Mouse IgG2a – isotype control antibodies

For research use only

Product	Content	Order no.
Mouse IgG2a-APC	for 30 tests	130-113-831
Mouse IgG2a-FITC	for 30 tests	130-113-833
Mouse IgG2a-FITC	for 100 tests	130-113-271
Mouse IgG2a-VioBright FITC	for 30 tests	130-113-840
Mouse IgG2a-VioBright FITC	for 100 tests	130-113-278
Mouse IgG2a-PE	for 30 tests	130-113-834
Mouse IgG2a-PE	for 100 tests	130-113-272
Mouse IgG2a-APC	for 100 tests	130-113-269
Mouse IgG2a-VioBlue	for 30 tests	130-113-839
Mouse IgG2a-VioBlue	for 100 tests	130-113-277
Mouse IgG2a-VioGreen	for 30 tests	130-113-841
Mouse IgG2a-VioGreen	for 100 tests	130-113-279
Mouse IgG2a-PerCP	for 30 tests	130-113-837
Mouse IgG2a-PerCP	for 100 tests	130-113-275
Mouse IgG2a-PE-Vio770	for 30 tests	130-113-836
Mouse IgG2a-PE-Vio770	for 100 tests	130-113-274
Mouse IgG2a-APC-Vio770	for 30 tests	130-113-832
Mouse IgG2a-APC-Vio770	for 100 tests	130-113-270
Mouse IgG2a-PerCP-Vio700	for 30 tests	130-113-838
Mouse IgG2a-PerCP-Vio700	for 100 tests	130-113-276

Technical data and background information

Antigen	Hapten NP
Clone	S43.10
Isotype	mouse IgG2a
Alternative names of antigen	Hapten NP (4-hydroxy-3-nitro-phenyl) acetyl
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

The Mouse IgG2a isotype control antibody clone S43.10 is specific for the hapten NP (4-hydroxy-3-nitro-phenyl) acetyl. This hapten is not expressed on cells or cell lines. Therefore, the antibody clone S43.10 can be used as a negative control to distinguish specific from non-specific binding of mouse IgG2a 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.

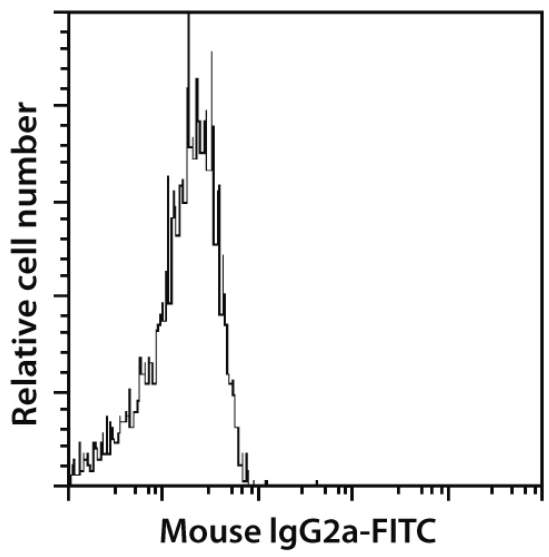
Protocol for cell surface staining

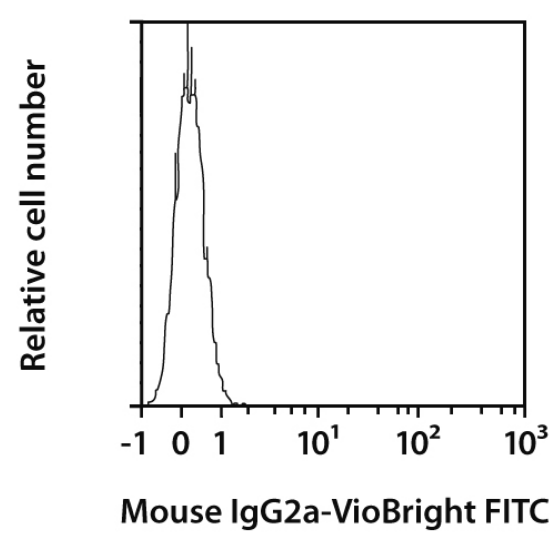
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

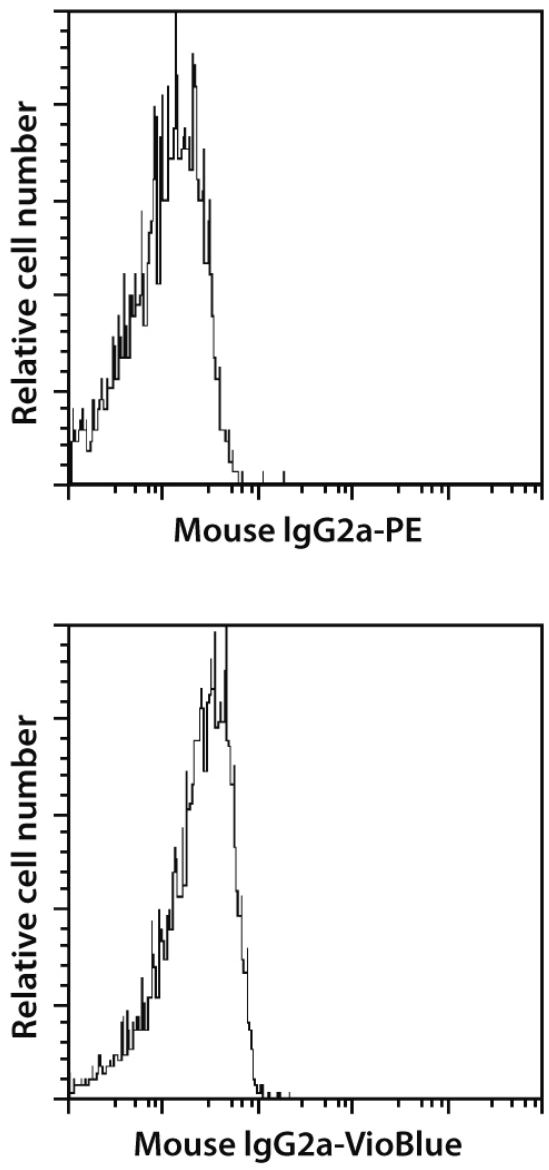
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 were stained with Mouse IgG2a antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide solution. Cells were gated on viable lymphocytes.






Relative cell number
Mouse IgG2a-PE
Mouse IgG2a-APC
Mouse IgG2a-VioBlue
Mouse IgG2a-VioGreen
Mouse IgG2a-PerCP
Mouse IgG2a-PE-Vio615
Mouse IgG2a-PE-Vio770
Mouse IgG2a-APC-Vio770
Mouse IgG2a-PerCP-Vio700
-1 0 1 10¹ 10² 10³

Warranty

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