Miltenyi Biotec

CD105 MicroBeads
human

Order no. 130-051-201

Contents

1. Description

Components	2 mL CD105 MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-human CD105 antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD105 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 $CD105^+$ cells are magnetically labeled with CD105 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 $CD105^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD105^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD105^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The CD105 MicroBeads are developed for the isolation of endothelial cells from endothelial tissue and mesenchymal stromal cells (MSCs) from bone marrow. The CD105 antigen serves as a receptor for the growth and differentiation factors TGF-β_1 and TGF-β_3. An epitope on CD105 is recognized by the SH-2 antibody[1], which was raised against human MSCs that show mesodermal differentiation capacity.[2]

Further, CD105 is also expressed on mature endothelial cells (endoglin) and on some leukemic cells of B lymphoid and myeloid origin. $CD105^+$ bone marrow cells also show multipotent differentiation *in vitro*[2] and the capacity to form bone *in vivo* without prior cultivation or differentiation.[3]

1.3 Applications

- Positive selection or depletion of cells expressing human CD105 antigen.
- Isolation or depletion of endothelial cells from single-cell suspensions of vascular endothelium, e.g. from human umbilical cord vein.
- Studies on mesengenesis.[2,3]
- *In vitro* investigations on hematological disorders.[4]

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: $CD105^+$ cells can be enriched by using MS or LS Columns (positive selection).

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, 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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling when using pheripheral blood samples.
- (Optional) Fluorochrome-conjugated CD105 antibodies for flow cytometric analysis, e.g., CD105-PE (# 130-094-941), CD105-APC (# 130-094-926), CD105-Biotin (# 130-094-916), or CD45-FITC (130-080-202). For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com.
- (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.

140-003-053.07

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

▲ A special protocol for "Isolation of human umbilical vein endothelial cells (HUVECs) from umbilical cord" is available at www.miltenyibiotec.com/protocols.

When working with anticoagulated peripheral blood or buffy coat or bone marrow aspirate, mononuclear cells (MNCs) 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, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Wash and centrifuge cell suspension. Aspirate supernatant completely.
3. Resuspend cell pellet in appropriate amount of buffer per 10^7 total cells.

   When labeling without using FcR Blocking Reagent, resuspend in 80 µL of buffer.

   When MACS **FcR Blocking Reagent, human** has been used resuspend in 60 µL of buffer.
4. Add 20 µL of CD105 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 200×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.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD105^+$ cells. For details refer to 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 refer to 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) To increase the purity of $CD105^+$ cells, 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 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 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.

140-003-053.07

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. For a standard separation choose the following program:

   Positive selection: Posseld2

   Collect positive fraction in row C 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 pos2.
3. For a standard separation choose the following program:

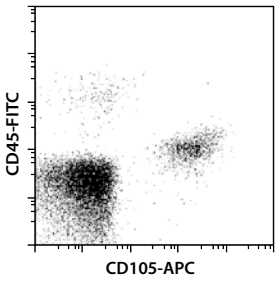
   Positive selection: Posseld2

   Collect positive fraction from outlet port pos2.

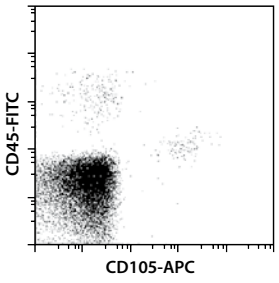
3. Example of a separation using the CD105 MicroBeads

$CD105^+$ human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical cord vein using the CD105 MicroBeads, an MS Column, and an appropriate MACS Separator. Cells were fluorescently stained with CD105-APC (# 130-094-926) and CD45-FITC (# 130-080-202) and analyzed by using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

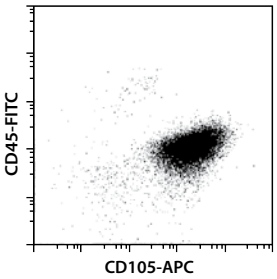
Before separation



$CD105^-$ cells



$CD105^+$ cells



4. References

1. Barry, F. P. *et al.* (1999) The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). Biochem. Biophys Res. Commun. 265: 134–139.
2. Majumdar, M. K. *et al.* (2000) Isolation, characterization, and chondrogenic potential of human bone marrow - derived multipotential stromal cells. J. Cell Physiol. 185: 98–106.
3. Aslan, H. *et al.* (2006) Osteogenic Differentiation of noncultured immunoisolated bone marrow-derived $CD105^+$ Cells. Stem Cells 24: 1728–1737.
4. Campioni, D. *et al.* (2003) Enrichment of $CD105^+$ and $fibroblast^+$ stromal cells from healthy donors and AML patients. MACS&more 7/1: 11–13.

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.

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.

140-003-053.07