FcR Blocking Reagent
mouse

Order no. 130-092-575

Index

1. **Description**
   - 1.1 **Background and product applications**
   - 1.2 **Reagent requirements**
2. **Protocol**
   - 2.1 **Sample preparation**
   - 2.2 **Use of FcR Blocking Reagent for antibody or MicroBead labeling of mouse cells**
3. **Example staining of cells with and without FcR Blocking Reagent**

1. Description

Product format	1 mL FcR Blocking Reagent, mouse: FcR Blocking Reagent is supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests or up to 10^9 total cells.
Reagent dilution	The FcR Blocking Reagent is used at a dilution of 1:10.
Storage	Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

Incubation with FcR Blocking Reagent increases the specificity of antibody and MicroBead labeling to their target cells, including extremely rare target cells such as hematopoietic progenitor cells, neural stem cells or regulatory T cells.

Product applications

- Blocking of the binding of antibodies and MicroBeads to the Fc receptor of mouse Fc receptor-expressing cells, e.g. monocytes and macrophages.

1.2 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 (4–8 °C).
  Required for MicroBead labeling: 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 mouse serum albumin, mouse serum, or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs, non-lymphoid tissue, or peripheral blood using standard methods For details see section General Protocols in the User Manuals or visit www.miltenyibiotec.com. For the preparation of single-cell suspensions from neural tissues, please refer to the data sheet of the Neural Tissue Dissociation Kit (# 130-092-628).

▲ **Note:** Dead cells may bind non-specifically to antibodies. 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 for antibody or MicroBead labeling of mouse 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 and magnetic labeling 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, # 130-041-407) to remove cell clumps which may clog the column.

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 90 µL of buffer.
4. Add 10 µL of FcR Blocking Reagent, mouse.
5. Mix well and refrigerate for 10 minutes (4–8 °C).
   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Add antibody or MicroBeads according to manufacturer's recommendation.
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.

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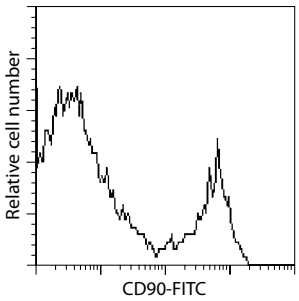
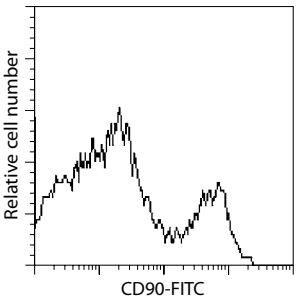
3. Example staining of cells with and without FcR Blocking Reagent

Mouse spleen cell suspensions were incubated with (a) and without (b) FcR Blocking Reagent and subsequently stained with CD90-FITC (# 130-091-602). Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(a) Mouse spleen cells with FcR Blocking Reagent



(b) Mouse spleen cells without FcR Blocking Reagent



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

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