



Anti-IgG antibodies human

Anti-IgG-FITC	130-093-192
Anti-IgG-PE	130-093-193
Anti-IgG-APC	130-093-194
Anti-IgG-Biotin	130-093-195
Anti-IgG pure	130-093-197

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1. Description

Components	1 mL Anti-IgG antibodies, human: monoclonal Anti-IgG 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	IS11-3B2.2.3 (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-IgG antibodies react with the IgG isotype of human immunoglobulins. The clone IS11-3B2.2.3 recognizes the Fab region of IgG and reacts with all subclasses of IgG. IgG is the most abundant and the longest lived immunoglobulin in humans. The monomeric 150 kDa molecule is produced by B cells and is expressed in secreted and membrane-associated forms. Functions of IgGs are, for example, promotion of the phagocytosis of antibody-coated particles, feedback inhibition of B cell activation, complement activation, or antibody-dependent cell-mediated cytotoxicity (ADCC).

1.2 Applications

- Identification and enumeration of IgG⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human IgG⁺ memory B cells can be isolated by using, for example, the CD19 MultiSort Kit, human (# 130-055-301) and Mouse Anti-Human IgG MicroBeads (# 130-047-501). A special protocol is available at www.miltenyibiotec.com/protocols.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-IgG 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
- Anti-IgG				
MicroBead-labeled cells	n. r.	1:11	1:11	n. r.

a) Given antibody dilutions are for a cell concentration of up to 10⁷ 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 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-IgG-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.
- (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-IgG antibody.

140-009-10201

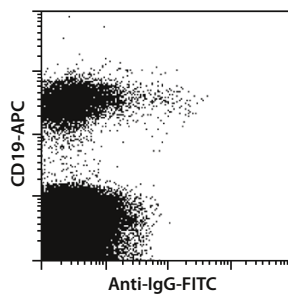


5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge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

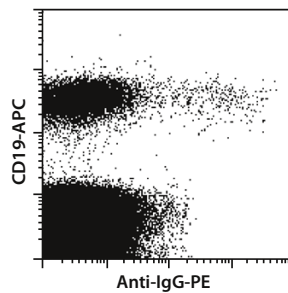
3. Examples of immunofluorescent staining with Anti-IgG antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-IgG antibodies conjugated to FITC (a), PE (b), or APC (c), as well as with CD19-PE and CD19-APC, respectively, and analyzed by flow cytometry. Cells stained with Anti-IgG-Biotin (d) were stained with Anti-Biotin-PE (# 130-090-756) as well as with CD19-APC. A lymphocyte gate was activated based on the scatter properties of the cells. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

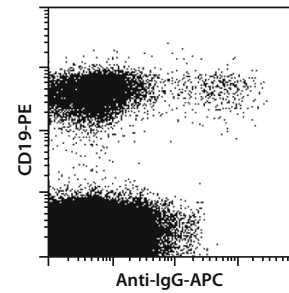
(a) Human PBMCs stained with Anti-IgG-FITC and CD19-APC.



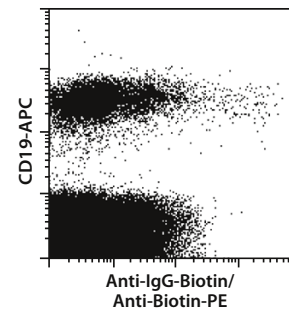
(b) Human PBMCs stained with Anti-IgG-PE and CD19-APC.



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



(d) Human PBMCs stained with Anti-IgG-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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