Miltenyi Biotec

Anti-TCR γ/δ MicroBeads
rat

Order no. 130-090-496

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1. Description

Components	2 mL Anti-TCR γ/δ MicroBeads, rat: MicroBeads conjugated to monoclonal mouse anti-rat Anti-TCR γ/δ antibodies (isotype: mouse IgG1, κ; clone: V45).
Size	For 10^9 total cells, up to 100 separations.
Product format	Anti-TCR γ/δ 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 TCR γ/δ$^+$ cells are magnetically labeled with Anti-TCR γ/δ 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 TCR γ/δ$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of TCR γ/δ$^+$ cells. After removal of the column from the magnetic field, the magnetically retained TCR γ/δ$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Anti-TCR (T cell receptor) γ/δ MicroBeads are developed for the positive selection or depletion of T cells, expressing TCR γ/δ from blood, lymphoid tissue and non-lymphoid tissue, like gut, skin or liver. Analysis of peripheral lymphoid organs has shown that the recognized epitope is expressed on about 80%–90% of the TCR γ/δ$^+$ T cells.[1, 2] By immunofluorescence staining it was shown that TCR γ/δ$^+$ T cells are OX-52$^+$, CD2$^+$, CD3$^+$, CD5$^+$ and almost all express CD8a.[1-3] The recognized TCR complex is also found on a subpopulation of intestinal intraepithelial lymphocytes.[4]

Examples of applications

- Positive selection or depletion of TCR γ/δ$^+$ cells from single-cell suspensions of lymphoid or non-lymphoid tisssue, or peripheral blood.

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^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: TCR γ/δ$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). Anti-TCR γ/δ MicroBeads can be used for depletion of TCR γ/δ$^+$ cells on LD, CS or D Columns. Cells which strongly express the TCR γ/δ 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 Anti-TCR γ/δ 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.

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2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation (e.g. Ficoll-Paque®, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** 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 separation. Pass cells through 30 µm 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×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD3 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 µL of CD3-FITC (# 130-080-401), 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×g for 10 minutes. Pipette off supernatant completely.
8. 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.
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 $CD3^+$ 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 µL 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: 3×500 µL 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".

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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"

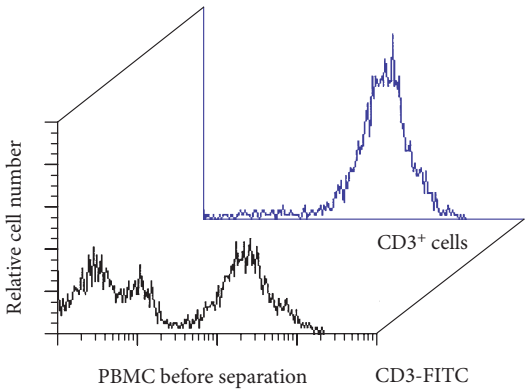
   Depletion: "Depletes"

   ▲ **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 $CD3^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the $CD3^-$ cell fraction.

3. Example of a separation using CD3 MicroBeads

Separation of PBMCs using CD3 MicroBeads and a MiniMACS™ Separator with an MS Column. The cells are fluorescently stained with CD3-FITC (# 130-080-401). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Kühnlein, P; Mitnacht, R; Torres-Nagel, NE; Herrmann, T; Elbe, A; Hünig, T (1996) The canonical T cell receptor of dendritic epidermal γδ T cells is highly conserved between rats and mice. Eur. J. Immunol. 26: 3092-3097.
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3. Lawetzky, A; Tiefenthaler, G; Kubo, R; Hunig, T (1990) Identification and characterization of rat T cell subpopulations expressing T cell receptors α/β and γ/δ. Eur. J. Immunol. 20: 343-9.
4. Todd, D; Singh, AJ; Greiner, DL; Mordes, PJ; Rossini, AA; Bortell, R (1999) A new isolation method for rat intraepithelial lymphocytes. J. Immunol. Meth. 224: 111-127.
5. Wildner, G; Hünig, T; Thurau, SR (1996) Orally Induced Peptide-Specific γ/δ TCR+ Cells Suppress Experimental Autoimmune Uveitis. Eur. J. Immunol. 26: 2140-2148. [354].

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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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