Miltenyi Biotec

StraightFrom® Leukopak® PBMC Isolation Kit
human

Order no. 130-123-456

Contents

1. Description

This product is for research use only.

Components	**4×2 mL Erythrocyte Depletion MicroBeads, human** **1×2 mL Granulocyte Depletion MicroBeads, human** **1× Multi-24 Column Block** 1× Multi-24 Column Block and 1× 24-well Deep Well Plate, sterile packed.
Capacity	For 50 mL fresh Leukopak® sample
Product format	MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store MicroBeads 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 erythrocytes and granulocytes in a Leukopak® sample are magnetically labeled with Erythrocyte Depletion MicroBeads and Granulocyte Depletion 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 erythrocytes and granulocytes are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted from erythrocytes and granulocytes. After removing the column from the magnetic field, the magnetically retained erythrocytes and granulocytes 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, enabling the separation of 50 mL Leukopak® sample.

- Columns are “flow stop” and do not run dry.
- Void volume per single column: 250 µL. Reservoir volume: 5 mL.
- 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® PBMC Isolation Kit, human has been developed for the isolation of peripheral blood mononuclear cells (PBMCs) directly from Leukopak® by using the MultiMACS Cell24 Separator Plus and MultiMACS X. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis.

1.4 Applications

- Isolation of PBMCs from Leukopaks®. The purified PBMCs are well suited for further cell separation, flow cytometric, functional, or molecular analysis.

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).
- Single-well Deep Well Plates (# 130-114-966).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

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2. Protocol

▲ The StraightFrom Leukopak® PBMC Isolation Kit, human has been developed for untouched isolation of peripheral blood mononuclear cells (PBMCs) from 50 mL Leukopak® sample containing approx. 5×10^9 cells.

▲ The hematocrit (HCT) value of the Leukopak® sample is given on the Certificate of Analysis (CoA). If the HCT level of the original sample is >3.5 (based on a 1:1 dilution factor), dilute sample with separation buffer. Use the same reagent volumes as indicated.

2.1 Preparation of Leukopak®

1. Divide the 50 mL Leukopak® sample into two collection tubes. If the volume per tube is less than 25 mL, fill up to 25 mL with separation buffer. Mix the Leukopak® well before distributing to conical tubes to avoid unequal distribution of erythrocytes caused by sedimentation.
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 given below are for one tube containing 25 mL sample.

▲ 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 Erythrocyte Depletion MicroBeads and 1 mL Granulocyte Depletion MicroBeads to both tubes containing 25 mL sample.
2. Mix gently by carefully inverting the tube 5–7 times.
3. Incubate for 15 minutes in the refrigerator (2–8 °C).
4. Proceed 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 the Multi-24 Column Block and two Deep Well Plates for magnetic separation with the StraightFrom Leukopak® PBMC Isolation Kit, human.

1. Switch on the MultiMACS Cell24 Separator Plus and select the program **Deplete**.
2. Position a waste plate (24-well Deep Well Plate) on the tip-touch plate.
3. Equilibrate the Multi-24 Column Block with 2 mL separation buffer per column.
4. Remove the waste plate and insert a new Single-well Deep Well Plate for collection of PBMCs (untouched fraction).
5. Carefully invert the tube containing the sample 2–3 times.
6. Add 2.5 mL of sample onto each column.
7. Let it run through until the reservoir is empty.
8. Wash each column with 1 mL separation buffer.
9. Pipette unlabeled cells from the Single-well Deep Well Plate into a fresh tube.
10. Rinse the Single-well Deep Well Plate with 10 mL separation buffer and add to the unlabeled cells in the tube.

    ▲ **Note:** Magnetically labeled cells (erythrocytes and granulocytes) are retained in the columns.
11. Centrifuge unlabeled cells at 200×g for 10 minutes. Aspirate supernatant carefully. The supernatant contains plasma and platelets.
12. Resuspend cell pellet in a suitable amount of buffer or medium for subsequent downstream experiments.

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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