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### 1. Description

<b>Components</b>	2 mL CD45 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD45 (Ly-5; isotype: rat IgG2b; clone: 30F11.1) antibody.
<b>Size</b>	For $2 \times 10^9$ total cells, up to 200 separations.
<b>Product format</b>	CD45 MicroBeads are supplied as a suspension containing stabilizer 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 CD45<sup>+</sup> cells are magnetically labeled with CD45 MicroBeads. Then, the cell suspension is loaded onto a MACS<sup>®</sup> Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD45<sup>+</sup> cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD45<sup>+</sup> cells. After removal of the column from the magnetic field, the magnetically retained CD45<sup>+</sup> cells can be eluted as the positively selected cell fraction.

#### 1.2 Background and product applications

CD45 MicroBeads are used for the positive selection or depletion of leukocytes from lymphoid and non-lymphoid tissues. The CD45 antigen is expressed on all cells of hematopoietic origin except erythrocytes and platelets.

#### Examples of applications

- Positive selection or depletion of CD45<sup>+</sup> leukocytes from spleen, lymph nodes, thymus, bone marrow, peripheral blood, body fluids or non-hematopoietic tissue.
- CD45 MicroBeads were used in combination with Anti-Ter-119 MicroBeads (# 130-049-901) for depletion of mouse leukocytes to enrich human cells from bone marrow of chimeric mice.<sup>1</sup>

### 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 mouse serum albumin, mouse 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: CD45<sup>+</sup> cells can be enriched by using MS, LS or XS Columns (positive selection). CD45 MicroBeads can be used for depletion of CD45<sup>+</sup> cells on LD, CS or D Columns. Cells which strongly express the CD45 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 CD45 antibodies, e.g. CD45-FITC (# 130-091-609), CD45-PE (# 130-091-610) or CD45-APC (# 130-091-811).
- (Optional) PI (propidium iodide) or 7-AAD for the 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](http://www.miltenyibiotec.com)).

▲ Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells we recommend to remove dead cells by density gradient centrifugation or using the Dead Cell Removal Kit (# 130-090-101).



### 2.2 Magnetic labeling

▲ Work fast, keep the 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  $\mu\text{m}$  nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge at  $300 \times g$  for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 90  $\mu\text{L}$  of buffer per  $10^7$  total cells.
4. Add 10  $\mu\text{L}$  of CD45 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  $\mu\text{L}$  of CD45-FITC (# 130-091-609), 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  $\mu\text{L}$  of buffer.
 

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

▲ **Note:** For depletion with LD columns, resuspend cell pellet in 500  $\mu\text{L}$  of buffer for up to  $1.25 \times 10^8$  cells.
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 CD45<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 $\mu\text{L}$	LS: 3 mL
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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: $3 \times 500 \mu\text{L}$	LS: $3 \times 3 \text{ mL}$
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 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
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▲ **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  $2 \times 1 \text{ mL}$  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 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 autoMACS™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.

- 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".

- When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CD45<sup>+</sup> cell fraction.

When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD45<sup>-</sup> cell fraction.

### 3. Example of a separation using CD45 MicroBeads

CD45<sup>+</sup> cells were isolated from a mouse spleen cell suspension using CD45 MicroBeads, a MiniMACS™ Separator and an MS Column. The cells are fluorescently stained with CD45-FITC (# 130-091-609). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

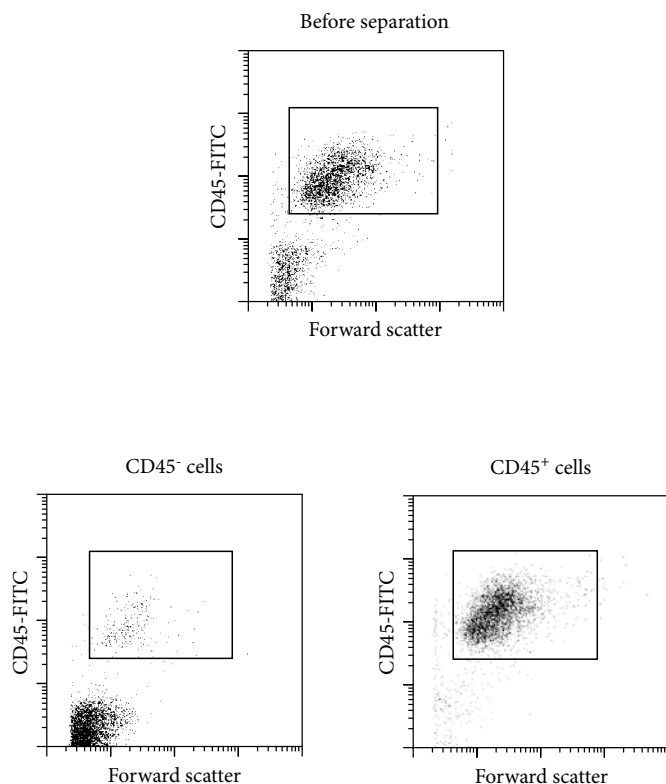
### Warnings

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.

### Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. MILTENYI BIOTEC GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. MILTENYI BIOTEC GmbH's liability is limited to either replacement of the products or refund of the purchase price. MILTENYI BIOTEC GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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### 4. References

- Schiedmeier, B, Kühlicke, K; Eckert, HG; Baum, C; Zeller, WJ; Fruehauf, S (2000) Quantitative assessment of retroviral transfer of the human multidrug resistance 1 gene to human mobilized peripheral blood progenitor cells engrafted in nonobese diabetic/severe combined immunodeficient mice. *Blood* 95: 1237–1248.