Miltenyi Biotec

StraightFrom® Spleen Naive CD4$^+$ T Cell Isolation Kit

mouse

Order no. 130-139-856

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1. Description

This product is for research use only.

Components	**1 mL StraightFrom Spleen Naive CD4$^+$ T Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antbodies **4 mL StraightFrom Spleen Naive CD4$^+$ T Cell MicroBead Cocktail, mouse:** MicroBeads conjugated to monoclonal mouse anti-biotin and monoclonal mouse anti-CD44 (isotype: recombinant human IgG1).
Capacity	For 20 spleens. The specified number of digestions is valid when digesting a spleen with an average weight of 80–140 mg following the protocol in chapter 2.1.
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 StraightFrom Spleen Naive Separation

Using the StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit, mouse naive CD4$^+$ T cells are isolated by depletion of non-target cells. Spleen is gently mechanically dissociated using the gentleMACS™ Octo Dissociator with Heaters. During the dissociation step, non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies as the primary labeling reagent while the gentleMACS Octo Coolers provide an optimal temperature for labeling the cells. Afterwards, anti-biotin monoclonal antibodies and CD44 antibodies conjugated to MicroBeads are used as secondary labeling reagent. In between the two labeling steps no washing steps are required. After dissociation and magnetic labeling, the sample is passed through a 70 µm filter to remove larger particles from the single-cell suspension. Then, the cell suspension is loaded onto MACS® Column placed in the magnetic field of a MACS Separator. 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 naive CD4$^+$ T cells run through the column.

1.2 Background information

The StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit, mouse has been developed for the isolation of naive CD4$^+$ T cells directly from mouse spleen by using the gentleMACS Octo Dissociator with Heaters in combination with MACS Technology, leading to purification of viable and functionally active cells that can directly be used for downstream experiments.

1.3 Applications

- Isolation of purified naive CD4$^+$ T cells for *in vitro* and *in vivo* studies, e.g., flow cytometry, cell-based assays, or cell culture under polarizing cytokine condition to study differentiation into Th1, Th2, or TH17 substes.

  ▲ **Note:** The StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit is optimized for mouse strain C57BL/6J, C57BL/6N, 129, and C3H. When working with different mouse strains, further optimisation of the components of the StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit is required.

1.4 Reagent and instrument requirements

- Separation 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). Always use freshly prepared buffer. Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** 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.

- Pre-Separation Filters (70 µm) (# 130-095-823)

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- gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS Octo Coolers (# 130-130-533): Pre-cool gentleMACS Octo Coolers for at least 12 hours in the refrigerator (+2 to +8 °C) before use.

  ▲ **Note:** Do not freeze the gentleMACS Octo Coolers.
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- 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
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS	2×10^8	4×10^9	autoMACS NEO Separator, autoMACS Pro Separator
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45 Antibody, anti-mouse, VioGreen™, REAfinity™, CD4 Antibody, anti-mouse, PE, REAfinity, CD3ε Antibody, anti-mouse, PerCP-Vio®770, CD62L Antibody, anti-mouse, APC, REAfinity, and CD44 anti-mouse (clone NIM–R8), FITC. For more information about antibodies refer to **www.miltenyibiotec.com/antibodies**.
- (Optional) Propidium Iodide Solution (# 130-093-233), 7-AAD Staining Solution (# 130-111-568), or DAPI Staining Solution (# 130-111-570) for flow cytometric exclusion of dead cells.

2. Protocol

▲ For details on the use of the gentleMACS Octo Dissociator with Heaters, refer to the user manual.

▲ Ensure that the gentleMACS Octo Dissociator with Heaters software is up-to-date and the required program is installed. The latest software update package can be requested at www.miltenyibiotec.com.

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ One mouse spleen is dissociated in a volume of approximately 1 mL of separation buffer containing StraightFrom Spleen Naive CD4$^+$ T Cell Biotin-Antibody Cocktail, mouse. The weight of one mouse spleen amounts to 80–140 mg.

▲ Remove fat tissue from the dissected mouse spleen before dissociation.

▲ Spleens from 6–12-week-old mice are recommended.

▲ The StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit is optimized for mouse strain C57BL/6J, C57BL/6N, 129, and C3H. When working with different mouse strains, further optimisation of the components of the StraightFrom Spleen Naive CD4+ T Cell Isolation Kit is required.

2.1 Spleen dissociation and magnetic labeling of cells

▲ Cells can be labeled with StraightFrom Spleen Naive CD4$^+$ T Cell Microbead Cocktail using the autolabeling function of the autoMACS NEO Separator. For more information refer to section 2.2.3.

▲ 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 given below are for one spleen. When working with more spleens (max. of three spleens) per C Tube, scale up all reagent volumes accordingly (e.g. for two spleens, use twice the volume of all indicated reagent volumes).

1. Add 950 µL separation buffer and 50 µL StraightFrom Spleen Naive CD4$^+$ T Cell Biotin-Antibody Cocktail, mouse into a gentleMACS C Tube.
2. Transfer one mouse spleen into the C Tube.

   ▲ **Note:** Remove excess buffer by placing spleen briefly at a paper towel before placing it into the C Tube.
3. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Octo Dissociator with Heaters.

   ▲ **Note:** Close C Tube tightly beyond the first resistance.

   ▲ **Note:** Ensure that the sample material is located in the area of the rotor/stator.
4. Place the pre-cooled gentleMACS Octo Cooler over the already installed C Tube.
5. Run the gentleMACS Program **4C_m_SF_SIK_1**.
6. After termination of the program, remove the gentleMACS Octo Cooler and detach the C Tube from the gentleMACS Octo Dissociator with Heaters.

   ▲ **Note:** If the spleen is not completey dissociated, repeat steps 4 and 5. Note that fragments of the fibrotic capsule of the spleen might still be visible.
7. Perform a short centrifugation step to collect the sample material at the bottom of the tube.

   ▲ **Note:** Do not exceed 300×g speed.

   ▲ **Note:** If a cell pellet is visible, carefully resuspend the cells by pipetting up and down.
8. (Optional) For automated magnetic labeling with StraightFrom Spleen Naive CD4$^+$ T Cell Microbead Cocktail followed by separation using the autoMACS NEO Separator, proceed directly to step 11.
9. Add 200 µL of StraightFrom Spleen Naive CD4$^+$ T Cell Microbead Cocktail and resuspend cell suspension gently.
10. Incubate for 10 minutes at +2 to +8 °C.
11. Place a Pre-Separation Filter (70 µm) on a 15 mL tube. Moisturize the Pre-Separation Filter (70 µm) by pipetting 0.5 mL of separation buffer into the reservoir and discard effluent.
12. Apply cell suspension on top of the Pre-Separation Filter (70 µm).
13. Apply 0.5 mL of separation buffer on the Pre-Separation Filter (70 µm). Tap the rim of the filter to elute the suspension completely.

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14. Proceed directly to magnetic separation (2.2).



2.2 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of non-naive $CD4^+$ T 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.

2.2.1 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 naive $CD4^+$ T cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive $CD4^+$ T cells, and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
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-naive $CD4^+$ T cells by firmly pushing the plunger into the column.

2.2.2 Magnetic separation with the MultiMACS Cell24 Separator Plus

Refer to the the MultiMACS Cell Separator Plus user manual for instructions on how to use the MultiMACS Cell24 Separator Plus.

2.2.3 Magnetic labeling and separation using the autoMACS NEO 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.

▲ 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. Dissociate and label the sample as described in section 2.1 Spleen dissociation and magnetic labeling of cells.
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 with the StraightFrom Spleen Naive $CD4^+$ T Cell Microbead Cocktail and separation

1. Dissociate the sample as described in section 2.1 Spleen dissociation and magnetic labeling of cells.
2. Prepare and prime the instrument.
3. Place the Chill Rack and MACS Reagent Rack 8 on the MACS MiniSampler S.
4. Select the same Chill Rack and MACS Reagent Rack 8 in the **Experiment** tab. An experiment is created automatically.
5. Tap to select sample position(s).
6. 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.
7. Unscrew the lids from the reagent vials and place the vials onto the designated positions on the MACS Reagent Rack 8.
8. Tap **Place reagent(s) on reagent rack** button in the dialog box.
9. 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**.
10. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
11. Tap **Run** to start the separation process.

2.2.4 Magnetic 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

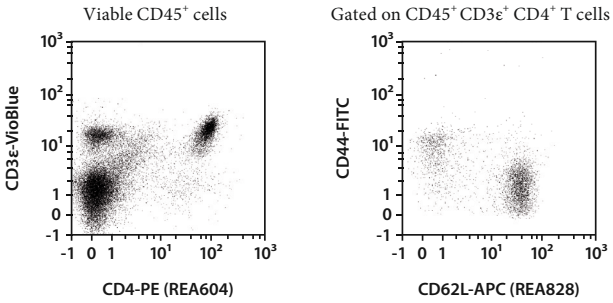
1. Dissociate and label the sample as described in section 2.1 Spleen dissociation and magnetic labeling of cells.
2. Prepare and prime the instrument.
3. Apply tube containing the sample.
4. For a standard separation choose one of the following programs:

   Depletion: Deplete2
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched naive CD4$^+$ T cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-CD4 T cells.
6. Tap **Run** to start the separation process.

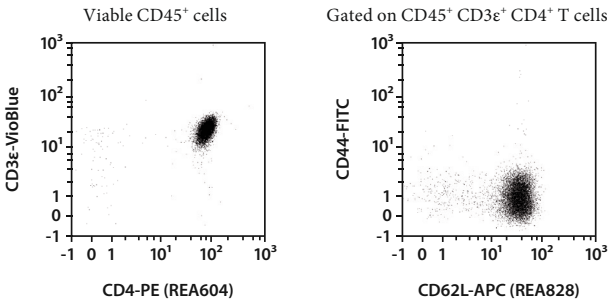
3. Example of a separation using the StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit, mouse

Separation of a spleen sample using the StraightFrom Spleen Naive CD4$^+$ T Cell Isolation Kit, mouse and the autoMACS NEO Separator. Cells were fluorescently stained with CD45-VioGreen, CD4-PE, CD62L-APC, CD3ε-VioBlue, and CD44-FITC (clone NIM–R8) and analyzed by flow cytometry using the MACSQuant® Analyzer 16. 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$^+$ cells.

A) Before separation



B) Isolated naive CD4$^+$ T cells



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.

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