

<b>Components</b>	<p><b>1 mL Naive Pan T Cell Biotin-Antibody Cocktail, human:</b> Cocktail of biotin-conjugated monoclonal antibodies against HLA-DR, CD14, CD15, CD16, CD19, CD25, CD36, CD56, CD57, CD45RO, CD123, CD244, and CD235a (Glycophorin A).</p> <p><b>2 mL Naive Pan T Cell MicroBead Cocktail, human:</b> MicroBeads conjugated to monoclonal anti-CD61 antibody (isotype: mouse IgG1) and anti-biotin antibody (isotype: mouse IgG1).</p> <p><b>1 mL Anti-TCR<math>\gamma</math>/<math>\delta</math>-Biotin, human:</b> For depletion of TCR<math>\gamma</math>/<math>\delta</math> T cells (optional).</p>
<b>Capacity</b>	For 10 <sup>9</sup> total cells.
<b>Product format</b>	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial labels.

## Safety information

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Before use, please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Cell separation protocols

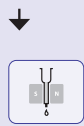


**Fully automated cell labeling and separation using the autoMACS<sup>®</sup> Pro Separator**

Alternatively:



**Manual magnetic labeling**



**Subsequent manual separation**

or



**Subsequent automated cell separation using the autoMACS<sup>®</sup> Pro Separator**

## General notes

- ▲ For tips concerning sample preparation, magnetic labeling and separation, visit [www.miltenyibiotec.com/faq](http://www.miltenyibiotec.com/faq) and [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

- ▲ For product-specific background information and applications of this product, refer to the respective product page at [www.miltenyibiotec.com/130-097-095](http://www.miltenyibiotec.com/130-097-095).

- ▲ This protocol includes an optional step for the depletion of TCR $\gamma$ / $\delta$  T cells. This step is not compatible with the autolabeling program using the autoMACS Pro Separator. If TCR $\gamma$ / $\delta$  T cells shall be depleted, use manual labeling and manual cell separation.

## 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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Degas buffer before use, as air bubbles could block the column.
- (Optional) Pre-Separation Filters (30  $\mu$ m) (# 130-041-407) to remove cell clumps.
- Choose the appropriate MACS Separator and MACS Columns:

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10 <sup>8</sup>	2×10 <sup>9</sup>	MidiMACS, QuadroMACS
autoMACS	2×10 <sup>8</sup>	4×10 <sup>9</sup>	autoMACS Pro

▲ **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.



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- ▲ Refer to the user manual for instructions on how to use the autoMACS<sup>®</sup> Pro Separator.

- ▲ All buffer temperatures should be  $\geq 10$  °C.

- ▲ Place tubes in the following Chill Rack positions:  
**position A** = sample, **position B** = negative fraction,  
**position C** = positive fraction.

- For appropriate resuspension volumes and cell concentrations, please visit [www.automacspro.com/autolabeling](http://www.automacspro.com/autolabeling).
- Switch on the instrument for automatic initialization.
- 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.

- Place sample and collection tubes into the Chill Rack.
- 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).
- Enter sample volume into the **Volume** submenu. Press **Enter**.
- Select **Run**.
- Collect enriched naive pan T cell fraction at position B = negative fraction.



### Manual magnetic labeling

- ▲ Work fast, keep cells cold, and use pre-cooled solutions (2–8 °C).
- ▲ Volumes for magnetic labeling given below are for up to  $10^7$  total cells. When working with fewer cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
- ▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling.

- Prepare cells and determine cell number.
- Resuspend cell pellet in 40  $\mu\text{L}$  of buffer per  $10^7$  total cells.  
▲ **Note:** If optional step 4 is included, please resuspend cell pellet in 30  $\mu\text{L}$  of buffer per  $10^7$  total cells.
- Add 10  $\mu\text{L}$  of Naive Pan T Cell Biotin-Antibody Cocktail per  $10^7$  total cells.
- (Optional) Add 10  $\mu\text{L}$  of Anti-TCR $\gamma/\delta$ -Biotin per  $10^7$  total cells.  
▲ **Note:** This step is not compatible with the autolabeling program using the autoMACS Pro Separator. Only subsequent manual cell separation can be done.
- Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
- Add 30  $\mu\text{L}$  of buffer per  $10^7$  total cells.
- Add 20  $\mu\text{L}$  of Naive Pan T Cell MicroBead Cocktail per  $10^7$  total cells.
- Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
- Proceed to subsequent magnetic cell separation.  
▲ **Note:** A minimum of 500  $\mu\text{L}$  is required for magnetic separation. If necessary, add buffer to the cell suspension.



### Subsequent manual cell separation

- ▲ Always wait until the column reservoir is empty before proceeding to the next step.
- Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
  - Prepare column by rinsing with 3 mL of buffer.
  - Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched naive pan T cells.
  - Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive pan T cells, and combine with the effluent from step 12.
  - (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.

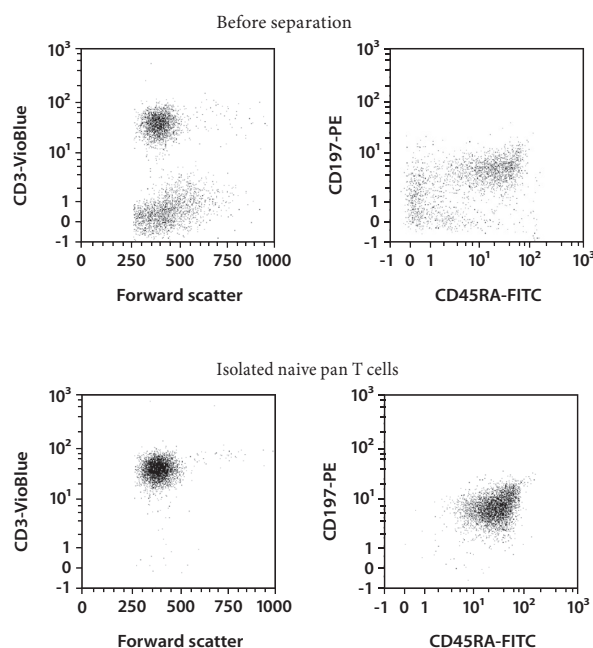


### Subsequent automated cell separation using the autoMACS® Pro Separator

- ▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.
  - ▲ All buffer temperatures should be  $\geq 10$  °C.
  - ▲ Place tubes in the following Chill Rack positions:  
**position A** = sample, **position B** = negative fraction, **position C** = positive fraction.
- Prepare and prime the instrument.
  - Follow the instructions that are given in the user manual.
  - The program “**Depletes**” is recommended. Collect enriched naive pan T cells at position B = negative fraction.

### 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, CD45RA-FITC, and CD197 (CCR7)-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.



For more information or assistance refer to our technical support.

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