Miltenyi Biotec

StraightFrom® Whole Blood PBMC Isolation Kit

human

2 mL	130-126-359
10 mL	130-139-976

Contents

1. Description

This product is for research use only.

Components	**#130-126-359:** **2×2 mL Erythrocyte Depletion MicroBeads** **2×2 mL Granulocyte Depletion MicroBeads** or **#130-139-976:** **2×10 mL Erythrocyte Depletion MicroBeads** **2×10 mL Granulocyte Depletion MicroBeads**
Capacity	For 500 mL peripheral whole blood (#130-126-359). or for 2500 mL peripheral whole blood (#130-139-976).
Product format	MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store reagents protected from light at +2 to +8 °C. Do not freeze. The expiration dates are indicated on the vial labels.

1.1 Principle of the StraightFrom Whole Blood PBMC Isolation Kit, human

Using the StraightFrom Whole Blood PBMC Isolation Kit, human, peripheral blood mononuclear cells (PBMCs) are isolated by depletion of non-PBMCs (untouched isolation) after a first erythrocyte sedimentation step. Granulocytes and erythrocytes are directly magnetically labeled with Granulocyte Depletion MicroBeads and Erythrocyte Depletion MicroBeads. The magnetically labeled non-PBMCs are depleted by retaining them on a MACS Column in the magnetic field of a separator, while the unlabeled PBMCs pass through the column.

1.2 Background information

The StraightFrom Whole Blood PBMC Isolation Kit, human has been developed for the negative selection of PBMCs directly from anticoagulated whole blood by using the autoMACS NEO Separator, the MultiMACS™ Cell24 Separator Plus, or manual separation. No density gradient centrifugation, erythrocyte lysis, or washing after labeling are required. The StraightFrom Whole Blood PBMC Isolation Kit, human eliminates erythrocytes via a sedimentation step and depletes all remaining non-PBMCs targeting granulocytes and erythrocytes with specific beads.

1.3 Applications

- Untouched isolation of PBMCs without density gradient centrifugation

1.4 Reagent and instrument requirements

- Sedimentation Kit II, human (# 130-126-357) or Sedimentation Kit II, human – Large Scale (# 130-132-321).
- 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). 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.

  ▲ **Note:** autoMACS Running Buffer (# 130-091-221) can be used alternatively.
- 0.5 M EDTA stock solution, pH 8.0.
- Automated separation:
  - autoMACS NEO Separator (# 130-120-327) or autoMACS Pro Separator (# 130-092-545) with serial number 1401 or higher and software version 2.4.8.0 or higher.
  - autoMACS Pro Starting Kit (# 130-092-545).
  - autoMACS Columns (# 130-021-101).

  or

  - MultiMACS Cell24 Separator Plus (# 130-098-637).
- Manual separation:

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- MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976).
- LS Columns (# 130-042-401).

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

▲ Volumes given below are for 1 mL whole blood sample. Scale up volumes according to sample volume.

Tube size	Maximal volume of whole blood/tube
15 mL	6 mL
50 mL	15 mL
225 mL	100 mL

Table 1: Maximal volumes of whole blood samples that can be prepared in different tube sizes.

▲ When working with samples older than 24 hours, recovery of target cells will be lower.

▲ Adjust Sedimentation Buffer II from the Sedimentation Kit II and samples to room temperature before use.

1. Add 0.8 mL Sedimentation Buffer II and 2 µL RBC Removal Antibodies from the Sedimentation Kit II, human to a tube.
2. Add 18 µL of 0.5 M EDTA stock solution to the tube containing Sedimentation Buffer II and RBC Removal Antibodies for a final concentration of 5 mM (including sample volume).
3. Mix whole blood well to avoid unequal distribution of erythrocytes caused by sedimentation.
4. Add 1 mL of whole blood sample to the prepared tube.
5. Mix gently by inverting the tube ten times.
6. Centrifuge at room temperature according to table 2.

   ▲ **Note:** It is recommended to centrifuge at full acceleration and full break.

Tube size	Centrifugation condition
15 mL	50×g, 2 min
50 mL	50×g, 2 min
225 mL	50×g, 5 min

Table 2: Centrifugation conditions for different tube sizes.

7. Carefully collect supernatant and transfer into a new tube. Discard cell pellet.
8. Fill up with separation buffer.
9. Invert tube twice and centrifuge at room temperature according to table 3.

Tube size	Centrifugation condition
15 mL	300×g, 10 min
50 mL	300×g, 10 min
225 mL	400×g, 10 min

Table 3: Centrifugation conditions for different tube sizes.

10. Discard supernatant and proceed to magnetic labeling.

2.2 Magnetic labeling

▲ Volumes given below are for 1 mL whole blood sample. Scale up volumes according to sample volume.

▲ The recommended incubation temperature is room temperature. Working on ice may require increased incubation times.

1. Resuspend cell pellet in 20 µL separation buffer.

   ▲ **Note:** Multiple cell pellets from one sample can be combined in one tube at this point for easier handling.
2. Add 8 µL of Erythrocyte Depletion MicroBeads and 8 µL of Granulocyte Depletion MicroBeads.
3. Mix gently and incubate sample for 5 minutes at room temperature.

   ▲ **Note:** Ensure that there are no large air bubbles in the tube.
4. Proceed to magnetic separation.

2.3 Magnetic separation

Magnetic separation with LS Columns

▲ Use one LS Column per 10 mL whole blood starting material.

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 2 mL of separation buffer.
3. Fill up sample with separation buffer to a total volume of 2 mL per 10 mL whole blood starting material and mix gently.
4. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
5. Wash column with 2 mL of separation buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3. This is the target cell fraction (PBMCs).

   ▲ **Note:** The positive fraction is retained in the column and contains the erythrocytes and granulocytes.

Magnetic separation with the MultiMACS Cell24 Separator

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

2.4 Magnetic labeling and separation using autoMACS Separators

▲ 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.

2.4.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ 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. Label the sample as described in section 2.2 Magnetic labeling.
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 and separation

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

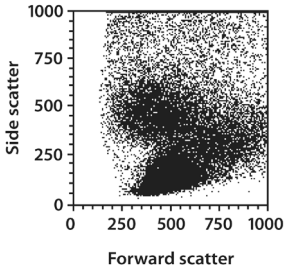
2.4.2 Magnetic labeling and separation using the autoMACS Pro Separator

For instructions on magnetic separation on the autoMACS Pro Separator, contact technical support. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

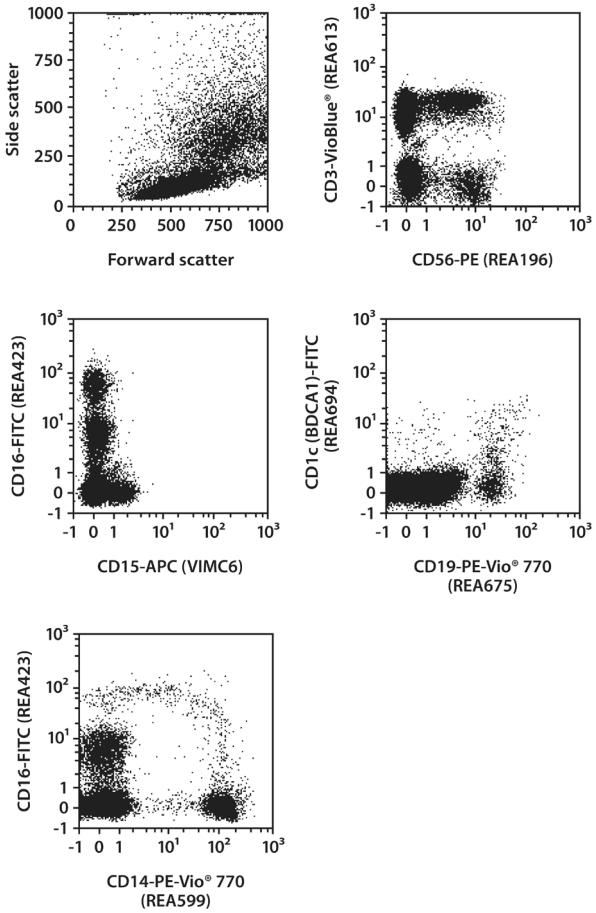
3. Example of a separation using the StraightFrom Whole Blood PBMC Isolation Kit, human

PBMCs were isolated from human whole blood using the StraightFrom Whole Blood PBMC Isolation Kit, human, the MultiMACS Cell24 Separator, and Multi-24 Column Blocks. Cells were fluorescently stained with CD45-VioGreen™, CD3-VioBlue®, CD56-PE, CD16-FITC, CD15-APC, and CD14-PE-Vio® 770, or with CD45-VioGreen, CD19-PE-Vio 770, and CD1c (BDCA1)-FITC and analyzed by flow cytometry using the MACSQuant® Analyzer.

A) Before separation



B) Isolated viable CD45$^+$ 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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