



Anti-IgA antibodies human

| | |
|-----------------|-------------|
| Anti-IgA-FITC | 130-093-071 |
| Anti-IgA-PE | 130-093-128 |
| Anti-IgA-APC | 130-093-113 |
| Anti-IgA-Biotin | 130-093-114 |
| Anti-IgA pure | 130-093-073 |

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1. Description

| | |
|-----------------------|--|
| Components | 1 mL Anti-IgA antibodies, human: monoclonal Anti-IgA antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. |
| Clone | IS11-8E10 (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

Anti-IgA antibodies react with the IgA isotype of human immunoglobulins. There are two forms of IgA, the serum IgA, which is mainly a monomer, and the secreted IgA, consisting of 2–4 IgA molecules that are connected via the J chain and the so-called secretory component. Secreted IgA is transported across the epithelial cells and secreted into the lumens of the respiratory and gastrointestinal tracts.

1.2 Applications

- Identification and enumeration of IgA⁺ cells by flow cytometry or fluorescence microscopy.
- Intracellular staining of IgA-producing cells.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

| Anti-IgA conjugate | FITC | PE | APC | Biotin |
|-----------------------------------|------|------|------|--------|
| Flow cytometry^a | | | | |
| - In general | 1:11 | 1:11 | 1:11 | 1:11 |
| - Formaldehyde-fixed cells | 1:11 | 1:11 | 1:11 | 1:11 |

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.

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 Anti-IgA-Biotin.
- (Optional) CD19-PE (# 130-091-247), CD19-APC (# 130-091-248), or CD19-FITC (# 130-091-328).
- (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-IgA antibody.

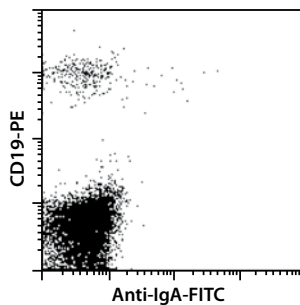


5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge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

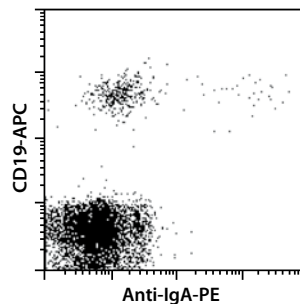
3. Examples of immunofluorescent staining with Anti-IgA antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgA antibodies conjugated to FITC (a), PE (b), or APC (c), as well as with CD19-PE or CD19-APC, and analyzed by flow cytometry. Cells stained with Anti-IgA-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856) as well as with CD19-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

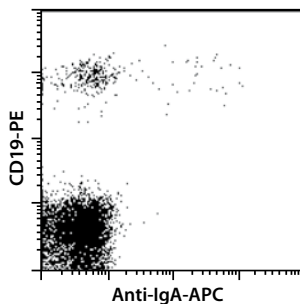
(a) Human PBMCs stained with Anti-IgA-FITC and CD19-PE.



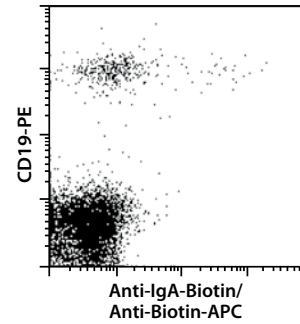
(b) Human PBMCs stained with Anti-IgA-PE and CD19-APC.



(c) Human PBMCs stained with Anti-IgA-APC and CD19-PE.



(d) Human PBMCs stained with Anti-IgA-Biotin, Anti-Biotin-APC, and CD19-PE.



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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