

Index

1. Description
 - 1.1 Principle of MACS® separation
 - 1.2 Background and product applications
 - 1.3 Reagent and instrument requirements
2. Protocol
 - 2.1 Sample preparation
 - 2.2 Magnetic labeling
 - 2.3 Magnetic separation
3. Example of a separation using CD20 MicroBeads
4. References

1. Description

Components	2 mL CD20 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD20 antibodies (isotype: mouse IgG1).
Size	For 10 ⁹ total cells, up to 100 separations.
Product format	CD20 MicroBeads are supplied as a suspension containing 0.1% 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 CD20⁺ cells are magnetically labeled with CD20 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 CD20⁺ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD20⁺ cells. After removal of the column from the magnetic field, the magnetically retained CD20⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD20 MicroBeads are developed for the selection of human cells based on the expression of the CD20 antigen. The CD20 antigen is a 33–37 kDa non-glycosylated transmembrane protein that becomes heavily phosphorylated upon activation^{1,2}. It is expressed exclusively on B cells (pre-B cells, naive- and memory B cells) and the majority of B cell lineage malignancies but not on early B cell progenitors or plasma cells^{2,3}. Unlike other anti-CD20 antibodies, including those therapeutically used, the specific antibody clone coupled to MACS MicroBeads induces neither apoptosis nor complement lysis⁴.

Examples of applications

- Positive selection or depletion of cells expressing the human CD20 antigen.
- Isolation or depletion of B cells from peripheral blood mononuclear cells (PBMCs), body fluids (e.g. bronchial lavage), or single-cell suspensions from tissue (e.g. lymphoid and tumor tissue).

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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD20⁺ cells can be enriched by using MS, LS or XS Columns (positive selection). CD20 MicroBeads can be used for depletion of CD20⁺ cells on LD, CS or D Columns. B cells which strongly express the CD20 antigen, such as in some malignancies can also be depleted using MS, LS or XS 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 VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated CD20 antibody for flow-cytometric analysis, e.g. CD20-FITC (# 130-091-108), CD20-PE (# 130-091-109).
- (Optional) PI (propidium iodide) 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/protocols).

▲ **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.

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** 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 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 cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD20 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.
▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add staining antibodies, e.g. add 10 µL of CD20-PE (# 130-091-109), and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. 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.
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 CD20⁺ cells (see table in section 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"

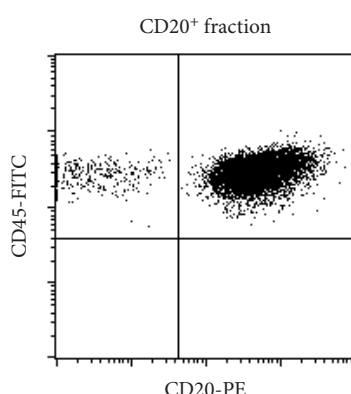
Depletion: "Depletes"

▲ **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 CD20⁺ cell fraction.
When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD20⁻ cell fraction.

3. Example of a separation using CD20 MicroBeads

Separation of PBMCs using CD20 MicroBeads and the autoMACS Separator with the program "Possel". The cells are fluorescently stained with CD20-PE (# 130-091-109) and CD45-FITC (# 130-080-202). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Chang, KL; Arbor, DA; Weiss, LM (1996) CD20: a review. *Applied Histochemistry* 4(1): 1–15
2. Polyak, MJ and Deans, JP (2002) CD20 Workshop Panel report. In: *Leucocyte typing VII*, Oxford University Press.
3. Countouriotis, A; Moore, TB; Sakamoto, KM (2002) Cell Surface Antigen and Molecular Targeting in the Treatment of Hematologic Malignancies. *Review. Stem cells* 20: 215–229
4. Cragg, MS; Asidipour, A; O'Brien, L; Tutt, A; Chan, C; Anderson, VA; Glennie, MJ (2002) Opposing properties of CD20 mAb. In: *Leucocyte typing VII*, Oxford University Press.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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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