Miltenyi Biotec

# Anti-TCR γ/δ MicroBead Kit
human

Order No. 130-050-701

## Index



## 1. Description

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|---|---|
| **Components** | **1 mL Anti-TCR γ/δ Hapten-Antibody** (isotype: mouse IgG1; clone 11F2),<br>**2 mL Anti-Hapten MicroBeads-FITC**: MicroBeads conjugated to FITC and a monoclonal anti-hapten antibody (isotype: mouse IgG2a). |
| **Size** | For $10^9$ total cells, up to 100 separations. |
| **Product format** | The Anti-Hapten 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

For magnetic isolation the TCR γ/δ$^+$ cells are labeled with the Anti-TCR γ/δ MicroBead Kit. First, the cells are incubated with Anti-TCR γ/δ Hapten-Antibodies, and then fluorescently and magnetically labeled with Anti-Hapten MicroBeads-FITC. Then the cell suspension is loaded on a 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, 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. The TCR γ/δ$^+$ cells are fluorescently stained for flow-cytometric analysis.

### 1.2 Background and product applications

The Anti-TCR γ/δ MicroBead Kit is an indirect magnetic and fluorescent labeling system for T cells expressing the γ/δ-variant of the T cell receptor (TCR γ/δ). TCR γ/δ is expressed by up to 10% of peripheral blood T cells.
The Anti-TCR γ/δ MicroBead Kit is a magnetic labeling system for the positive selection or depletion of TCR γ/δ expressing T cells from peripheral blood, lymph nodes, thymus, spleen and cultured cells. TCR γ/δ$^+$ T cells isolated with the Anti-TCR γ/δ MicroBead Kit have been expanded and used for proliferation and cytotoxicity assays.[1,2,3]

**Examples of applications**

- Positive selection or depletion of cells expressing human TCR γ/δ.
- Isolation or depletion of TCR γ/δ expressing T cells from peripheral blood mononuclear cells (PBMC), lymph nodes, thymus, spleen and epithelial tissue.

### 1.3 Reagent and instrument requirements

- Buffer (degassed): PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% 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 human serum albumin, human 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). The Anti-TCR γ/δ MicroBead Kit can be used for depletion of TCR γ/δ$^+$ cells on LD, CS or D Columns. Since the Anti-TCR γ/δ MicroBead Kit enables strong magnetic labeling of TCR γ/δ expressing T cells, effficient depletion is possible even on positive selection 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 | $10^7$ | $2\times10^8$ | MiniMACS, OctoMACS, VarioMACS, SuperMACS |
| LS | $10^8$ | $2\times10^9$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| XS | $10^9$ | $2\times10^{10}$ | SuperMACS |
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| CS | $2\times10^8$ | | VarioMACS, SuperMACS |
| D | $10^9$ | | SuperMACS |
| **Positive selection or depletion** | | | |
| autoMACS | $2\times10^8$ | $4\times10^9$ | autoMACS |

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) Additional fluorochrome-conjugated staining antibodies, e.g. CD3-PE (# 130-091-374) and CD8-APC (# 130-091-076).


**Miltenyi Biotec B.V. & Co. KG**
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
**www.miltenyibiotec.com**





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- (Optional) PI (propidium iodide) or 7-AAD for the flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filter (# 130-041-407).

## 2. Protocol

### 2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **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).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation (e.g. Ficoll-Paque™) 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 $2\times10^7$ 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 Filter # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per $10^7$ total cells.
4. Add 10 µL Anti-TCR γ/δ Hapten-Antibody per $10^7$ total cells.

   ▲ **Note:** Cells labeled with the Anti-TCR γ/δ MicroBead Kit cannot be additionally stained with another flourochrome-conjugated anti-TCR γ/δ antibody against the same epitope, since the Anti-TCR γ/δ Hapten-Antibody ocuppies most of the epitopes.
5. Mix well and incubate for 10 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. Add 30 µL of buffer and 20 µL of MACS Anti-Hapten MicroBeads-FITC per $10^7$ total cells.
7. 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.
8. Wash cells by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300xg for 10 minutes. Pipette off supernatant completely.
9. 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 cell pellet in 500 µL of buffer for up to $1.25\text{x}10^8$ cells.
8. 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 TCR $\gamma/\delta^+$ cells (see table in section 1.3).

▲ The efficiency of the separation of TCR $\gamma/\delta^+$ cells is easily evaluated by flow cytometry or fluorescence microscopy because of FITC-conjugated Anti-Hapten MicroBeads.

#### 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: 3x500 µL  LS: 3x3 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 second, 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 2x1 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.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Possel"

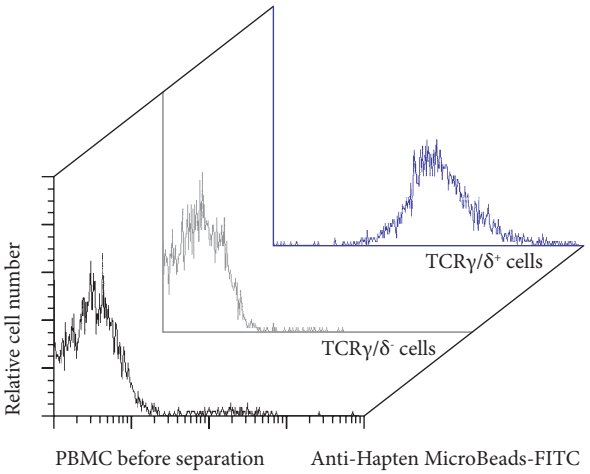
   Depletion: "Depletes"

   ▲ **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 TCR γ/δ$^+$ cell fraction.

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

## 3. Example of a separation using the Anti-TCR γ/δ MicroBead Kit

Separation of human PBMC using Anti-TCR γ/δ MicroBead Kit and a MiniMACS™ Separator with an MS Column. Cells are fluorescently stained with Anti-Hapten MicroBeads-FITC.




## 4. References

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2. Schilbach,K; Geiselhart, A; Handgretinger, R (2001) Induction of proliferation and augmented cytotoxicity of γ/δ T lymphocytes by bisphosphonate clodronate. Blood 97: 2917–2918. [2287]
3. Rothenfusser, S; Hornung, V; Krug A; Towarowski, A; Krieg, AM; Endres, S; Hartmann, G (2001) Distinct CpG oligonucleotide sequences activate human γ/δ T cells via interferon-α/-β. Eur. J. Immunol. 31: 3525–3534. [2010]

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.

### 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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