Miltenyi Biotec

CD25 MicroBead Kit
mouse

Order no. 130-091-072

Contents

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.

1. Description

This product is for research use only.

Components	**0.2 mL CD25-PE, mouse:** monoclonal CD25 antibody conjugated to R-Phycoerythrin (PE) (isotype: rat IgM, kappa). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-xy antibodies (isotype: mouse IgG).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the $CD25^+$ cells are indirectly magnetically labeled with CD25-PE and Anti-PE 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 $CD25^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD25^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD25^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the $CD25^+$ cells must be separated over a second column.

1.2 Background information

CD25, the low affinity interleukin-2 receptor α chain (IL-2Rα), is expressed in the early phase ($CD4^-CD8^-$) 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 β chain (CD122) and γ chain (CD132). A subpopulation of $CD4^+CD25^+$ T cells is supposed to act as regulatory T cells upon activation.[1]
The 7D4 antibody does not inhibit the binding of IL-2.[2,3]

1.3 Applications

- Positive selection or depletion of $CD25^+$ 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 $CD4^+CD25^+$ T cells the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit (# 130-091-041) is recommended.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), 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 (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **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 bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: $CD25^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD or D Columns. Cells which strongly express the CD25 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, SuperMACS II
D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ II Separators. For details refer to the respective MACS Separator

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data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ 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 labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 98 µL of buffer per 10^7 total cells.
4. Add 2 µL of CD25-PE per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
8. Add 10 µL of Anti-PE MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. 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.

12. 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 $CD25^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of $CD25^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 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

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.

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3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

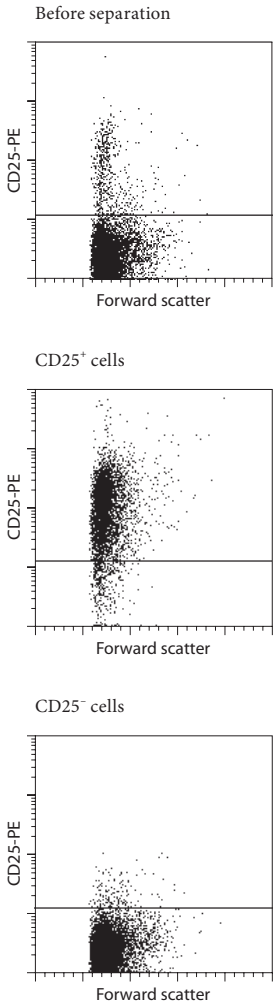
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using the CD25 MicroBead Kit

$CD25^+$ cells were isolated from mouse spleen using the CD25MicroBead Kit, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD25-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Sakaguchi, S. *et al.* (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). J. Immunol. 155: 1151–1164.
2. Ortega, R. G. *et al.* (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, T. R. *et al.* (1983) Identification and initial characterization of a rat monoclonal antibody reactive with the murine interleukin 2 receptor-ligand complex. Immunology 80: 5694–5698.

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.

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