Miltenyi Biotec

CD4 (TIL) MicroBeads
mouse

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Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
   - 2.4 Cell separation with the autoMACS® Pro Separator
3. Example of a separation using CD4 (TIL) MicroBeads

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	1 mL CD4 (TIL) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD4 antibodies (isotype: recombinant human IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD4 (TIL) MicroBeads 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 $CD4^+$ cells are magnetically labeled with CD4 (TIL) 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 $CD4^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD4^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD4^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the $CD4^+$ cells must be separated over a second column.

1.2 Background information

CD4 (TIL) MicroBeads, mouse have been developed for the isolation of $CD4^+$ TILs (tumor-infiltrating lymphocytes) from single-cell suspensions of solid mouse tumors. The CD4 antigen is expressed on T helper cells, regulatory T cells, and at lower levels on subpopulations of NKT cells and dendritic cells.

1.3 Applications

- Positive selection of $CD4^+$ T cells from solid mouse tumors, e.g., CT26.WT, 4T1, or B16-F10.

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. Always use freshly prepared buffer.

  ▲ **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: $CD4^+$ cells can be enriched by using MS or LS Columns. Positive selection can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator Plus.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	4×10^7	5×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II,
	4×10^7	5×10^7	MultiMACS Cell24 Separator Plus
autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24 Column Block (per column)	2×10^7	2.5×10^7	MultiMACS Cell24 Separator Plus

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

▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- Tumor Dissociation Kit, mouse (# 130-096-730) for the generation of single-cell suspension from tumor tissues.

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- gentleMACS™ Dissociator (# 130-093-235), gentle MACS Octo Dissociator (# 130-095-937), gentle MACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Fluorochrome-conjugated REA (REAfinity™ antibodies: recombinantly engineered, lacking Fcγ-binding site) CD4 and CD3 antibodies for flow cytometric analysis, e.g., CD4-PE and CD3ε-APC-Vio® 770. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** Due to expression of Fcγ receptors on tumor-infiltrating leukocytes REA antibodies are recommended.
- (Optional) Propidium Iodide Solution (# 130-093-233), DAPI Staining Solution (# 130-111-570), 7-AAD Staining Solution (# 130-111-568) or Viobility™ Fixable Dyes (# 130-109-812, # 130-109-814, # 130-109-816) 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.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For preparation of a single-cell suspension from solid mouse tumors use the Tumor Dissociation Kit, mouse (# 130-096-730) in combination with the gentleMACS™ Dissociators.

For details refer to www.gentlemacs.com/protocols

▲ Dead cells may bind non-specifically to MACS® MicroBeads. To remove dead cells, we recommend using the Dead Cell Removal Kit (# 130-090-101).



2.2 Magnetic labeling

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.1.

▲ 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 (MACS SmartStrainers (30 µm), # 130-098-458). 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 90 µL of buffer per 10^7 total cells.

   ▲ **Note:** Always use freshly prepared buffer.
4. Add 10 µL of CD4 (TIL) MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendations.
7. Add buffer to a final volume of 500 µL for up to 5×10^7 cells.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Wash cells with 1 mL of buffer per 10^7 cells. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
9. Resuspend up to 5×10^7 cells in 500 µL of buffer.

   ▲ **Note:** If more cells were used, split the sample onto multiple columns during magnetic separation.
10. 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 $CD4^+$ cells. For details refer to the table in section 1.4.

▲ **Note:** MS Columns are recommended for highest purity of $CD4^+$ cells. LS Columns are recommended for highest recovery of $CD4^+$ cells.

▲ 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 (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ 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: 2×500 µL LS: 2×1 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.

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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: 3 mL

7. (Optional) To increase the purity of $CD4^+$ 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 the MultiMACS™ Cell24 Separator

Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

2.4 Cell separation with the autoMACS® Pro Separator

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

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

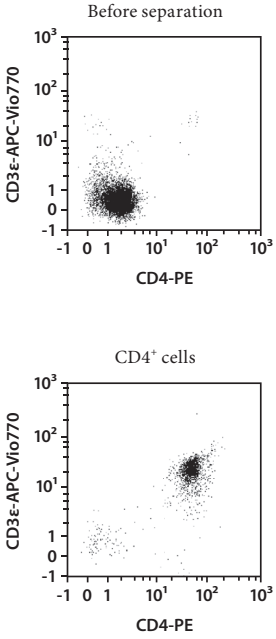
2.4.2 Magnetic separation using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose one the following programs:

   Positive selection:
   Posseld2 for highest purity
   or
   Possels for highest recovery
   Collect positive fraction in row C of the tube rack.

3. Example of a separation using CD4 (TIL) MicroBeads

A tumor induced by the B16-F10 cell line was dissociated using the gentleMACS™ Octo Dissociator with Heaters in combination with the Tumor Dissociation Kit, mouse. $CD4^+$ TILs were isolated from the single-cell suspension using CD4 (TIL) MicroBeads, optional wash step, two MS Columns, and a MiniMACS™ Separator.
Cells were fluorescently stained with CD4-PE and CD3ε-APC-Vio®770 and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and DAPI fluorescence.



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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