Miltenyi Biotec

Anti-Fibroblast MicroBeads
human

Order no. 130-050-601

Contents

1. Description

Components	2 mL Anti-Fibroblast MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-fibroblast antibodies (isotype: mouse IgG2a).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-Fibroblast MicroBeads are supplied in buffer 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 the MACS® Separation

First, the fibroblasts are magnetically labeled with Anti-Fibroblast 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 fibroblasts are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of fibroblasts. After removing the column from the magnetic field, the magnetically retained fibroblasts can be eluted as the positively selected cell fraction.

1.2 Background information

Anti-Fibroblast MicroBeads have been developed for the positive selection or depletion of human fibroblasts using a fibroblast-specific antigen.[1] This antigen is also known to be expressed on mesenchymal stromal cells (MSCs) in the bone marrow and has been used for their isolation.[2–4]

1.3 Applications

- Removal of fibroblastic cells from bone marrow cultures or other primary tissue cultures.
- Depletion of fibroblasts can be an effective step in the establishment of primary tumor cell lines in order to prevent fibroblastic overgrowth within the tissue culture.
- Isolation of MSCs from bone marrow aspirate.
- Depletion of fibroblasts that are used as feeder cells to support the growth of ESC or iPS cells.

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). 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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Fibroblasts can be enriched by using MS, LS, or XS Columns or depleted with the use of CS or D Columns. Cells which strongly express the fibroblast antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection or depletion			
MS	3×10^6	10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	3×10^7	10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	3×10^8	10^9	SuperMACS II
autoMACS	up to 10^8	up to 10^9	autoMACS Pro, autoMACS
Depletion			
CS	6×10^7		VarioMACS, SuperMACS II
D	3×10^8		SuperMACS II

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

- (Optional) Fluorochrome-conjugated anti-fibroblast antibodies for flow cytometric analysis. For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.

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- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion 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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to 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).

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 labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Anti-Fibroblast MicroBeads per 10^7 total cells.
5. Mix well and incubate for 30 minutes at room temperature (19–25 °C).
6. (Optional) Add staining Anti-Fibroblast antibody at the titer recommendend by the manufacturer and incubate for additional 5–10 minutes atroom temperature (19–25 °C) in the dark.
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 up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
9. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Column capacity is dependent on starting material.

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of fibroblasts. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of 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
7. (Optional) If high purity of Anti-Fibroblast MicroBeads-labeled cells is desired, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

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

Depletion with CS Columns

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

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Depletion with D Columns

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

Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the cell source, the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. **a:** For cell frequency >5% choose the following programs:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

   b: For cell frequency <5% choose the following programs:

   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. **a:** For cell frequency >5% choose the following programs:

   Positive selection: Possel
   Collect positive fraction from outlet port pos1.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

   b: For cell frequency <5% choose the following programs:

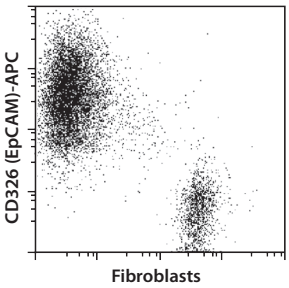
   Positive selection: Posseld
   Collect positive fraction from outlet port pos2.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

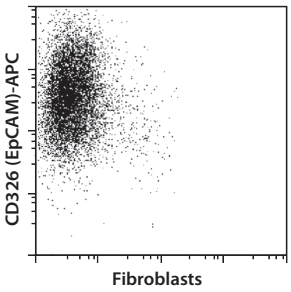
3. Example of a separation using the Anti-Fibroblast MicroBeads

Human iPS cells were co-cultured with human fibroblasts. Depletion of the fibroblasts was achieved using the Anti-Fibroblast MicroBeads, a LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD326 (EpCAM)-APC (# 130-091-254) and CD44-PE (# 130-095-180) as a fibroblast marker and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

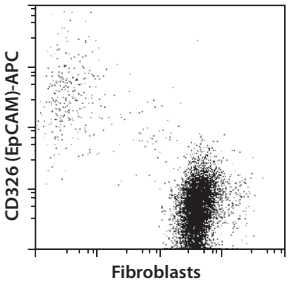
Before separation



Negative fraction



Positive fraction



4. References

1. Fearns, C. and Dowdle, E. B. (1992) The desmoplastic response: induction of collagen synthesis by melanoma cells *in vitro*. Int. J. Cancer 50: 621–627.
2. Jones, E. A. *et al.* (2002) Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis and Rheumatism 46: 3349–3360.
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4. Jones, E. A. *et al.* (2007) Purification of proliferative and multipotential marrow stromal cells (MSCs) from bone marrow aspirate by selection for CD271 (LNGFR) expression. MACS&more 11-1: 22–25.

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.

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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