Miltenyi Biotec

Mouse IgG1
isotype control antibodies

Contents

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.

1. Description

This product is for research use only.

Components Monoclonal Mouse IgG1 antibodies, conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-092-213	130-098-847
VioBright™ FITC	130-104-513	130-104-562
PE	130-092-212	130-098-845
APC	130-092-214	130-098-846
VioBlue®	130-094-670	130-099-756
VioGreen™	130-096-919	–
PerCP	130-094-968	–
PE-Vio® 615	130-107-452	130-107-506
PE-Vio770	130-096-654	130-098-563
APC-Vio770	130-096-653	130-100-098
PerCP-Vio700	130-097-561	–
Biotin	130-093-018	–

Clone IS5-21F5 (isotype: mouse IgG1).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 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

- Expression patterns: 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, for example 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

The recommended antibody dilution for all Mouse IgG1 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Mouse IgG1-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

140-001-521.08

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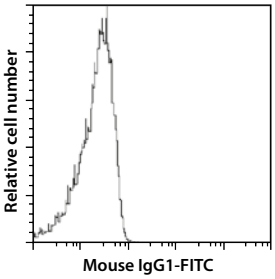
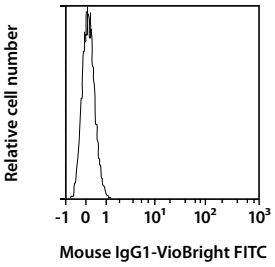
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Mouse IgG1 isotype control antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

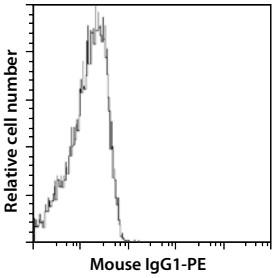
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Mouse Ig-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, 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.

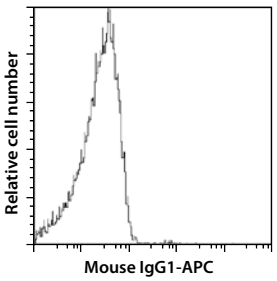
3. Examples of immunofluorescent staining with Mouse IgG1 isotype control antibodies

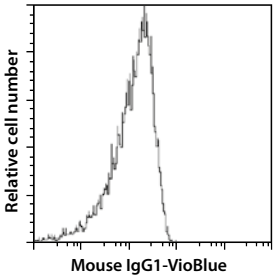
Human peripheral blood lymphocytes were stained with Mouse IgG1 isotype control antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Cells are gated on viable lymphocytes.

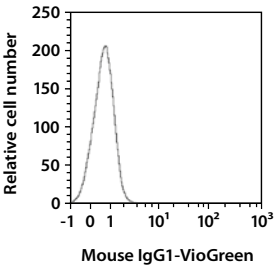


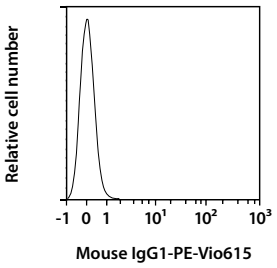


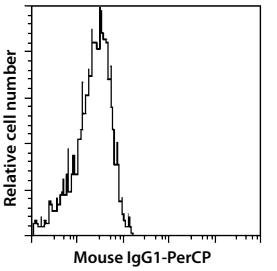


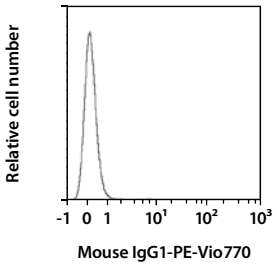




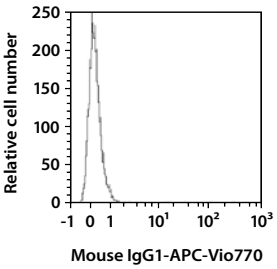




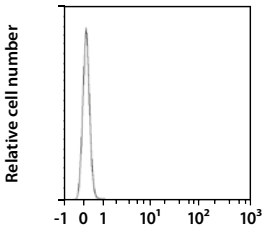




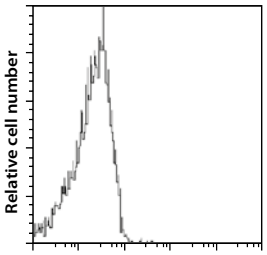
Mouse IgG1-PE-Vio770



Mouse IgG1-APC-Vio770



Mouse IgG1-PerCP-Vio700



Mouse IgG1-Biotin/
Anti-Biotin-APC

All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warranty

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