Miltenyi Biotec

Streptavidin MicroBeads

2 mL Streptavidin MicroBeads	Order no. 130-048-101
1 mL Streptavidin MicroBeads	Order no. 130-048-102

Index

1. Description

Components	**2 mL Streptavidin MicroBeads** (# 130-048-101) or **1 mL Streptavidin MicroBeads** (# 130-048-102): MicroBeads conjugated to streptavidin.
Size	**2 mL** for 2×10^9 total cells, up to 200 separations or **1 mL** for 10^9 total cells, up to 100 separations.
Product format	Streptavidin MicroBeads are 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 Principle of MACS® Separation

First, the cells are stained with a biotinylated primary antibody or ligand. Subsequently, the cells are magnetically labeled with Streptavidin MicroBeads. Then the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained in the column while the unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Streptavidin MicroBeads are developed for the separation of cells according to surface markers labeled with biotinylated antibodies or ligands.

Streptavidin MicroBeads have been utilized for the isolation of human cells such as isotype specific[1] or allergen specific[2] B cells, epithelial cells[3], follicular dendritic cells[4,5,6], or stromal cells[7]. They have also been used for the isolation of mouse cells such as MHC class I^+ cells[8], isotype specific B cells[9], liver sinusoidal endothelial cells[10], or for the sequential isolation of mouse dendritic cells, macrophages, and medullary and cortical epithelial cells[11]. Further, Streptavidin MicroBeads have been used for the isolation of rat T cells[12] and rat retinal ganglion cells[13], rhesus monkey T cells[14], sheep B cell subpopulations[15], fish (carp) B cells[16] or plant (potato) protoplasts[17].

Example applications

- Positive selection or depletion of cells labeled with biotinylated antibodies.
- Positive selection or depletion of cells labeled with biotinylated ligands.

1.3 Reagent and instrument requirements

- **Labeling buffer**: Prepare a solution containing phosphate buffered saline (PBS) pH 7.2, supplemented with 2 mM EDTA, optionally also supplemented with 0.5% **biotin-free** bovine serum albumin (BSA). Keep buffer cold (4–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** For magnetic labeling, use **biotin-free** labeling buffer only, for example, buffer without BSA or buffer supplemented with biotin-free BSA. If the BSA preparation is contaminated with biotin, free biotin competes with biotinylated antibody for the binding of the Streptavidin MicroBeads. The use of cell culture medium is not recommended as it may contain free biotin. Alternatively, use Anti-Biotin MicroBeads (# 130-090-485) which do not bind to free biotin.

- **Separation buffer**: Prepare a solution containing PBS pH 7.2, 0.5% 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). 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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

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- MACS® Columns and MACS Separators: Cells labeled with Streptavidin MicroBeads can be enriched by using MS, LS or XS Columns (positive selection). Streptavidin MicroBeads can be used for depletion of cells on LD, CS or D Columns. Cells which strongly express the biotin labeled antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using the autoMACS™ Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- Biotinylated primary antibody or ligand.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), Anti-Biotin-APC (# 130-090-856) or fluorochrome conjugated streptavidin for flow cytometric analysis.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the labeling and discrimination of dead cells by flow cytometry.

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, e.g., using Ficoll-Paque™. For details see section General Protocols in the user manuals or visit 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, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** 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 Magnetic labeling

▲ 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 2×10^7 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

▲ The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet and label cells with biotinylated antibody at time and titer recommended by the manufacturer. Typically, labeling for 10 minutes is sufficient.

   ▲ **Note:** The biotinylated antibody should be used at its optimal titer, i.e., with optimal labeling intensity and no background labeling.
4. Wash cells to remove unbound primary antibody by adding 1–2 mL of **labeling buffer** per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. (Optional) Repeat washing step.
6. Resuspend cell pellet in 90 µL of **labeling buffer** per 10^7 total cells.
7. Add 10 µL of Streptavidin MicroBeads per 10^7 total cells.
8. Mix well and refrigerate for 15 minutes (4–8 °C).

   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
9. (Optional) Add 10 µL of Anti-Biotin-fluorochrome per 10^7 cells and refrigerate for 5 minutes in the dark (4–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of **separation buffer**.

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

    ▲ **Note:** For depletion with LD columns, resuspend cell pellet in 500 µl of buffer for up to 1.25×10^8 cells.
12. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS® Column and MACS Separator according to the number of total cells and the number of magnetically labeled cells. For details see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of **separation buffer**:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of **separation buffer**. Perform washing steps by adding **separation buffer** three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an appropriate amount of **separation buffer** onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL

   ▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells over a new, freshly prepared column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of **separation buffer**.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash with 2×1 mL of **separation buffer**. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of **separation buffer**. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash with 30 mL of **separation buffer** from top. Collect total effluent. This is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Magnetic separation with autoMACS™ Separator

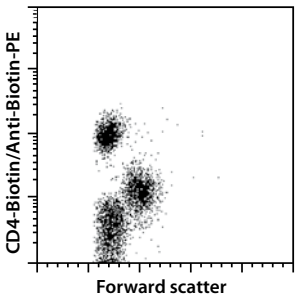
▲ Refer to the autoMACS™ user manual for instructions on how to use the autoMACS Separator.

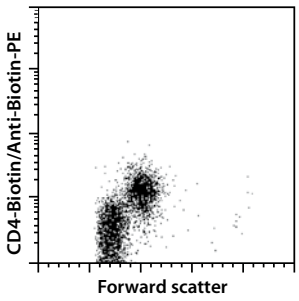
1. Prepare and prime autoMACS Separator.
2. Place tube containing magnetically labeled cells in the autoMACS Separator. Choose a separation program according to the recommendations in the autoMACS user manual.

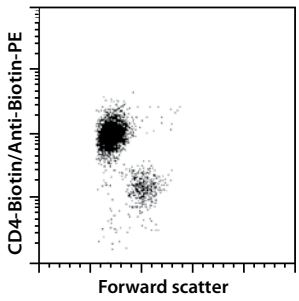
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details see autoMACS user manual, section autoMACS Cell Separation Programs.

3. Example of a separation using Streptavidin MicroBeads

Separation of human peripheral blood mononuclear cells (PBMCs) using biotinylated anti-human CD4 antibody, Streptavidin MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.







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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.

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.

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