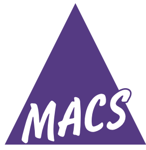


Whole Blood CD138 MicroBeads
human

Order no. 130-093-062

Contents

1. **Description**
    - 1.1 **Principle of the MACS® Separation**
    - 1.2 **Background information**
    - 1.3 **Applications**
    - 1.4 **Reagent and instrument requirements**
2. **Protocols**
    - 2.1 **Preparation of whole blood**
    - 2.2 **Preparation of bone marrow**
    - 2.3 **Magnetic labeling**
    - 2.4 **Magnetic separation**
3. **Example of a separation using the Whole Blood CD138 MicroBeads**
4. **Appendix**
    - 4.1 **Intracellular staining of cells in suspension**
    - 4.2 **Intracellular staining of cells on slides**
5. **References**

1. Description

Components 2 mL Whole Blood CD138 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD138 antibodies (isotype: mouse IgG1).

MACS® Whole Blood MicroBeads are developed for use with the autoMACS™ Pro Separator, the autoMACS Separator or the Whole Blood Column Kit.

Capacity For 40 mL human whole blood or bone marrow.

Product format Whole Blood 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 whole blood or bone marrow sample are magnetically labeled with Whole Blood CD138 MicroBeads. Then, the cell suspension is applied 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 and are collected as the negative fraction; this cell fraction is thus depleted of CD138⁺ cells. After removal of the column from the magnetic field, the magnetically retained CD138⁺ cells are eluted as the positively selected cell fraction.

1.2 Background information

Whole Blood CD138 MicroBeads are developed for the positive selection of human plasma cells directly from anticoagulated whole blood or bone marrow by using the autoMACS Pro Separator, the autoMACS 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]. In the peripheral blood of healthy donors approximately 50% of plasma cells express this antigen. 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

- Positive selection of plasma cells from bone marrow or peripheral blood from healthy donors or patients with plasma cell disorders, for example, plasma cell malignancies, such as Multiple Myeloma. Isolated plasma cells can be used for molecular biology studies such as 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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Automated separation:
    - autoMACS Pro Starting Kit (# 130-092-545) or autoMACS Starting Kit (# 201-01).
    - 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, QuadroMACS Separator, VarioMACS Separator, SuperMACS Separator, or SuperMACS II Separator.
    - LS Column Adapter (# 130-090-544) for use with VarioMACS Separator or SuperMACS Separator, or Adapter for MS, LS, and LD Columns for use with SuperMACS II Separator.
- HEPES-buffered cell culture medium (e.g. IMDM), supplemented with 100 U/mL heparin for the preparation of bone marrow cells.
- Filter (100 µm pore size) to remove bone fragments and cell clumps.

140-001-968.04

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0 Fax +49 2204 85197
macs@miltenyibiotec.de

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871 Fax +1 530 888 8925
macs@miltenyibiotec.com

- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps from whole blood.
- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, for example, CD138-PE (# 130-081-301), CD138-APC (# 130-091-250), CD38-FITC (# 130-092-259), CD38-PE (# 130-092-260), CD38-APC (# 130-092-261), CD19-FITC (# 130-091-328), CD19-PE (#130-091-247), CD19-APC (# 130-091-248), CD56-PE (# 130-090-755), CD56-APC (# 130-090-843), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or CD45-APC (# 130-091-230). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

2. Protocols

▲ MACS Whole Blood MicroBeads have been developed for positive selection of target cells from anticoagulated whole blood or bone marrow samples, ranging in volume from **0.25 mL to 15 mL (autoMACS Pro Separator or Whole Blood Column Kit)** or **0.25 mL to 3 mL (autoMACS Separator)**.

2.1 Preparation of whole blood

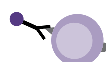
▲ Anticoagulants such as EDTA, heparin-EDTA, citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD) can be used. For subsequent molecular biology applications use EDTA as an anticoagulant.

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

1. Collect up to 15 mL of venous blood in a collection tube containing an appropriate anticoagulant.
2. Proceed to magnetic labeling (2.3).

2.2 Preparation of bone marrow cells

1. Collect 2–10 mL of bone marrow from the upper iliac crest or the sternum using an aspiration needle.
2. Transfer bone marrow into a 50 mL conical tube containing an equal volume of HEPES-buffered cell culture medium.
3. Pass cells through filter (100 µm pore size) in order to remove bone fragments or cell clumps. Wet filter with separation buffer (autoMACS Running Buffer) before use.
4. Centrifuge at 445×g for 10 minutes at 20 °C in a swinging bucket rotor without brake.
5. Aspirate supernatant carefully. Do not disturb cell pellet. Dilute cell pellet to the original volume with separation buffer (autoMACS Running Buffer). The final volume should not exceed the starting volume used in step one.
6. Proceed to magnetic labeling (2.3).
   ▲ **Note:** If cells cannot be separated on the day of harvest, store cells at 4 °C.



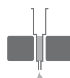
2.3 Magnetic labeling

▲ 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 1 mL of anticoagulated whole blood or bone marrow. 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).

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Add 50 µL Whole Blood CD138 MicroBeads per 1 mL anticoagulated whole blood or bone marrow.
2. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
3. Wash cells by adding 2–5 mL of separation buffer (autoMACS Running Buffer) per 1 mL of whole blood or bone marrow and centrifuge at 445×g for 10 minutes at room temperature in a swinging bucket rotor without brake.
4. Aspirate supernatant carefully. Do not disturb the cell pellet. Leave a residual volume of supernatant (approximately 1–2 mm in height) to avoid cell loss.
5. Resuspend cell pellet by adding one volume of separation buffer (autoMACS Running Buffer) to a total volume of 1 mL.
6. Proceed to magnetic separation (2.4).



2.4 Magnetic separation

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥ 10 °C.

Magnetic separation with the autoMACS™ Pro Separator

▲ 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. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. Choose program sequence "Posselwb/Rinse". Collect positive fraction in row C of the tube rack.
4. Choose program "Sleep" after all samples have been processed. The autoMACS Pro Separator can be switched off now.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube below the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. Choose program "Posseld2". Collect positive fraction from outlet port pos2.
4. Choose program "Rinse" before proceeding to the next separation.
5. Choose program "Sleep" after all samples have been processed. The autoMACS™ Separator can be switched off now.

Magnetic separation with the Whole Blood Column Kit

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

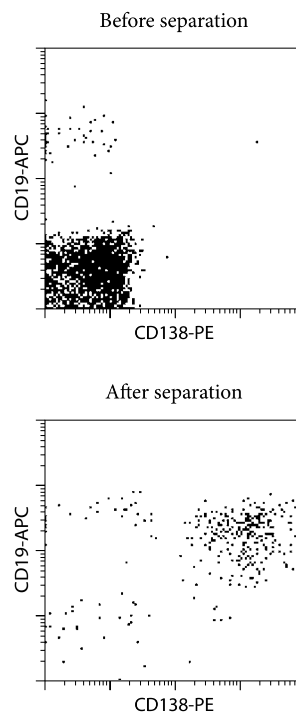
   ▲ **Note:** The reservoir of the Whole Blood Column contains a maximum of 7.5 mL. Samples greater than 7.5 mL should be applied in aliquots to the column.
4. Wash Whole Blood Column with 3×3 mL separation buffer (autoMACS Running Buffer). Collect unlabeled cells that pass through and combine with the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

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove Whole Blood Column from the separator and place it on a new collection tube.
6. Pipette 5 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. (Optional) To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a new, freshly prepared MS Column (# 130-042-201, for up to 10^7 magnetically labeled cells) or LS Column (# 130-042-401, for up to 10^8 magnetically labeled cells).

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

3. Example of a separation using the Whole Blood CD138 MicroBeads

Separation of a whole blood sample using Whole Blood CD138 MicroBeads and the autoMACS Separator. Cells are fluorescently stained with CD45-FITC (# 130-080-202), CD138-PE (# 130-081-301), and CD19-APC (# 130-091-248). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Dot plots shown are gated on CD45$^+$ cells.



4. Appendix

4.1 Intracellular staining of cells in suspension

Reagent and instrument reqirements

- Buffer: autoMACS Running Buffer (# 130-091-221), alternatively, 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). 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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- Inside Stain Kit (# 130-090-477).
- Antibodies specific for intracellular antigens, for example, anti-human κ-FITC or anti-human λ-FITC (Southern Biotechnology Associates).

Protocol

1. Resuspend cells in 250 µL of buffer.
2. Add 250 µL of Inside Fix. Mix well and incubate for 20 minutes at room temperature.

140-001-968.04

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

3. Centrifuge cells at 300×g for 5 minutes.
4. Aspirate supernatant carefully and resuspend pellet in 1 mL of buffer.
5. Centrifuge cells at 300×g for 5 minutes and aspirate supernatant completely.
6. Dilute staining antibody with Inside Perm at appropriate titer (according to the manufacturer's instructions) to a final volume of 100 µL and resuspend cell pellet in diluted staining antibody.
7. Incubate for 10 minutes at room temperature.
8. Add 1 mL of Inside Perm.
9. Centrifuge cells at 300×g for 5 minutes.
10. Aspirate supernatant carefully and resuspend cell pellet in 0.5–1 mL of buffer.
11. Proceed to flow cytometric analysis.

4.2 Intracellular staining of cells on slides

Reagent and instrument requirements

- Buffer: autoMACS Running Buffer (# 130-091-221), alternatively, 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). 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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- Inside Stain Kit (# 130-090-477).
- PBS, pH 7.2.
- Antibodies specific for intracellular antigens, for example, anti-human κ-FITC or anti-human λ-FITC (Southern Biotechnology Associates).
- Mounting medium (e.g. Fluoromount-G, Southern Biotechnology Associates).
- Cytocentrifuge.
- Slides and coverslips.
- Staining trough, for example, type Hellendahl.
- Humidity chamber.

Protocol

1. Resuspend an appropriate number of cells in buffer at a final concentration of 10^5–10^6 cells/mL.
2. Transfer an aliquot of 50–100 µL onto a slide using a cytocentrifuge.
3. Let slides dry for 20 minutes at 20–25 °C.
4. Fix cells in Inside Fix for 20 minutes at 20–25 °C using a staining trough.
5. Wash slides in buffer using a staining trough.

   ▲ **Note:** Slides can be stored in buffer supplemented with 0.05% NaN_3 at 4 °C in the dark until staining.
6. Incubate slides in Inside Perm for 10 minutes at 20–25 °C using a staining trough.
7. Wipe slides dry but exclude cell area.
8. Dilute antibody solution with Inside Perm according to the manufacturer's instructions.
9. Add 250 µL of diluted antibody solution to the cell area and incubate for 10 minutes in the dark at 20–25 °C using a humidity chamber .
10. Wash slides in PBS using a staining trough.
11. Let slides dry. Then cover cells with mounting medium and coverslip.
12. Proceed to fluorescence analysis.

5. References

1. Bernfield, M. *et al.* (1992) Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. Annu. Rev. Cell Biol. 8: 365–393. [9658]
2. Wijdenes, J. *et al.* (1996) A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br. J. Haematol. 94: 318–323. [9657]
3. Pellat-Deceunynck, C. *et al.* (1994) Expression of CD28 and CD40 in human myeloma cells: a comparative study with normal plasma cells. Blood 84: 2597–2603. [9656]
4. Draube, A. *et al.* (2001) Immunomagnetic enrichment of CD138 positive cells from weakly infiltrated myeloma patients samples enables the determination of the tumor clone specific IgH rearrangement. Ann. Hematol. 80: 83–89. [989]
5. Zhan, F. *et al.* (2003) Gene expression profiling of human plasma cell differentiation and classification of multiple myeloma based on similarities to distinct stages of late-stage B-cell development. Blood 101: 1128–1140. [4367]
6. Kumar, S. *et al.* (2004) Bone marrow angiogenic ability and expression of angiogenic cytokines in myeloma: evidence favoring loss of marrow angiogenesis inhibitory activity with disease progression. Blood 104: 1159–1165. [5196]
7. Shaughnessy, J. *et al.* (2001) Cyclin D3 at 6p21 is dysregulated by recurrent chromosomal translocations to immunoglobulin loci in multiple myeloma. Blood 98: 217–223. [1237]

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

MACS is a registered trademark and autoMACS, MidiMACS, QuadroMACS, SuperMACS, and VarioMACS are trademarks of Miltenyi Biotec GmbH.

Copyright © 2008 Miltenyi Biotec GmbH. All rights reserved.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.