



# Anti-IgG1 antibodies human

Anti-IgG1-PE	130-093-188
Anti-IgG1-APC	130-093-189
Anti-IgG1-Biotin	130-093-190

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## 1. Description

<b>Components</b>	1 mL Anti-IgG1 antibodies, human: monoclonal Anti-IgG1 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
<b>Clone</b>	IS11-12E4.23.20 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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-IgG1 antibodies react with the IgG1 isotype of human immunoglobulins. The clone IS11-12E4.23.20 recognizes the Fc region of IgG1. In humans, IgG1 is the most abundant IgG subclass in serum. One function of IgG1 is, for example, the binding of antigens which facilitates phagocytosis.

### 1.2 Applications

- Identification and enumeration of IgG1<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgG1 conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	n. r.	n. r.	n. r.

a) Given antibody dilutions are for a cell concentration of up to 10<sup>7</sup> cells/100 µL of buffer.  
n. r.: not recommended

## 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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>™</sup> 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<sup>2+</sup> or Mg<sup>2+</sup> 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-IgG1-Biotin.
- (Optional) CD19-APC (# 130-091-248) or CD19-PE (# 130-091-247).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> 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<sup>7</sup> nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-IgG1 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 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-IgG1-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.

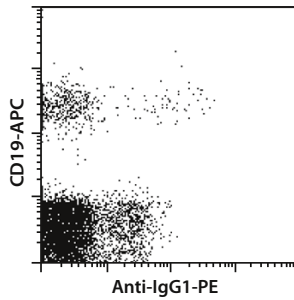
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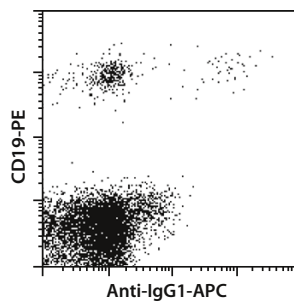
### 3. Examples of immunofluorescent staining with Anti-IgG1 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgG1 antibodies conjugated to PE (a) or APC (b), as well as with CD19-APC and CD19-PE, respectively, and analyzed by flow cytometry. Cells stained with Anti-IgG1-Biotin (c) 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.

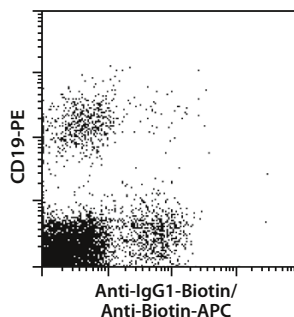
(a) Human PBMCs stained with Anti-IgG1-PE and CD19-APC.



(b) Human PBMCs stained with Anti-IgG1-APC and CD19-PE.



(c) Human PBMCs stained with Anti-IgG1-Biotin, Anti-Biotin-APC, and CD19-PE.



All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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