Miltenyi Biotec

Epidermal Langerhans Cell MicroBead Kit
mouse

Order no. 130-095-408

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1. Description

Components	**1 mL Epidermal Langerhans Cell MicroBeads, mouse:** MicroBeads conjugated to monoclonal rat antibodies against antigens expressed by mouse epidermal Langerhans cells (isotype: rat IgG2b). **1 mL FcR Blocking Reagent, mouse**
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All components 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, Langerhans cells from mouse epidermis single-cell suspension are magnetically labeled with the Epidermal Langerhans Cell 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 epidermal Langerhans cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of epidermal Langerhans cells. After removing the column from the magnetic field, the magnetically retained epidermal Langerhans cells can be eluted as the positively selected cell fraction.

1.2 Background information

The Epidermal Langerhans Cell MicroBead Kit is designed for the isolation of Langerhans cells (LCs) from single-cell suspension of epidermis. Epidermal LCs are the principal dendritic cell population in the epidermis and play a pivotal role in the initiation and maintenance of primary immune responses in the skin. Immature LCs are highly specialized in antigen uptake. *In vivo* LCs exposed to pathogens migrate from the skin to the draining lymph nodes, where they elicit antigen-specific T cell responses.[1–8]

1.3 Applications

- Positive selection or depletion of mouse epidermal Langerhans cells from single-cell suspension prepared from epidermis using the Epidermis Dissociation Kit, mouse.

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 mouse serum albumin, mouse 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: Mouse epidermal Langernhans cells can be enriched by using MS or LS Columns or depleted with the use of LD 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
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) MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD11c-PE (# 130-091-830) or Anti-MHC Class II-APC (# 130-091-806). For more information about fluorochrome-conjugated 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 (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For highest recovery and purity of Langerhans cells from mouse epidermis, a single-cell suspension has to be prepared by enzymatic disaggregation using the Epidermis Dissociation Kit, mouse.

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

▲ Magnetic labeling should be performed immediately after the epidermal dissociation procedure to prevent cell aggregation.

▲ 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). It is recommended to incubate up to 2×10^7 cells in 1.5 mL tubes to allow for stringent removal of buffer after centrifugation steps. Adjust the buffer volume for washing the cells after magnetic labeling (step 7) accordingly.

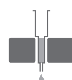
▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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 10 µL of FcR Blocking Reagent per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of Epidermal Langerhans Cell MicroBeads per 10^7 total cells.
7. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C). Vortex every 5 minutes during the incubation period to prevent sedimentation and cell aggregation, e.g., using the MACSmix Tube Rotator.
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 5×10^7 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.
10. 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 epidermal Langerhans cells. For details refer to 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
7. (Optional) To increase the purity of epidermal Langerhans 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.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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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. Program recommendations below refer to separation of mouse epidermal cells.

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.

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® Separator

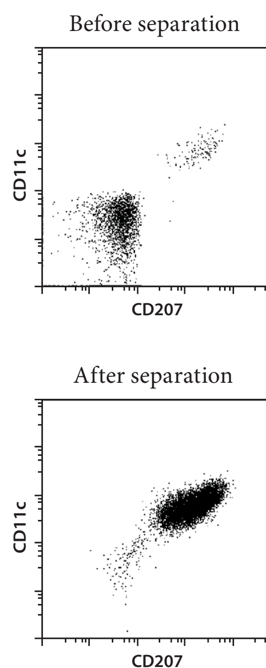
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and pos1.
3. For a standard separation choose one of the following programs:

   Positive selection: Possel
   Collect positive fraction from outlet pos1.

   Depletion: Deplete
   Collect negative fraction from outlet port neg1.

3. Example of a separation using the Epidermal Langerhans Cell MicroBead Kit

A single-cell suspension was prepared using the Epidermis Dissociation Kit, mouse (# 130-095-928). Langerhans cells were isolated from mouse epidermis single-cell suspension by using the Epidermal Langerhans Cell MicroBead Kit, one MS Column, and a MiniMACS™ Separator. The cells were fluorescently stained with CD207 and CD11c-PE (# 130-091-830) 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.



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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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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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