Miltenyi Biotec

Pan T Cell Isolation Kit
human

Order no. 130-096-535

Contents

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 Pan T Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (Glycophorin A). **2 mL Pan T Cell MicroBead Cocktail, human:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1) and monoclonal anti-CD61 antibody (isotype: mouse IgG1).
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 Pan T Cell Isolation Kit, human 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 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 T cells run through the column.

1.2 Background information

The Pan T Cell Isolation Kit has been developed for the isolation of untouched T cells from human peripheral blood mononuclear cells (PBMCs). Non-target cells, i.e., monocytes, neutrophils, eosinophils, B cells, stem cells, dendritic cells, NK cells, granulocytes, or erythroid cells are labeled by using a cocktail of biotin-conjugated antibodies. The cocktail contains antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (Glycophorin A). Subsequently, non-target cells are magnetically labelled with the Pan T Cell MicroBead Cocktail. Isolation of highly pure T cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Studies on signal requirements for T cell activation, induction of T cell proliferation, induction of T cell anergy, etc.
- Studies on signal transduction in T cells.
- Studies on regulation of 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 (FBS). 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
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

  ▲ **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-FITC (# 130-080-401). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

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- (Optional) Anti-FoxP3-APC (# 130-093-013) or Anti-FoxP3-PE (# 130-093-014) and FoxP3 Staining Buffer Set (# 130-093-142).
- (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 refer to 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. 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 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of 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 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-FITC (# 130-080-401), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
10. Proceed to magnetic separation (2.3).

▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and the number of total cells. For details refer to 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 T cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched 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-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.

2.4 Cell separation with the autoMACS® Pro Separator

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

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

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

2.4.1 Cell separation with the autoMACS® Pro Separator using autolabeling for a fully automated procedure

1. Turn on the instrument for automatic initialization (automated preparation and priming procedure).
2. Program autolabeling by selecting Read Reagent in the "reagent menu" tab and scan the 2D barcode on each reagent vial with the barcode scanner on the autoMACS Pro instrument. Place the reagent into the appropriate space on the reagent rack.

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3. Place sample and collection tubes into the sample rack. Sample tube should be in row A, and the collection tubes in rows B and C.
4. 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.
6. Select run.

For more details on complete walk away automation on the autoMACS Pro Separator, please refer to the user manual.

2.4.2 Magnetic separation with the autoMACS® Pro Separator 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 tube in row A of the tube rack and the fraction collection tubes in rows B and C.
4. For a standard separation choose the following program:

   Depletion: Depletes

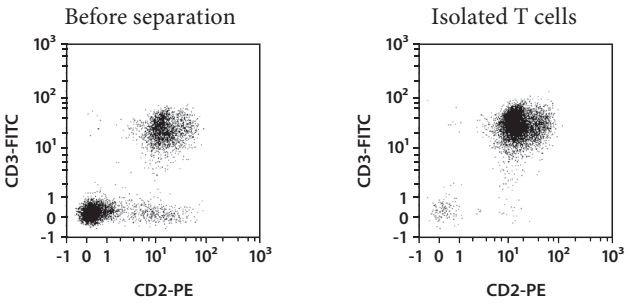
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched T cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non–T cells.

2.5 (Optional) Evaluation of T cell purity

The purity of the enriched T cells can be evaluated by flow cytometry, e.g. using the MACSQuant® Analyzer, or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against a T cell marker, e.g., CD3-FITC (# 130-080-401), as recommended in the respective data sheet. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-T cells with the Biotin-Antibody Cocktail can be visualized by counter-staining with fluorochrome-conjugated anti-biotin antibodies, 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 Pan T Cell Isolation Kit

Untouched T cells were isolated from human PBMCs by using the Pan T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD3-FITC (# 130-080-401) and CD2-PE (# 130-091-115) and and analyzed by flow cytometry using the MACSQuant Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols.