Miltenyi Biotec

Rat IgM
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 IgM isotype control antibodies

Conjugate	Order no. 30 µg in1 mL (200 tests)	Order no. 9 µg in 300 µL (60 tests)
FITC	130-102-671	130-103-048
VioBright™ FITC	130-105-158	130-105-217
PE	130-102-672	130-103-049
APC	130-102-673	130-103-047
PE-Vio770™	130-102-665	130-103-038
APC-Vio770™	130-104-666	130-103-051
PerCP-Vio700™	130-104-667	130-103-039
Biotin	130-101-971	130-103-063

Clone ES26-13D3.4 (isotype: rat IgM)

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 IgM isotype control antibody clone ES26-13D3.4 is specific for KLH (keyhole limpet hemocyanin). This protein is not expressed on human cells or cell lines. Therefore, the antibody clone ES26-13D3.4 can be used as a negative control to distinguish specific from non-specific binding of Rat IgM 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

- Isotype control antibody for flow cytometric analysis of cells

1.3 Recommended antibody dilution

The recommended antibody dilution for all Rat IgM 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 IgM-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.
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 IgM isotype control antibody.
5. Mix well and incubate for 10 minutes in the dark in

140-004-009.03

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

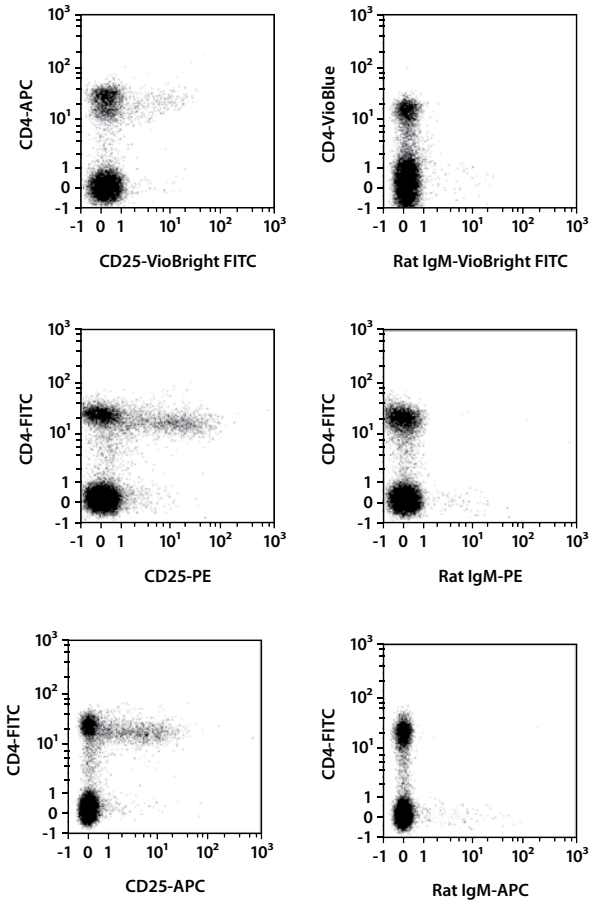
the refrigerator (2–8 °C).

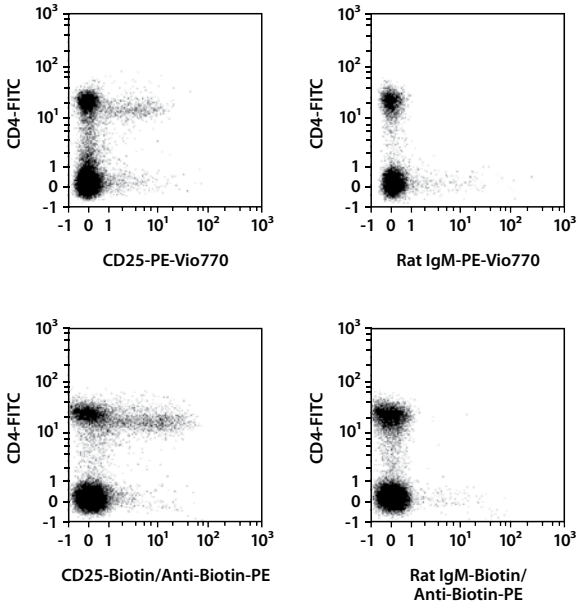
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) Rat IgM-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 IgM isotype control antibodies

Splenocytes from Balb/c mice were stained with Rat IgM antibodies or with the CD25 antibodies of the same isotype (left images) as well as with CD4-FITC or CD4-VioBlue. Flow cytometry was performed using the the MACSQuant® Analyzer. Cells labeled with Rat IgM-Biotin or CD25-Biotin were stained with Anti-Biotin-PE as well. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.





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

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

140-004-009.03