Miltenyi Biotec

Isolation of CD10$^+$ B cells from human bone marrow or lysed blood

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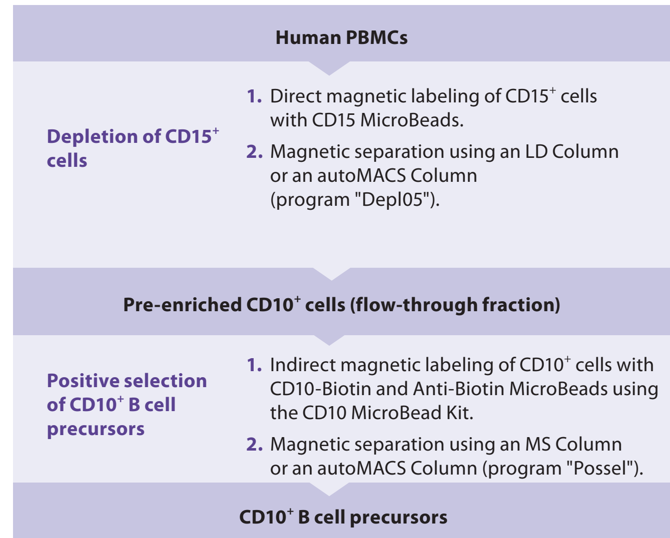
1. Description

1.1 Principle of the MACS® Separation

The isolation of CD10$^+$ B cell precursors is performed in a two-step procedure. First, CD15$^+$ cells are directly magnetically labeled with CD15 MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, CD10$^+$ cells are indirectly labeled with CD10-Biotin and Anti-Biotin MicroBeads and isolated by positive selection from the pre-enriched CD15$^-$ cell fraction.
After removing the column from the magnetic field, the magnetically retained CD10$^+$ B cell precursors can be eluted as the positively selected cell fraction.



1.2 Background information

CD10, the human common acute lymphoblastic leukemia antigen (CALLA), is a 100 kDa cell surface molecule identical to human membrane-associated neutral endopeptidase (NEP) and also known as neprilysin or enkephalinase. Human CD10 is expressed on a wide variety of normal and neoplastic cell types from different tissues including neural and hematopoietic cells.
CD10 is expressed on pre– and pro–B cells and is involved in B cell development and differentiation. The antigen is also present on mature neutrophils, T cell precursors, and some T cell leukemias/lymphomas. Furthermore, CD10 is found on neoplastic cells of several B lymphoid leukemias/lymphomas.[1,2]
For isolation of CD10$^+$ pre–B cells from bone marrow aspirates or lysed blood, prior depletion of CD15$^+$ granulocytes abundant in these specimens is highly recommended.

1.3 Application

- Pre-depletion of CD15$^+$ granulocytes and isolation of CD10$^+$ B cell precursors from bone marrow aspirates.
- Isolation of CD10$^+$ B cells from patients with B cell leukemia/lymphoma.

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:** 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.

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

- CD10 MicroBead Kit, human (# 130-093-452) containing CD10-Biotin and Anti-Biotin MicroBeads.
- CD15 MicroBeads, human (# 130-046-601).
- MACS Columns and MACS Separators: Depletion of CD15+ cells is performed on an LD Column. The subsequent positive selection of CD10+ cells is performed on an MS Column. Depletion and positive selection can also be performed by using the autoMACS™ Separator or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
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™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-FITC (# 130-091-328) and Anti-Biotin-APC (# 130-090-856). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (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 (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

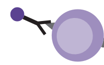
When working with bone marrow aspirates, bone marrow mononuclear cells (BM MNCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **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.

When working with lysed blood, prepare a single-cell suspension using standard methods.

For details see the protocols section at www.miltenyibiotec.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 of CD15+ cells

▲ 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).

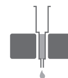
▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

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

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 CD15 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. 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.
8. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of CD15+ cells

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details 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 that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled granulocyte-depleted cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of CD10+ B cell precursors.

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Depletion 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.

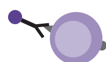
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Depletion 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 the following program:
   Depletion: "Depl05"
   Collect negative fraction in row B of the tube rack. This is the pre-enriched CD10⁺ cell fraction.

Depletion 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 the following program:
   Depletion: "Depl05"
   Collect negative fraction from outlet port neg1. This is the pre-enriched CD10⁺ cell fraction.



2.4 Magnetic labeling of CD10⁺ cells

▲ Volumes for magnetic labeling of pre-enriched CD10⁺ cells given below are for an initial starting cell number of up to 10^7 cells.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 40 µL of buffer.
3. Add 10 µL of CD10-Biotin.
4. Mix well and incubate for 10 minutes in the refrigerator (2−8 °C).
5. Add 30 µL of buffer and 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
6. Mix well and incubate for 15 minutes 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 (2.5).

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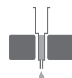

2.5 Magnetic separation: Positive selection of CD10⁺ B cell precursors

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see the MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent 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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled CD10⁺ cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of CD10⁺ cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection 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.

Positive selection 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 the following program:
   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

Positive selection 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 the following program:
   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos1.

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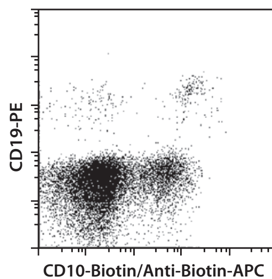
2.6 (Optional) Evaluation of CD10$^+$ cell purity

The purity of enriched CD10$^+$ cells or any intermediate fraction can be evaluated by flow cytometry. Stain aliquots of cell fractions with fluorochrome-conjugated antibodies against CD19, e.g., CD19-PE (# 130-091-247) and against Biotin, e.g., Anti-Biotin-APC (# 130-090-856).

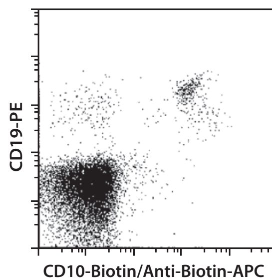
3. Example of a separation using CD15 MicroBeads and the CD10 MicroBead Kit

CD10$^+$ B cell precursors were isolated from human bone marrow mononuclear cells (BM MNCs) using CD15 MicroBeads, the CD10 MicroBead Kit, an LD and an MS Column, and appropriate MACS Separators. Cells were fluorescently stained with CD19-PE (# 130-091-247) and Anti-Biotin-APC (# 130-090-856) and analyzed using the MACSQuant™ Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

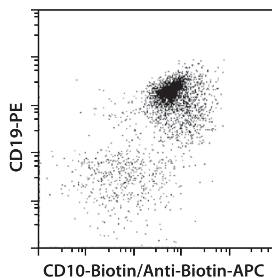
Before separation



Pre-enriched CD10$^+$ cells



CD10$^+$ B cell precursors



4. References

1. Shipp, M. A. and Look, A. T. (1993) Hematopoietic differentiation antigens that are membrane-associated enzymes: cutting is the key! Blood 82: 1052–1070.
2. Kalled, S. L. *et al.* (1995) The distribution of CD10 (NEP 24.11, CALLA) in human and mice is similar in non-lymphoid organs but differs within the hematopoietic system: absence on murine T and B lymphoid progenitors. Euro. J. Immunol. 25: 677–687.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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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