SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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BASOSTEP KIT
Basophil Activation Test

Reference	Size
BSTP-100T	100 test

PRODUCT DESCRIPTION

Reagent provided: monoclonal antibodies combination.
Tested application: flow cytometry
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN_3).
Recommended usage: Immunostep's BASOSTEP Kit, is intended for quantitative determination of the degranulation of basophilic granulocytes in heparinized human whole blood. BASOSTEP is intended for flow cytometry test of IgE-mediated allergic reactions by the analysis of CD63 antigen surface on basophils upon allergen stimulation using flow cytometry.
This reagent is effective for direct immunofluorescence staining for flow cytometric analysis using $\leqslant$20 µl/10^6 cells.
Presentation: liquid
Reagents supplied and preparation:

- **Stimulation Buffer:** Containing calcium, heparin and IL-3. 3 vials lyophilized. Reconstitute with 10 ml of demineralized water. After reconstitution store at 2-8° C for a week or aliquot and freeze.

- **Stimulation Control**: Containing fMLP and anti-IgE. 1 vial lyophilized. Reconstitute with 0,4 ml of demineralized water. After reconstitution store at 2-8 ° C for a week or aliquot and freeze.

- **Staining Reagent:**

  Mix of antibodies: CD63 FITC; CD203c; PE/HLA-DR PerCP; CD123 APC

CLINICAL RELEVANCE

In susceptible people, IgE is produced by B cells in response to specific antigens such as foods, pollens, latex, and drugs. This antigenspecific (or allergen-specific) IgE circulates in the serum and binds to high-affinity IgE receptors on immune effector cells such as mast cells located throughout the body.
Upon subsequent exposure to the same allergen, IgE receptors cross-link and initiate downstream signaling events that trigger mast cell degranulation and an immediate allergic response—hence the term immediate hypersensitivity.

Basophils are among the least abundant populations of circulating leukocytes, but by virtue of their sensitization with allergen-specific IgE, they represent a significant effector population in allergic pathogenesis. Basophils are known to be an early and abundant source of Th2 cytokines and other mediators of Th2 inflammation. There is also growing recognition of their capacity to modulate adaptive immunity.

The kit is based on the method described by Sainte-Laudy et al. 1994 where basophil activation by allergens is detected by flow cytometry measured by the increase of CD63 (gp53) at the cellular surface.
BASOSTEP allows the quantitative determination of human basophils degranulation. The test included the chemotactic peptide N-formyl-Met-Leu-Phe (fMLP) as positive control and a cocktel of for antibodies for detection and determination of degranulated basophils activation. Therefore BASOSTEP assay is based on in-vitro stimulation of basophils using fMLP as positive control and subsequent flow cytometry analysis of membrane expression of CD63 on basophils after degranulation.

Using defined allergens, this test provides information concerning the releasability of IgE-bearing basophils. When IgE-receptors on basophils are cross-linked by an allergen the cells undergo degranulation throught cytoplasmatic 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.

ANTIGEN DETAILS

Large description: CD63 clone TEA3/18 is a 53 kDa, type III lysosomal glycoprotein, expressed on activated platelets, monocytes and macrophages. CD63 contains four hydrophobic transmembrane domains with a major extracellular region of 95 amino acids between transmembrane segments 3 and 4.

CD203c clone NP4D6 is expressed on basophils and mast cells. CD203c expression is upregulated on basophils activated by allergen or IgE crosslinking. Therefore, CD203c is used as an indicator of basophil activation in various IgE-mediated allergic responses. CD203c is also known as ectonucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3). ENPP family members catalyze the hydrolysis of pyrophosphate and phosphodiester bonds to nucleoside 5-monophosphates.

HLA-DR clone GRB1 is directed against the HLA-DR-antigen. The antibody reacts with the cells of the monocytic lineage, with myeloblasts and promyelocytes and the cells of B lymphocyte lineage. Basophils are found negative. HLA-DR is present on B cells lymphocytes, Haemopoietic precursor cells, activated T-cells, monocytic cells and macrophages.

CD123 clone AC145 monoclonal antibody reacts with human CD123, the α-chain of the IL-3 receptor. This 60-70 kDa transmembrane protein binds to IL-3 with low affinity by itself, and when associated with CD131 (common β chain) binds IL-3 with high affinity. CD123 is expressed by myeloid precursors, macrophages, dendritic cells, mast cells, basophils, and megakaryocytes.

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Crystals may be formed during storage at 2-8° C and should be dissolved at 18-28° C prior to use.
Store in the dark.
Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be

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explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com).

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed.

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS ⚠

a) The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR RESEARCH USE ONLY.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE PREPARATION

- Stimulation buffer: 3 bottle of lyophilized stimulation buffer to be reconstituted in 10 ml of ultrapure, apyrogenic water provides 30 ml ready to use 1X Stimulation buffer.

- Stimulation control: 1 vial containing the chemotactic peptide fMLP and Anti-IgE human lyophilized to be reconstituted in 0,4 ml of ultrapure, apyrogenic water provides 40 test.

1. Mix the anti-coagulated heparinized human whole blood sample by inverting the venepuncture several times.

2. For each patient label de next tubes:
   - Control positive tube
   - Control negative tube
   - A1 for allergen 1. We recommended testing several dilution of each Allergen (e.g. 1:100; 1:1000; 1:10.000; 1:100.000; 1:1.000.000).
   - A2 for allergen 2 ...

3. A range allergen dilution should be prepared in basophil medium. Prepare several tenfold serial dilutions of allergen stimulant between 10 µg/ml and 1 ng/ml in 100 ul of Stimulation buffer. Add 100 ul of Stimulation buffer to Negative control tube and 90 ul of Stimulation buffer + 10 ul of positive control to Positive control tube.

4. Add 100 ul of the heparinized whole blood and mix gently (by vortex).

5. The samples are incubated for 20 minutes at 37° C in a water bath.

6. Add 20 ul of Staining Reagent to each tube. Vortex and incubate for 20 minutes at 2-8° C or in ice.

7. Add Lysing Reagent into the tubes and incubete according to the manufacturer.

8. Centrifuge tubes for 5 minutes at 540x g.

9. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.

10. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.

11. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.

12. Remove the supernatant and resuspend the pellet in 0,3 ml of PBS buffer.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis. If samples are not analyzed immediately, vortex thoroughly just before acquisition.

FLOW CYTOMETRY ANALYSIS

Before acquiring samples, verify the cytometer is correctly aligned and standardized for light scatter (FSC and SSC parameters must be set on linear amplification) and fluorescence intensity (FL1, FL2, FL3 FL4... parameters must be set on logarithmic amplification) and colour compensation has been set following the instructions of the cytometer manufacturer.

Before acquiring samples, set to the minimum the Threshold or Discriminator in parameter FSC to minimize debris and ensure population of interest are included.

Gently mix the samples manually immediately prior to running on the flow cytometer to ensure thorough resuspension of cells and microspheres.

Set on the cytometer to store only the events in the region R1 as imagen below:

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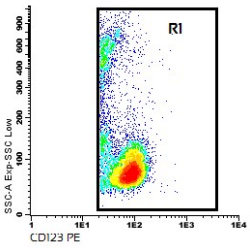
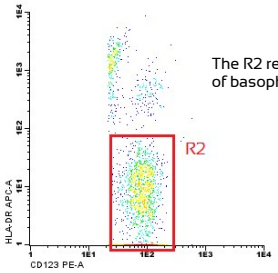


Acquire and store all R1 events possible. It is recommended to acquire at a low or medium speed to avoid cell aggregates.

Analysis example:

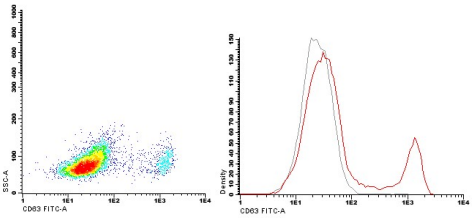
The following histograms correspond to the analysis of a blood sample from a healthy individual using the Basostep:

Step 1: Select the population of R2



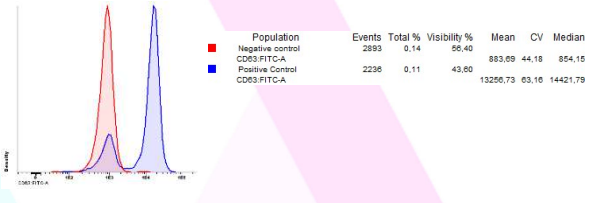
The R2 region contains the population of basophils

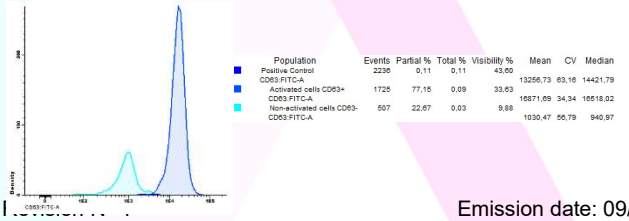
Step 2: Using the fluorescence intensity of CD63 analyze the results.



The histogram is a representations of a lysate normal whole blood sample gated on CD123+/CD203c+/HLA-DR-. Control Negative are represented by the grey histogram and Positive Control by red histogram.

Example of data analysis:





WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

REFERENCES

1. Sainte-Laudy, J, et al. Analysis of membrane expression of the CD63 human basophil activation marker. Applications to allergologic diagnosis. Allerg. Immunol. Paris 26, 211-4 (1994)

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3. Chirumbolo et al. Differential response of human basophil activation markers: a multi-parameter flow cytometry approach. Clinical and Molecular Allergy 2008, 6:12

4. Peter Valent et al. Assay for measuring in vitro basophil activation induced by recombinant allergens. Methods 32 (2004) 265-270

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