



# CD25 MicroBead Kit

## mouse

Order No. 130-091-072

## Magnetic cell sorting

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### 1. Description

<b>Components</b>	<b>1 mL CD25-PE, mouse:</b> monoclonal anti-mouse CD25 antibody conjugated to R-Phycoerythrin (PE) (isotype: rat IgM, kappa; clone: 7D4)
	<b>1 mL Anti-PE MicroBeads:</b> MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1)
<b>Size</b>	For 10 <sup>9</sup> (100×10 <sup>7</sup> ) total cells
<b>Product format</b>	The products are supplied in a solution 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

For MACS® separation, cells are incubated with Anti-CD25-PE and Anti-PE MicroBeads, by which CD25 expressing cells are magnetically labeled. Subsequently cells are isolated by separation over a column which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD25<sup>+</sup> cells are retained on the column, while the unlabeled CD25<sup>-</sup> cells are collected in the flow through. After removal of the column from the magnetic field, the retained CD25<sup>+</sup> cells are eluted.

#### 1.2 Background and product applications

CD25, the low affinity interleukin-2 receptor  $\alpha$  chain (IL-2R $\alpha$ ), is expressed in the early phase (CD4<sup>+</sup>CD8<sup>-</sup>) of thymic T cell development, as well as on activated T and B cells, and at a lower level on activated monocytes. CD25 forms the high affinity IL-2 receptor complex together with the  $\beta$  chain (CD122) and  $\gamma$  chain (CD132). A subpopulation of CD4<sup>+</sup>CD25<sup>+</sup> T cells is supposed to act as regulatory T cells upon activation.<sup>1</sup> The 7D4 antibody does not inhibit the binding of IL-2.<sup>2,3</sup>

### Example of applications

- Positive selection or depletion of CD25<sup>+</sup> cells from single-cell suspensions of spleen, thymus, lymph nodes, etc. for functional, biochemical or molecular analyses, for example.
  - ▲ **Note:** For the isolation of regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells we recommend the CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit (# 130-091-041).

#### 1.3 Reagent and instrument requirements

- Buffer (degassed): phosphate buffered saline (PBS), pH 7.2, supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA. 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 gelatine, murine serum or fetal calf serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Pre-Separation Filters (# 130-041-407)
- MACS Columns and MACS Separators:

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 or SuperMACS. For details, see "MACS Separator data sheets".

140-090-772/02



## 2. Protocol

### 2.1 Sample preparation

Prepare single-cell suspension from lymphoid tissues by using standard methods.



### 2.2 Magnetic labeling

▲ Work fast, keep cells cold, 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  $10^7$  total cells (without erythrocytes). 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 an 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 columns.

- Determine cell number.
- Centrifuge cells at  $300 \times g$  for 10 minutes. Pipette off supernatant completely.
- Resuspend cell pellet in 100  $\mu\text{L}$  of buffer per  $10^7$  total cells.
- Add 10  $\mu\text{L}$  of CD25-PE antibody per  $10^7$  total cells.
- Mix well and incubate for 10 minutes in the dark at  $4-8^\circ\text{C}$ .  
▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
- Wash cells by adding 1–2 mL of buffer and centrifuge at  $300 \times g$  for 10 minutes. Pipette off supernatant completely.
- Resuspend cell pellet in 90  $\mu\text{L}$  of buffer per  $10^7$  total cells.
- Add 10  $\mu\text{L}$  of Anti-PE MicroBeads.
- Mix well and incubate for 15 minutes in the dark at  $4-8^\circ\text{C}$ .  
▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
- Wash cells by adding 1–2 mL of buffer and centrifuge at  $300 \times g$  for 10 minutes. Pipette off supernatant completely.
- Resuspend cell pellet in 500  $\mu\text{L}$  of buffer.  
▲ **Note:** Use a maximum of  $10^8$  cells per 500  $\mu\text{L}$  of buffer. For larger cell numbers, scale up 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.
- 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 CD25<sup>+</sup> cells (see table 1.3).

#### Magnetic separation with MS or LS Columns

- Place the column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
- Prepare column by rinsing with appropriate amount of buffer:  
MS: 500  $\mu\text{L}$       LS: 3 mL
- Apply cell suspension onto the column.
- Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times once the column reservoir is empty.  
MS:  $3 \times 500 \mu\text{L}$       LS:  $3 \times 3 \text{ mL}$ .  
Collect total effluent. This is the unlabeled cell fraction (CD25<sup>-</sup> cells).
- Remove column from the separator and place it on a collection tube.
- Pipette appropriate amount of buffer onto the column.  
MS: 1 mL      LS: 5 mL.  
Immediately flush out fraction of the magnetically labeled cells (CD25<sup>+</sup> cells) by firmly applying the plunger, supplied with the column.  
▲ **Note:** For a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
- To increase the purity of the enriched CD25<sup>+</sup> cells, repeat the magnetic separation procedure as described in steps 1. - 6. by using a new 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

- Place LD Column in the magnetic field of a suitable MACS Separator (see "LD Column data sheet").
- Prepare column by rinsing with 2 mL of buffer.
- Apply cell suspension onto the column.
- 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 (CD25<sup>-</sup> cells).

#### Depletion with CS Columns

- Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (see "CS Column data sheet").
- 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").
- Apply cell suspension onto the column.
- Collect unlabeled cells which pass through and wash with 30 mL buffer from top. Collect total effluent. This is the unlabeled cell fraction (CD25<sup>-</sup> cells).

## 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 the autoMACS Separator.
2. Place the tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose from the following separation programs:

Positive selection: "Posseld".

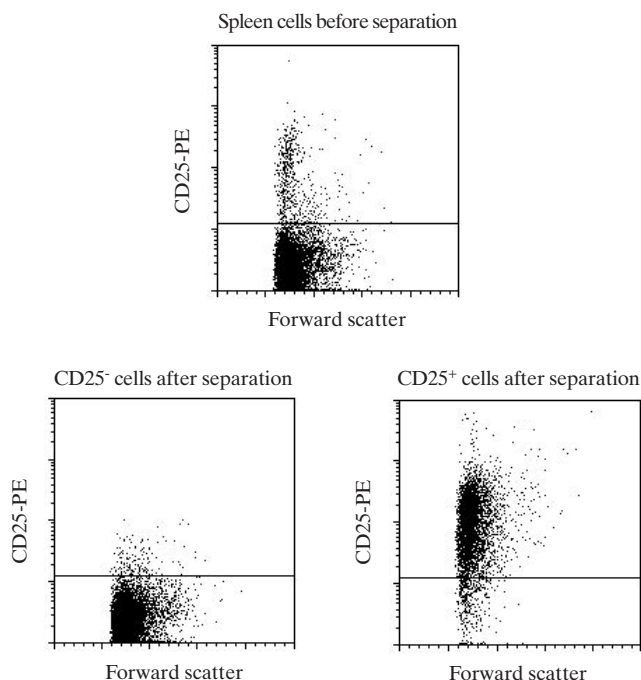
Depletion: "Depletes".

▲ **Note:** The program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details, see the "autoMACS User Manual", chapter "autoMACS Cell Separation Programs".

3. When using the program "Posseld", collect positive fraction. This is the purified CD25<sup>+</sup> fraction (outlet port "pos2").  
When using the program "Depletes", collect unlabeled fraction. This is the CD25<sup>-</sup> cell fraction (outlet port "neg1").

## 3. Example of a separation using the CD25 MicroBead Kit

Separation of mouse spleen cells using the CD25 MicroBead Kit and a MiniMACS™ Separator with two MS Columns. Cells were fluorescently stained with CD25-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.



## 4. References

1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor  $\alpha$ -chains (CD25). J. Immunol. 155: 1151–1164.
2. Ortega RG, Robb RJ, Shevach EM, Malek TR (1984) I. Monoclonal antibodies that define distinct functional epitopes on activated T cells and react with activated B cells. J. Immunol. 133: 1970–1975.
3. Malek TR, Robb RJ, Shevach EM (1983) Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor-ligand complex. Immunology 80: 5694–5698.

## 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

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