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1. Description

Components	1 mL CD23 antibodies, human: monoclonal CD23 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	M-L23.4 (isotype: mouse IgG1).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
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

CD23, also known as FcεRII, IgE Fc receptor, or low-affinity IgE receptor, is a 45 kDa type-II transmembrane glycoprotein that belongs to the Ig family. It is expressed at most mature B cells, monocytes, follicular dendritic cells, and on low levels on T cells, Langerhans cells, eosinophils, and platelets. CD23 is thought to play a role in the regulation of B cell activation and IgE response. It is up-regulated upon B cell activation. The proteolytic cleavage of membrane CD23 can result in the release of soluble forms of the antigen (sCD23) with a reported biological activity.

1.2 Applications

- Identification and enumeration of CD23⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human B cells can be isolated by using, for example, CD19 MicroBeads, human (# 130-050-301), the IgM⁺ Memory B Cell Isolation Kit (# 130-093-619), or the B Cell Isolation Kit II (# 130-091-151).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD23 conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 μL of buffer.
b) For optimal results, cells must be stained prior to fixation.

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. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD23-Biotin.
- (Optional) CD19-FITC (# 130-091-328) or CD19-PE (# 130-091-247). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (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 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).

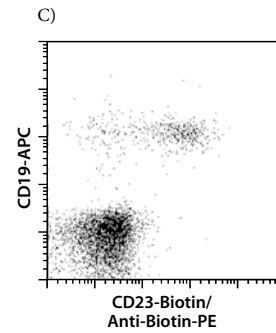
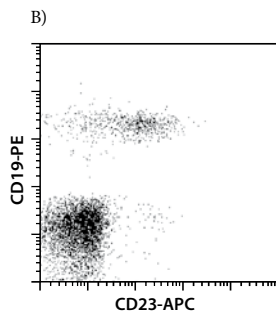
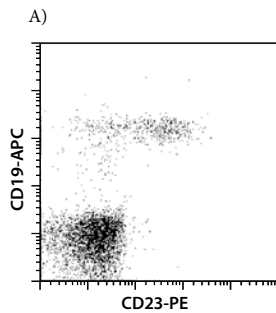
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 μL of buffer.

4. Add 10 μ L of the CD23 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
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 CD23-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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 CD23 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD23 antibodies conjugated to PE (A) or APC (B), as well as with CD19-APC (# 130-091-248) and CD19-PE (# 130-091-247) and analyzed by flow cytometry. Cells labeled with CD23-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD19-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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.

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