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1. Description

Components	1 mL monoclonal Anti-FcεRIα antibodies, human conjugated to various dyes.												
	<table border="0"> <tr> <td>VioBlue®</td> <td>130-095-981</td> </tr> <tr> <td>FITC</td> <td>130-095-978</td> </tr> <tr> <td>PE</td> <td>130-095-979</td> </tr> <tr> <td>APC</td> <td>130-095-980</td> </tr> <tr> <td>Biotin</td> <td>130-095-982</td> </tr> <tr> <td>pure – functional grade</td> <td>130-096-004</td> </tr> </table>	VioBlue®	130-095-981	FITC	130-095-978	PE	130-095-979	APC	130-095-980	Biotin	130-095-982	pure – functional grade	130-096-004
VioBlue®	130-095-981												
FITC	130-095-978												
PE	130-095-979												
APC	130-095-980												
Biotin	130-095-982												
pure – functional grade	130-096-004												
Clone	CRA1 (isotype: mouse IgG2b).												
Capacity	100 tests or up to 10 ⁹ total cells. The functional grade antibody is supplied at a concentration of 100 µg/mL.												
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/µg of protein. <i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i>												
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.												

1.1 Background information

Clone CRA1 detects the alpha subunit of the human high affinity Fc receptor for IgE. The receptor is composed of one alpha-, one beta- and two disulphide-linked gamma-chains, of which the alpha subunit binds IgE¹. FcεRIα is expressed on mast and basophil cells, is upregulated in the presence of IgE^{2,3}, and is found on a subset of blood dendritic cells⁴. CRA1 does not inhibit the binding of IgE⁵, it induces mast cell activation followed by a histamine release⁶. The FcεRI complex plays an important role in triggering IgE-mediated allergic reactions.

1.2 Applications

- Identification and enumeration of FcεRIα⁺ cells by flow cytometry or fluorescence microscopy.
- The Anti-FcεRIα pure – functional grade antibody is suited for functional assays.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-FcεRIα conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °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 bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., APC (# 130-090-856) as secondary antibody reagent in combination with Anti-FcεRIα-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD123-FITC (# 130-090-897), CD123-PE (# 130-090-899), or CD123-APC (# 130-090-901). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG2b isotype control antibodies conjugated to, e.g., PE (# 130-092-215). For more information about isotype control antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

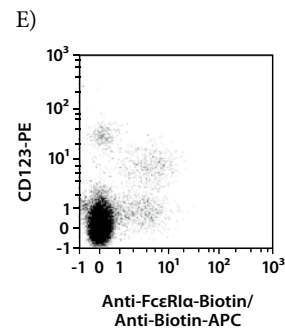
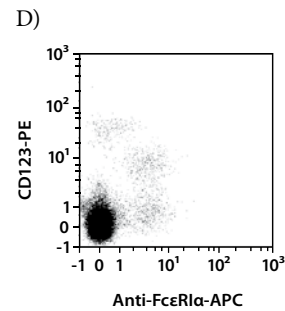
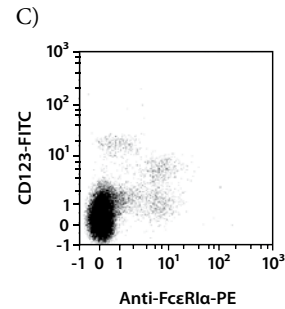
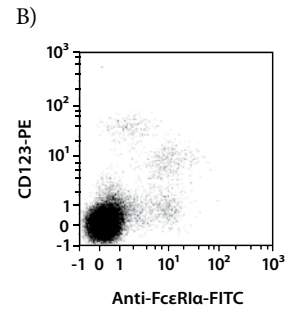
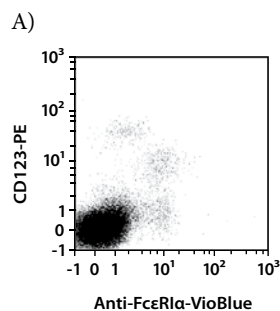
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 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 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-Fc ϵ RI α antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Fc ϵ RI α -Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-Fc ϵ RI α antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-Fc ϵ RI α antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) as well as with CD123-PE (# 130-090-899) or CD123-APC (# 130-090-901) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-Fc ϵ RI α -Biotin (E) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD123-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Hasegawa, S. *et al.* (1999) Functional expression of the high affinity receptor for IgE (Fc ϵ RI) in human platelets and its intracellular expression in human megakaryocytes. *Blood* 93: 2543–2551.
2. Suzukawa, M. *et al.* (2005) IgE- and Fc ϵ RI-mediated migration of human basophils. *Int. Immunol.* 17: 1249–1255.
3. Hakimi, J. *et al.* (1990) The alpha subunit of the human IgE receptor (Fc ϵ RI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* 265: 22079–22081.
4. Hammad, H. *et al.* (2010) Inflammatory dendritic cells—not basophils—are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J. Exp. Med.* 207: 2097–2111.

5. Takai, T. *et al.* (2000) Epitope analysis and primary structures of variable regions of anti-human FcepsilonRI monoclonal antibodies, and expression of the chimeric antibodies fused with human constant regions. *Biosci. Biotechnol. Biochem.* 64: 1856–1867.
6. Yokoi, H. *et al.* (2008) Inhibition of FcepsilonRI-dependent mediator release and calcium flux from human mast cells by sialic acid-binding immunoglobulin-like lectin 8 engagement. *J. Allergy Clin. Immunol.* 121: 499–505.e1.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

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