Miltenyi Biotec

Mouse IgG2a
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 IgG2a antibodies, conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-091-837	130-098-877
VioBright™ FITC	130-104-512	130-104-561
PE	130-091-835	130-098-849
APC	130-091-836	130-098-850
VioBlue®	130-094-671	130-098-898
VioGreen™	130-096-923	130-099-817
PerCP	130-094-967	130-099-190
PE-Vio® 615	130-107-465	130-107-518
PE-Vio770	130-096-638	130-098-564
APC-Vio770	130-096-637	130-099-758
PerCP-Vio700	130-097-563	–

Clone S43.10 (isotype: mouse IgG2a).

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 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.

1.2 Applications

- Mouse IgG2a isotype control antibodies are suitable for assessing the level of background staining for flow cytometric cell analysis of cells.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Mouse IgG2a conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and 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) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/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.

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.

140-001-948.11

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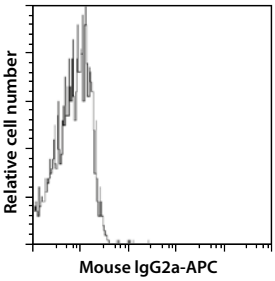
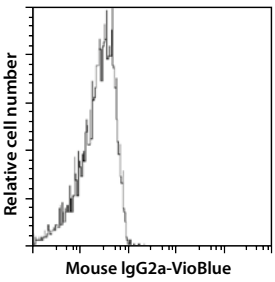
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Mouse IgG2a isotype control antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

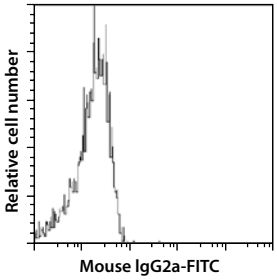
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

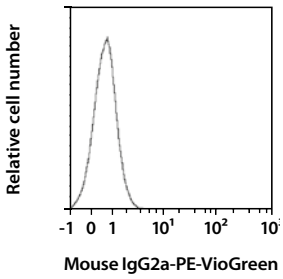
3. Examples of immunofluorescent staining with Mouse IgG2a isotype control antibodies

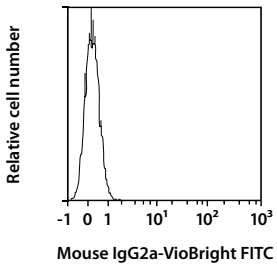
Human peripheral blood lymphocytes were stained with Mouse IgG2a isotype control antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Cells were gated on viable lymphocytes.

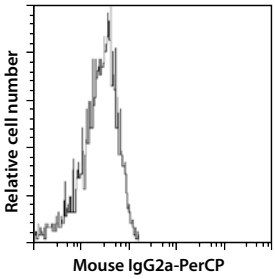


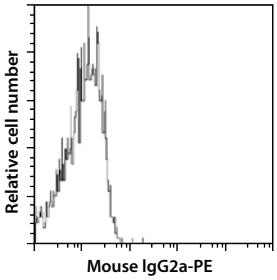


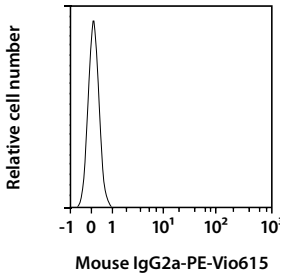


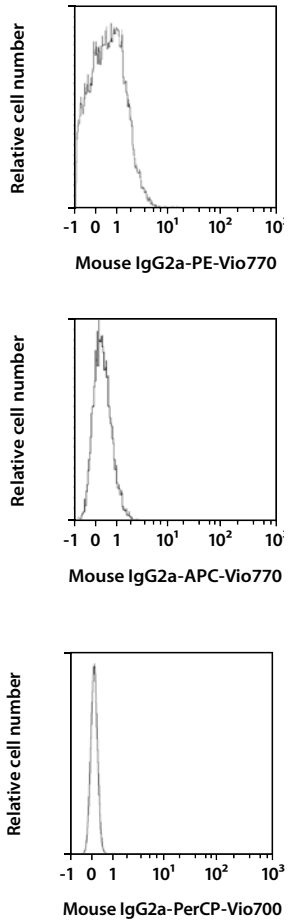












All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warranty

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