Miltenyi Biotec

MACSprep™ Multiple Myeloma CD138 MicroBeads human

Order no. 130-111-744

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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	2 mL MACSprep™ Multiple Myeloma CD138 MicroBeads, human: Ultrapure MicroBeads conjugated to monoclonal anti-human CD138 antibodies (isotype: mouse IgG1). **MACSprep Multiple Myeloma MicroBeads have been developed for use with the autoMACS® Pro Separator or the Whole Blood Column Kit.**
Capacity	For 40 mL human bone marrow or whole blood.
Product format	MACSprep Multiple Myeloma CD138 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the CD138$^+$ cells in a bone marrow or whole blood sample are magnetically labeled with MACSprep Multiple Myeloma CD138 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD138$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD138$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD138$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

MACSprep Multiple Myeloma CD138 MicroBeads have been developed for the positive selection of CD138$^+$ cells directly from anticoagulated bone marrow or whole blood by using the autoMACS Pro Separator or the Whole Blood Column Kit. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The CD138 antigen, also known as syndecan-1[1], is primarily found on normal and malignant plasma cells in the bone marrow[2]. The CD138 antigen is not expressed by naive B cells, germinal center B cells, memory B cells, T cells, or monocytes.[2,3]

1.3 Applications

- Isolation of CD138$^+$ cells from bone marrow or whole blood. The enriched CD138$^+$ cells are well suited for further flow cytometric, functional, or molecular analysis such as FISH, PCR analysis[4,6], microarrays[5,7], and protein analysis[6], and for analysis of cell surface markers or intracellular antigens, for example, immunoglobulin light chains[4].

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 (2–8 °C). 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.

- Automated separation:
  - autoMACS Pro Starter Kit (# 130-092-545)
  - autoMACS Columns (# 130-021-101)
- Manual separation:
  - Whole Blood Column Kit (# 130-093-545), including Whole Blood Columns and Whole Blood Column Elution Buffer.
  - MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976).
- MACS® SmartStrainers (100 µm) (# 130-098-463) to remove bone fragments and cell clumps.
- (Optional) HEPES-buffered cell culture medium (e.g. IMDM or RPMI 1640), supplemented with 100 U/mL heparin for the

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preparation of bone marrow cells.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD138-APC, CD38-FITC, 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.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

▲ MACSprep™ Multiple Myeloma MicroBeads have been developed for positive selection of target cells directly from anticoagulated bone marrow or whole blood samples, ranging in volume from **0.25 mL to 15 mL (autoMACS® Pro Separator or Whole Blood Column Kit)**.

2.1 Preparation of bone marrow or whole blood samples

▲ Anticoagulants, such as EDTA, heparin, citrate dextrose formula-A (ACD-A), or citrate phosphate dextrose (CPD) can be used. For subsequent molecular biology applications use EDTA as an anticoagulant. However, replacing EDTA can decrease purity and/or recovery.

1. Collect 2–15 mL of bone marrow from the upper iliac crest or the sternum using an aspiration needle or up to 15 mL of venous blood in a collection tube containing an appropriate anticoagulant.
2. When working with bone marrow samples: Pass cells through MACS® SmartStrainers (100 µm) in order to remove bone fragments or cell clumps; when working with whole blood samples: Proceed to magnetic labeling (2.3).
3. Proceed to magnetic labeling (2.3)

2.2 (Optional) Washing of bone marrow samples

▲ To further remove debris or dead cells in the bone marrow samples before magnetic labeling, the bone marrow samples can be washed. However, the cell yield might be decreased.

1. Transfer bone marrow into a 50 mL conical tube containing an equal volume of cell culture medium or autoMACS Running Buffer.
2. Centrifuge at 445×g for 10 minutes at 20 °C in a swinging bucket rotor without brake.
3. Resuspend cell pellet with cell culture medium or autoMACS Running Buffer to the original volume used in step 1.
4. Proceed to magnetic labeling (2.3).



2.3 Magnetic labeling

▲ Cells can be labeled with MACSprep Multiple Myeloma CD138 MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ Volumes for magnetic labeling given below are for 1 mL of anticoagulated bone marrow or whole blood. When working with larger volumes, scale up all reagent volumes and total volumes accordingly (e.g. for 2 mL, use twice the volume of all indicated reagent volumes and total volumes). When working with volumes below 1 mL (minimal volume 0.25 mL), scale down all reagent and total volumes accordingly.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm) # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Add 50 µL MACSprep Multiple Myeloma CD138 MicroBeads per 1 mL anticoagulated bone marrow or whole blood.
2. Mix well and incubate for 15 minutes at room temperature (+19–25 °C).
3. Proceed to magnetic separation (2.4).



2.4 Magnetic separation

2.4.1 Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ (Optional) Pre-cool Chill Racks. Operating at room temperature is recommended for optimal results.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

Fully automated cell labeling and separation

▲ When using the autolabeling feature of the autoMACS Pro Separator manual magnetic labeling of samples (section 2.3) is not necessary.

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.

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6. Select **Run**.
7. Collect positive fraction in row C of the tube rack. Spin down the cells at 300×g 5 minutes and remove supernatant. The cells are ready for downstream application.

Magnetic separation using manual labeling

▲ When working with the program "Posselwb" on the autoMACS® Pro Separator, samples will be automatically diluted 1:3 with autoMACS Running Buffer before sample uptake. For example, when processing 1 mL, the sample will be diluted with 2 mL of buffer. Make sure that the capacities of the sample tubes and fraction collection tubes are sufficient for the total volumes.

1. Label the sample as described in section 2.3 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. Choose program sequence **Posselwb/Clean**. Collect positive fraction in row C of the tube rack.
5. Choose program **Sleep** after all samples have been processed. The autoMACS Pro Separator can be switched off now.
6. Spin down the cells at 300×g 5 minutes and remove supernatant. The cells are ready for downstream application.

2.4.2 Magnetic separation with the Whole Blood Column Kit

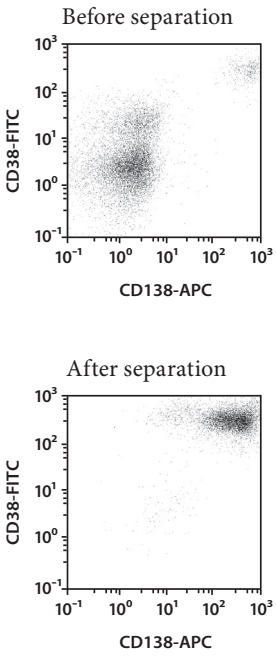
1. Place Whole Blood Column in the magnetic field of a suitable MACS Separator. For details refer to the Whole Blood Column Kit data sheet.
2. Prepare column by rinsing with 3 mL separation buffer.
3. Apply magnetically labeled cell suspension onto the prepared Whole Blood Column. Collect flow-through containing unlabeled cells.

   ▲ **Note:** The reservoir of the Whole Blood Column contains a maximum of 7.5 mL. Samples greater than 7.5 mL should be applied in aliquots to the column.
4. Wash Whole Blood Column with 2×2 mL separation buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove Whole Blood Column from the separator and place it on a new collection tube.
6. Pipette 4 mL **Whole Blood Column Elution Buffer** onto the Whole Blood Column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. Spin down the cells at 300×g 5 minutes and remove supernatant. The cells are ready for downstream application.

3. Example of a separation using MACSprep™ Multiple Myeloma CD138 MicroBeads

Separation of a bone marrow sample using MACSprep™ Multiple Myeloma CD138 MicroBeads and the autoMACS® Pro Separator. Cells were fluorescently stained with CD138-APC, CD38-FITC, and CD45-VioBlue® and 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.



4. References

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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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