Miltenyi Biotec

StraightFrom® Buffy Coat PBMC Isolation Kit

human

Isolation Kit (LS)	130-126-453
Isolation Kit	130-126-448

Contents

1. Description

This product is for research use only.

Components	**StraightFrom Buffy Coat PBMC Isolation Kit (LS), human (# 130-126-453):** 2× 2 mL Erythrocyte Depletion MicroBeads, human 1× 2 mL Granulocyte Depletion MicroBeads, human 2× 12 LS Columns: columns and plungers, sterile packed. **StraightFrom Buffy Coat PBMC Isolation Kit, human (# 130-126-448):** 2× 2 mL Erythrocyte Depletion MicroBeads, human 1× 2 mL Granulocyte Depletion MicroBeads, human
Capacity	For two buffy coats (each 40–100 mL) from max. 1 L whole blood.
Product format	MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store MicroBeads protected from light at +2 to +8 °C. Do not freeze. Store LS Columns dry at +10 to +35 °C and protected from light. The expiration date is indicated on the vial or box label.

1.1 Principle of the MACS® Separation

Using the StraightFrom Buffy Coat PBMC Isolation Kits, 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, human and Erythrocyte Depletion MicroBeads, human. 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 Buffy Coat PBMC Isolation Kits, human have been developed for the negative selection of PBMCs directly from buffy coat by using an autoMACS® NEO Separator, autoMACS Pro Separator, the MultiMACS™ Cell24 Separator Plus, or manual separation. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The StraightFrom Buffy Coat PBMC Isolation Kits, human eliminate erythrocytes via a sedimentation step and deplete all remaining non-PBMCs targeting granulocytes and erythrocytes with specific MicroBeads.

1.3 Applications

- Untouched isolation of PBMCs from buffy coat samples without density gradient centrifugation.

1.4 Reagent and instrument requirements

- 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). Alternatively, use autoMACS Running Buffer. Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). 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)
  - Chill 15 Rack (# 130-092-952) or Chill 50 Rack (# 130-092-953)
  - autoMACS Columns (# 130-021-101)
  - autoMACS Running Buffer (# 130-091-221)
  - autoMACS Washing Solution (# 130-092-987)

  or

  - MultiMACS Cell24 Separator Plus (# 130-098-637)
  - MultiMACS 12× Single-Column Adapter LS (# 130-108-816) with LS Columns (# 130-042-401) or Multi-24 Column Block (# 130-095-692)

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-006-876.01

  - Single-well Deep Well Plates (# 130-114-966), 2-well Deep Well Plates (# 130-120-010), 3-well Deep Well Plates (# 130-120-009), or 24-well Deep Well Plates (# 130-110-500) depending on the number of target cell fractions.
- Manual separation:
  - LS Columns (# 130-042-401)
  - QuadroMACS Separator (# 130-090-976)
  - MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052)
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocol

2.1 Sample preparation

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

▲ When working with more than 40 mL buffy coat, split buffy coat and fill up with buffer.

Tube size	Maximal volume of buffy coat per tube
15 mL	4–6 mL
50 mL	15 mL
225 mL	40 mL (filled up to 80 mL with buffer)

Table 1: Maximal volumesof buffy coat 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 buffy coat well to avoid unequal distribution of erythrocytes caused by sedimentation.
4. Add 1 mL of buffy coat 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 tube 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).

2.2 Magnetic labeling

▲ Volumes given below are for one buffy coat (40–100 mL). Scale down volumes according to sample volume, when working with smaller buffy coat samples.

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

1. Resuspend and combine cell pellets from a full buffy coat after preparation steps (see 2.1), using a total of 4 mL separation buffer for reconstitution.
2. Add 2 mL Erythrocyte Depletion MicroBeads, human and 1 mL Granulocyte Depletion MicroBeads, human.
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 directly to magnetic separation (2.3).

2.3 Magnetic separation

Manual magnetic separation with LS Columns

▲ Use 12 LS Columns per one buffy coat starting material. Divide the sample equally between the 12 LS Columns.

1. Fill up sample with separation buffer to a total volume of 24 mL per buffy coat and mix gently. Divide the sample equally between the 12 LS Columns (2 mL per column).
2. Place one column in the magnetic field of a QuadroMACS Separator. For details refer to the respective MACS Column data sheet.
3. Prepare one column by rinsing with 2 mL of separation buffer.
4. Apply respective sample volume onto one 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.
6. Repeat steps 2–5 with for each column.

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.

▲ The MultiMACS Cell24 Separator Plus, including the MACS Elution Station, has to be used with the Single-Column Adapter and LS Columns or Multi-24 Column Blocks, and elution plate for magnetic separation with Granulocyte Depletion MicroBeads, human and Erythrocyte Depletion MicroBeads, human.

▲ Buffer volumes per column are as follows:

Equilibration: 2 mL (separation buffer)
Wash: 2 mL (separation buffer)

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

▲ Divide the sample equally between the 12 LS Columns, e.g., when starting volume is 24 mL, add 2 mL onto each column.

▲ (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

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

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.

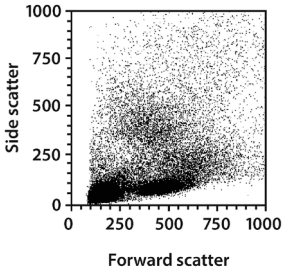
2.4.2 Magnetic labeling and separation using the autoMACS Pro Separator

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.

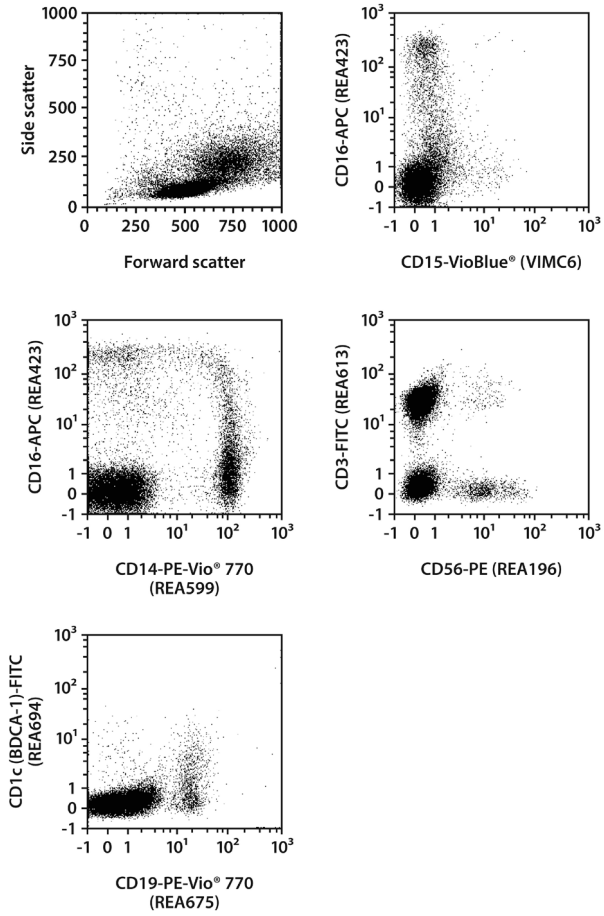
3. Example of a separation using the StraightFrom Buffy Coat PBMC Isolation Kit, human

Separation of a buffy coat sample using the StraightFrom Buffy Coat PBMC Isolation Kit, human and the MultiMACS Cell24 Separator Plus with the Single-Column Adapter and LS Columns. Cells were fluorescently stained with CD15-VioBlue®, CD16-APC, CD14-PE-Vio® 770, CD3-FITC, CD56-PE, CD19-PE-Vio 770, and CD1c (BDCA-1)-FITC and analyzed by flow cytometry using the MACSQuant® Analyzer.

A) Before separation



B) Isolated PBMCs



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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