Mouse IgG1 – isotype control antibodies

For research use only

One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
Mouse IgG1-PerCP-Vio700	for 100 tests	130-113-204
Mouse IgG1-FITC	for 30 tests	130-113-761
Mouse IgG1-FITC	for 100 tests	130-113-199
Mouse IgG1-VioBright FITC	for 30 tests	130-113-768
Mouse IgG1-VioBright FITC	for 100 tests	130-113-206
Mouse IgG1-PE	for 30 tests	130-113-762
Mouse IgG1-PE	for 100 tests	130-113-200
Mouse IgG1-APC	for 30 tests	130-113-758
Mouse IgG1-APC	for 100 tests	130-113-196
Mouse IgG1-VioBlue	for 30 tests	130-113-767
Mouse IgG1-VioBlue	for 100 tests	130-113-205
Mouse IgG1-VioGreen	for 30 tests	130-113-769
Mouse IgG1-VioGreen	for 100 tests	130-113-207
Mouse IgG1-PerCP	for 30 tests	130-113-765
Mouse IgG1-PerCP	for 100 tests	130-113-203
Mouse IgG1-PE-Vio615	for 30 tests	130-113-763
Mouse IgG1-PE-Vio615	for 100 tests	130-113-201
Mouse IgG1-PE-Vio770	for 30 tests	130-113-764
Mouse IgG1-PE-Vio770	for 100 tests	130-113-202
Mouse IgG1-APC-Vio770	for 30 tests	130-113-759
Mouse IgG1-APC-Vio770	for 100 tests	130-113-197
Mouse IgG1-PerCP-Vio700	for 30 tests	130-113-766
Mouse IgG1-VioBright 515	for 30 tests	130-113-770
Mouse IgG1-VioBright 515	for 100 tests	130-113-208
Mouse IgG1-Biotin	for 30 tests	130-113-760
Mouse IgG1-Biotin	for 100 tests	130-113-198

Technical data and background information

Antigen	KLH
Clone	IS5-21F5
Isotype	mouse IgG1κ
Alternative names of antigen	keyhole limpet hemocyanin

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

The Mouse IgG1 isotype control antibody clone IS5-21F5 is specific for keyhole limpet hemocyanin (KLH). 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.

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) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (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.

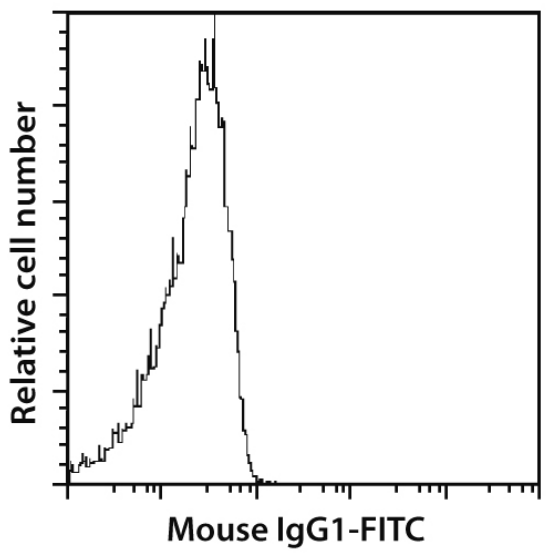
Protocol for cell surface staining

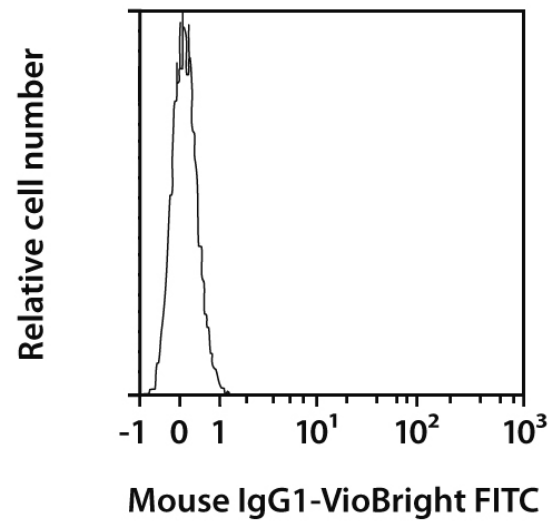
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the 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 biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Human peripheral blood lymphocytes (PBLs) 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. Gates were set on viable lymphocytes.


Relative cell number
Mouse IgG1-FITC


Relative cell number
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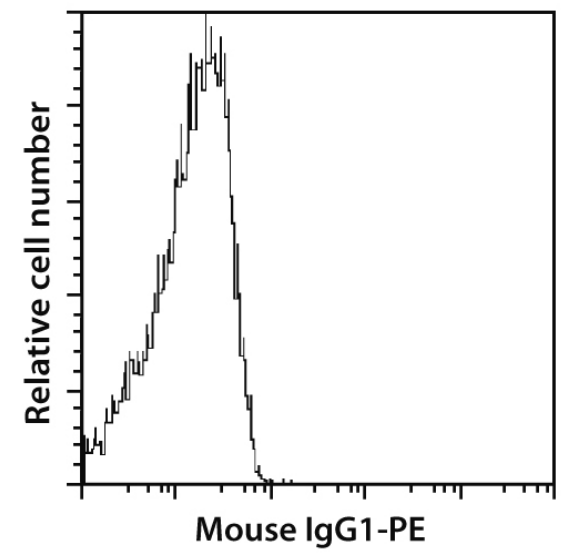
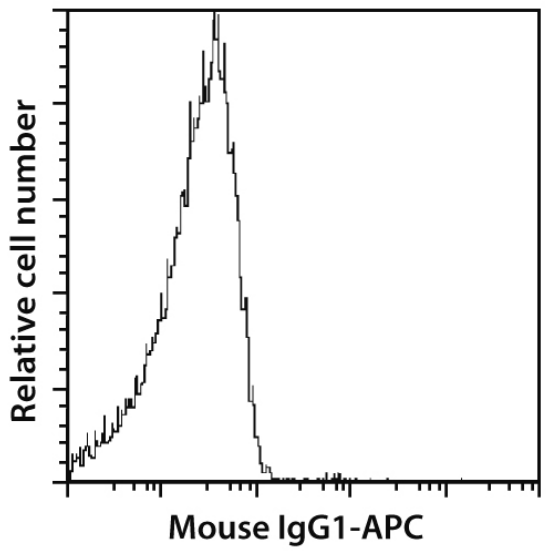
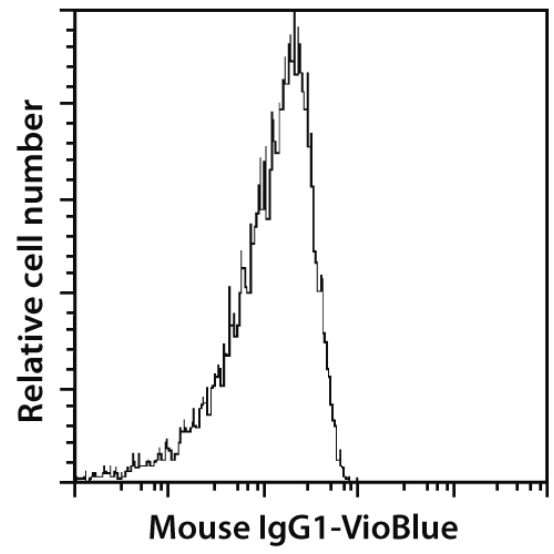
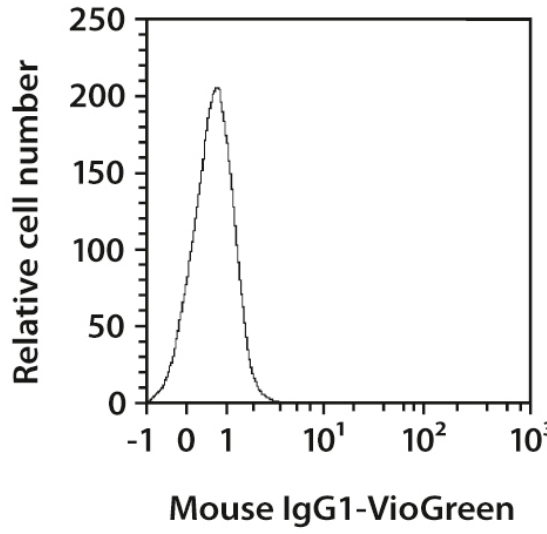
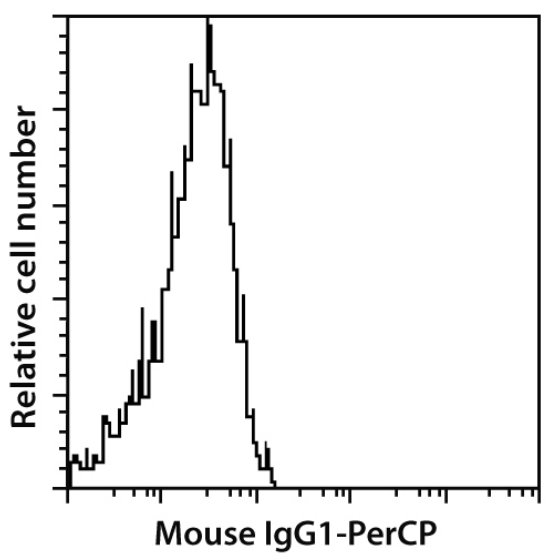
Mouse IgG1-VioBright FITC

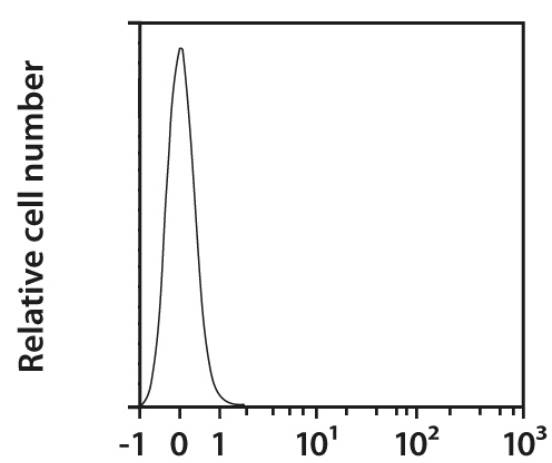

Relative cell number
Mouse IgG1-PE


Relative cell number
Mouse IgG1-APC


Relative cell number
Mouse IgG1-VioBlue


Relative cell number
250
200
150
100
50
0
-1 0 1 10¹ 10² 10³
Mouse IgG1-VioGreen


Relative cell number
Mouse IgG1-PerCP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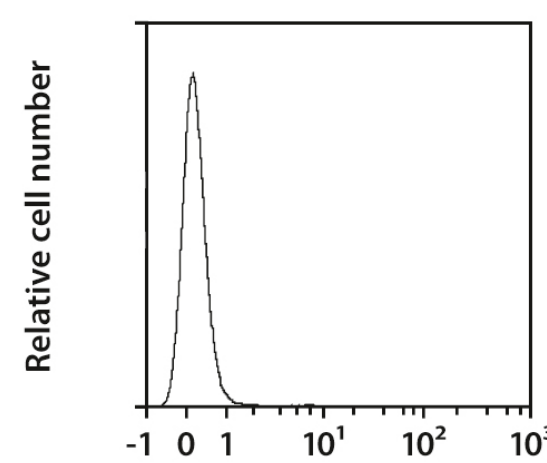
Mouse IgG1-PE-Vio615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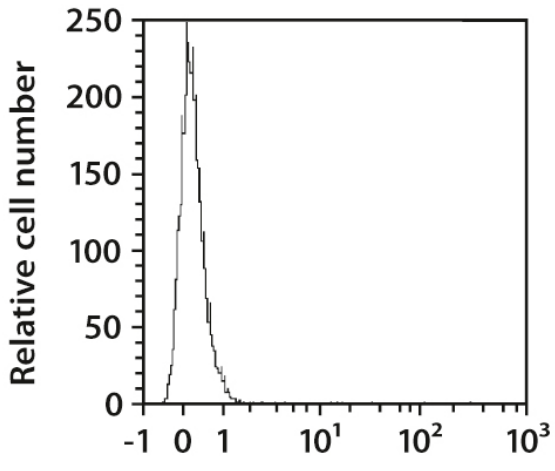
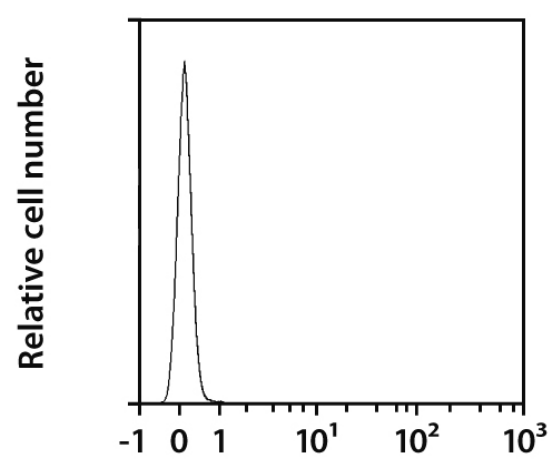
Mouse IgG1-PE-Vio770


Relative cell number
250
200
150
100
50
0
-1 0 1 10¹ 10² 10³
Mouse IgG1-APC-Vio770


Relative cell number
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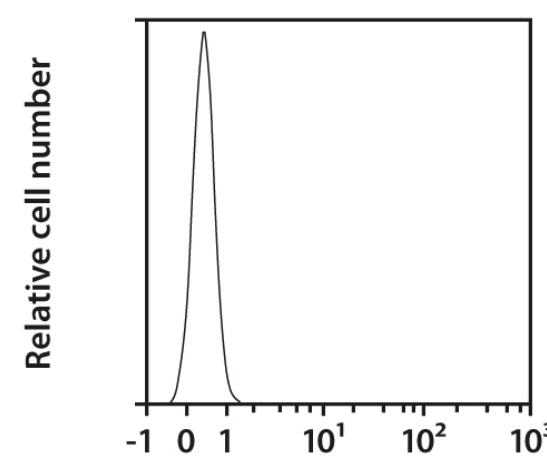
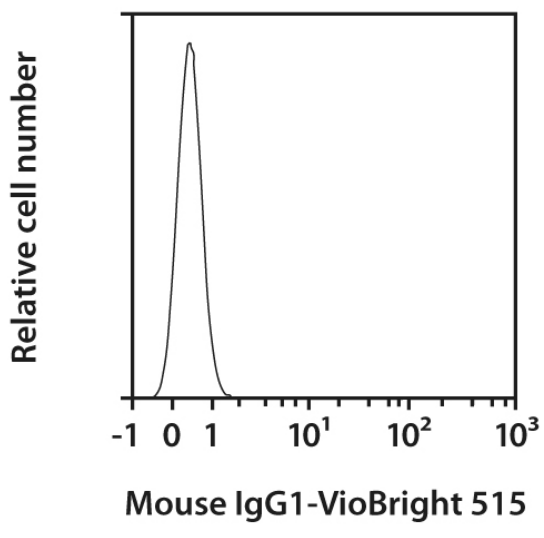
Mouse IgG1-PerCP-Vio700


Relative cell number
-1 0 1 10¹ 10² 10³
Mouse IgG1-Vio515


Relative cell number
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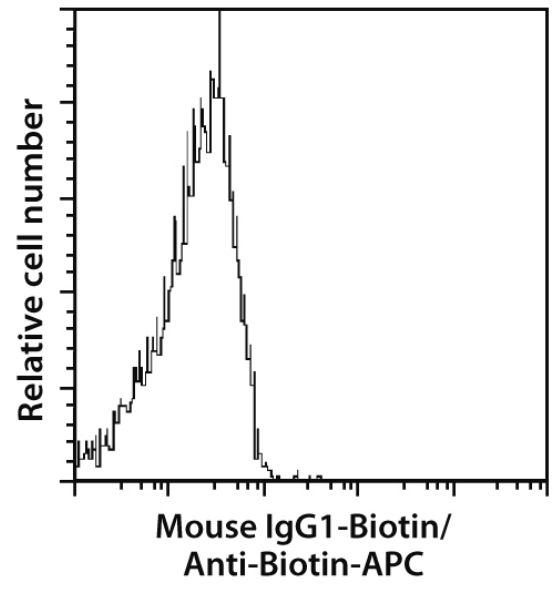
Mouse IgG1-VioBright 515


Relative cell number
Mouse IgG1-Biotin/
Anti-Biotin-APC

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