Miltenyi Biotec

Mouse IgG2b
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 IgG2b antibodies, conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-092-216	130-099-119
VioBright™ FITC	130-104-575	130-104-649
PE	130-092-215	130-098-875
APC	130-092-217	130-098-890
VioBlue®	130-098-591	130-098-601
VioGreen™	130-096-935	–
PerCP	130-098-594	130-098-604
PE-Vio770™	130-096-825	130-098-562
APC-Vio770™	130-096-822	130-100-408
PerCP-Vio700™	130-097-567	–
Biotin	130-092-466	130-099-759

Clone IS6-11E5.11 (isotype: mouse IgG2b).

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 IgG2b isotype control antibody clone IS6-11E5.11 is specific for KLH (keyhole limpet hemocyanin). This protein is not expressed on human cells or cell lines. Therefore, the antibody clone IS6-11E5.11 can be used as a negative control, to distinguish specific from non-specific binding of mouse IgG2b 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 IgG2b 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 IgG2b conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

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) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Mouse IgG2b-Biotin.
- (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.

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2. General protocol for immunofluorescent staining

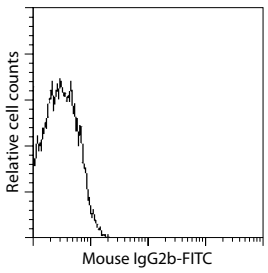
▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Mouse IgG2b 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 IgG2b-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 IgG2b antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Mouse IgG2b antibodies conjugated to FITC (A), VioBright FITC (B), PE (C), APC (D), VioBlue (E), VioGreen (F), PerCP (G), PE-Vio770 (H), APC-Vio770 (I), or PerCP-Vio700 (J) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Mouse IgG2b-Biotin (K) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)



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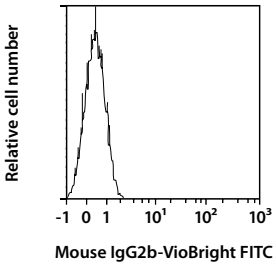

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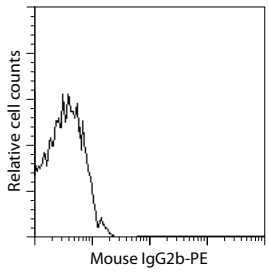

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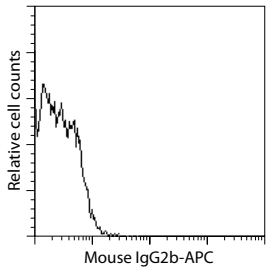

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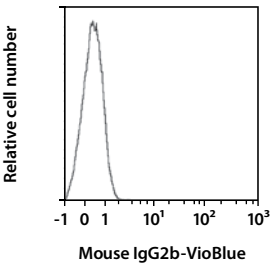

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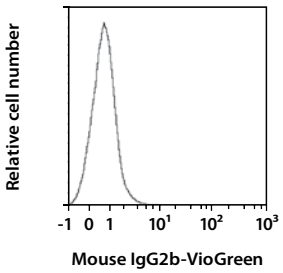

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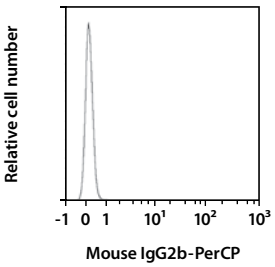
G)



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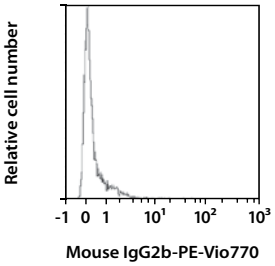

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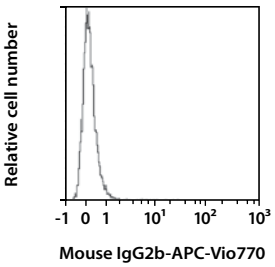

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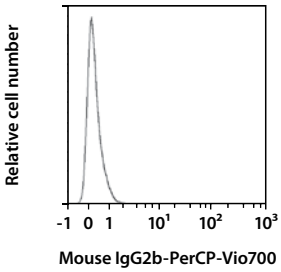

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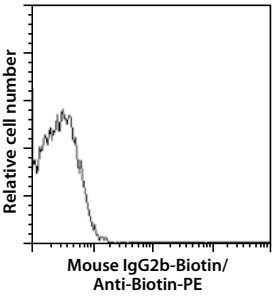

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