Miltenyi Biotec

CD171 (L1CAM) MicroBead Kit
mouse

Order no. 130-101-548
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Contents

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	**50 µL CD171 (L1CAM) MicroBeads, mouse (# 130-101-548)** or **1 mL CD171 (L1CAM) MicroBeads, mouse (# 130-101-549):** MicroBeads conjugated to monoclonal CD171 (L1CAM) antibodies (isotype: rat IgG2a). **50 µL FcR Blocking Reagent, mouse (# 130-101-548)** or **1 mL FcR Blocking Reagent, mouse (# 130-101-549)**
Capacity	For 5×10^7 total cells, up to 5 separations (# 130-101-548) or for 10^9 total cells, up to 100 separations (# 130-101-549).
Product format	All reagents 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, Fc receptors are blocked with FcR Blocking Reagent, mouse. Then, the CD171 (L1CAM)$^+$ cells are magnetically labeled with CD171 (L1CAM) MicroBeads. The cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD171 (L1CAM)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD171 (L1CAM)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD171 (L1CAM)$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected fraction containing the CD171 (L1CAM)$^+$ cells can be separated over a second column.

1.2 Background information

CD171 (L1CAM) MicroBeads (L1CAM: L1 cell adhesion molecule) have been developed for the separation of mouse cells based on the expression of the CD171 (L1CAM) antigen.
Expression of the cell adhesion molecule CD171 is found on tetanus-toxin positive neurons, endothelial cells, certain epithelial cells, reticular fibroblasts, and several malignant tumors, including colon and breast carcinomas, colon melanoma, and tumor cells of neuronal and mesothelial origin, where a role of CD171 in augmenting tumor growth has been demonstrated.
CD171 plays a vital role in cell adhesion and signal transduction. It is involved in the development of the nervous system and regulates processes such as neuron–neuron adhesion, myelination, axonal guidance, and neuronal migration.
CD171 (L1CAM) MicroBeads were especially optimized for the separation of neurons based on the expression of CD171 (L1CAM). The isolation was optimized on dissociated postnatal CD-1® mouse brain tissue derived from animals younger than postnatal day eight (P8).
Up to 99.5% purity was obtained with P7 cerebellum tissue because here the expression of CD171 is found almost exclusively on neurons. Purity decreases with age: P9 up to 80%; P12 up to 64%. For other brain regions, the depletion of glial cells might become necessary if high purities are required.
The CD171 (L1CAM) epitope shows papain sensitivity. Therefore, the Neural Tissue Dissociation Kit (T) is recommended prior to cell isolation.

1.3 Applications

- Positive selection or depletion of cells expressing the mouse CD171 (L1CAM) antigen.
- Isolation or depletion of CD171 (L1CAM)$^+$ neurons from dissociated mouse brain tissue derived from mice younger than eight days (<P8).

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1.4 Reagent and instrument requirements

- DPBS/BSA buffer: Prepare a solution containing Dulbecco's phosphate-buffered saline (DPBS) with Ca^{2+} and Mg^{2+} and 0.5% bovine serum albumin (BSA) by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with DPBS. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS).

- The Neural Tissue Dissociation Kit (T) (# 130-093-231) is recommended for the generation of single-cell suspensions of neural cells from mouse brain tissue because the CD171 (L1CAM) epitope shows papain sensitivity.
- Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- MACS Columns and MACS Separators: CD171 (L1CAM)$^+$ 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 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro

  ▲ **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 Labeling Check Reagents to stain labeled cells for flow cytometric analysis, e.g., Labeling Check Reagent-PE (# 130-095-228). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ **Note:** The use of CD171 antibodies, clone 555, is not recommended for analysis of cells that are labeled with CD171 (L1CAM) MicroBeads.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) MACS Neuro Medium (# 130-093-570) and MACS NeuroBrew®-21 (# 130-093-566) for cultivation.
- (Optional) gentleMACS™ Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937) and gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) MACSmix™ Tube Rotator (# 130-090-753).

2. Protocol

2.1 Sample preparation

For the preparation of single-cell suspensions from neural tissues refer to the data sheet of the Neural Tissue Dissociation Kit (T) (# 130-093-231), which can be used in combination with the gentleMACS Dissociator (# 130-092-235).

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

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. For best performance it is recommended to use at least 5×10^6 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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) 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 10 µL of FcR Blocking Reagent per 10^7 total cells.
5. Mix well. Do not vortex. Incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 10 µL of CD171 (L1CAM) MicroBeads per 10^7 total cells.
7. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).

   ▲ **Note:** (Optional) The MACSmix Tube Rotator can be used for continuous mixing of larger volumes. Operate MACSmix Tube Rotator on permanent run at a speed of approximately 4 rpm.

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 10^7 cells in 500 µL of buffer.

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

10. Proceed to magnetic separation (2.3 or 2.4).

2.3 Magnetic separation

▲ Choose an appropriate MACS® Column and MACS Separator according to the number of total cells and the number of CD171 (L1CAM)$^+$ cells. For details refer to the table in section 1.4.

▲ **Note:** MS Columns are recommended for highest purity of CD171 (L1CAM)$^+$ cells. LS Columns are recommended for highest recovery of CD171 (L1CAM)$^+$ cells.

▲ 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 CD171 (L1CAM)$^+$ 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.

   ▲ **Note:** Elution of the cells from the column after the separation should be performed with cell culture medium if cells are to be taken directly into culture, otherwise elute with buffer as before.

   ▲ **Note:** Keep handling time of cells in PBS/BSA buffer to a minimum.

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.

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.

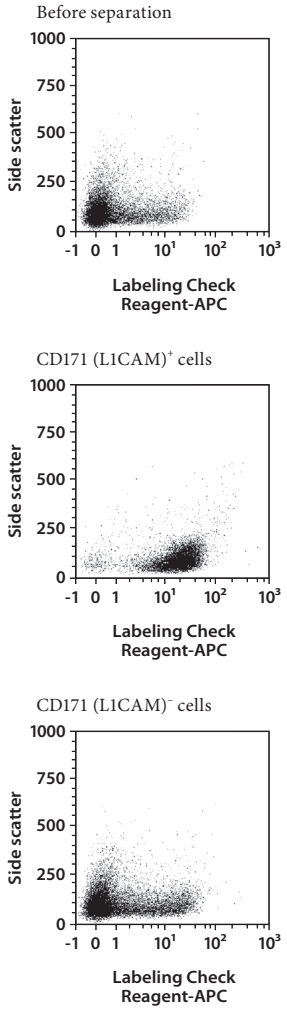
1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
4. For a standard separation choose one of the following programs:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

   Depletion: Depl025
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using the CD171 (L1CAM) MicroBead Kit

CD171 (L1CAM)$^+$ cells were isolated from P1 CD-1 mouse cerebellum tissue using the Neural Tissue Dissociation Kit (T), the gentleMACS™ Dissociator, the CD171 (L1CAM) MicroBead Kit, a MidiMACS™ Separator, and an LS Column. Cells were fluorecently stained with Labeling Check Reagent-APC 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.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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