



Anti-IgM antibodies human

Anti-IgM-PE	130-093-075
Anti-IgM-APC	130-093-076
Anti-IgM-Biotin	130-093-077
Anti-IgM pure	130-093-078

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1. Description

Components	1 mL Anti-IgM antibodies, human: monoclonal Anti-IgM antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	PJ2-22H3 (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

The Anti-IgM antibody clone PJ2-22H3 reacts with soluble as well as membrane-bound human IgM. It does not react with other human Ig isotypes. IgM is the first immunoglobulin made by B cells in the immune response. Surface IgM is expressed on immature and mature B cells, while IgM heavy chain is expressed intracellularly in pre-B cells. The secreted form of IgM forms a pentamer. IgM serves as the naive B cell antigen receptor and is involved in B cell maturation and complement activation.

1.2 Applications

- Identification and enumeration of IgM⁺ cells by flow cytometry or fluorescence microscopy.
- Intracellular staining of IgM-producing cells.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human IgM⁺ B cells can be isolated by using, for example, the Anti-IgM MicroBeads, human (# 130-093-230).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgM conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:22	1:11	1:11
- Anti-IgM			
MicroBead-labeled cells	1:11	1:11	1:11

- a) Given antibody dilutions are for a cell concentration of 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-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-IgM-Biotin.
- (Optional) CD19-FITC (# 130-091-328) or CD19-PE (# 130-091-247).
- (Optional) Propidium iodide (PI) 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 µL of the Anti-IgM antibody.

▲ **Note:** See table for exceptions.

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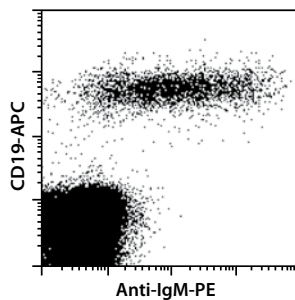


5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-IgM-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (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.

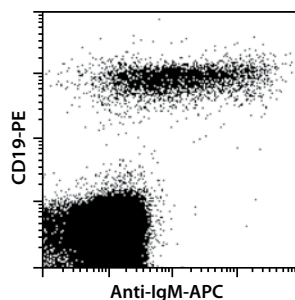
3. Examples of immunofluorescent staining with Anti-IgM antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgM antibodies conjugated to PE (a), or APC (b), as well as with CD19-APC or CD19-PE, and analyzed by flow cytometry. Cells stained with Anti-IgM-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 PI fluorescence.

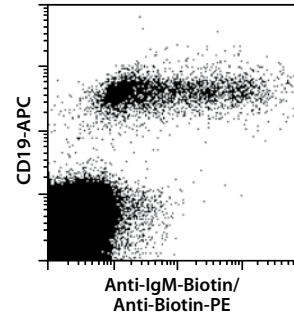
(a) Human PBMCs stained with Anti-IgM-PE and CD19-APC.



(b) Human PBMCs stained with Anti-IgM-APC and CD19-PE.



(c) Human PBMCs stained with Anti-IgM-Biotin, Anti-Biotin-PE, and CD19-APC.



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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