Miltenyi Biotec

CD207 (Langerin) MicroBeads
human

Order no. 130-097-898

Contents

1. Description

Components	2 mL CD207 (Langerin) MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-human CD207 antibodies (mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD207 (Langerin) 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 CD207$^+$ cells are magnetically labeled with CD207 (Langerin) 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 CD207$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD207$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD207$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Langerin (CD207 antigen), a 40 kDa, glycosylated type II transmembrane C-type lectin receptor, is described as the state-of-the-art marker for identifying Langerhans cells (LCs) in humans and mice[4]. LCs are a subset of dendritic cells (DCs) cells that reside in epithelia; the best studied example of these antigen-presenting cells are LCs of the epidermis. Their unique identifying feature are organelles called Birbeck Granules (BGs), which can be detected by electron microscopy. CD207 is expressed at both the surface membrane and in the cytoplasmatic BGs. CD207 plays a role as an endocytic receptor. It is directly involved in antigen capture and its endocytosis and induces the formation of BGs in immature DCs[5].

1.3 Applications

- Positive selection of CD207$^+$ dendritic cells from single-cell suspensions prepared from skin[1], mucosa, and other non-lymphoid tissues.
- Purification of CD207$^+$ dendritic cells from cultured cells after *in vitro* differentiation from CD14$^+$ monocytes[2] or CD34$^+$ cells[3].

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: CD207$^+$ cells can be enriched by using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

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
Positive selection			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

  ▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti HLA-DR-FITC, human (# 130-095-295), Anti-HLA-DR-PE, human (# 130-091-231) or Anti-HLA-DR-APC,human (# 130-091-232) and CD1a-FITC, human (# 130-097-903), CD1a-PE, human (# 130-097-868), CD1a-APC, human (# 130-097-875), CD207 (Langerin)-FITC (# 130-098-349), CD207 (Langerin)-PE (# 130-098-355), CD207 (Langerin)-APC (# 130-098-364), or CD207 (Langerin)-Biotin (# 130-098-344). 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.

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- (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.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

Prepare pa single-cell suspension from human epidermis, for example, the protocol "Isolation of Skin Dendritic Cells from Mouse and Man", Stoizner *et al.*, 2010.[1]

▲ 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 CD207 (Langerin) 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 CD207 (Langerin)-FITC (# 130-098-349), 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 $CD207^+$ 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 only when 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

Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ 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.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro 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 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: Possel
   Collect positive fraction in row C of the tube rack.

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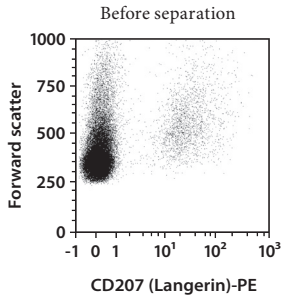
Magnetic separation with the autoMACS® Separator

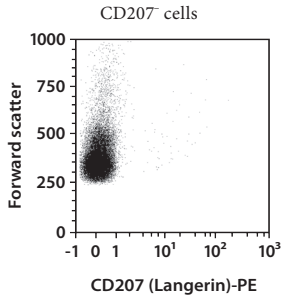
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose one of the following programs:

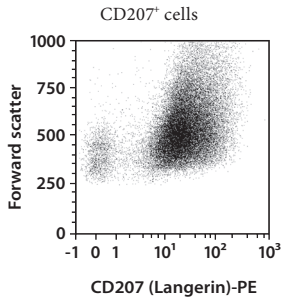
   Positive selection: Possel
   Collect positive fraction from outlet port pos1.

3. Example of a separation using CD207 (Langerin) MicroBeads

Human peripheral blood mononuclear cells (PBMCs) were spiked with CD207-transfected 1881 cells to obtain a 5% initial frequency of CD207-positive cells. CD207-positive cells were isolated using CD207 (Langerin) MicroBeads, an MS Column and a MiniMACS™ Separator. Cells were stained with CD207 (Langerin)-PE (# 130-098-355) 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

1. Stoizner, P. *et al.* (2010) Isolation of skin dendritic cells from mouse and man, Dendritic Cell Protocols, Methods Mol. Biol. 595: 235–248.
2. Geissmann, F. *et al.* (1998) Transforming growth factor β1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. J. Exp. Med. 187(6): 961.
3. Rozis, G. *et al.* (2008) Human Langerhans' cells and dermal-type dendritic cells generated from CD34 stem cells express different toll-like receptors and secrete different cytokines in response to toll-like receptor ligands. Immunology 124(3): 329–338.
4. Valladeau, *et al.* (2000) Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. Immunity 12(1): 71–81.
5. Romani, *et al.* (2010) Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol. Rev. 234(1): 120–141.

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.

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.

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