Miltenyi Biotec

Pan T Cell Isolation Kit
human

Order no. 130-096-535

Contents

1. Description

This product is for research use only.

Components	**1 mL Pan T Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (GlycophorinA). **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
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +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 to +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
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS NEO

  ▲ **Note:** When using this kit the unwanted cell fraction is labeled and the target cells remain unlabeled. Depending on the target cell frequency, the labeled fraction can therefore represent the majority of the total cells.
  To avoid blocking of the column, do not exceed the max. number of labeled cells per column. Estimate the number of labeled cells in the sample, split the sample if necessary and use the appropriate number of separation columns.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD3 Antibody, anti-human. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) FoxP3 Antibody, anti-human, APC or FoxP3 Antibody, anti-human, PE and the FoxP3 Staining Buffer Set (# 130-093-142).
- (Optional) Propidium Iodide Solution or 7-AAD for flow cytometric exclusion of dead cells.

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- (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 using a MACS PBMC Isolation Kit or 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.

For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use 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.

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS NEO or autoMACS Pro Separators. For more information refer to section 2.3.

▲ 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.

▲ 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).

▲ The recommended incubation temperature is +2 to +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 to +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 to +8 °C).
9. (Optional) Add staining antibodies, e.g., CD3 Antibody anti-human, FITC, according to manufacturer's recommendation.
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 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.

2.3.1 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.

2.3.2 Magnetic labeling and separation using the autoMACS NEO Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

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

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction,

position C = labeled (positive) fraction.

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Place the Chill Rack on the MACS MiniSampler S.
4. Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
5. Assign a reagent to each sample.
6. Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
7. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
8 The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.
9. Place the sample(s) and empty tubes to the Chill Rack.
10. Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

1. Prepare and prime the instrument.
2. Place the Chill Rack and MACS Reagent Rack 8 on the MACS MiniSampler S.
3. Select the same Chill Rack and MACS Reagent Rack 8 in the **Experiment** tab. An experiment is created automatically.
4. Tap to select sample position(s).
5. To assign a reagent to each sample, tap **Scan reagent** and scan the reagent barcode. Alternatively, tap on a free position of the MACS Reagent Rack 8 for selection out of the reagent list.
6. Unscrew the lids from the reagent vials and place the vials onto the designated positions on the MACS Reagent Rack 8.
7. Tap **Place reagent(s) on reagent rack** button in the dialog box.
8. Automated labeling is set automatically if autolabeling is supported and a reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Auto**.
9. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
10. Tap **Run** to start the separation process.

2.3.3 Magnetic labeling and separation using the autoMACS Pro Separator

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

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

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction,

position C = labeled (positive) fraction.

Magnetic separation after 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.
4. For a standard separation choose one of the following programs:

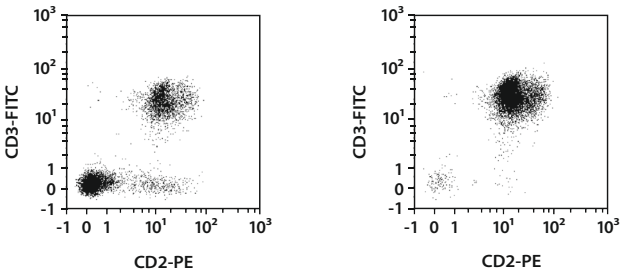
   Depletion: Depletes
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched T cells.
5. Tap **Run** to start the separation process.

Fully automated magnetic 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. Tap **Run** to start the separation process.

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 and CD2-PE 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.



For more information or assistance refer to our technical support.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

140-003-378.07

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