

BasoFlowEx Kit

Cat.No. ED7043

1. Intended purpose

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. Commercially available allergens can be used for the stimulation.

Context of a physiological or pathological state

The crucial point in allergy diagnostics is to determine the causal allergen. Reliability of the commonly used method of the 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 for most allergens, its sensitivity is usually about 75% only. Moreover, for food and drug allergens both sensitivity and specificity of sIgE detection is much lower. By contrast, the basophil activation test (BAT) shows very high specificity and also high sensitivity, including many problematic allergens.

2. Test principle

The BAT assay is based on *in vitro* stimulation of basophils using sensitizing allergen and subsequent flow cytometry analysis of externalized CD63 antigen exposition on basophil surface after degranulation. If there is sufficient quantity of allergen-specific IgE molecules bound to the basophil cell surface high-affinity FcεRI receptors, allergen molecules crosslink the receptors leading to full activation of the basophil. As a consequence, cytoplasmic granules containing the CD63 transmembrane protein fuse with the plasma membrane and release inflammatory mediators. Therefore, the CD63 antigen is exposed as a marker of basophil activation (degranulation). The exposed CD63 antigen is detected by monoclonal antibody staining (clone MEM-259, FITC labeled). The basophil population is identified by staining using anti-CD203c monoclonal antibody (clone NP4D6, R-phycoerythrin (PE) labeled). A positive control sample is stimulated using a monoclonal antibody against IgE molecule which mimics the allergen crosslinking process of IgE molecules on the basophil surface and using a chemotactic peptide N-formyl-Met-Leu-Phe (fMLP) which activates basophils via fMLP receptor (FPR1).

3. 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.

4. Materials required but not provided

Allergens
Suitable 5ml test tubes for blood staining (e.g. 12 × 75 mm)
PBS buffer
Ultrapure demineralized water

5. Equipment required

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)

6. Storage and handling

Store the BasoFlowEx Kit at 2-8 °C. Keep away from sunlight. Expiration date is stated on a vial labels and on the box.
See section 9, "Procedure-Reagent Preparation" for information about storage of reconstituted reagents and their In Use Stability.

7. Warnings, precautions and limitations of use

- Intended for In Vitro Diagnostic use in laboratories outside USA and Canada.
- This CE-IVD kit is in conformity with the European Directive 98/79/EC.
- Do not use reagents after their expiration date.
- Avoid reagents contamination.
- Blood samples are considered as potentially infectious and must be handled with care.
- Avoid Staining Reagent (ED7043-3) prolonged exposure to light.
- The Lysing Solution (ED7043-4) contains

formaldehyde and methanol.

H-phrases

H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled.
H317: May cause an allergic skin reaction.
H351: Suspected of causing cancer.

P-phrases

P270: Do not eat, drink or smoke when using this product.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302+P352: IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501: Dispose of contents/container to authorized facility for dangerous wastes.

- See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.
- Blood sample may contain insufficient number of basophils.
- Basophils in 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 not-correctly compensated or if gates were set inaccurately.
- Stained samples should be analyzed within standardized time frame but no later than 2 hours after lysis.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure stable sensitivity of detectors.
- Any non-performance of assay procedure may produce false results.

8. Specimen

Use human heparinized whole blood for examination.

Blood must be collected into a tube containing heparin. Follow the instructions of the tube manufacturer to ensure that the correct filling volume is achieved.

Anticoagulants EDTA and citrate negatively affect results of the analysis.

Blood samples should be stored at room temperature.

Blood samples must be treated within 8 hours after collection if stored at room temperature.

Aeroallergens (pollen, mites, dust) may contaminate open tubes in laboratory and cause an increased background release.

9. Procedure

Reagent preparation

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

Examination procedure

For the examination of one blood sample prepare the test tubes for **negative control**, **positive control** and for **samples to be stimulated with different allergens**. Various commercially available allergens may be used for stimulation. Prepare samples according to following procedure.

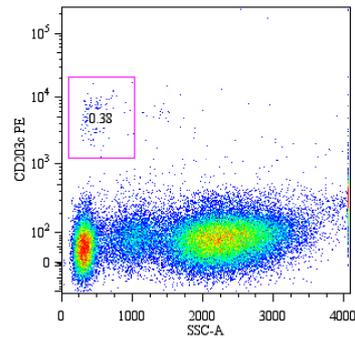
- Add in the tube of:
 - allergen-stimulated sample - 10 µl of allergen,
 - negative control sample - nothing,
 - positive control sample - 10 µl of Stimulation Control.
- Add 100 µl of Stimulation Buffer into all tubes.
- Add 100 µl of heparinized whole blood into all tubes and vortex gently.
- Incubate tubes at 37 °C for 15 minutes in water bath or 25 minutes in air incubator.
- Add 20 µl of Staining Reagent into all tubes, vortex gently, and incubate for 20 minutes at 2-8 °C or on ice.

- Add 300 µl of Lysing Solution into all tubes, vortex gently, and incubate for 5 minutes at room temperature.
- 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.
- Centrifuge tubes for 5 minutes at 300x g.
- Remove the supernatant and resuspend the pellet in 0.2-0.4 ml of PBS buffer.
- Analyze samples using a flow cytometer within 2 hours after staining.

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. Compensate your data (FITC and PE channels) prior to or after the 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 set individually for each sample.

Fig. 1 Delimitation of basophil population (CD203c^{pos} / SSC^{low}).



Then plot the gated basophils as a histogram shown in figures 2a,b,c where the X-axis represents fluorescence intensity in FITC channel (FL1). Then plot the gated basophils as a histogram shown in figures 2a,b,c where the X-axis represents fluorescence intensity in FITC channel (FL1). Set the gate for non-stimulated basophils (CD63^{dim}) using the negative control sample.

Fig. 2a Histogram of allergen-stimulated basophils.

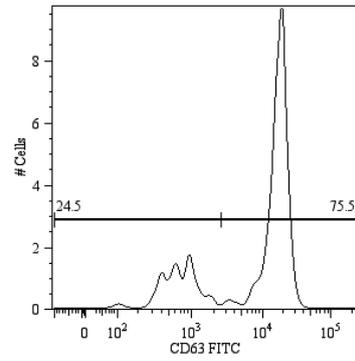


Fig. 2b Histogram of negative control basophils.

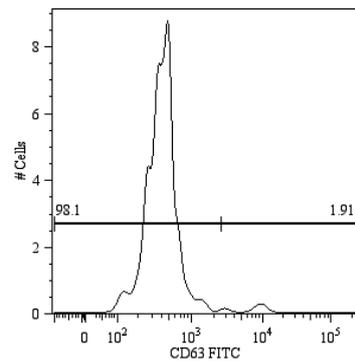
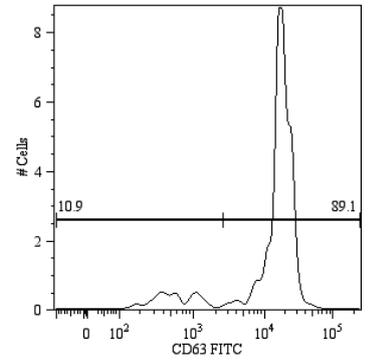


Fig. 2c Histogram of positive control basophils.



Calculation and interpretation of analytical results

Calculate the percentage of activated basophils (CD63^{bright}) in the allergen-stimulated samples and positive control sample.

Individuals are considered as allergic to the tested allergens when the percentage of activated basophils exceeds 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 < 20 %, the allergen-stimulated samples cannot be interpreted.

10. Analytical performance

Precision (repeatability and reproducibility)

Repeatability and reproducibility of the assay was determined from the data measured by five operators using six sensitizing allergens in two parallels on one blood sample under the same experimental conditions. Using Analysis of variance the following parameters were calculated:

| | |
|-------------------------------|---------|
| CV _{repeatability} | = 2.4 % |
| CV _{reproducibility} | = 3.4 % |

11. Clinical performance

Specificity and Sensitivity

Specificity and Sensitivity of the assay was determined by comparison of BasoFlowEx Kit with two competitor BAT tests commercially available on the market. The comparison was performed in routine clinical laboratory on 100 tests.

| Parameter | BasoFlowEx vs. Comp. A | BasoFlowEx vs. Comp. B | Comp. B vs. Comp. A |
|-----------------|------------------------|------------------------|---------------------|
| Sensitivity (%) | 96.6 | 85.9 | 94.7 |
| Specificity (%) | 82.9 | 79.3 | 69.2 |

Expected values

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

| Parameter | Mean (%) | SD | CV (%) |
|---------------------|----------|------|--------|
| Activated basophils | 77.3 | 19.4 | 25.1 |

12. References

1. Sainte-Laudy J, Sabbah A, Vallon C, Guerin JC (1998) Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm Res*. 47(10): 401-408
2. Sanz ML, Sánchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, Diéguez I, De Weck AL (2001) Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. *Clin. Exp. Allergy* 31(7): 1007-1013
3. Erdmann SM, Heussen N, Moll-Slodowy S, Merk HF, Sachs B (2003) CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. *Clin. Exp. Allergy* 33(5): 607-614
4. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ (2004) In vitro allergy diagnosis: should we follow the flow? *Clin. Exp. Allergy* 34(3): 332-339
5. Valent P, Hauswirth AW, Natter S, Sperr WR, Bühring HJ, Valenta R (2004) Assays for measuring in vitro basophil activation induced by recombinant allergens. *Methods* 32(3): 265-270

13. Manufacturer

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14. Trademarks

n/a

15. Revision History

- Version 1, ED7043_IFU_v1
Initial Release
- Version 2, ED7043_IFU_v2
A text change in the Precautions section : Text "Intended for professional use only" removed and replaced with "Intended for In Vitro Diagnostic use in laboratories outside USA and Canada. This CE-IVD kit is in conformity with the European In Vitro Diagnostic Medical Device Directive 98/79/EC".
- Version 3, ED7043_IFU_v3
Text of Warning was changed from "The Lysing Solution (ED7043-4) contains < 5 % formaldehyde" to "The Lysing Solution (ED7043-4) contains formaldehyde and methanol". R- and S-phrases and hazard symbol were changed to H- and P-phrases and GHS symbols according to Regulation (EC) No 1272/2008.
- Version 4, ED7043_IFU_v4
Text "e.g. used for prick tests" was removed from Intended use section.
Text "(e.g. for prick tests)" was removed from Necessary material not supplied section.
Text "e.g. prick test allergens at suitable dilution (1-10x)" was removed from Assay procedure section.
- Version 5, ED7043_IFU_v5
The company logo changed.
IFU layout changed.
The "keep away from sunlight" phrase added to the Storage section.
The text "Follow the instructions of the tube manufacturer to ensure that the correct filling volume is achieved" was added to the Specimen section.
The text "See section 9, "Procedure-Reagent Preparation" for information about storage of reconstituted reagents and their In Use Stability." was added to the Storage and handling section.
Manufacturer postal code changed from 25242 to 25250.
The text "See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product." was added to the Warnings, precautions and limitations of use section.
- Version 6, ED7043_IFU_v6
Product Use Limitation text was refined.

exbio

BasoFlowEx Kit

100 tests | Cat.No. ED7043



Instructions for Use

Version ED7043_IFU_v6_EN
Date of Issue: 20-01-2020



Symbols

| | |
|--|------------------------------------|
| | Catalogue number |
| | Batch code |
| | Use-by date |
| | Temperature limits |
| | Keep away from sunlight |
| | In vitro diagnostic medical device |
| | CE marking of conformity |
| | Consult instructions for use |
| | Manufacturer |

The product is intended for In Vitro Diagnostic Use. In vivo diagnostic or therapeutic applications are strictly forbidden.

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