Miltenyi Biotec

Anti-Biotin MACSiBead™ Particles

cell culture grade

Order no. 130-092-357

Contents

1. Description

This product is for research use only.

Components	2 mL Anti-Biotin MACSiBead™ Particles, cell culture grade, corresponding to 4×10^8 MACSiBead Particles.
Product format	Anti-Biotin MACSiBead Particles are supplied in an azide-free buffer containing stabilizer. Low endotoxin.
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

Anti-Biotin MACSiBead Particles are designed for the activation, expansion, or differentiation of cells, and can be used in a flexible manner. The Anti-Biotin MACSiBead Particles are in a first step loaded with the appropriate biotinylated primary antibodies. Loaded Anti-Biotin MACSiBead Particles can subsequently be used to stimulate cells. Each potential application requires preparatory work in order to determine the optimal test conditions.

Anti-Biotin MACSiBead Particles show no autofluorescence and normally do not need to be removed prior to flow cytometric analysis. However, scatter properties of cells may be altered after a short term stimulus for up to 24 hours due to the strong interaction between cells and MACSiBead Particles.

If desired, removal of Anti-Biotin MACSiBead Particles is easily achieved by using the MACSiMAG™ Separator (refer to 2.3).

1.2 Reagent and instrument requirements

- One or more biotinylated primary antibodies for the specific application of interest. For more information about biotin-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** Anti-Biotin MACSiBead Particles cannot be loaded with enzymatically biotinylated molecules like commonly used peptide-MHC-complexes.

- 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.

- Humidified incubator
- MACSmix™ Tube Rotator (# 130-090-753) for loading of MACSiBead Particles.
- (Optional) Cell culture medium, e.g., TexMACS™ Medium (# 130-097-196).
- (Optional) MACSiMAG Separator (# 130-092-168) for removal of Anti-Biotin MACSiBead Particles.

  ▲ **Note:** Do not remove MACSiBead Particles with MACS® Columns and autoMACS, MidiMACS™, MiniMACS™, OctoMACS™, QuadroMACS™, SuperMACS™ II, or VarioMACS™Separators.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis. For information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

2. Protocol

▲ For appropriate use of Anti-Biotin MACSiBead Particles, it is essential to determine the optimal primary antibody concentration as well as the ideal bead-to-cell ratio for each specific application. This is important as the efficiency of the desired application can depend on the differentiation status of the cells, which will often be heterogenous. An over-stimulation of cells can, for example, carry a risk of activation-induced cell death.

▲ All steps in the protocol have to be performed under sterile conditions.

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.

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

For details refer to www.miltenyibiotec.com/gentlemacs.

2.2 Loading of Anti-Biotin MACSiBead™ Particles

▲ Resuspend Anti-Biotin MACSiBead™ Particles thoroughly by vortexing before use, to obtain a homogenous suspension.

▲ Anti-Biotin MACSiBead Particles are supplied without preservative. Remove aliquots under aseptic conditions.

▲ It is recommended to load Anti-Biotin MACSiBead Particles in batches of 1×10^8 Anti-Biotin MACSiBead Particles.

1. Pipette an appropriate aliquot of biotinylated primary antibodies into a sealable 2 mL tube.

   ▲ **Note:** Depending on the application, more than one biotinylated primary antibody can be used. All primary antibodies must be mixed thoroughly before adding the Anti-Biotin MACSiBead Particles. The total antibody volume should not exceed 500 µL.

   ▲ **Note:** The loading capacity of the Anti-Biotin MACSiBead Particles amounts to a maximum of 30 µg total biotinylated primary IgG antibody per 1×10^8 Anti-Biotin MACSiBead Particles.

2. Resuspend Anti-Biotin MACSiBead Particles thoroughly by vortexing.

3. Remove 500 µL of Anti-Biotin MACSiBead Particles (1×10^8 Anti-Biotin MACSiBead Particles) and add to the antibodies.

4. Add buffer to adjust to a total volume of 1 mL.

5. Incubate for 2 hours at 2–8 °C under constant, gentle rotation by using the MACSmix™ Tube Rotator at slowest permanent run program. The loaded Anti-Biotin MACSiBead Particles can be stored at this point. For storage, do not remove the loaded Anti-Biotin MACSiBead Particles from the antibody mix.

   ▲ **Note:** It is recommended to store the loaded Anti-Biotin MACSiBead Particles at 2–8 °C. The storage life must be experimentally determined.

6. Resuspend loaded Anti-Biotin MACSiBead Particles (1×10^8 Anti-Biotin MACSiBead Particles/mL) thoroughly and transfer an appropriate aliquot to a suitable tube. Add an adequate volume of culture medium and centrifuge at 300×g for 5 minutes.

   ▲ **Note:** Add 100–200 µL cell culture medium per 25 µL loaded Anti-Biotin MACSiBead Particles. For larger aliquots, scale up volume of culture medium.

7. Remove supernatant and resuspend the loaded Anti-Biotin MACSiBead Particles in a suitable volume of fresh culture medium.

   The loaded Anti-Biotin MACSiBead Particles can now be used for various applications. For each application, the optimal bead-to-cell ratio must first be determined. For example, activation of human T cells with Anti-Biotin MACSiBead Particles loaded with CD2-, CD3-, and CD28-Biotin requires a bead-to-cell ratio of 1:2. For more details, refer to the data sheet of the T Cell Activation/Expansion Kit, human (# 130-091-441).

2.3 Removal of Anti-Biotin MACSiBead™ Particles

▲ Removal of MACSiBead™ Particles used for cell activation or expansion may be required before restimulation with different agents or antigens, or before magnetic separation of cells with MACS® MicroBeads.

1. Harvest cells and transfer to a 5 mL, 15 mL, or 50 mL tube and wash once with buffer.

2. Resuspend cells in buffer at a density of up to 2×10^7 cells per 1 mL and vortex thoroughly.

3. Place tube in the magnetic field of the MACSiMAG™ Separator.

   ▲ **Note:** Use tube rack to insert 5 mL tube into the magnetic field of the separator. For more details, refer to the MACSiMAG Separator data sheet .

4. Allow the MACSiBead Particles to adhere to the wall of the tube:

5 mL tubes:	2 minutes
15 mL or 50 mL tubes:	4 minutes

5. While retaining the tube in the magnet, carefully remove the supernatant containing the MACSiBead-depleted cells and place in a new tube.

6. Remove the tube from the separator and add buffer to the same volume as before.

7. Vortex sample, replace tube in the MACSiMAG Separator, and repeat steps 4–5.

8. Collected cells can now be further processed as required.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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