individual variations.

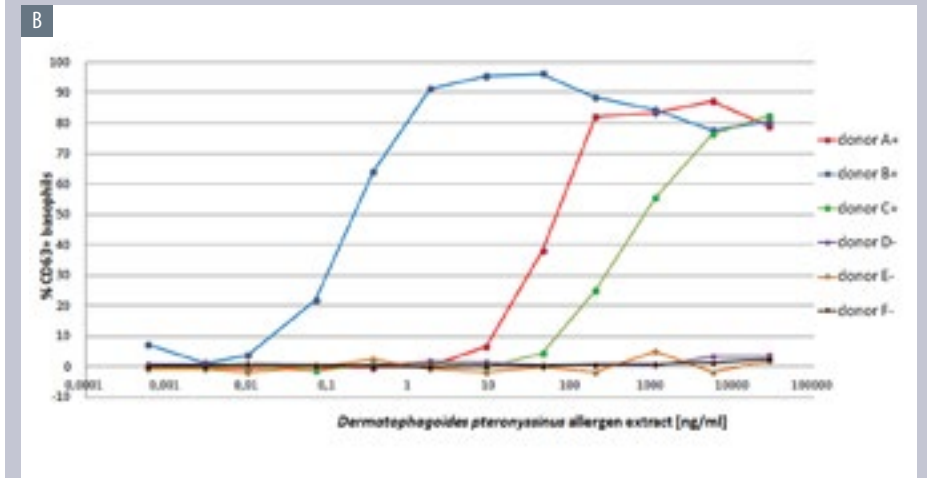
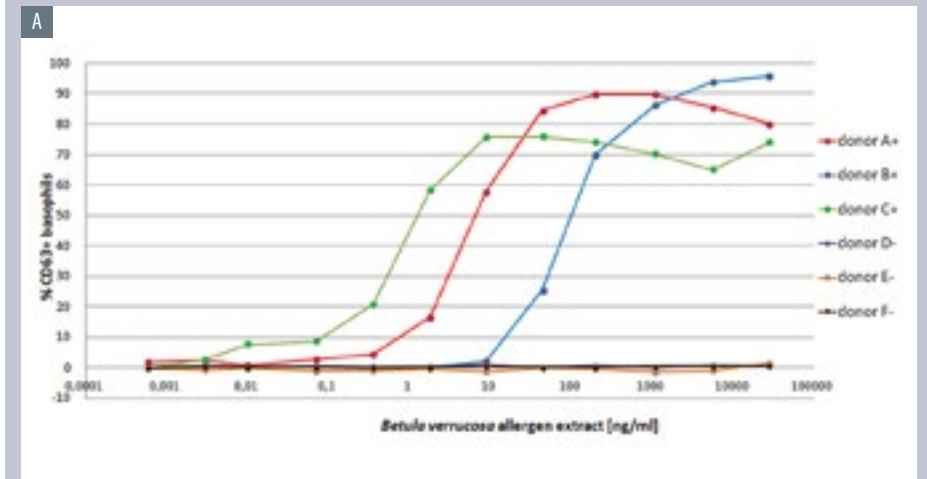
The set of titration experiments has been conducted in order to evaluate the basophil activation profile of donors with known allergy history by using four different allergen extracts and **BasoFlowEx® Kit** as analytical tool. The extracts of *Betula verrucosa*, *Phleum pratense*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were tested. Testing the other types of allergen extracts, for example of inhalant, food and hymenoptera venom allergy, is currently under process.



Experimental Data

Data demonstrate that patients reactive to the same antigen can differ substantially in the course of antigen dose-dependent activation curve. Percentage of activated basophils (% CD63⁺) is plotted against log concentration of allergen extract. Both allergic and healthy donors were taken into analysis. A suppression of basophil response appeared for some samples at very high concentrations of allergen.

- A Allergen extract of *Betula verrucosa* (Common silver birch).
- B Allergen extract of *Dermatophagoides pteronyssinus* (European house dust mite).



anti-CD203c

Antigen	Clone	Host	Isotype	Reactivity	Application
CD203c	NP4D6	Ms	IgG1	Hu	FC, ICC

Regulatory status | RUO

Formats: Purified | FITC | PE | APC

For currently available formats see particular product page at www.exbio.cz.

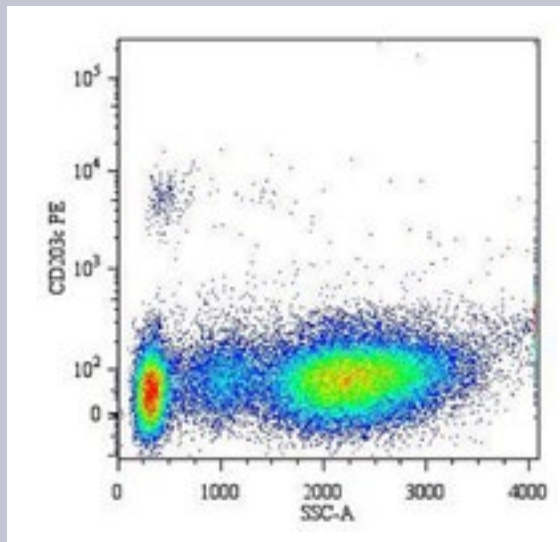
CD203c, also known as ENPP-3 (ectonucleotide pyrophosphatase/phosphodiesterase 3), an important marker of basophils, is an integral membrane ectoenzyme, that hydrolyses nucleotide triphosphates and thus modulates purinergic signaling. CD203c is expressed on basophils and mast cells and is up-regulated in response to IgE-receptor cross-linking.

It is overexpressed on neoplastic mast cells in patients with systemic mastocytosis. Besides role of CD203c as a basophil marker, measurement of its induced enhancement on the plasma membrane is useful for diagnostics of allergies. Externalization of CD203c reflects different stimulatory pathways compared to the externalization of CD63, thus simultaneous evaluation of both these markers increases the amount of information concerning particular tested sample.

CD203c in BAT: Optimal marker for basophil gating, supplementary basophil activation marker.

Example Data

Flow cytometry analysis of basophils (surface staining) in allergen-stimulated human whole blood by anti-CD203c antibody (NP4D6). Basophils identified as CD203c positive SSC negative population.



anti-CD63 / LAMP-3

Antigen	Clone	Host	Isotype	Reactivity	Application
CD63 / LAMP-3	MEM-259	Ms	IgG1	Hu	FC, IP, IHC(P), ICC

Regulatory status | RUO

Formats: Purified | biotin | FITC | Alexa Fluor 488® | PE | APC | PE-Cy™5 | PerCP | PerCP-Cy™5.5

For currently available formats see particular product page at www.exbio.cz.

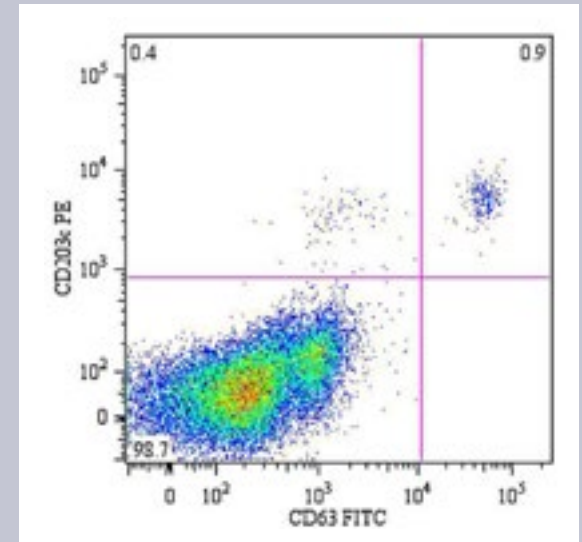
CD63 / LAMP-3 (lysosome-associated membrane protein-3), a glycoprotein of tetraspanin family, is present in late endosomes, lysosomes and secretory vesicles of various cell types. It interacts with integrins and affects phagocytosis and cell migration. Upon basophil activation, CD63 is quickly exposed on the cell surface and this process well correlates with histamine release. Hence, it has become a widely used basophil activation

marker. In mast cells, however, CD63 exposure does not need their activation. Percentage of CD63⁺ basophils detected after stimulation with particular allergen gives information about reactivity to this allergen. To interpret the results correctly, however, positivity thresholds must be set for particular groups of allergens (e.g. 5% for drug allergens, 10% for hymenoptera venom, 15% for inhaled allergens, etc.)

CD63 / LAMP3 in BAT: Main basophil activation marker.

Example Data

Flow cytometry analysis of human peripheral blood lymphocytes from a patient with allergy to bee venom after stimulation with bee venom, stained with anti-human CD63 (MEM-259) FITC.



anti-CD193 / CCR3

Antigen	Clone	Host	Isotype	Reactivity	Application
CD193/CCR3	5E8	Ms	IgG2b	Hu	FC

Regulatory status **RUO**

Formats: **Purified** | **PE** | **APC**

CD193 / CCR3 (chemokine receptor 3) is a G-protein coupled receptor for several chemokines, namely CCL11 (eotaxin), CCL26 (eotaxin-3), CCL7 (MCP-4), or CCL5 (RANTES). It is highly expressed on eosinophils and basophils, and is also detected in TH1 and TH2 cells, as well as in airway epithelial cells. This recep-

tor may contribute to the accumulation and activation of eosinophils and other inflammatory cells in the allergic airway. CD193 / CCR3 may be used as a basophil marker in BAT, however, it is less specific compared to CD203c.

CD193 / CCR3 in BAT: Not so specific marker for basophil gating

anti-IgE

Antigen	Clone	Host	Isotype	Reactivity	Application
anti-IgE	BE5	Ms	IgG1	Hu	FC, ELISA, stimulation
	4G7	Ms	IgG	Hu	ELISA
	4H10	Ms	IgG1	Hu	FC, ELISA

Regulatory status **RUO**

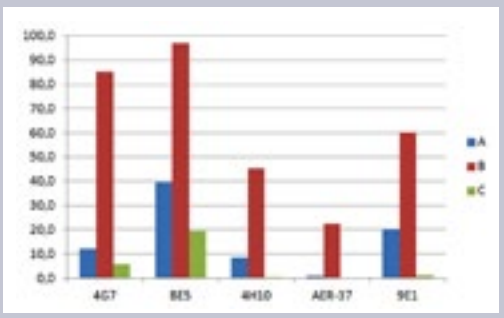
For currently available formats see particular product page at www.exbio.cz.

Basophils may be also detected through IgE. However, one must keep in mind it is also attached to other cell types, e.g. to monocytes, and that the antibody binding can cause artificial basophil activation. On the other hand, some anti-IgE

antibodies are good positive controls of basophil activation.

IgE in BAT: Target for basophil activation, but not recommended for basophil gating.

Comparison of five different mouse monoclonal anti-IgE antibodies (4G7, BE5, 4H10, AER-37, 9E1) regarding their use as a stimulation control in a basophil activation test. Percentage of activated basophils in peripheral blood samples of three independent individuals (A, B, C) indicated. Activation done at 37 °C for 15 min. and evaluated by mouse monoclonal anti-CD63 (MEM-259) FITC.



Quality statement

EXBIO Praha, a.s. is dedicated to its customers and to providing them with the products and services that are of the highest quality and that facilitate successful research. 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.

Regulatory Notes

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



- **RUO** reagents are intended for *research use only*. Not for use in diagnostic or therapeutic procedures.



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