

<b>Components</b>	2 mL CD4 (L3T4) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD4 antibodies (L3T4; isotype: rat IgG2b).
<b>Capacity</b>	For $2 \times 10^9$ 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® Pro Separator**

Alternatively:



**Manual magnetic labeling**



Subsequent manual separation

or



Subsequent automated cell separation using the autoMACS® 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/130-117-043](http://www.miltenyibiotec.com/130-117-043).

### 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). Degas buffer before use, as air bubbles could block the column.

- (Optional) Pre-Separation Filters (30 µm) (# 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
<b>Positive selection</b>			
MS	$10^7$	$2 \times 10^8$	MiniMACS, OctoMACS
LS	$10^8$	$2 \times 10^9$	MidiMACS, QuadroMACS
<b>Depletion</b>			
LD	$10^8$	$5 \times 10^8$	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
<b>Positive selection or depletion</b>			
autoMACS	$2 \times 10^8$	$4 \times 10^9$	autoMACS Pro



**Fully automated cell labeling and 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.

1. For appropriate resuspension volumes and cell concentrations, please visit [www.automacspro.com/autolabeling](http://www.automacspro.com/autolabeling).
2. Switch on the instrument for automatic initialization.
3. 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.
4. Place sample and collection tubes into the Chill Rack.
5. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation (**Possel**), and wash protocols will be selected automatically for **positive selection** by default).
  - ▲ **Note:** Choose program **Possels** if higher recovery is required at costs of purity or contact technical support [macstec@miltenyibiotec.de](mailto:macstec@miltenyibiotec.de).
  - ▲ **Note:** If depletion of target cells is desired, please select program **Deplete** from the menu. Choose program **Depletes** if stronger depletion is required or contact technical support [macstec@miltenyibiotec.de](mailto:macstec@miltenyibiotec.de).
6. Enter sample volume into the **Volume** submenu. Press **Enter**.
7. Select **Run**.
8. Collect enriched CD4<sup>+</sup> T cell fraction at position C = positive fraction.
9. (Optional) Collect negative cell fraction at position B containing unlabeled cells that are depleted from CD4<sup>+</sup> T cells.



### 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 90  $\mu$ L of buffer per  $10^7$  total cells.
3. Add 10  $\mu$ L of CD4 (L3T4) MicroBeads per  $10^7$  total cells.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. 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.
6. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
  7. Prepare column by rinsing with the appropriate amount of buffer:
 

MS: 500 $\mu$ L	LS: 3 mL
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  8. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the CD4<sup>-</sup> cell fraction.
  9. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 8.
 

MS: 2x500 $\mu$ L	LS: 1x3 mL
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  10. Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled CD4<sup>+</sup> T cells by firmly pushing the plunger into the column.
 

MS: 1 mL	LS: 5 mL
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  11. (Optional) To increase the purity of CD4<sup>+</sup> T cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 6 to 10 by using a new column.



### Subsequent automated cell 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.

6. Prepare and prime the instrument.
7. Follow the instructions that are given in the user manual.
8. For a standard separation choose one of the following programs:

#### Positive selection: Possel

Collect positive fraction from row C of the tube rack.

- ▲ **Note:** Choose program **Possels** if higher recovery is required at costs of purity or contact technical support [macstec@miltenyibiotec.de](mailto:macstec@miltenyibiotec.de).

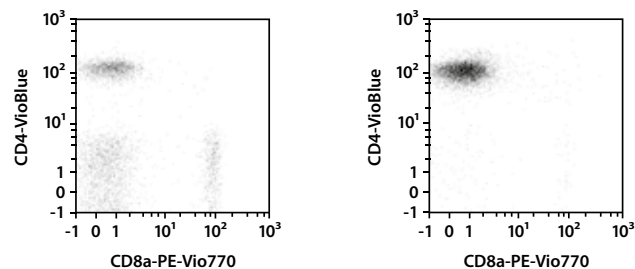
#### Depletion: Deplete

Collect negative fraction in row B of the tube rack.

- ▲ **Note:** Choose program **Depletes** if stronger depletion is required or contact technical support [macstec@miltenyibiotec.de](mailto:macstec@miltenyibiotec.de).

### Example of a separation using CD4 (L3T4) MicroBeads

A single-cell suspension from mouse spleen was prepared using the gentleMACS<sup>™</sup> Dissociator. CD4<sup>+</sup> T cells were isolated from this single-cell suspension using the CD4 (L3T4) MicroBeads, an LS Column, and a QuadroMACS<sup>™</sup> Separator. Cells were fluorescently stained with REAfinity<sup>™</sup> Antibodies CD45-VioGreen<sup>™</sup>, CD4-VioBlue<sup>®</sup>, and CD8a-PE-Vio<sup>®</sup> 770 and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Viable leukocytes were gated for analysis based on scatter signals, 7-AAD Staining Solution fluorescence, and CD45 expression.



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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