Miltenyi Biotec

Anti-CLA MicroBead Kit
human

Order no. 130-092-464

Index

1. Description

Components	**1 mL Anti-CLA-PE, human:** Monoclonal anti-human CLA antibody conjugated to R-phycoerythrin (PE) (clone: HECA-452; isotype: rat IgM). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	Anti-CLA-PE is supplied in a solution containing stabilizer and 0.05% sodium azide. Anti-PE MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First the CLA$^+$ cells are labeled with Anti-CLA-PE. Subsequently, the cells are magnetically labeled with Anti-PE MicroBeads. The cell suspension is then loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled CLA$^+$ cells are retained in the column. The unlabeled cells run through, this cell fraction is depleted of CLA$^+$ cells. After removing the column from the magnetic field, the magnetically retained CLA$^+$ cells can be eluted as the positively selected cell fraction. To increase purity, the positively selected cell fraction containing the CLA$^+$ cells is separated over a second column.

1.2 Background and product applications

The cutaneous lymphocyte-associated antigen (CLA) is an inducible carbohydrate modification of P-selectin glycoprotein ligand 1 (PSGL-1) and functions as ligand for E-selectin. CLA is a unique skin-homing receptor and is predominantly found on a minor subset of human T cells that infiltrate the skin. This post-translational modification of PSGL-1 is discussed to serve as mechanism to regulate homing of CD4$^+$ and CD8$^+$ memory/effector T cells from peripheral blood to the skin, which plays an essential role during many inflammatory and certain malignant skin diseases.[1–3]
In peripheral blood, CLA is not only found on skin-homing memory/effector T cells. The E-selectin ligand is also expressed on memory/effector B cells,[4] NK cells,[7] blood dendritic cells,[8] and on monocytes[1,5]. CLA is furthermore found on Langerhans cells in the skin.[6]

Example of applications

- Isolation of CLA$^+$ cells from peripheral blood or skin for phenotypical or functional characterization.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate-buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin), 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 (4–8 °C).

  ▲ **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 gelatine, human serum, or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CLA$^+$ cells can be enriched by using MS, LS, or XS Columns (positive selection). The Anti-CLA MicroBead Kit can also be used for depletion of CLA$^+$ cells on LD, CS, or D Columns. Positive selection or depletion can also be performed by using the autoMACS Separator.

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

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Additional staining reagents, such as CD4-FITC or CD4-APC, CD8-FITC, CD8-APC, CD14-FITC or CD14-APC, and CD19-FITC or CD19-APC.

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- (Optional) CD8$^+$ T Cell Isolation Kit II (# 130-091-154), B Cell Isolation Kit II (# 130-091-151) or Monocyte Isolation Kit II (# 130-091-153).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 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 (e.g. Ficoll-Paque™, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

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 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 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge the cells at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 100 µL of buffer per 10^7 cells.
4. Add 10 µL of Anti-CLA-PE per 10^7 cells.
5. (Optional) Add additional staining antibodies, e.g. 10 µL of CD4-FITC or CD4-APC.

   ▲ **Note:** Do not use tandem conjugates of phycoerythrin, like BD Cy-Chrome™ (Pharmingen), PE-Cy5 (Serotec), ECD, PC5 (Coulter-Immunotech) etc., they may also be recognized by the Anti-PE MicroBeads.
6. Mix well and incubate for 10 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 cells.
9. Add 20 µL of Anti-PE MicroBeads per 10^7 cells.
10. Mix well and incubate for additional 15 minutes at 4–8 °C.
11. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
12. 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.
13. 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 CLA$^+$ cells (see table 1.3).

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL.
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared 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 (see "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 which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (see "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 (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 30 mL buffer from the top. Collect total effluent. 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™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Possel"

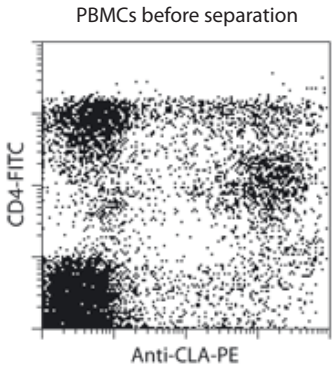
   Depletion: "Depletes"

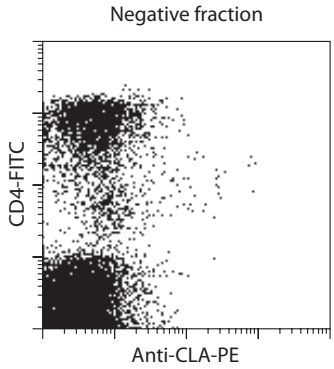
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CLA$^+$ cell fraction.

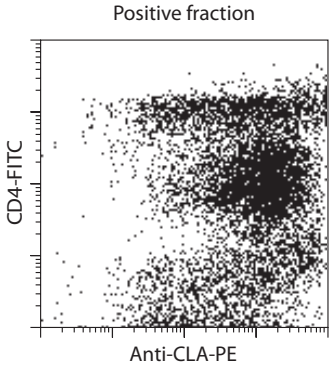
   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CLA$^-$ cell fraction.

3. Example of a separation using the Anti-CLA MicroBead Kit

CLA$^+$ cells were isolated from human PBMCs by using the Anti-CLA MicroBead Kit, an MS Column, and a MiniMACS™ Separator. The cells are fluorescently stained with CD4-FITC and Anti-CLA-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.







4. References

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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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