Miltenyi Biotec

CD144 (VE-Cadherin) MicroBeads
human

Order no. 130-097-857

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1. Description

Components	2 mL CD144 (VE-Cadherin) MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD144 antibodies.
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD144 (VE-Cadherin) 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 $CD144^+$ cells are magnetically labeled with CD144 (VE-Cadherin) 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 $CD144^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD144^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD144^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD144 (VE-Cadherin) MicroBeads have been developed for the separation of ES- or iPS-derived human endothelial cells based on the expression of CD144 (VE-Cadherin, Cadherin-5).
CD144 is a member of the cadherin family of calcium-dependent adhesion molecules. The antigen is expressed on endothelial cells *in vivo* and *in vitro*[1,2] and has been used for isolation of endothelial cells derived from embryonic stem cells and induced pluripotent stem cells[2,3]. It is known to be involved in homophilic cell interactions contributing to the cohesion and organization of the intercellular junctions of the vascular endothelium. It is thought to play a role in vascular permeability and remodeling.
CD144 (VE-Cadherin) MicroBeads react with a calcium-independent epitope on CD144.

1.3 Applications

- Positive selection or depletion of cells expressing human CD144 antigen.
- Isolation of $CD144^+$ endothelial cells differentiated from pluripotent stem cells.[3]

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:** 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: $CD144^+$ cells can be enriched by using LS Columns or depleted with the use of LD Columns. Cells that strongly express the CD144 antigen can also be depleted using LS 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			
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS, SuperMACS II
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro, autoMACS

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

- CellMates™ Accutase® Cell Detachment Solution (# 130-095-545)
- Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD31-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

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2. Protocol

2.1 Sample preparation

Harvesting of endothelial cells differentiated from human embryonic stem cells (ES) or induced pluripotent cells (iPS)

Different protocols have been described to differentiate pluripotent stem cells into CD144$^+$ endothelial cells.[2,3] Here, harvesting and magnetic isolation of CD144$^+$ cells has been optimized based on the protocol described by Patsch *et al.*[3]

1. Remove culture medium and wash culture plates with PBS.
2. Add 5 mL of pre-warmed CellMates™ Accutase® Cell Detachment Solution per 10 cm culture dish for 5 minutes at 37 °C.
3. Dissociate cell clumps to single-cell suspension by pipetting up and down using a 5 mL serological pipette.
4. Add 10 mL of buffer.
5. 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 or culture medium before use.



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.

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

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 CD144 (VE-Cadherin) 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, e.g., 10 µL of CD31-APC, 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 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

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD144$^+$ cells. 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 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 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **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 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of CD144$^+$ cells, the eluted fraction can be enriched over a second LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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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.

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 one of the following programs:

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

   ▲ **Note:** Program Posseld2 can be used to further increase purity if necessary.

   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. For a standard separation choose one of the following programs:

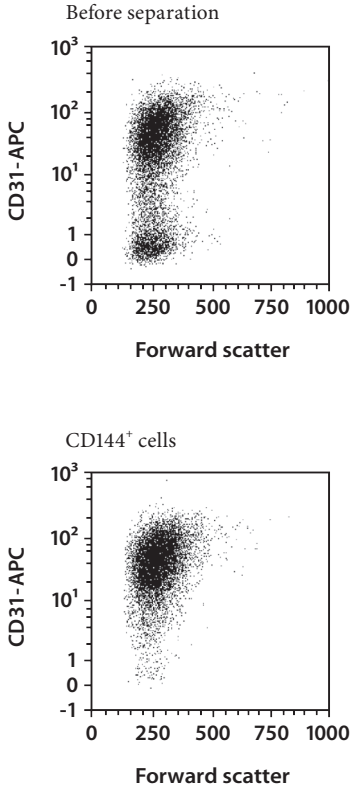
   Positive selection: Possel
   Collect positive fraction from outlet port pos1.

   ▲ **Note:** Program Posseld2 can be used to further increase purity if necessary.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

3. Example of a separation using CD144 (VE-Cadherin) MicroBeads

CD144-expressing endothelial cells were isolated from a heterogeneous culture of differentiated human ES cells using CD144 (VE-Cadherin) MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD31-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris, dublets, and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Rufaihah, A. J. *et al.* (2011). Endothelial cells derived from human iPSCS increase capillary density and improve perfusion in a mouse model of peripheral arterial disease. Arterioscler. Thromb. Vasc. Biol. 31(11): e72–79.
2. Tatsumi, R. *et al.* (2011). Simple and highly efficient method for production of endothelial cells from human embryonic stem cells. Cell Transplant. 20(9): 1423–1430.
3. Patsch, C. *et al.* (2012) Highly efficient vascular induction of human pluripotent stem cells into endothelial cells by a novel GSK3ß inhibitor. Manuscript submitted.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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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