Miltenyi Biotec

CD326 (EpCAM) MicroBeads
mouse

Order no. 130-105-958

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1. Description

This product is for research use only.

Components	2 mL CD326 (EpCAM) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD326 (EpCAM) antibodies (isotype: rat IgG1).
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	CD326 (EpCAM) MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +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

CD326 (EpCAM) is a 40 kDa transmembrane glycoprotein involved in cell adhesion, cell proliferation, and tumor progression. It functions as a homotypic calcium-independent cell adhesion molecule. EpCAM is broadly expressed on epithelial cells including definitive endoderm cells, keratinocytes, immature thymocytes, langerhans cells, lymph node dendritic cells, thymic dendritic cells, splenic dendritic cells, and tumor cells including almost all carcinomas.[1,2] Moreover, EpCAM is expressed on mouse embryonic stem cells and was described as a marker for the identification of successfully reprogrammed induced pluripotent stem cells (iPS).[3]

1.3 Applications

- Positive selection of cells expressing mouse CD326 (EpCAM) antigen.

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 to +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: CD326 (EpCAM)$^+$ cells can be enriched by using LS Columns. Positive selection can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator Plus.

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Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
LS	4×10^7	5×10^7	MidiMACS, QuadroMACS, SuperMACS II,
	4×10^7	5×10^7	MultiMACS Cell24 Separator Plus
autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24 Column Block (per column)	2×10^7	2.5×10^7	MultiMACS Cell24 Separator Plus

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

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD326 (EpCAM)-APC and CD45-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) for tissue dissociation when working with primary tissue.
- (Optional) Tumor Dissociation Kit, mouse (# 130-096-730).
- (Optional) CD45 MicroBeads, mouse (# 130-052-301).

2. Protocol

2.1 Sample preparation

When working with lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.com/protocols.

Besides its presence on epithelial cells, tumor cells, and embryonic stem cells, CD326 (EpCAM) is expressed on leukocyte subtypes. Therefore, these cells have to be depleted, e.g., by using CD45 MicroBeads, mouse (# 130-052-301), beforehand when epithelial or tumor cells should be isolated from dissociated primary tissue.

Depletion of $CD45^+$ cells

1. Resuspend cells in 90 µL of buffer per 10^7 total cells.
2. Add 10 µL of CD45 MicroBeads per 10^7 total cells.
3. Mix well and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
4. Wash cells by adding 1 mL of buffer per 10^7 cells and centrifuge at 300 × g for 5 minutes. Aspirate supernatant completely.
5. Place LS Column in the magnetic field of a suitable MACS Separator.

   ▲ **Note:** Automated separation can be performed by using the autoMACS Pro Separator with the following program: **Depletes**.
6. Prepare column by rinsing with 3 mL of buffer.
7. Resuspend cells in 500 µL of buffer and apply cell suspension onto the column.
8. Collect unlabeled cells that pass through. Perform three washing steps with 3 mL of buffer each.
9. Collect total effluent; this is the $CD45^-$ fraction.
10. (Optional, if $CD45^+$ cells are needed) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
11. Proceed with enrichment of $CD326\ (EpCAM)^+$ cells.

▲ 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 to +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 CD326 (EpCAM) MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendation.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300 × g for 10 minutes. Aspirate supernatant

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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 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 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 as soon as 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 CD326 (EpCAM)$^+$ 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.

Magnetic separation with the MultiMACS™ Cell24 Separator

Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

Magnetic separation with the autoMACS® Pro Separator

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

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

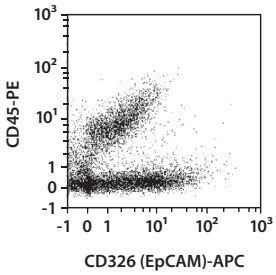
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

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

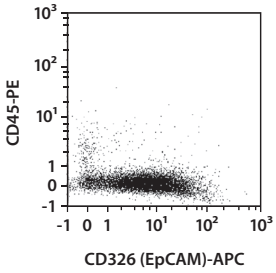
3. Example of a separation using CD326 (EpCAM) MicroBeads

Tumors induced by 4T1 cell line were dissociated by using the Tumor Dissociation Kit, mouse (# 130-096-730) and the gentleMACS Octo Dissociator (# 130-095-937). CD45$^+$ cells were depleted by using the CD45 MicroBeads, a MidiMACS™ Separator, and a LS Column. CD326 (EpCAM)$^+$ cells were isolated by using the CD326 (EpCAM) MicroBeads, a MidiMACS Separator, and a LS Column. The cells were fluorescently stained with CD45-PE and CD326 (EpCAM)-APC 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



Isolated CD45$^-$CD326 (EpCAM)$^+$ cells



4. References

1. Nelson, A. J. *et al.* (1996) The murine homolog of human Ep-CAM, a homotypic adhesion molecule, is expressed by thymocytes and thymic epithelial cells. Eur. J. Immunol. 26: 401–408.
2. Borkowski, T. A. *et al.* (1996) Expression of gp40, the murine homologue of human epithelial cell adhesion molecule (Ep-CAM), by murine dendritic cells. Eur. J. Immunol. 26: 110–114.
3. Chen, H. F. *et al.* (2011) Surface marker epithelial cell adhesion molecule and E-cadherin facilitate the identification and selection of induced pluripotent stem cells. Stem Cell Rev. 7 (3): 722–735.

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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