Miltenyi Biotec

CD326 (EpCAM) MicroBeads
human

Order no. 130-061-101

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 CD326 (EpCAM) MicroBeads, human: MicroBeads conjugated to monoclonal antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 20 separations.
Product format	CD326 (EpCAM) 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 CD326 (EpCAM)$^+$ cells are magnetically labeled with CD326 (EpCAM) 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 CD326 (EpCAM)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD326 (EpCAM)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD326 (EpCAM)$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD326 (EpCAM)$^+$ cells must be separated over a second column.

1.2 Background information

Epithelial cell adhesion molecule (EpCAM), which is also known as CD326 and human epithelial antigen (HEA), is broadly expressed on cells of epithelial origin and on epithelial derived tumor cells[1]. CD326 (EpCAM) MicroBeads are used for the positive selection of viable epithelial tumor cells from peripheral blood, bone marrow, lymphoid tissue and serous effusions of patients with carcinomas. In order to prevent FcR-mediated non-specific labeling of non-epithelial cells it is strongly recommended to use FcR Blocking Reagent before magnetically labeling.

1.3 Applications

- Enrichment of disseminated carcinoma cells from peripheral blood, bone marrow, and lymphoid tissue, for example, of patients with epithelial cancer for subsequent analysis, e.g., enumeration, cultivation, or RT-PCR.
- Purification of CD326 (EpCAM)$^+$ cells from epithelial tissues (e.g. carcinomas).

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.

  ▲ **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: CD326 (EpCAM)$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD326 (EpCAM) antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

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

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D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

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

- (Optional) Fluorochrome-conjugated REA (REAfinity™ antibodies: Recombinantly engineered, lacking Fcγ-binding site) CD326 antibodies for flow cytometric analysis, e.g., CD326 (EpCAM)-FITC, CD326 (EpCAM)-PE, or CD326 (EpCAM)-Vio® Bright 515 and CD45 conjugated to a different fluorochrome, e.g., CD45-Vio Bright FITC, CD45-PE, or CD45-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233), DAPI Staining Solution (# 130-111-570), 7-AAD Staining Solution (# 130-111-568) or Viobility™ Fixable Dyes (# 130-109-812, # 130-109-814, # 130-109-816) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.
- (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.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.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.

▲ Always include positive and negative controls when working with patient samples. For a positive control, PBMCs spiked with tumor cells should be used. For a negative control, simply use PBMCs from a healthy donor.

▲ 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 300 µL of buffer per 5×10^7 total cells.
4. Add 100 µL of FcR Blocking Reagent per 5×10^7 total cells and mix well.

   ▲ **Note:** FcR Blocking Reagent should be used to block Fc receptor-mediated labeling of non-epithelial cells.
5. Add 100 µL of CD326 (EpCAM) MicroBeads per 5×10^7 total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (2–8 °C).
7. (Optional) Add staining antibodies according to manufacturer's recommendation.
8. Wash cells by adding 5–10 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. 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.
10. 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 CD326 $(EpCAM)^+$ 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: 4×500 µL LS: 4×3 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.

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 CD326 (EpCAM)$^+$ 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 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 column assembly and separation refer to the D Column data sheet.

Magnetic separation with the autoMACS® Pro Separator

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

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

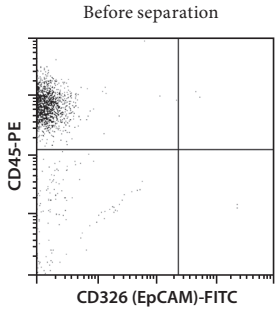
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

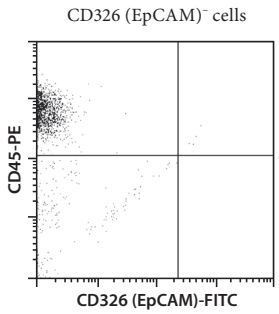
   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack.

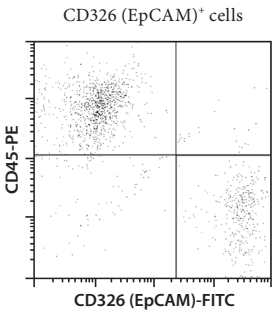
   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using CD326 (EpCAM) MicroBeads

CD326 (EpCAM)$^+$ cells were isolated from PBMCs spiked with cells from a breast cancer cell line (BT474) using CD326 (EpCAM) MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD326 (EpCAM)-FITC and CD45-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.







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5. Appendix

Preparation of bone marrow cells

1. Collect bone marrow in 50 mL tubes containing 5 mL PBS, pH 7.2, containing 2 mM EDTA, or 0.6% ACD-A, or 200 U/mL heparin.

   ▲ **Note:** Store cells at 4 °C if the cells cannot be processed immediately.

2. For preparation of a single-cell suspension of bone marrow cells dilute with 10× the volume of RPMI 1640 containing 0.02% collagenase B and 100 U/mL DNase and shake gently at room temperature for 45 minutes.
3. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407), in order to remove cells clumps. Wet filter with buffer before use.
4. Carefully layer 35 mL of diluted cell suspension over 15 mL Ficoll-Paque in a 50 mL conical tube.
5. Centrifuge at 400×g for 35 minutes at 20 °C in a swinging-bucket rotor without brake.
6. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
7. Carefully transfer the interphase cells (lymphocytes, monocytes, and thrombocytes) to a new 50 mL conical tube.
8. Fill the conical tube with PBS containing 2 mM EDTA or 0.6% ACD-A, mix, and centrifuge at 300×g for 10 minutes at 20 °C. Carefully aspirate supernatant completely.
9. Repeat step 8.

   ▲ **Note:** This step increases the purity of the CD326 (EpCAM) cell separation.

10. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 10^8 total cells. Proceed to magnetic labeling (2.2).

Preparation of cryopreserved cells

1. Thaw frozen cells (usually in 1–2 mL vials containing 10^7–10^8 cells) quickly at 40 °C in a waterbath by shaking the tube. Take the tube from the waterbath when there is still a very small piece of ice in the suspension and continue shaking for some seconds until the ice has disappeared.
2. Place cells immediately on ice.
3. Transfer cells to a 50 mL conical tube and dilute the cells (1–2 mL) slowly with cold (4 °C) Dulbecco's PBS (pH 7.0–7.2), containing 0.5 mM $MgCl_2$ and 1 mM $CaCl_2$, 0.5% BSA, 100 U/mL DNase to a final volume of 20 mL.
4. Incubate cells for 10 minutes on ice (4 °C).
5. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C.
6. Carefully remove supernatant.
7. Resuspend cell pellet in 15 mL of Dulbecco's PBS (pH 7.0–7.2), containing 0.5 mM Mg^{2+} and 1 mM Ca^{2+}, 0.5% BSA, and 100 U/mL DNase and centrifuge at 300×g for 10 minutes at 4 °C.
8. Carefully remove supernatant completely.
9. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 5×10^7 total cells. Proceed to magnetic labeling (2.2).

Preparation of cells from lymphoid tissue

1. Isolate single-cell suspension from lymphoid tissue by a standard preparation method.
2. Centrifuge cells at 300×g for 10 minutes in a 15 mL conical tube.
3. Carefully aspirate supernatant completely.
4. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 5×10^7 total cells. Proceed to magnetic labeling (2.2).

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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