

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 CD45RA MicroBeads
4. References

1. Description

Components	2 mL CD45RA MicroBeads, rat: MicroBeads conjugated to monoclonal mouse anti-rat CD45RA antibodies (isotype: mouse IgG1, κ ; clone: OX-33).
Size	For 10^9 total cells, up to 100 separations.
Product format	CD45RA MicroBeads are supplied as a suspension containing 0.1% gelatine 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 CD45RA⁺ cells are magnetically labeled with CD45RA 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 CD45RA⁺ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of labeled cells. After removal of the column from the magnetic field, the magnetically retained CD45RA⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Rat CD45RA MicroBeads are used for the positive selection or depletion of rat B cells from blood, peripheral lymphoid organs like spleen or lymph node and single-cell suspensions from inflamed non-lymphoid tissue. The CD45RA isoform (molecular mass 240 kDa) is B cell lineage specific, and neither expressed on thymocytes nor any peripheral T cell subset.^{1,2} CD45RA was not detected on B cell progenitors in bone marrow.¹

Examples of applications

- Positive selection or depletion of CD45RA⁺ cells from single-cell suspensions of lymphoid or non-lymphoid tissue, or peripheral blood.³

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 in 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 rat serum albumin, rat serum or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD45RA⁺ cells can be enriched by using MS, LS or XS Columns (positive selection). CD45RA MicroBeads can be used for depletion of labeled cells on LD, CS or D Columns. Cells which strongly express the CD45RA antigen 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 CD45RA antibody for flow-cytometric analysis.
- (Optional) PI (propidium iodide) or 7-AAD for flow-cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cells clumps.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs, non-lymphoid tissue or peripheral blood using standard methods (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101) is recommended



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 μ m 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 $300 \times g$ for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 μ L of buffer per 10^7 total cells.
4. Add 20 μ L of CD45RA 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 according to manufacturer's recommendation 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 $300 \times g$ for 10 minutes. Pipette off supernatant completely.
8. Resuspend up to 10^8 cells in 500 μ L 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 μ L 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 CD45RA⁺ 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 μ L 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: $3 \times 500 \mu$ L 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"

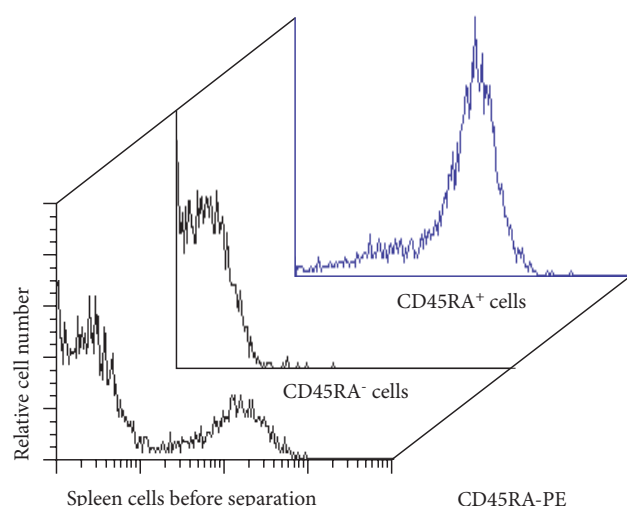
Depletion: "Depletes"

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

3. Example of a separation using CD45RA MicroBeads

Positive selection of CD45RA⁺ cells from rat spleen using CD45RA MicroBeads, a MiniMACS™ Separator and an MS Column. Cells were stained with CD45RA-PE. Histogram shows staining of gated live leukocytes.



4. References

1. Woodlett, GR; Barclay, AN; Puklavec, M; Williams AF (1985) Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. Eur. J. Immunol. 15: 168-173.
2. McCall, MN; Shotton, DM; Barclay, AN (1992) Expression of soluble isoforms of rat CD45. Analysis by electron microscopy and use in epitope mapping of anti-CD45R monoclonal antibodies. Immunology 76: 310-317.
3. Yan Y, Devos T, Yu L, Xia G, Rutgeerts O, Goebels J, Segers C, Lin Y, Vandeputte M, Waer M. (2003) Pathogenesis of autoimmunity after xenogeneic thymus transplantation. J Immunol. 15; 170(12): 5936-46. [3787]

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.

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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