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## 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** 2 mL FcR Blocking Reagent, human

**Product format** FcR Blocking Reagent is supplied in a solution 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<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

## 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™.

▲ **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.

For details refer to the protocols section at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to [www.miltenyibiotec.com/gentlemacs](http://www.miltenyibiotec.com/gentlemacs).

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

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

▲ 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 for magnetic labeling given below are for up to  $10^7$  total 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  $2 \times 10^7$  total 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 labeling. Pass cells through 30  $\mu$ m nylon mesh (Pre-Separation Filters (30  $\mu$ m), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  total cells per 60  $\mu$ L of buffer.
4. Add 20  $\mu$ L of FcR Blocking Reagent per  $10^7$  total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add amount of MicroBeads according to manufacturer's recommendation and follow the corresponding protocol.

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

▲ 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 for fluorescent labeling given below are for up to  $10^7$  total 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  $2 \times 10^7$  total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  total cells per 80  $\mu$ L of buffer.
4. Add 20  $\mu$ L of FcR Blocking Reagent.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add amount of antibody according to manufacturer's recommendation and follow the corresponding protocol.

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit [www.miltenyibiotec.com/local](http://www.miltenyibiotec.com/local) to find your nearest Miltenyi Biotec contact.

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