

# Mouse IgG1 isotype control antibodies

Mouse IgG1-FITC	130-092-213
Mouse IgG1-PE	130-092-212
Mouse IgG1-APC	130-092-214
Mouse IgG1-Biotin	130-093-018
Mouse IgG1-VioBlue®	130-094-670
Mouse IgG1-PerCP	130-094-968

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## 1. Description

<b>Components</b>	1 mL Mouse IgG1 antibodies: monoclonal Mouse IgG1 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), biotin, VioBlue®, or peridinium chlorophyll protein (PerCP). The antibody concentrations are 55 µg/mL (FITC-conjugate), 22 µg/mL (PE-conjugate), 22 µg/mL (APC-conjugate), 11 µg/mL (Biotin-conjugate), 55 µg/mL (VioBlue-conjugate), or 55 µg/mL (PerCP-conjugate).
<b>Clone</b>	IS5-21F5 (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

The Mouse IgG1 isotype control antibody clone IS5-21F5 is specific for KLH (keyhole limpet hemocyanin). This protein is not expressed on human cells or cell lines. Therefore, the antibody clone IS5-21F5 can be used as a negative control, to distinguish specific from non-specific binding of mouse IgG1 fluorochrome-conjugated antibodies to human cells, e.g. via Fc receptors, or due to interactions of the fluorochrome with the cell surface.

### 1.2 Applications

- Mouse IgG1 isotype control antibodies are suitable for assessing the level of background staining for flow cytometric cell analysis of human cells.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

Mouse IgG1 conjugate	FITC	PE	APC	Biotin	VioBlue	PerCP
<b>Flow cytometry<sup>a</sup></b>						
- In general	1:11	1:11	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10<sup>7</sup> 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<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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

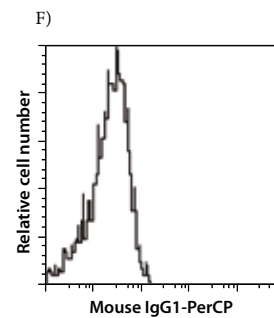
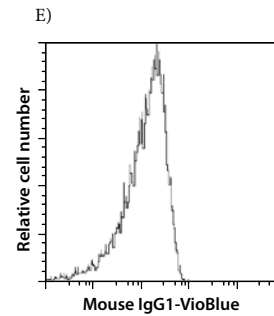
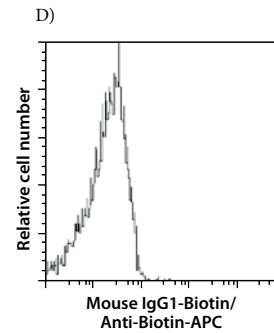
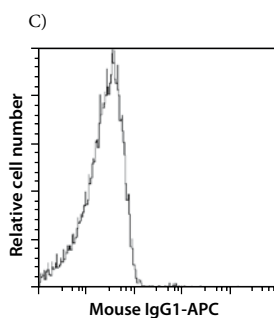
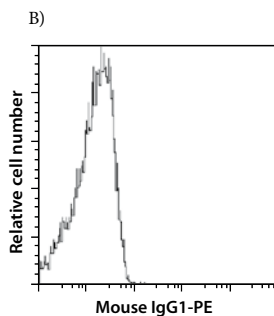
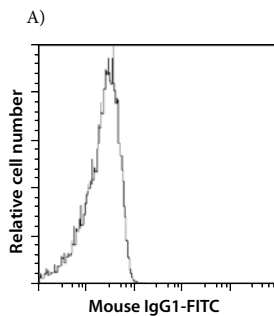
▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use twice 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 Mouse 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 and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. (Optional) If Mouse IgG1-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L 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 Mouse IgG1 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Mouse IgG1 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (E), or PerCP (F) and analyzed by flow cytometry. Cells stained with Mouse IgG1-Biotin (D) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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.

#### Warranty

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