FcR Blocking Reagent
human

Order no. 130-059-901

Contents

1. Description

Components	2 mL FcR Blocking Reagent, human.
Product format	FcR Blocking Reagent is 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

Incubation with FcR Blocking Reagent increases the specificity of antibody or MicroBead labeling and thereby improves the purity of target cells, including extremely rare target cells such as antigen-specific B cells, fetal cells in maternal blood, stem cells, or disseminated epithelial tumor cells.

1.2 Applications

- Blocking of the binding of MACS® MicroBeads to the Fc receptor of human Fc receptor-expressing cells.
- Blocking of the binding of antibodies to the Fc receptor of human Fc receptor-expressing cells

1.3 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). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, wee recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ 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).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation or antibody labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

2.2 Use of FcR Blocking Reagent and direct MicroBeads for the magnetic labeling of human cells

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. Add 20 µL of FcR Blocking Reagent per 10^7 total cells.
5. Add 20 µL of direct MicroBeads.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

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

140-000-088.06

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0 Fax +49 2204 85197

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

7. (Optional) Add staining antibodies and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
10. Proceed to magnetic separation.

2.3 Use of FcR Blocking Reagent and indirect MicroBeads for the magnetic labeling of human cells

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 60 µL of buffer per 10^7 total cells.
4. Add 20 µL of FcR Blocking Reagent per 10^7 total cells.
5. Add primary antibody according to manufacturer´s recommedations and adjust the volume of cell suspension to 100 µL per 10^7 total cells.
6. Mix well and incubate according to manufacturer's recommendations.
7. Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per 10^7 cells and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
9. Add 20 µL of indirect MicroBeads per 10^7 total cells.
10. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

    ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
11. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

    ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
13. Proceed to magnetic separation.

2.4 Use of FcR Blocking Reagent and antibodies for the labeling of human cells

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 80 µL of buffer.
4. Add 20 µL of FcR Blocking Reagent.
5. Add antibodies according to manufacturer's recommendation.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

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

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.

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.

140-000-088.06