

CD203c antibodies

human

CD203c-PE	130-092-343
CD203c-APC	130-092-344
CD203c-Biotin	130-092-345
CD203c pure	130-092-392

Index

1. Description
 - 1.1 Background and product applications
 - 1.2 Examples of staining concentrations
 - 1.3 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD203c antibodies
4. References

1. Description

Clone	FR3-16A11 (isotype: mouse IgG1).
Product format	1 mL CD203c antibodies, human: monoclonal CD203c antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), biotin (Biotin), or as unconjugated antibody (pure). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests (for up to 10 ⁹ total cells).
Storage	Store protected from light at 4–8°C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The CD203c antigen is a glycosylated type II transmembrane molecule (Mw = 270 kDa, unreduced; 130–150 kDa, reduced). The antigen belongs to the family of ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP3) enzymes that catalyze the hydrolysis of oligonucleotides, nucleoside phosphates, and NAD. Among hematopoietic cells, expression of CD203c is restricted to basophils, mast cells, and their precursors, and has been described as a specific marker for this lineage.¹ Protein and/or mRNA expression of CD203c have also been found in solid tissues such as uterus or prostate.² Basophils and mast cells are key producers of mediators that drive the onset of inflammatory responses, e.g. in allergy or upon parasite infections. Allergen challenge leads to a rapid up-regulation of activation markers such as CD203c or CD63.³ Due to its restricted expression pattern CD203c is discussed as a specific marker to monitor the allergen-induced activation of basophils, e.g. in flow cytometric basophil activation tests of the peripheral blood.^{3–5}

Product applications

- Identification and enumeration of human basophils in peripheral blood or bone marrow by flow cytometry or fluorescence microscopy. If preferred, cells may be counterstained with CD123 antibodies and identified as CD203c⁺CD123^{bright} cells.
- Analysis of antigen-induced activation of human basophils.
- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Human basophils can be isolated by using the Basophil Isolation Kit II (# 130-092-662).

1.2 Examples of staining concentrations for human cells.

CD203c conjugate	PE	APC	Biotin
	Recommended antibody dilution		
Flow cytometry^a			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells ^b	1:11	1:11	1:11
Immunohistochemistry^c			
a) Given antibody dilutions are for a cell concentration of up to 1×10 ⁸ cells/mL buffer.			
b) For optimal results, cells have to be stained prior to fixation.			
c) For immunohistochemical staining the optimal antibody dilution has to be tested.			

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).
 - ▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- FcR Blocking Reagent, human (# 130-059-901): Fc receptor-mediated fluorescent staining can be avoided by blocking of Fc receptor using FcR Blocking Reagent, human.
- (Optional) CD123-FITC (# 130-090-897), CD123-PE (# 130-090-899), or CD123-APC (# 130-090-901) for counterstaining.
- (Optional) CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), or CD303 (BDCA-2)-APC (# 130-090-905) for discrimination of plasmacytoid dendritic cells (CD203c⁻CD303⁺CD123^{bright})
- (Optional) Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD203c-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

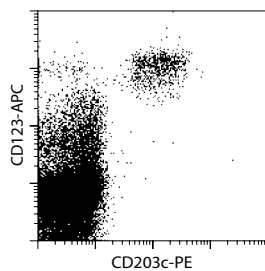
1. Resuspend up to 10⁷ nucleated cells per 80 µL of buffer.
2. Add 20 µL of FcR Blocking Reagent.
3. Add 10 µL of CD203c antibodies.

4. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
6. For fluorescent labeling of CD203c-Biotin resuspend cell pellet in 100 µL buffer, add 10 µL Anti-Biotin fluorochrome, e.g. Anti-Biotin-PE (# 130-090-756), and continue as described in step 4 to 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

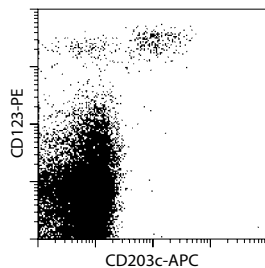
3. Examples of immunofluorescent staining with CD203c antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD203c antibodies conjugated to PE (a), APC (b), or Biotin and Anti-Biotin-PE (c), counterstained with CD123-PE (b) or CD123-APC (a, c) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. CD123^{bright}CD203⁻ cells are plasmacytoid dendritic cells, which can be excluded from the analysis after counterstaining of CD303 (BDCA-2) (data not shown).

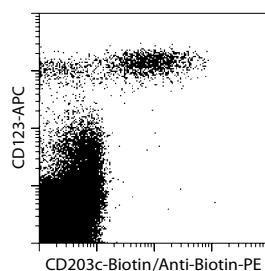
(a) Human PBMCs stained with CD203c-PE and CD123-APC.



(b) Human PBMCs stained with CD203c-APC and CD123-PE.



(c) Human PBMCs stained with CD203c-Biotin, Anti-Biotin-PE and CD123-APC.



4. References

1. Bühring *et al.* (1999) The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 94: 2343–2356.
2. Goding *et al.* (2000) Ecto-enzymes: physiology meets pathology. *J. Leukoc. Biol.* 67: 285–311.
3. Bühring *et al.* (2004) The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol.* 133(4): 317–29.
4. Kahlert *et al.* (2003) Measurement of basophil-activating capacity of grass pollen allergens, allergoids and hypoallergenic recombinant derivatives by flow cytometry using anti-CD203c. *Clin Exp Allergy* 33: 1266–1272.
5. Hauswirth *et al.* (2002) Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol.* 110: 102–109.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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