

## 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 CD4 MicroBeads
4. References

## 1. Description

<b>Components</b>	2 mL CD4 MicroBeads, rat: MicroBeads conjugated to monoclonal mouse anti-rat CD4 antibodies (isotype: mouse IgG2a, κ; clone: OX-38).
<b>Size</b>	For 10 <sup>9</sup> total cells, up to 100 separations.
<b>Product format</b>	CD4 MicroBeads are supplied as a suspension containing 0.1% gelatine and 0.05% sodium azide.
<b>Storage</b>	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 CD4<sup>+</sup> cells are magnetically labeled with CD4 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 CD4<sup>+</sup> cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD4<sup>+</sup> cells. After removal of the column from the magnetic field, the magnetically retained CD4<sup>+</sup> cells can be eluted as the positively selected cell fraction.

### 1.2 Background and product applications

CD4 MicroBeads are developed for the positive selection or depletion of CD4<sup>+</sup> cells. CD4 is expressed on most thymocytes, T helper cells and, at a lower level, on macrophages and monocytes.<sup>1</sup> CD4<sup>+</sup> cells can be isolated from blood, lymphoid organs, like spleen, thoracic duct and lymph node, and also from peritoneal fluid or single-cell suspensions of inflamed lung, liver, kidney, nerve and heart tissue. The antigen recognized by the CD4 MicroBeads was shown to be the same as recognized by clone W3/25.<sup>1,2</sup>

### Examples of applications

- Positive selection or depletion of CD4<sup>+</sup> cells from single-cell suspensions of lymphoid or non-lymphoid tissue, or peripheral blood.<sup>3,4</sup>

## 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 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- MACS Columns and MACS Separators: CD4<sup>+</sup> cells can be enriched by using MS, LS or XS Columns (positive selection). CD4 MicroBeads can be used for depletion of CD4<sup>+</sup> cells on LD, CS or D Columns. Cells which strongly express the CD4 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
<b>Positive selection</b>			
MS	10 <sup>7</sup>	2×10 <sup>8</sup>	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10 <sup>8</sup>	2×10 <sup>9</sup>	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10 <sup>9</sup>	2×10 <sup>10</sup>	SuperMACS
<b>Depletion</b>			
LD	10 <sup>8</sup>	5×10 <sup>8</sup>	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10 <sup>8</sup>		VarioMACS, SuperMACS
D	10 <sup>9</sup>		SuperMACS
<b>Positive selection or depletion</b>			
autoMACS	2×10 <sup>8</sup>	4×10 <sup>9</sup>	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 CD4 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 cell 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](http://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  $2 \times 10^7$  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  $\mu$ 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  $\mu$ L of buffer per  $10^7$  total cells.
4. Add 20  $\mu$ L of CD4 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  $\mu$ L of buffer.

▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

▲ **Note:** For depletion with LD Columns, resuspend up to  $1.25 \times 10^8$  cells in 500  $\mu$ 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 CD4<sup>+</sup> 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  $\mu$ 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:  $3 \times 3$  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  $2 \times 1$  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"

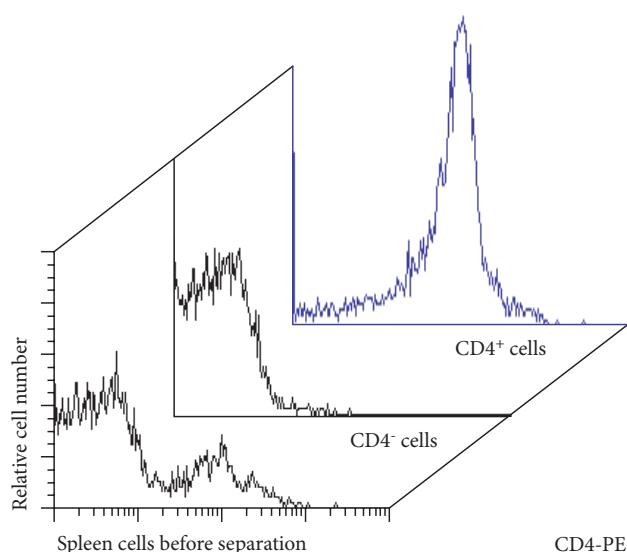
Depletion: "Depletes"

▲ **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 CD4 MicroBeads

Positive selection of CD4<sup>+</sup> cells from rat spleen using CD4 MicroBeads, a MidiMACS™ Separator and an LS Column. Cells are stained with CD4-PE. Histogram shows staining of gated live leukocytes.



## 4. References

1. Jefferies, WA; Green, JR; Williams, AF (1985) Authentic T Helper CD4 (W3/25) Antigen on Rat Peritoneal Macrophages. *J. Exp. Med.* 162: 117-127.
2. Williams, AF; Galfrè, G; Milstein, C (1977) Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. *Cell* 12: 663-673.
3. Yan Y, Devos T, Yu L, Xia G, Rutgeerts O, Goebels J, Segers C, Lin Y, Vandeputte M, Waer (2003) M. Pathogenesis of autoimmunity after xenogeneic thymus transplantation. *J Immunol.* 15; 170(12): 5936-46. [3787]
4. Kataoka M, Margenthaler JA, Ku G, Flye MW. (2003) Development of infectious tolerance after donor-specific transfusion and rat heart transplantation. *J Immunol.* 1; 171(1): 204-11. [3785]

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit [www.miltenyibiotec.com/local](http://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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