Miltenyi Biotec

Naive Pan T Cell Isolation Kit
human

Order no. 130-097-095

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1. Description

Components

1 mL Naive Pan T Cell Biotin-Antibody Cocktail, human:
Cocktail of biotin-conjugated monoclonal anti-human antibodies against HLA-DR, CD14, CD15, CD16, CD19, CD25, CD36, CD56, CD57, CD45RO, CD123, CD244, and CD235a (Glycophorin A).

2 mL Naive Pan T Cell MicroBead Cocktail, human:
MicroBeads conjugated to monoclonal anti-CD61 antibody (isotype: mouse IgG1) and anti-biotin antibody (isotype: mouse IgG1).

1 mL Anti-TCR γ/δ-Biotin for Naive Pan T Cell Isolation Kit, human:
Monoclonal Anti-TCR γ/δ antibodies conjugated to biotin (isotype: mouse IgG1; clone 11F2).

Capacity For 10^9 total cells, up to 100 separations.

Product format All components 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

Using the Naive Pan T Cell Isolation Kit, human naive pan T cells are isolated by depletion of non-target cells (negative selection). Non-target cells are labeled with a cocktail of biotin-conjugated monoclonal antibodies and the Naive Pan T Cell MicroBead Cocktail. In between and after the two labeling steps no washing steps are required. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled naive pan T cells run through the column.

1.2 Background information

The Naive Pan T Cell Isolation Kit has been developed for the isolation of untouched naive $CD4^+$ and $CD8^+$ T cells from human peripheral blood mononuclear cells (PBMCs). Non-target cells, i.e., memory T cells, regulatory T cells, monocytes, granulocytes, NK cells, B cells, or erythrocytes are labeled by using a cocktail of biotin-conjugated antibodies. The cocktail contains antibodies against HLA-DR, CD14, CD15, CD16, CD19, CD25, CD36, CD56, CD57, CD45RO, CD123, CD244, and CD235a (Glycophorin A). Optionally, TCR γ/δ T cells can also be depleted. Subsequently, non-target cells are magnetically labelled with the Naive Pan T Cell MicroBead Cocktail. Isolation of highly pure naive T cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Studies on signal requirements and transduction for activation of naive T cells.
- Studies on *in vitro* priming of naive T cells.
- Studies on cytokine expression of naive T cells upon differentiation.

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 (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ 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.

140-003-503.01

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

▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD3-VioBlue® (# 130-094-363), CD45RA-FITC (# 130-092-247), CD4-APC (# 130-091-232), CD8-PE-Vio770™ (# 130-096-556), or CD8-APC-Vio770 (# 130-096-561). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

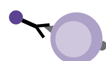
When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

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

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details see the protocols section at www.miltenyibiotec.com/protocols.

▲ 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 labeling. 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.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

2.2.1 Without depletion of TCR γ/δ T cells (compatible with autolabeling)

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 Naive Pan T Cell Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 30 µL of buffer per 10^7 cells.
7. Add 20 µL of Naive Pan T Cell MicroBead Cocktail per 10^7 cells.
8. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
9. (Optional) Add staining antibodies, e.g., 10 µL of CD3-VioBlue (# 130-094-363), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
10. Adjust the volume to a minimum of 500 µL of buffer.
    ▲ **Note:** Resuspend up to 10^8 cells in 500 µL of buffer. For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

2.2.2 With depletion of TCR γ/δ T cells (not compatible with autolabeling)

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 30 µL of buffer per 10^7 total cells.
4. Add 10 µL of Naive Pan T Cell Biotin-Antibody Cocktail per 10^7 total cells.
5. Add 10 µL of Anti-TCR γ/δ-Biotin per 10^7 total cells.
6. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
7. Add 30 µL of buffer per 10^7 cells.
8. Add 20 µL of Naive Pan T Cell MicroBead Cocktail per 10^7 cells.
9. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
10. (Optional) Add staining antibodies, e.g., 10 µL of CD3-VioBlue (# 130-094-363), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
11. Adjust the volume to a minimum of 500 µL of buffer.
    ▲ **Note:** Resuspend up to 10^8 cells in 500 µL of buffer. For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

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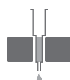


2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of labeled cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with 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 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched naive pan T cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive pan T cells, and combine with the flow-through from step 3.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-naive pan T cells by firmly pushing the plunger into the column.

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® Pro Separator or the autoMACS® Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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® 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.
   This fraction represents the enriched naive pan T cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-naive pan T cells.

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:

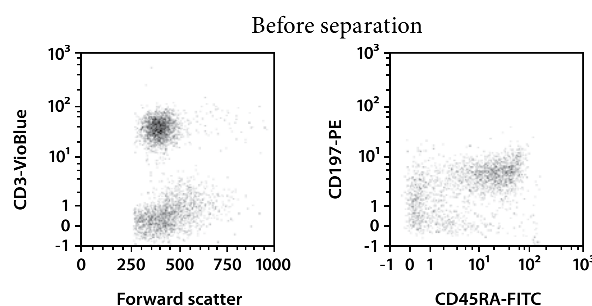
   Depletion: Depletes
   Collect negative fraction from outlet port neg1.
   This fraction represents the enriched naive pan T cells.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-naive pan T cells.

2.4 (Optional) Evaluation of naive pan T cell purity

The purity of the enriched naive pan T cells can be evaluated by flow cytometry, e.g. using the MACSQuant® Analyzer, or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-conjugated antibodies, e.g., CD3-VioBlue, CD45RA-FITC, and CD197 (CCR7)-PE as recommended in the respective data sheet and optional against the T cell markers CD4 and CD8. Analyze cells by flow cytometry or fluorescence microscopy. To distinguish between effector memory ($CD45RA^+CD197^-$) and naive ($CD45RA^+CD197^+$) CD8 T cells it is important to gate on $CD45RA^+CD197^+$ double positive cells.

3. Example of a separation using the Naive Pan T Cell Isolation Kit

Untouched naive pan T cells were isolated from human PBMCs using the Naive Pan T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD3-VioBlue (# 130-094-363) and CD45RA-FITC (# 130-092-247) and analyzed by flow cytometry using the MACSQuant Analyzer. The cells are already labeled with CD197 (CCR7)-PE during the separation procedure. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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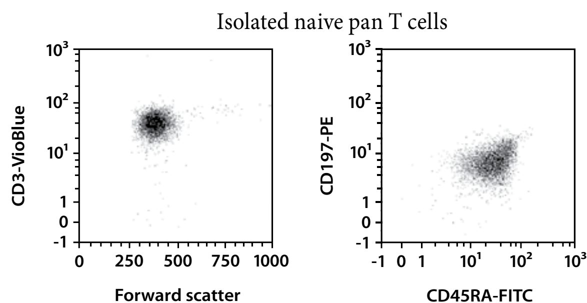

4. References

1. Dietz, L. *et al.* (2010) Tracking human contact allergens: from mass spectrometric identification of peptide-bound reactive small chemicals to chemical-specific naive human T-cell priming. Toxicol. Sci. 117: 336–347.

2. Jedema, I. *et al.* (2011) Successful generation of primary virus-specific and anti-tumor T-cell responses from the naive donor T-cell repertoire is determined by the balance between antigen-specific precursor T cells and regulatory T cells. Haematologica 96(8): 1204–1212.

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.

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