Isolation of naive CD4$^+$ T cells from non-human primate PBMCs

Contents

The below mentioned reagents are 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 has not been tested with other non-human primates.

1. Description

1.1 Principle of the MACS® Separation

The isolation of naive CD4$^+$ T cells can be performed by using three reagents:

- CD4$^+$ T Cell Isolation Kit, non-human primate (# 130-092-144), which contains a Biotin-Antibody Cocktail and Anti-Biotin MicroBeads,
- CD95-PE, human (# 130-092-146), and
- Anti-PE MicroBeads (# 130-048-801).

Naive CD4$^+$ T cells are enriched by depletion of non-CD4$^+$ T cells and subsets of memory T cells in a single step. Undesired cells are labeled with a cocktail of selected biotin-conjugated monoclonal antibodies contained in the CD4$^+$ T Cell Isolation Kit, and CD95-PE as primary labeling reagents. For indirect magnetic labeling, Anti-Biotin MicroBeads and Anti-PE MicroBeads are used. The magnetically labeled cells are depleted by retaining within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled naive CD4$^+$ T cells pass through the column.

1.2 Background information

Untouched CD4$^+$ T cells from non-human primate peripheral blood mononuclear cells (PBMCs) can be isolated by depletion of non-CD4$^+$ T cells, i.e., CD8$^+$ T cells, B cells, NK cells, monocytes, and granulocytes. This is achieved by indirect magnetic labeling using a cocktail of biotin-conjugated antibodies against CD8, CD11b, CD16, CD20, CD56, and CD66abce, and Anti-Biotin MicroBeads. Naive T cells can be distinguished from memory T cells by their expression of CD95. Naive T cells are CD95^{low}, whereas subsets of CD4$^+$ and CD8$^+$ memory T cells are CD95^{high}.[1] Therefore, the depletion of CD4$^+$ memory T cells is possible by indirect magnetic labeling using PE-conjugated CD95 and Anti-PE MicroBeads.

1.3 Applications

- Functional studies on naive CD4$^+$ T 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 CD4$^+$ T cell anergy, etc.
- Studies on signal transduction in CD4$^+$ T cells.
- Studies on regulation of CD4$^+$ T cell cytokine expression.

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 human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-CD4$^+$ T cells is performed on LS Columns or using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

▲ **Note:** Column adapters are required to insert LS Columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- CD4$^+$ T Cell Isolation Kit, non-human primate (# 130-092-144).
- CD95-PE (# 130-092-416).
- Anti-PE MicroBeads (# 130-048-801).
- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g., CD4-FITC (# 130-080-501), CD4-APC (# 130-091-232), CD45-FITC (# 130-091-898), CD45-APC (# 130-091-900), CD45RA-FITC (# 130-092-247), or CD45RA-APC (# 130-092-249). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

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- (Optional) Propidium iodide Solution (# 130-093-233) or 7-AAD 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 (# 130-041-407) to remove cell clumps.

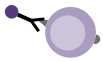
2. Protocol

2.1 Sample preparation

When working with rhesus monkey anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation. For details see the protocols section at 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.

▲ 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. Wet filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. 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. Aspirate 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. Add 5 µL of CD95-PE per 10^7 total cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Add 60 µL of buffer per 10^7 total cells.
9. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
10. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
11. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
12. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
13. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
14. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

Magnetic separation with an LS Column

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see table in section 1.4 and the respective MACS Column data sheet.
2. Prepare LS Column by rinsing with 3 mL of buffer:
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×3 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Collect entire effluent in the same tube as effluent of step before. This fraction represents the enriched $CD4^+CD95^-$ T cells.
7. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-$CD4^+$ T cells.

Magnetic separation with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Separator

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 at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:
   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1.

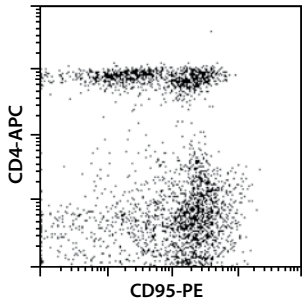
Magnetic separation with the autoMACS™ Pro Separator

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

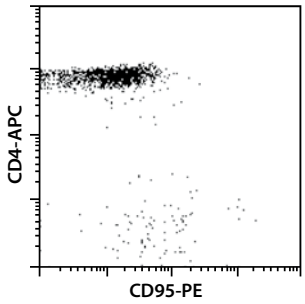
3. Example of a separation using the CD4$^+$ T Cell Isolation Kit, CD95-PE, and Anti-PE MicroBeads

Isolation of naive CD4$^+$ T cells from rhesus monkey PBMCs using the CD4$^+$ T Cell Isolation Kit, CD95-PE, Anti-PE MicroBeads, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD4-APC (# 130-091-232) and CD95-PE (# 130-092-416). Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

PBMCs before separation



Isolated naive CD4$^+$ T cells



4. Reference

1. Pitcher, C. J. *et al.* (2002) Development and Homeostasis of T Cell Memory in Rhesus Macaque. J. Immunol. 168(1): 29–43.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

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