Miltenyi Biotec

CD146 (LSEC) MicroBeads
mouse

Order no. 130-092-007

Contents

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.

1. Description

This product is for research use only.

Components	2 mL CD146 (LSEC) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse LSEC antibodies (isotype: rat IgG2a).
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	CD146 (LSEC) 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, liver sinusoidal endothelial cells (LSECs) are magnetically labeled with CD146 (LSEC) 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 LSECs are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of LSECs. After removing the column from the magnetic field, the magnetically retained LSECs can be eluted as the positively selected cell fraction.

1.2 Background information

CD146 is a transmembrane glycoprotein and belongs to the IgG superfamily of cell adhesion molecules.[1] CD146 (LSEC) MicroBeads, formerly termed Anti-LSEC MicroBeads, have been developed to isolate mouse liver sinusoidal endothelial cells (LSECs). LSECs are microvascular endothelial cells lining the hepatic sinusoidal wall and are supposed to mainly contribute to the control of immune responses against circulating soluble antigens in the liver. Their strategic positioning favors a tight interaction with lymphocytes migrating through the liver. LSECs possess a high capacity for antigen uptake and processing but express, in contrast to professional antigen-presenting cells (e.g. dendritic cells), only low levels of costimulatory molecules.[2]
The CD146 (LSEC) antibody also binds to endothelial cells from a various range of organs, such as skin, liver, kidney, brain, spleen, lymph node, intestine, heart and skeletal muscle as well as on blood vessel structures, such as pulmonary arteries, veins, and the capillary network of the alveolar walls but not on lymphatic endothelium. In contrast to humans that express CD146 also on T cells or follicular dendritic cells, murine CD146 expression was only found on a subset of $NK1.1^+$ cells at low levels.[3]

1.3 Applications

- Isolation or depletion of LSECs from mouse liver.
- Isolation of endothelial cells from vascular tissues.

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.
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **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 mouse serum albumin, mouse 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: $CD146^+$ cells can be enriched by using MS, LS, or XS or depleted with the use of LD, CS, or D Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

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

▲ **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.

- Liver Dissociation Kit, mouse (# 130-105-807)
- (Optional) Fluorochrome-conjugated CD146 (LSEC) antibodies for flow cytometric analysis, e.g., CD146 (LSEC)-FITC (# 130-092-026). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (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

Dissociate mouse liver according to the protocol of the Liver Dissociation Kit, mouse using the gentleMACS™ Dissociators.

▲ 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.

▲ 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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD146 (LSEC) 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 CD146 (LSEC)-FITC (# 130-092-026), 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.

   ▲ **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 $CD146^+$ 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 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 flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

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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

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 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.

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 flow-through; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on the 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.

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.

   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 pos1.
3. For a standard separation choose one of the following programs:

   Positive selection: Possel
   Collect positive fraction from port pos1.

   Depletion: Depletes
   Collect negative fraction from port neg1.

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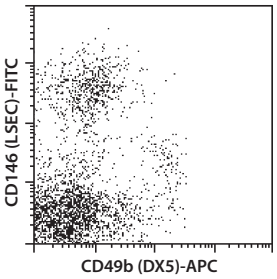
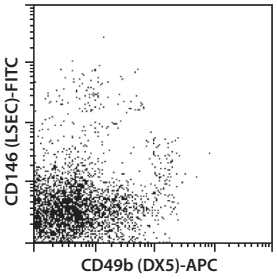
3. Example of a separation using CD146 (LSEC) MicroBeads

LSECs were isolated from mouse liver cell suspension using CD146 (LSEC) MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD146 (LSEC)-FITC (# 130-092-026) and CD49b (DX5)-APC (# 130-091-813) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

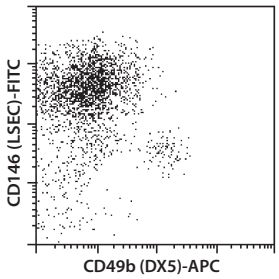
Mouse liver cells before separation



Mouse liver cells depleted of LSECs



Isolated LSECs



4. References

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2. Diehl, L. *et al.* (2008) Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent $CD8^+$ T cell tolerance. Hepatology 47: 296–305.
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4. Hegenbart, S. *et al.* (2006) Efficient isolation of liver sinusoidal endothelial cells (LSECs) by immunomagnetic separation. MACS&more 10: 8–10.

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.

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