Miltenyi Biotec

StraightFrom® Leukopak® CD8 MicroBead Kit
human

Order no. 130-117-019

Contents

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	**4 mL StraightFrom® Leukopak® CD8 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD8 antibodies (isotype: mouse IgG2a). **1× 50 mL Whole Blood Column Elution Buffer** **1× Multi-24 Column Block** 1× Multi-24 Column Block and 1× 24-well Deep Well Plate, sterile packed.
Capacity	For one ½ Leukopak®.
Product format	StraightFrom® Leukopak® 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® Leukopak® CD8 MicroBeads and Whole Blood Column Elution Buffer protected from light at 2–8 °C. Do not freeze. Store Multi-24 Column Block 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 $CD8^+$ T cells in a Leukopak® sample are magnetically labeled with StraightFrom® Leukopak® CD8 MicroBeads. Then, the cell suspension is loaded onto a Multi-24 Column Block, which is placed in the magnetic field of a MultiMACS™ Cell24 Separator Plus. The magnetically labeled $CD8^+$ T cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD8^+$ T cells. After removing the column from the magnetic field, the magnetically retained $CD8^+$ T cells can be eluted as the positively selected cell fraction.

1.2 Technical specifications of the Multi-24 Column Block

One Multi-24 Column Block is a unit of 24 columns, enabeling up to 24 separations in parallel.

- Column capacity: 1×10^8 magnetically labeled cells from up to 1×10^9 total cells per single column.
- Columns are “flow stop” and do not run dry.
- Void volume per single column: 250 µL. Reservoir volume: 5 mL.

  ▲ **Note:** If sample volume exceeds 5 mL per column, apply sample in aliquots.
- Multi-24 Column Blocks are for single use only.

1.3 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.
The StraightFrom® Leukopak® CD8 MicroBead Kit has been developed for the positive selection of $CD8^+$ T cells directly from a Leukopak® by using the MultiMACS Cell24 Separator Plus. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. In human whole blood 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

- Isolation of $CD8^+$ T cells from Leukopaks®. The purified $CD8^+$ T 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 (# 130-091-221). 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.

- 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., CD3-PE, CD8-APC, and 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.

2. Protocol

▲ The StraightFrom® Leukopak® CD8 MicroBead Kit has 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 higher volumes per ½ Leukopak®, please contact our Technical Support team.

2.1 Preparation of Leukopak®

1. Transfer up to 200 mL of a ½ Leukopak® into a collection tube. If the volume is less than 200 mL, fill up to 200 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 200 mL sample. 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 StraightFrom® Leukopak® CD8 MicroBeads and half of the Multi-24 Column Block).

▲ 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 4 mL StraightFrom® Leukopak® CD8 MicroBeads to the tube containing 200 mL sample.
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

▲ 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 a Multi-24 Column Block and three Deep Well Plates for magnetic separation with StraightFrom® Leukopak® CD8 MicroBeads.

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

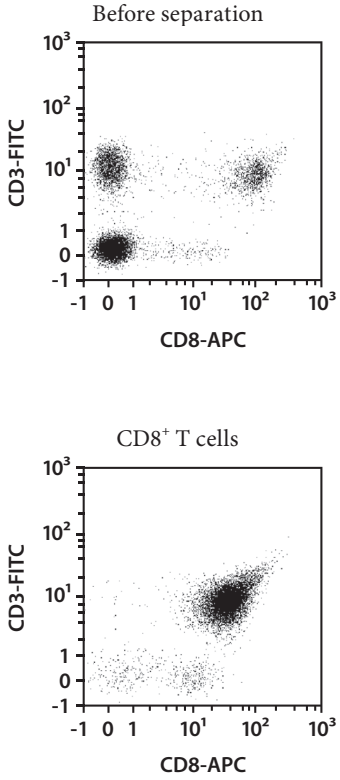
▲ Divide the sample equally between the 24 columns of the Multi-24 Column Block, e.g., when starting volume is 204 mL (200 mL Leukopak® and 4 mL StraightFrom® Leukopak® CD8 MicroBeads), add 8.5 mL onto each column. Load the sample in two steps onto the columns with max. 5 mL per step, e.g., 2× 4.25 mL/column.

▲ Select the program **POSSEL2** 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.

3. Example of a separation using the StraightFrom® Leukopak® CD8 MicroBead Kit

Separation of a Leukopak® sample using the StraightFrom® Leukopak® CD8 MicroBead Kit and the MultiMACS Cell24 Separator Plus with the Multi-24 Column Block. Cells were fluorescently stained with CD3-PE, CD8-APC, as well as 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/local to find your nearest Miltenyi Biotec contact.

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