Miltenyi Biotec

StraightFrom® LRSC CD4/CD8 MicroBead Kit
human

Order no. 130-121-307

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	**2 mL StraightFrom® LRSC CD4/CD8 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD4 and CD8 antibodies (isotype: mouse IgG1 and IgG2a, respectively). **50 mL Whole Blood Column Elution Buffer** **12 LS Columns** columns and plungers, sterile packed.
Capacity	For one LRSC.
Product format	StraightFrom LRSC CD4/CD8 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide. Whole Blood Column Elution Buffer contains stabilizer and 0.09% sodium azide.
Storage	Store StraightFrom LRSC CD4/CD8 MicroBeads and Whole Blood Column Elution Buffer protected from light at 2–8 °C. Do not freeze. Store LS Columns dry at 10–35 °C and protected from light. The expiration date is indicated on the vial or box label.

1.1 Principle of the MACS® Separation

First, the $CD4^+$ and $CD8^+$ cells in an LRSC sample are magnetically labeled with StraightFrom LRSC CD4/CD8 MicroBeads. Then, the cell suspension is loaded onto an LS Column, which is placed in the magnetic field of a MACS® Separator. The magnetically labeled $CD4^+$ and $CD8^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD4^+$ and $CD8^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD4^+$ and $CD8^+$ cells can be eluted as the positively selected cell fraction.

1.2 Technical specifications of LS Columns

- Columns are “flow stop” and do not run dry.
- Void volume: 400 µL. Reservoir volume: 8 mL.
- LS Columns are for single use only.
- Use Whole Blood Column Elution Buffer to elute positive cell fraction from column.

▲ To remove clumps and to prevent aggregates in the sample, pass cells through 30 µm nylon mesh (MACS SmartStrainers (30 µm), # 130-098-458) before separation.

1.3 Background information

Leukoreduction system chambers (LRSCs), also known as buffy cones, arise after standard plateletpheresis procedures, where leukocytes are filtered out into the LRSCs. They contain highly concentrated leukocytes in a small volume.
The StraightFrom LRSC CD4/CD8 MicroBead Kit has been developed for the simultaneous positive selection of $CD4^+$ and $CD8^+$ cells directly from LRSCs by using the MultiMACS™ Cell24 Separator Plus or the MidiMACS™ Separator or QuadroMACS™ Separator. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The CD4 antigen is expressed on T helper (T_H) cells and at a lower level on monocytes and dendritic cells. The CD4 antigen is an accessory molecule in the recognition of MHC class II/peptide complexes by the TCR heterodimer on $CD4^+$ T_H cells. The CD8 antigen is expressed on cytotoxic T cells and on a subset of $CD16^+$ NK cells. The CD8 antigen acts as an accessory molecule in the recognition of MHC class I/peptide complexes by the TCR heterodimer on $CD8^+$ cytotoxic T cells.

1.4 Applications

- Simultaneous isolation of $CD4^+$ and $CD8^+$ cells from LRSCs. The purified $CD4^+$ and $CD8^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.

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1.5 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 (2–8 °C). Alternatively, use autoMACS Running 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.

- Semi-automated separation:
  - MultiMACS™ Cell24 Separator Plus (# 130-098-637) with the MultiMACS 12× Single-Column Adapter LS (# 130-108-816)
  - Single-well Deep Well Plates (# 130-114-966) or 24-well Deep Well Plates (# 130-110-500)
- Manual separation:
  - MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976)
  - MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052)
- (Optional) Fluorochrome-conjugated REAfinity™ Antibodies for flow cytometric analysis, e.g., CD4-PE-Vio® 770, CD8-APC, as well as CD45-VioBlue®. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.

2. Protocol

2.1 Preparation of LRSCs

1. Transfer content of one LRSC into a collection tube. Fill up to 40 mL with separation buffer.

   ▲ **Note:** LRSC can be rinsed with separation buffer to maximize cell recovery from the LRSC. Do not exceed 40 mL sample volume in total.

2. Proceed to magnetic labeling (2.2).



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.

▲ Volumes for magnetic labeling given below are for one entire LRSC (filled up to 40 mL sample volume). When working with fractions of LRSC, scale down all reagents and total volumes accordingly (e.g. for half an LRSC, fill up to 20 mL and use 1 mL StraightFrom® LRSC MicroBeads as well as 6 LS Columns).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (MACS SmartStrainers (30 µm), # 130-098-458) to remove cell clumps which may clog the column. Moisten filter with separation buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Add 2 mL StraightFrom LRSC CD4/CD8 MicroBeads per 40 mL sample volume.
2. Mix well by inverting the tube and incubate for 15 minutes in the refrigerator (2–8 °C).
3. Proceed directly to magnetic separation (2.3).



2.3 Magnetic separation

2.3.1 Semi-automated magnetic separation with the MultiMACS™ Cell24 Separator Plus

▲ For more detailed instructions on how to use the MultiMACS™ Cell24 Separator Plus, please refer to the user manual.

▲ The MultiMACS Cell24 Separator Plus, including the MACS Elution Station, has to be used with the Single-Column Adapter, LS Columns, and Deep Well Plates for magnetic separation with StraightFrom LRSC MicroBeads.

▲ **Note:** To reach maximum cell recovery, rinse the Deep Well Plate of the positive fraction and combine with the positive fraction.

▲ Buffer volumes are as follows:

Equilibration: 3 mL (separation buffer)
Wash: 2×2 mL (separation buffer)
Elution: 4 mL (Whole Blood Column Elution Buffer)

▲ Divide the sample equally between the 12 LS Columns, e.g., when starting volume is 42 mL (40 mL sample and 2 mL StraightFrom LRSC MicroBeads), add 3.5 mL onto each column.

▲ If equilibration solution and negative fraction should be collected in the same Deep Well Plate as waste, select the program **POSSEL_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ If equilibration solution and negative fraction with wash fractions should be collected in separate Deep Well Plates, select the program **POSSEL2_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ After the separation, centrifuge positive fraction at 200×g for 10 minutes. Aspirate supernatant carefully. Resuspend cell pellet in a suitable amount of buffer or medium for subsequent analysis.

2.3.2 Manual magnetic separation with the MidiMACS™ Separator or QuadroMACS™ Separator

▲ The protocol given below is for one LS Column. Repeat steps for each of the 12 LS Columns.

▲ Divide the sample equally between the LS Columns, e.g., when starting volume is 42 mL, add 3.5 mL onto each column.

1. Place LS Column in the magnetic field of a MidiMACS™ Separator or QuadroMACS™ Separator. Insert LS Column with the column wings to the front into the separator.
2. Prepare column by rinsing with 3 mL separation buffer.
3. Apply magnetically labeled cell suspension onto the prepared LS Column. Collect flow-through containing unlabeled cells.

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4. Wash LS Column with 2×2 mL separation buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

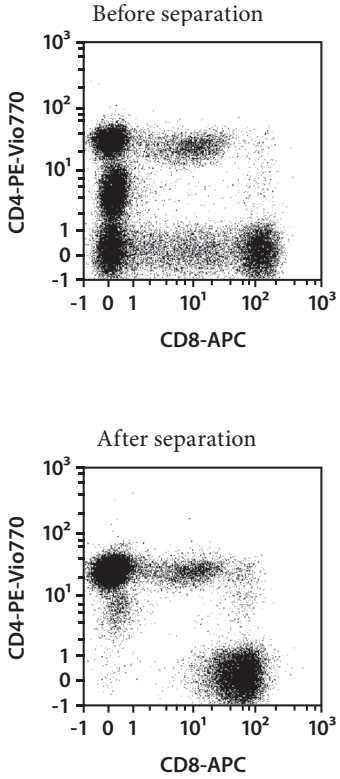
5. Remove LS Column from the separator and place it on a new collection tube.

6. Pipette 4 mL **Whole Blood Column Elution Buffer** onto the LS Column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

7. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant carefully. Resuspend cell pellet in a suitable amount of buffer or medium for subsequent analysis.

3. Example of a separation using the StraightFrom® LRSC CD4/CD8 MicroBead Kit

Separation of an LRSC sample using the StraightFrom® LRSC CD4/CD8 MicroBead Kit and the MultiMACS™ Cell24 Separator Plus with the Single-Column Adapter and LS Columns. Cells were fluorescently stained with REAfinity™ Antibodies CD4-PE-Vio® 770, CD8-APC, as well as CD45-VioBlue® and analyzed by flow cytometry using the MACSQuant® Analyzer. Viable leukocytes were gated for analysis based on scatter signals, propidium iodide fluorescence, and CD45 expression.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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