DATA SHEET

BasoFlowEx® Kit

Cat. No.: ED7036

(100 tests)

1. Intended use

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

2. Introduction

The crucial point in allergy diagnostics is to determine the causal allergen. Reliability of the commonly used method of skin prick test (SPT) is low and this test can promote the patient's allergy disease. Hence *in vitro* tests, such as detection of specific IgE in serum (sIgE), or analysis of basophil activation markers, are advisable. Although specificity of sIgE detection is sufficient in case of most antigens, its sensitivity is usually about 75% only. Moreover, in case of some allergens (namely of food and drugs) both sensitivity and specificity of sIgE detection is much lower. On the contrary, the basophil activation test (BAT) is noted not only for very high specificity, but also for high sensitivity, including many problematic allergens.

3. Principle

This assay is based on *in vitro* stimulation of basophils using sensitizing allergen and subsequent flow cytometry analysis of CD63 antigen exposition on the cell surface. If a basophil has allergen-specific IgE molecules bound to the cell surface, allergen bridges near IgE molecules leading to the activation of the basophil. As a consequence, cytoplasmatic granules containing the CD63 transmembrane protein are fused to the cellular membrane and release inflammatory mediators. Therefore the CD63 antigen is exposed to the extracellular matrix as a marker of basophil activation (degranulation). The exposition of CD63 antigen is followed by a monoclonal antibody staining (clone MEM-259; FITC labeled). Basophil population is identified by staining using a monoclonal antibody to CD203c (clone NP4D6) labeled with R-phycoerythrin (PE). Positive control sample is stimulated using a monoclonal antibody against IgE molecule which mimics the alergen bridging process of IgE molecules on the basophil surface, and also by a chemotactic peptide N-formyl-Met-Leu-Phe (fMLP) which activates basophils via unspecific way.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

4. Precautions

-For laboratory research only, not for drug, diagnostic or other use.
-Blood must be collected into a tube containing heparin. Anticoagulants EDTA and citrate negatively affect results of the analysis.
-Blood samples should be treated within 24 hours after collection.
-Stained samples should be analyzed within 2 hours after staining.
-Do not use reagents after expiration date stated on vial labels.
-Avoid Staining Reagent (ED7036-3) prolonged exposure to light.
-Avoid contamination of reagents.
-Aeroallergens (pollen, mites, dust) in laboratory may contaminate open tubes and cause an increased background release.
-Any non-performance of staining protocol may produce false results.
-Blood samples are considered as potentially infectious and must be handled with care.
-Avoid any contact of the sample with the skin, eyes and mucosa.
-Lysing Solution (ED7036-4) contains formaldehyde.

5. Reagents provided

-ED7036-1 **Stimulation Buffer** – 5 lyophilized vials, 1 vial is intended for stimulation of 20 tubes.
-ED7036-2 **Stimulation Control** – 2 lyophilized vials, 1 vial is intended for stimulation of 25 positive controls.
-ED7036-3 **Staining Reagent** – 1 vial containing 2 ml of premixed antibody cocktail: anti-CD63, FITC labeled + anti-CD203c, PE labeled.
-ED7036-4 **Lysing Solution** – 30 ml.

6. Necessary material not supplied

-Allergens (e.g. for prick tests)
-Suitable 5ml test tubes for blood staining (e.g. 12 × 75 mm)
-PBS buffer
-Ultrapure demineralized water
-Automatic pipettes with disposable tips
-Vortex mixer
-Thermostat able to incubate test tubes at 37 °C
-Centrifuge with rotor suitable for test tubes
-Flow cytometer - blue laser excitation at 488 nm, light emission at 525 nm (FITC) and 575 nm (PE)

7. Storage

Store the BasoFlowEx® Kit at 2-8 °C. Expiration date is stated on a vial labels and on the box.

8. Assay procedure

Use heparinized whole blood for examination. Prepare reagents according to following instructions.

Preparation of reagents before assay

1. Reconstitute lyophilized **Stimulation Buffer** using 2 ml of demineralized water. Unused volume of the buffer store at 2-8 °C up to **5 days**. Alternatively, the buffer can be aliquoted, frozen once, and stored at ≤ -20 °C for another use. Avoid repeated freeze/thaw cycles.
2. Reconstitute lyophilized **Stimulation Control** using 0.25 ml of demineralized water. Unused volume of the reagent store at 2-8 °C up to **30 days**. Alternatively, the reagent can be aliquoted, frozen once, and stored at ≤ -20 °C for another use. Avoid repeated freeze/thaw cycles.
3. Other reagents (**Staining Reagent**, **Lysing Solution**) are ready to use.

Assay procedure

For examination of one blood sample prepare test tubes for **negative control**, **positive control** and for **samples to be stimulated with allergens**. Various commercially available allergens may be use for stimulation, e.g. prick test allergens at suitable dilution (1-100x). Prepare samples according to following procedure:

1. In to the tubes destined for:
-**allergen-stimulated sample** add 10 µl of allergen,
-**negative control sample** add nothing,
-**positive control sample** add 10 µl of Stimulation Control.
2. Add 100 µl of Stimulation Buffer into all tubes.
3. Add 100 µl of heparinized whole blood into all tubes and vortex gently.
4. Incubate tubes at 37 °C for 15 minutes in water bath or 25 minutes in air incubator.
5. Add 20 µl of Staining Reagent into all tubes, vortex gently, and incubate for 20 minutes at 2-8 °C or on ice.
6. Add 300 µl of Lysing Solution into all tubes, vortex gently, and incubate for 5 minutes at room temperature.
7. Add 3-4 ml of demineralized water into all tubes, vortex gently, and incubate for 5-10 minutes at room temperature until the red blood cells are lysed.
8. Centrifuge tubes for 5 minutes at 300 g.
9. Remove the supernatant and resuspend the pellet in 0.2-0.4 ml of PBS buffer.
10. Analyze samples using a flow cytometer within 2 hours after staining.

9. Flow cytometric analysis

Analyze stained samples using flow cytometer. In order to analyze sufficient number of basophils (>200), acquire 50,000-100,000 events per sample. Make compensation of fluorescent signals prior or after data acquisition.
Visualize compensated data on the side-scatter (SSC) versus fluorescence intensity in PE channel (FL2) dot-plot. Set the gate for basophil population (CD203c positive, SSC low) as shown in figure 1. The gate must be positioned individually for each sample. Then bring the

gated basophils to histogram as shown in figures 2a,b,c where the X-axis represents fluorescenceintensity in FITC channel (FL1). Using the negative control sample set the gate for non-stimulated basophils (CD63dim). Then calculate the percentage of activated basophils (CD63bright) in allergen-stimulated samples and positive control sample.

10. Interpretation of results

Individuals are considered as allergic to the tested allergens when the percentages of activated basophils get over the cut-off value. Recommended cut-off values for allergen groups are as follows:
Inhalant and food allergens ≥15 %
Hymenoptera venoms ≥10 %
Drugs ≥5 %
If the positive control sample shows activation of basophils <10 %, measured results are regarded as nonevaluable.

11. Expected values

In theory, the number of activated basophils in allergen-stimulated sample may vary in range 0-100 %. Average number of activated basophiles measured in 20 positive control samples is listed below.

Parameter	Mean (%)	SD	CV (%)
Activated basophils	77,3	19,4	25,1

12. Limitations

-Blood sample may contain insufficient amount of basophiles.
-Basophiles of some blood samples are not susceptible to stimulation.
-Reliability of the assay depends on the quality of the allergen.
-Flow cytometer may produce false results if the device has not been aligned and maintained appropriately.
-Data may be incorrectly interpreted if fluorescent signals were compensated wrongly or if gates were positioned inaccurately.

Example data

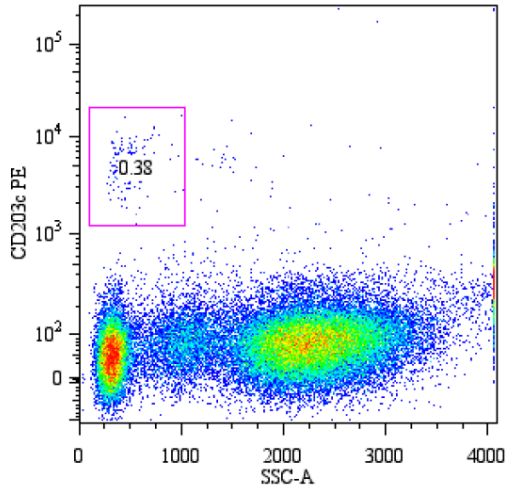

Fig. 1 Delimitation of basophil population (CD203c^{pos}/ SSC^{low}).
Obr. 1 Ohraničení populace bazofilů (CD203c^{pos}/ SSC^{low}).
Obr. 1 Ohraničenie populácie bazofilov (CD203c^{pos}/ SSC^{low}).

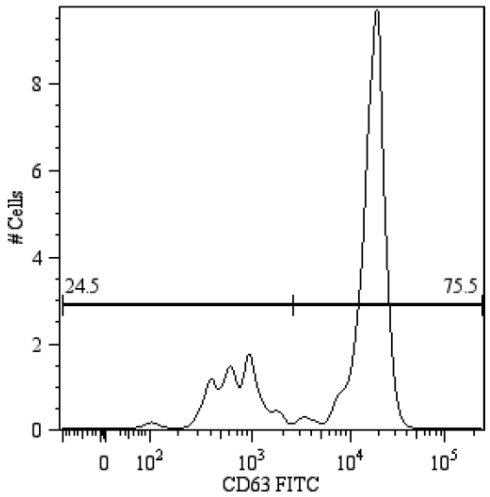

Fig. 2a Histogram of allergen-stimulated basophils.
Obr. 2a Histogram populace bazofilů po stimulaci alergenem.
Obr. 2a Histogram populácie bazofilov po stimulácii alergénom.

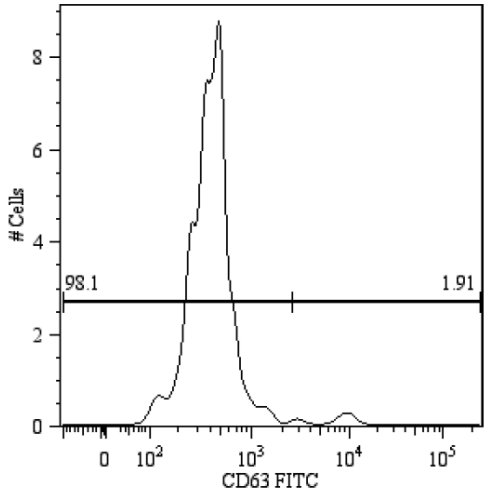

Fig. 2a Histogram of negative control basophils.
Obr. 2a Histogram populace bazofilů negativní kontroly.
Obr. 2a Histogram populácie bazofilov negatívnej kontroly.

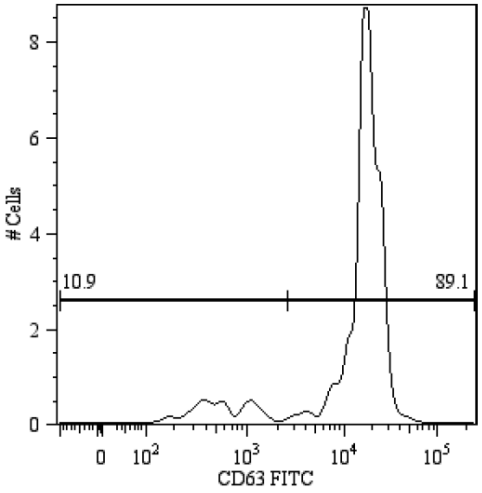

Fig. 2c Histogram of positive control basophils.
Obr. 2c Histogram populace bazofilů pozitivní kontroly.
Obr. 2c Histogram populácie bazofilov pozitívnej kontroly.

Explanation of symbols

REF	Catalog number Katalogové číslo Katalógové číslo
	Manufacturer identification Výrobce Výrobca
2 °C 8 °C	Store within temperature limits Rozmezí skladovacích teplot Rozmedzie skladovacích teplôt
LOT	Batch code Číslo šarže
	Use by Použitelné do Použiteľné do

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium