Miltenyi Biotec

CD1a MicroBeads
human

Order no. 130-051-001

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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 CD1a MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-human CD1a antibodies (mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD1a 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 CD1a$^+$ cells are magnetically labeled with CD1a 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 CD1a$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD1a$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD1a$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD1a$^+$ cells must be separated over a second column.

1.2 Background information

CD1a MicroBeads have been developed for positive selection of CD1a$^+$ dendritic cells from the epidermis (Langerhans cells), dermis, lung, mucosa, and other non-lymphoid tissues, or for further purification of CD1a$^+$ dendritic cells from cultured cells after *in vitro* differentiation from hematopoietic progenitor cells[1–5] or monocytes[6]. The CD1a antigen is a member of the CD1 family of proteins, which are structurally related to MHC class I proteins and mediate the presentation of non-peptide antigens to T cells.[7] CD1a belongs to the C-type lectins and has a molecular mass of 49 kDa.

1.3 Applications

- Positive selection of CD1a$^+$ dendritic cells from single-cell suspension prepared from skin, mucosa, and other non-lymphoid tissues.
- Purification of CD1a$^+$ dendritic cells from cultured cells after *in vitro* differentiation from CD34$^+$, CD133$^+$ hematopoietic progenitor cells[1–5], or CD14$^+$ monocytes[6].
- Isolation of pure, viable, and functionally intact epidermal Langerhans cells from human skin.[8]

1.4 Reagent and instrument requirements

- 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). 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.

- MACS Columns and MACS Separators: CD1a$^+$ cells can be enriched by using MS or LS Columns. Positive selection can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator Plus.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro
Multi-24 Column Block	10^8	10^9	MultiMACS Cell24 Separator Plus

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

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

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▲ **Note:** If using the MultiMACS Cell24 Separator Plus with the Single-Column Adapter, please refer to the user manual for column capacities.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD1a-FITC (# 130-097-903), CD1a-PE (# 130-097-868), CD1a-APC (# 130-097-875), CD1a-VioBlue® (# 130-097-905), CD1a-APC-Vio770™ (# 130-100-224), or CD1a-Biotin (# 130-097-908). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



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 for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ 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.

▲ 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. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD1a MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of CD1a-FITC (# 130-097-903), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD1a^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

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 the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of $CD1a^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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Magnetic separation with the MultiMACS™ Cell24 Separator Plus

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

Magnetic separation with the autoMACS® Pro Separator

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

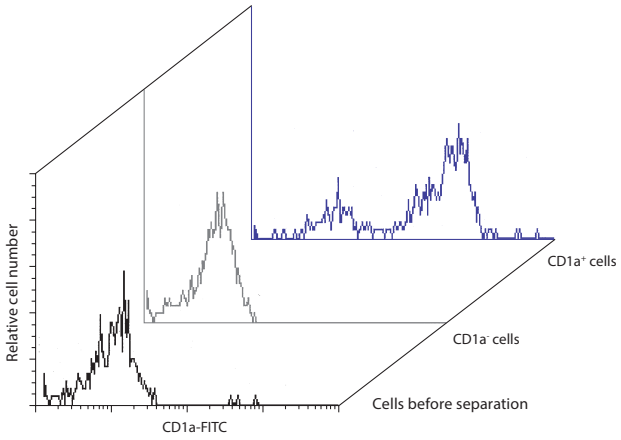
▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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 the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

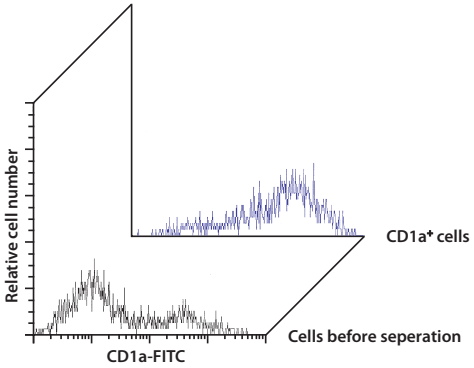
   Positive selection:
   • if target cell frequency > 5%: **Possel**
   • if target cell frequency < 5%: **Posseld2**
   Collect positive fraction in row C, unlabeled fraction in row B of the tube rack.

3. Example of a separation using CD1a MicroBeads

1. Separation of human CD1a$^+$ dendritic cells from single-cell suspension of human epidermis (Langerhans cells) using the CD1a MicroBeads and an autoMACS Separator. Cells were fluorescently stained with CD1a-FITC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



2. CD133$^+$ hematopoietic progenitor cells were isolated using the CD133 MicroBead Kit (# 130-050-801) and were cultured for 12 days in the presence of Flt-3 ligand, TGF-β, TNF-α, GM-CSF, and SCF. Thereafter, CD1a$^+$ dendritic cells were isolated using the CD1a MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD1a-FITC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Appendix: Preparation of single-cell suspensions from human skin

Reagent and instrument requirements

- Sterile scapels and forceps.
- Sterile petri dishes with a diameter of 90 mm.
- Cell suspension filter or cell strainer with a mesh size of 70 µm, e.g., Pre-Separation Filters, 70 µm (# 130-095-823).
- Cell culture medium, e.g., RPMI 1640 (# 130-091-440).
- 0.05% trypsin solution in PBS.
- Fetal bovine serum (FBS).
- Ficoll-Paque™.

Protocol for dissociation of epidermis

1. Collect skin biopsies in a tube containing sterile RPMI 1640.
2. Transfer pieces of skin biopsies in a sterile petri dish using sterile forceps.
3. Cut biopsy material into square pieces of approximately 5×5 mm using sterile forceps and a scalpel.
4. Add appropriate amount of 0.05% trypsin solution so that the pieces are covered with fluid.
5. Incubate the skin pieces overnight at 4 °C.
6. Separate the epidermis from the dermis using forceps and discard the dermis.
7. Stop trypsin digestion by adding the same volume of RPMI 1640 medium containing 15% FBS.
8. Dissociate the epidermis pieces by pipetting up and down.
9. Apply crude suspension of cells to a cell strainer with a mesh size of 70 µm and push the residual aggregates carefully through the filter using a syringe plunger.
10. Layer the cell suspension over Ficoll-Paque (ρ = 1.077).

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11. Centrifuge at 20–25 °C for 30 minutes at 400×g.
12. Collect the cells of the interphase and resuspend them in RPMI 1640 containing 10% FBS.
13. Centrifuge cells for 10 minutes at 300×g.
14. Aspirate supernatant and resuspend cells in Ca^{2+} and Mg^{2+} free PBS containing 0.5% BSA and 10 mM EDTA.
15. Proceed to magnetic labeling (2.2).

For other general protocols refer to the protocols section at www.miltenyibiotec.com/protocols.

5. References

1. Cao, Y. *et al.* (2008) Local accumulation of $FOXP3^+$ regulatory T cells: evidence for an immune evasion mechanism in patients with large condylomata acuminata.J. Immunol. 180: 7681–7686.
2. Caux, C. *et al.* (1996) $CD34^+$ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF^+TNF alpha. J. Exp. Med. 184: 695–706.
3. Meyer, R.G. *et al.* (2007) Prophylactic transfer of CD8-depleted donor lymphocytes after T-cell–depleted reduced-intensity transplantation. Blood 109: 258–264.
4. Ye, Z. *et al.* (1997) *In vitro* expansion and characterization of dendritic cells derived from human bone marrow $CD34^+$ cells. Bone Marrow Transplant. 16: 997–1008.
5. de Wynter, E. A. *et al.* (1998) $CD34^+AC133^+$ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. Stem Cells 16: 387–396.
6. Pickl, W. F. *et al.* (1996) Molecular and functional characteristics of dendritic cells generated from highly purified $CD14^+$ peripheral blood monocytes. J. Immunol. 157: 3850–3859.
7. Brigl, M. and Brenner, M. B. (2004) CD1: antigen presentation and T cell function. Annu. Rev. Immunol. 22: 817–890.
8. Peiser, M. *et al.* (2003) CD1a and CD1c cell sorting yields a homogeneous population of immature human Langerhans cells. J. Immunol. Methods 279: 41–53.

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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