Anti-IgA2 antibodies
human

Anti-IgA2-FITC	130-093-069
Anti-IgA2-PE	130-093-070
Anti-IgA2-APC	130-093-079
Anti-IgA2-Biotin	130-093-080
Anti-IgA2 pure	130-093-081

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1. Description

Components	1 mL Anti-IgA2 antibodies, human: monoclonal Anti-IgA2 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	IS11-21E11 (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-IgA2 antibodies react with the IgA2 subtype of human IgA immunoglobulins. IgA is produced as serum IgA, which is mainly a monomer, and as secreted IgA, consisting of 2–4 IgA molecules connected via the J chain and the so-called secretory component. IgA forms two subclasses: IgA1 and IgA2. IgA1 is produced by bone marrow B cells and mostly found in serum. IgA2 is produced by B cells located in the mucosae and is the most abundant immunoglobulin in mucosal secretions. The main structural difference between IgA1 and IgA2 is the extended, highly glycosylated hinge region of IgA1 that is lacking in IgA2.

1.2 Applications

- Identification and enumeration of $IgA2^+$ cells by flow cytometry or fluorescence microscopy.
- Intracellular staining of IgA2-producing cells.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgA2 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 10^7 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 Ca^{2+} or Mg^{2+} 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-IgA2-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 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×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-IgA2 antibody.

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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 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

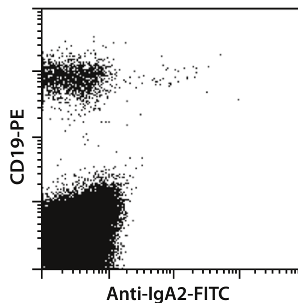
7. (Optional) If Anti-IgA2-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

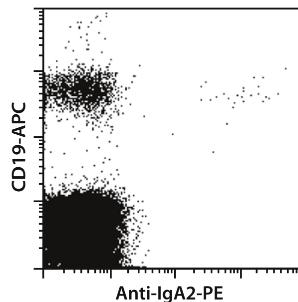
3. Examples of immunofluorescent staining with Anti-IgA2 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgA2 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-IgA2-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.

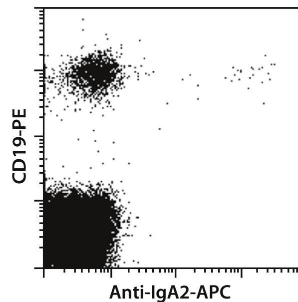
(a) Human PBMCs stained with Anti-IgA2-FITC and CD19-PE.



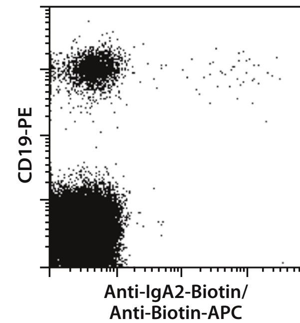
(b) Human PBMCs stained with Anti-IgA2-PE and CD19-APC.



(c) Human PBMCs stained with Anti-IgA2-APC and CD19-PE.



(d) Human PBMCs stained with Anti-IgA2-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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