

<b>Components</b>	<p><b>1 mL Naive CD4<sup>+</sup> T Cell Biotin-Antibody Cocktail, mouse:</b> Cocktail of biotin-conjugated monoclonal antibodies against CD8a, CD11b, CD11c, CD19, CD25, CD45R (B220), CD49b (DX5), CD105, Anti-MHC class II, Ter-119, and TCR<math>\gamma/\delta</math>.</p> <p><b>2 mL Anti-Biotin MicroBeads:</b> MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).</p> <p><b>1 mL CD44 MicroBeads, mouse:</b> MicroBeads conjugated to monoclonal anti-mouse CD44 antibodies (isotype: rat IgG2b).</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

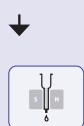


**Fully automated cell labeling and separation using the autoMACS<sup>®</sup> Pro Separator**

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/130-104-453](http://www.miltenyibiotec.com/130-104-453).

## 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.



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

▲ 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 CD4<sup>+</sup> 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.

1. Prepare cells and determine cell number.
2. Resuspend cell pellet in 40  $\mu$ L of buffer per  $10^7$  total cells.
3. Add 10  $\mu$ L of Biotin-Antibody Cocktail per  $10^7$  total cells.
4. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
5. Add 20  $\mu$ L of buffer per  $10^7$  total cells.
6. Add 20  $\mu$ L of Anti-Biotin MicroBeads per  $10^7$  total cells.
7. Add 10  $\mu$ L of CD44 MicroBeads per  $10^7$  total cells.
8. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
9. (Optional) For highest recovery wash cells by adding 1–2 mL of buffer per  $10^8$  total cells and centrifuge at  $300\times g$  for 10 minutes. Aspirate supernatant completely. Resuspend up to  $10^8$  cells in 500  $\mu$ L of buffer.
10. Proceed to subsequent magnetic cell separation.

▲ **Note:** A minimum of 500  $\mu$ 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.

11. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
12. Prepare column by rinsing with 3 mL of buffer.
13. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched naive CD4<sup>+</sup> T cells.
14. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive CD4<sup>+</sup> T cells, and combine with the effluent from step 13.
15. (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 CD4<sup>+</sup> T cells by firmly pushing the plunger into the column.



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

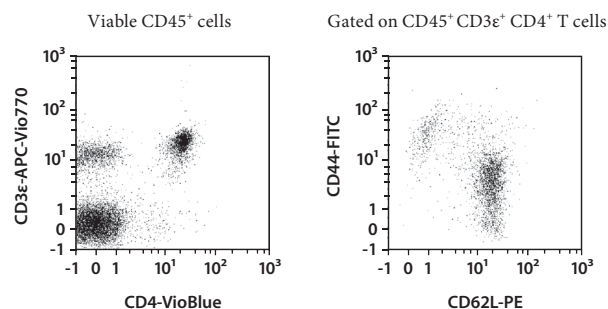
- ▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.
- ▲ All buffer temperatures should be  $\geq 10$  °C.
- ▲ Place tubes in the following Chill Rack positions:  
**position A** = sample, **position B** = negative fraction,  
**position C** = positive fraction.

11. Prepare and prime the instrument.
12. Follow the instructions that are given in the user manual.
13. The program “Depletes” is recommended. Collect enriched naive CD4<sup>+</sup> T cells at position B = negative fraction.

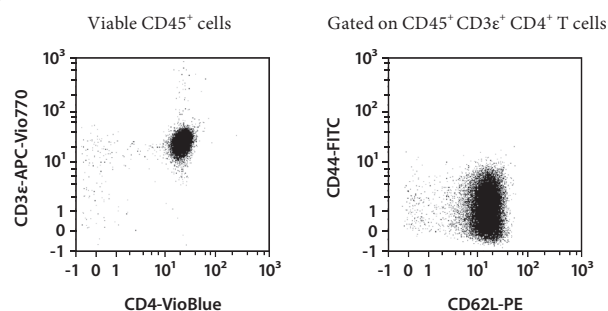
### Example of a separation using the Naive CD4<sup>+</sup> T Cell Isolation Kit

Naive CD4<sup>+</sup> T cells were isolated from a single-cell suspension from mouse spleen using the Naive CD4<sup>+</sup> T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD45-VioGreen, CD4-VioBlue, CD62L-PE, CD3 $\epsilon$ -APC-Vio770, and CD44-FITC 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 and a gate was set on CD45<sup>+</sup> cells.

#### A) Before separation



#### B) Isolated naive CD4<sup>+</sup> T cells



For more information or assistance refer to our technical support.

Check out Miltenyi Biotec's flow cytometry solutions at [www.miltenyibiotec.com/MACSQuant](http://www.miltenyibiotec.com/MACSQuant) and explore the extensive antibody portfolio at [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).

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