Miltenyi Biotec

Rat 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 Rat IgG2b antibodies conjugated to:

Conjugate	Order no. 30 µg in 1 mL (200 tests)	Order no. 9 µg in 300 µL (60 tests)
FITC	130-102-662	130-103-088
VioBright™ FITC	130-105-111	130-105-172
PE	130-102-663	130-103-089
APC	130-102-664	130-103-085
VioBlue®	130-102-661	130-103-083
VioGreen™	130-102-659	130-103-082
PE-Vio770™	130-102-656	130-103-091
APC-Vio770™	130-102-657	130-103-090
PerCP-Vio700™	130-102-658	130-103-084
Biotin	130-101-970	130-102-053

Clone ES26-5E12.4 (isotype: rat IgG2b).

Capacity One test corresponds to labeling of 10^6 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

- Antigen: keyhole limpet hemocyanin (KLH)
- Expression patterns: The Rat IgG2b isotype control antibody clone ES26-5E12.4 is specific for KLH (keyhole limpet hemocyanin). This protein is not expressed on mammalian cells or cell lines. Therefore, the antibody clone ES26-5E12.4 can be used as a negative control to distinguish specific from non-specific binding of Rat IgG2b fluorochrome-conjugated antibodies to human and mouse cells, for example, via Fc receptors, or due to interactions of the fluorochrome with the cell surface.

1.2 Applications

- Isotype control antibody for flow cytometric analysis of cells

1.3 Recommended antibody dilution

The recommended antibody dilution for all Rat IgG2b conjugates is **1:10 for up to 10^6 cells/50 µL** of buffer for labeling of cells and subsequent 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) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Rat IgG2b-Biotin.
- (Optional) For antibodies for additional staining, 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 10^6** 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 (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.

140-004-008.02

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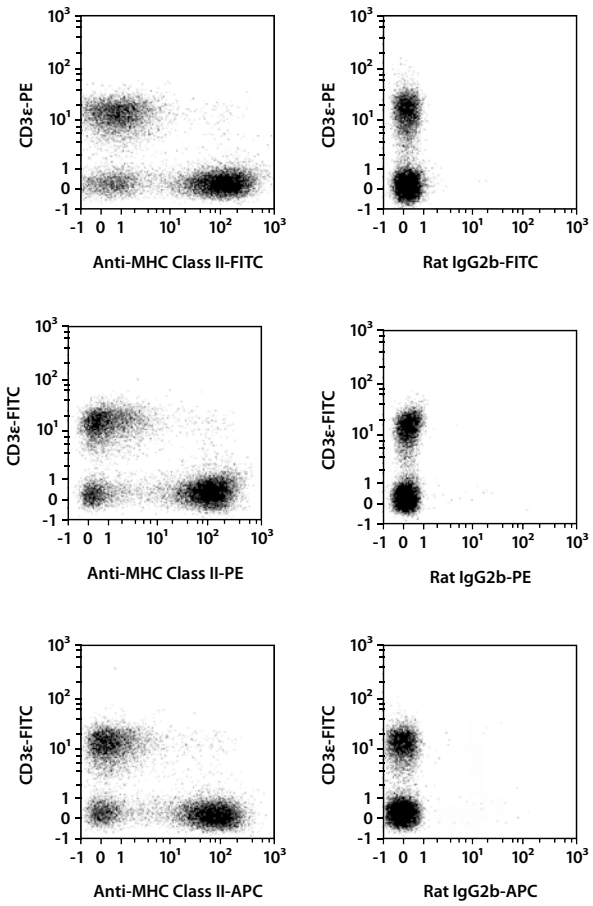
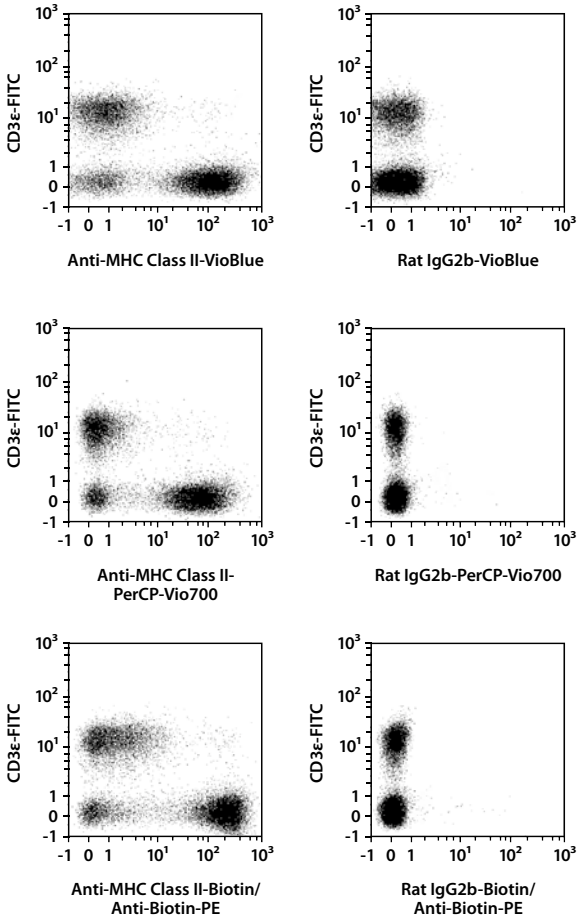
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the Rat IgG2b isotype control antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
   ▲ **Note:** 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) Rat 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 Rat IgG2b isotype control antibodies

Splenocytes from BALB/c mice were stained with Rat IgG2b antibodies or with the corresponding Anti-MHC Class II antibodies (left images). Flow cytometry was performed with the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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