Miltenyi Biotec

StraightFrom® Leukopak® CD4/CD8 MicroBeads
human

Order no. 130-131-607

Contents

1. Description

This product is for research use only.

Components	**4 mL StraightFrom Leukopak® CD4/CD8 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD4/CD8 antibodies (isotype: mouse IgG1).
Capacity	For one ½ Leukopak®.
Product format	StraightFrom Leukopak® CD4/CD8 MicroBeads, human are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store StraightFrom Leukopak® CD4/CD8 MicroBeads, human protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial or box label.

1.1 Principle of the MACS® Separation

First, the CD4/CD8$^+$ cells in a Leukopak® sample are magnetically labeled with StraightFrom Leukopak® CD4/CD8 MicroBeads, human. Then, the cell suspension is loaded onto a Multi-24 Column Block, which is placed in the magnetic field of a MACS® Separator. The magnetically labeled CD4/CD8$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD4/CD8$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD4/CD8$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

During leukapheresis white blood cells are separated from whole blood and collected as highly concentrated leukocytes in Leukopaks®, which are ideal for the isolation of large numbers of various leukocyte subsets.

StraightFrom Leukopak® CD4/CD8 MicroBeads, human have been developed for the positive selection of CD4/CD8$^+$ cells directly from one Leukopak® by using the MultiMACS™ Cell24 Separator Plus. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The CD4 antigen is expressed on T helper (TH) 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$^+$ TH 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.

1.3 Applications

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

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). 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.
- 1× Whole Blood Column Elution Buffer (# 130-131-889).
- 2× Multi-24 Column Block (# 130-095-692).
- MultiMACS Cell24 Separator Plus (# 130-098-637).
- 24-well Deep Well Plates (# 130-110-500) or Single-well Deep Well Plates (# 130-114-966).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4 Antibody, anti-human, CD8 Antibody, anti-human, or CD45 Antibody, anti-human. 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.

2. Protocol

▲ StraightFrom Leukopak® CD4/CD8 MicroBeads, human have been developed for positive selection of target cells from one ½ Leukopak® with up to 200 mL (5×10^9–1×10^{10} cells). When working with smaller volumes, scale down all reagents and total volumes accordingly (e.g. for ¼ Leukopak®, fill up to 100 mL and use 2 mL

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StraightFrom Leukopak® CD4/CD8 MicroBeads, human and one Multi-24 Column Block). When working with higher volumes per ½ Leukopak®, contact Technical Support.

2.1 Preparation of Leukopak®

1. Divide a ½ Leukopak® equally into two collection tubes (max. 100 mL per tube). If the volume per tube is less than 100 mL, fill up to 100 mL with separation buffer.
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 tube containing 100 mL sample. Repeat labeling and separation steps for each tube one after the other.

▲ The recommended incubation temperature is +2 to +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 Leukopak® CD4/CD8 MicroBeads, human the tube containing 100 mL sample.
2. Mix well by inverting the tube and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
3. Proceed directly to magnetic separation (2.3).

2.3 Magnetic separation

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

▲ The MultiMACS Cell24 Separator Plus, including the MACS Elution Station, has to be used with two Multi-24 Column Block and four Deep Well Plates for magnetic separation with StraightFrom Leukopak® MicroBeads.

▲ **Note:** For equilibration of the second column block use the Deep Well Plate from the first column block equilibration. Use one Deep Well Plate for collection of the positive fractions from both column blocks. To reach maximum cell recovery, rinse the Deep Well Plate after removal of the positive fraction and combine with the positive fraction. For collection of the negative fraction and the wash fractions, use two Deep Well Plates.

▲ Buffer volumes per column are as follows:

Equilibration: 2 mL separation buffer
Wash: 3×1 mL separation buffer
Elution: 1 mL Whole Blood Column Elution Buffer

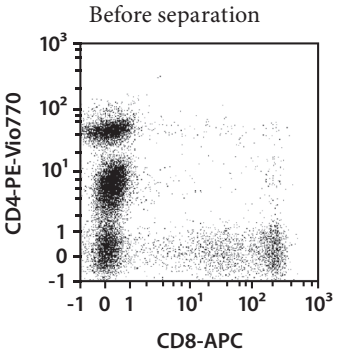
▲ Divide the sample equally between the 24 columns of the Multi-24 Column Block, e.g., when the starting volume is 102 mL (100 mL Leukopak® and 2 mL StraightFrom Leukopak® MicroBeads), add 4.5 mL onto each column. Repeat steps for the second tube.

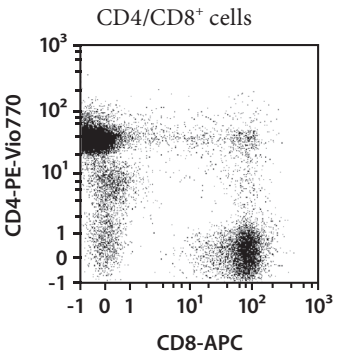
▲ Select the program **POSSEL2** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ After separation, centrifuge the 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.

3. Example of a separation using StraightFrom Leukopak® CD4/CD8 MicroBeads, human

Separation of a Leukopak® sample using StraightFrom Leukopak® CD4/CD8 MicroBeads, human and the MultiMACS Cell24 Separator Plus with the Multi-24 Column Blocks. Cells were fluorescently stained with CD4-PE-Vio® 770, CD8-APC, and CD45-VioBlue® 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.





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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