



Anti-Biotin MACSiBead™ Particles

Order no. 130-091-147

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1. Description

Components	2.5 mL Anti-Biotin MACSiBead™ Particles; MACSiBead Particles (appr. 3.5 µm diameter) conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1); anti-biotin antibodies do not bind to free biotin.
Capacity	For separation of 5×10^8 total cells.
Product format	Anti-Biotin MACSiBead Particles are supplied in buffer containing 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 Principle of a separation using Anti-Biotin MACSiBead™ Particles

First, the cells are labeled with biotinylated primary antibodies. In a second step, the biotin-labeled cells are magnetically labeled with Anti-Biotin MACSiBead™ Particles. Subsequently, the cell suspension is placed in the magnetic field of a MACSiMAG™ Separator. The bead-labeled cells migrate towards the magnet and adhere to the wall of the tube. The non-labeled cells in the supernatant are pipetted off and collected as the non-labeled target cell fraction.

1.2 Background information

Anti-Biotin MACSiBead Particles have been developed for depletion of non-target cells using (cocktails of) biotinylated antibodies. Depletion of non-target cells using Anti-Biotin MACSiBead Particles can be combined with positive selection using MACS® MicroBeads.

1.3 Applications

- Depletion of non-target cells from peripheral blood mononuclear cells (PBMCs) or lymphoid tissue.
- Two-step magnetic cell separation by depletion with Anti-Biotin MACSiBead Particles followed by positive selection with MACS MicroBeads.

1.4 Reagent and instrument 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- 5 mL tubes for up to 1×10^8 total cells or 15 mL tubes for $>1 \times 10^8$ total cells.
- MACSmix™ Tube Rotator (# 130-090-753) for incubation of cells with Anti-Biotin MACSiBead Particles.
- MACSiMAG Separator for removal of cells labeled with Anti-Biotin MACSiBead Particles.
▲ **Note:** Do not use MACSiBead Particles with MACS Columns and MiniMACS™, MidiMACS™, OctoMACS™, QuadroMACS, VarioMACS™, SuperMACS™, or autoMACS™ Separators.
- (Optional) Fluorochrome-conjugated antibodies, for example Anti-Biotin-FITC (# 130-090-875), Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856).

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 $200 \times g$ 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 protocols section at www.miltenyibiotec.com/protocols.

▲ 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).

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2.2 Magnetic labeling

▲ Resuspend Anti-Biotin MACSiBead Particles thoroughly before use, to obtain a homogenous dispersion of MACSiBead Particles in solution.

▲ When working with up to 1×10^8 total cells, optimal magnetic labeling is achieved using a 5 mL tube (up to 2 mL total volume for magnetic labeling). When working with higher cell numbers, use a 15 mL tube (>2 mL total volume for magnetic labeling).

- Determine cell number.
- Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
- Resuspend cell pellet and label cells with the primary biotinylated antibody at a final concentration of 1–5 $\mu\text{g}/\text{mL}$ or at the titer recommended by the manufacturer.

▲ **Note:** It is recommended to label up to 5×10^7 cells in a total volume of 500 μL . When working with higher cell numbers, scale up all reagent and buffer volumes.

▲ **Note:** The biotinylated antibody should be used at its optimal titer, i.e. with optimal labeling intensity and no background labeling.
- Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
- Wash cells by adding 5 mL of buffer per 5×10^7 cells and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
- Repeat washing step 5 and aspirate supernatant completely. (Optional) For immunofluorescent staining take an aliquot of the starting material before labeling with Anti-Biotin MACSiBead Particles.

▲ **Note:** The aliquot can be stained with fluorochrome-conjugated anti-biotin antibodies (for details, please refer to the Anti-Biotin-FITC, -PE or -APC data sheets.)
- For up to 5×10^7 cells, resuspend cells in 750 μL of buffer and add 250 μL of Anti-Biotin MACSiBead Particles.

▲ **Note:** When working with higher cell numbers, scale up all reagent and buffer volumes accordingly (e.g for 1×10^8 total cells use twice the volume of buffer and Anti-Biotin MACSiBead Particles). When working with fewer than 5×10^7 total cells, use the same volumes as for 5×10^7 total cells.
- Mix well and incubate for 15 minutes using the MACSmix™ Tube Rotator (medium speed/8 rpm).
- Proceed to magnetic separation (2.3).

2.2 Magnetic separation

- Place the tube with cells labeled with Anti-Biotin MACSiBead Particles in the magnetic field of a MACSiMAG Separator. Use tube rack to insert tubes from 1.5 mL to 5 mL in size. For more details, see MACSiMAG Separator data sheet.

▲ **Note:** Carefully resuspend cells. To avoid unintended detachment of MACSiBead Particles from magnetically labeled cells, do not vortex.
- Allow the MACSiBead-labeled cells to adhere to the wall of the tube:

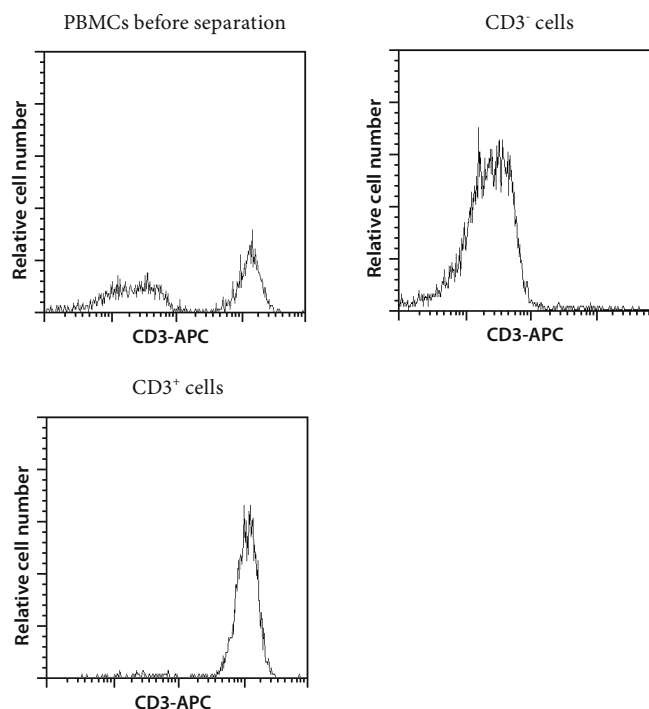
0.5 mL, 1.5 mL, 2 mL, or 5 mL tubes:	2 minutes
15 mL or 50 mL tubes:	4 minutes
- Retaining the tube in the MACSiMAG Separator, carefully pipette supernatant containing the non-labeled target cells into a new tube.

- (Optional) To remove residual MACSiBead Particles, replace tube with non-labeled target cells in the MACSiMAG Separator and repeat steps 2 and 3.

▲ **Note:** The non-labeled target-cell fraction or an aliquot thereof can be stained with fluorochrome-conjugated Anti-Biotin antibodies (for details, please refer to the Anti-Biotin-FITC, -PE, or -APC data sheets).

3. Example of a T cell depletion using the Anti-Biotin MACSiBead™ Particles

Depletion of T cells from human PBMCs using CD3-Biotin and Anti-Biotin MACSiBead Particles. Cells are fluorescently stained with CD3-APC. The magnetically labeled cells were stained with CellTracker™ CMFDA (Molecular Probes) for flow cytometric discrimination of viable cells from dead cells and Anti-Biotin MACSiBead Particles.



All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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