Miltenyi Biotec

CD4+ T Cell Isolation Kit
non-human primate

Order no. 130-092-144

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1. Description

Components	**1 mL CD4+ T Cell Biotin-Antibody Cocktail:** Cocktail of biotin-conjugated monoclonal antibodies against CD8, CD11b, CD16, CD20, CD56, and CD66abce. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations
Product format	The Biotin-Antibody Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads are supplied as a suspension containing stabilizer 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.

This product is applicable for the separation of cells from rhesus monkey (*Macaca mulatta*). The antibodies have been tested to cross-react with cynomolgus monkey (*Macaca fascicularis*). Cross-reactivity with other non-human primates has not been tested.

1.1 Principle of MACS® separation

Using the non-human primate CD4+ T Cell Isolation Kit, CD4+ T helper cells are isolated by depletion of non-CD4+ T cells (negative selection). Non-CD4+ T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The magnetically labeled non-CD4+ T cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled T helper cells pass through the column.

1.2 Background and product applications

The CD4+ T Cell Isolation Kit is an indirect magnetic labeling system for the isolation of untouched CD4+ T helper cells from rhesus monkey peripheral blood mononuclear cells (PBMCs). Non-CD4+ T cells, i.e. CD8+ T cells, B cells, NK cells, monocytes and granulocytes, are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies against CD8, CD11b, CD16, CD20, CD56, and CD66abce, and Anti-Biotin MicroBeads. Isolation of highly pure CD4+ T cells is achieved by depletion of magnetically labeled cells.

Examples of applications

- Functional studies on T helper cells in which effects due to minimal antibody-cross-linking of cell surface proteins should be avoided.
- Studies on signal requirements for CD4+ T cell activation, induction of T helper cell proliferation, induction of CD4+ T cell anergy, etc.
- Studies on signal transduction in CD4+ T cells.
- Studies on regulation of CD4+ T cell cytokine expression.

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 with 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 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: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	max. number of labeled cells	max. number of total cells	Separator
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
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 antibodies (e.g. CD4-PE # 130-091-231; CD4-APC # 130-091-232; CD3-PE # 130-092-009; CD3-APC # 130-091-998; CD45-FITC # 130-091-898; Anti-Biotin-PE # 130-090-756, Anti-Biotin-APC # 130-090-856).
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) PI (propidium iodide) or 7-AAD for the flow cytometric exclusion of dead cells.

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2. Protocol

2.1 Sample preparation

When working with rhesus monkey anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation, as with human samples (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 lymphoid 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 or the Dead Cell Removal Kit (# 130-090-101).



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 up to 10^7 total cells. When working with fewer than 10^7 cells, use 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 columns.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension 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 of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 minutes at 4–8 °C.
6. 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.
7. Add 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and incubate for an additional 15 minutes at 4–8 °C.
10. 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.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total cells (see table in section 1.3).

Magnetic separation with MS and 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.

   Allow the cells to pass through and collect effluent as fraction with unlabeled cells, representing the enriched $CD4^+$ T cell fraction.
4. 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 entire effluent in the same tube as effluent of step 3. This fraction represents the enriched $CD4^+$ T cells.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-$CD4^+$ T cells.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

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 the autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose program "Deplete".
3. Collect negative fraction (outlet port "neg1"). This fraction represents the enriched $CD4^+$ T cells.
4. (Optional) Collect positive fraction (outlet port "pos1"). This fraction represents the magnetically labeled non-$CD4^+$ T cells.

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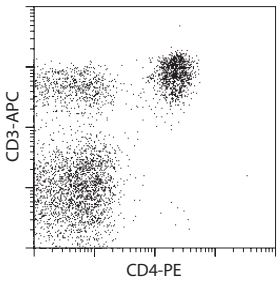
2.4 (Optional) Evaluation of CD4+ T cell purity

The purity of the enriched CD4+ T cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against CD45, e.g. CD45-FITC (# 130-091-898), CD4, e.g. CD4-PE (# 130-091-231) and optionally, against the T cell marker CD3, e.g. CD3-APC (# 130-091-998) as recommended in the respective antibody data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-CD4+ T cells with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g. Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

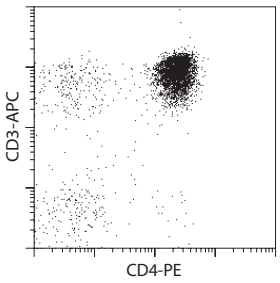
3. Example of a separation using the CD4+ T Cell Isolation Kit

Isolation of untouched CD4+ T cell from rhesus monkey PBMCs using the CD4+ T Cell Isolation Kit and an LS Column. Cells are fluorescently stained with CD4-PE (# 130-091-231) and CD3-APC (# 130-091-998). Plots show CD45+ leukocytes. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

PBMCs before separation



Isolated CD4+ T cells



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.notices

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