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1. Description

1.1 Principle of the MACS® Separation

The isolation of highly pure CD138⁺ plasma cells can be performed in a two-step procedure. First, CD20⁺ B cells are magnetically labeled with CD20 MicroBeads. The magnetically labeled cells are subsequently depleted by separation over a MACS® Column.

In a second step the CD138⁺ plasma cells are magnetically labeled with CD138 MicroBeads for positive selection. The magnetically labeled CD138⁺ plasma cells are retained within a MACS Column and eluted after removal of the column from the magnetic field.

1.2 Background information

The frequency of CD138⁺ plasma cells in PBMCs is normally very low. To obtain best purities and recoveries by magnetic separation, we recommend the depletion of CD20⁺ B cells with CD20 MicroBeads prior to positive selection of CD138⁺ plasma cells with CD138 MicroBeads. The CD20 antigen is a 33–37 kDa non-glycosylated transmembrane protein, which is exclusively expressed on B cells (pre-B cells, naive and memory B cells) but not on early B cell progenitors or plasma cells^{1,2}. CD138, also known as Syndecan-1, is primarily expressed on plasma cells in the bone marrow. It is also expressed on a subset of CD138⁺ plasma cells in peripheral blood and some lymphoid tissues.³

1.3 Application

- Isolation of CD138⁺ plasma cells from human PBMCs for further phenotypical characterization or functional analysis.

1.4 Reagent and instrument requirements

- CD20 MicroBeads (# 130-091-104).
- CD138 MicroBeads (# 130-051-301).
- 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. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- MACS Columns and MACS Separators: Depletion of CD20⁺ B cells is performed on an LD Column. The subsequent positive selection of CD138⁺ plasma cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS 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, autoMACS Pro

▲ **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-APC (# 130-091-248) and CD138-PE (130-081-301). For more information about other fluorochrome conjugates 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

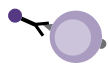
2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **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 tissues or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available 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 CD20⁺ B 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 2×10⁷ total cells. When working with fewer than 2×10⁷ 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 4×10⁷ 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 separation. Pass cells through 30 μm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Wet 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 2×10⁷ total cells.
4. Add 20 μL of CD20 MicroBeads per 2×10⁷ 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 2×10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. Resuspend up to 10⁸ 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 1.25×10⁸ cells in 500 μL of buffer.

8. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of CD20⁺ B cells

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 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 CD138⁺ plasma cells.

Depletion with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

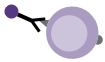
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Depletion with the autoMACS™ 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:
Depletion: “Depl05”
Collect negative fraction from outlet port neg1. This is the pre-enriched CD138⁺ plasma cell fraction.
4. Proceed to 2.4 for the isolation of CD138⁺ plasma cells.

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 the following program:
Depletion: “Depl05”
Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the isolation of CD138⁺ plasma cells.



2.4 Magnetic labeling of CD138⁺ plasma cells

▲ Volumes for magnetic labeling of pre-enriched CD138⁺ plasma cells given below are for an initial starting cell number of up to 2×10⁷ cells.

1. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of CD138 MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Add staining antibodies, e.g., 10 µL of CD138-PE (# 130-081-301), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 500 µL of buffer.
8. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of CD138⁺ plasma cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells (CD138⁺ plasma cells) by firmly pushing the plunger into the column.
7. To increase the purity of CD138⁺ cells, the eluted fraction should 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™ Separator or the autoMACS™ Pro Separator

- ▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.
- ▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro 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™ 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 pos2.
3. For a standard separation choose one of the following programs:

Positive selection: “Posseld2”
Collect positive fraction from outlet port pos2.

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 one of the following programs:

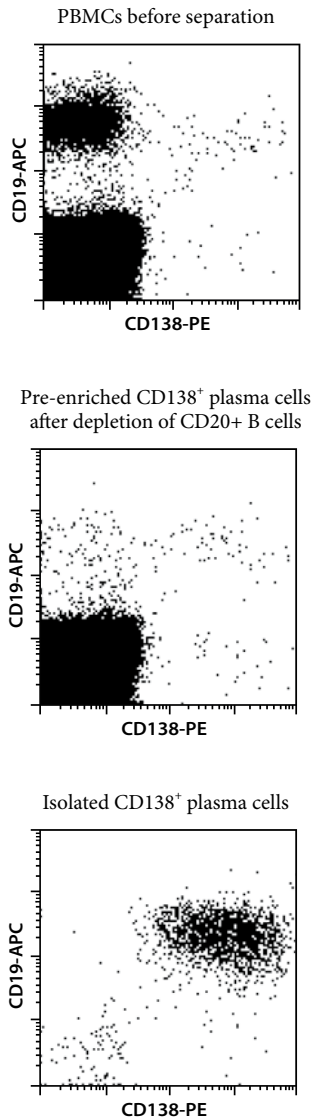
Positive selection: “Posseld2”
Collect positive fraction in row C of the tube rack.

2.6 (Optional) Evaluation of CD138⁺ plasma cells

The purity of enriched CD138⁺ plasma 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-APC (# 130-091-248) and against CD138, e.g., CD138-PE (# 130-081-301).

3. Example of a separation using CD20 and CD138 MicroBeads

CD138⁺ plasma cells were isolated from human PBMCs using CD20 and CD138 MicroBeads, an LD and two MS Columns, and appropriate MACS Separators. Cells are fluorescently stained with CD138-PE (# 130-081-301) and CD19-APC (# 130-091-248). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Polyak, M. J. and Deans, J. P. (2002) CD20 Workshop Panel report; in Mason, D. *et al.* (eds.) Leucocyte typing VII, Oxford University Press.
2. Countouriotis, A. *et al.* (2002) Cell surface antigen and molecular targeting in the treatment of hematologic malignancies. *Stem Cells* 20: 215–229.
3. Medina, F. *et al.* (2002) The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. *Blood* 99: 2154–2161.

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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