Miltenyi Biotec

CD11b (Microglia) MicroBeads
human and mouse

1 mL	130-093-634
100 µL	130-093-636

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	**1 mL CD11b (Microglia) MicroBeads, human and mouse** or **100 µL CD11b (Microglia) MicroBeads, human and mouse – small size:** MicroBeads conjugated to monoclonal anti-human/mouse CD11b (Mac-1α) antibodies (isotype: rat IgG2b).
Capacity	1 mL for 1×10^9 total cells, up to 100 separations or 100 µL for 1×10^8 total cells, up to 10 separations.
Product format	CD11b (Microglia) 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, a single-cell suspension of human or mouse brain is prepared according to an appropriate protocol, such as our protocols for the dissociation of neural tissues with the Neural Tissue Dissociation Kits (P≤7) or the Adult Brain Disassociation Kit (P>7). The CD11b$^+$ cells are magnetically labeled with CD11b (Microglia) MicroBeads. The cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD11b$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD11b$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD11b$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD11b$^+$ cells must be separated over a second column.

1.2 Background information

Microglia, often called brain macrophages, are the resident immune-effector cells in the central nervous system (CNS). In addition, activated microglia serve as the major antigen-presenting cells in the CNS. They are morphologically, immunophenotypically, and functionally related to cells of the monocyte/macrophage lineage. The most characteristic feature of microglial cells is their rapid activation in response to injury, inflammation, neurodegeneration, infection, and brain tumors. A paucity of microglial antigens has hindered microglial identification. However, CD11b has been widely used as marker for microglial identification and is also suitable for their immunomagnetic isolation from human and mouse brain tissue. CD11b (Microglia) MicroBeads have been optimized to isolate CD11b$^+$ cells from single-cell suspensions of brain or brain tumor tissue.

1.3 Applications

- Positive selection or depletion of CD11b$^+$ cells from single-cell suspensions of human or mouse brain tissue.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (#130-091-376) 1:20 with PBS. 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/human serum albumin, mouse/human serum, or fetal bovine serum (FBS).

- MACS Columns and MACS Separators: CD11b$^+$ cells can be enriched by using MS or LS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD11b antigen can also be depleted using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^7	MiniMACS, OctoMACS
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS

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Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24	2×10^7	4×10^7	MultiMACS Cell24

- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628)
- (Optional) Neural Tissue Dissociation Kit (T) (# 130-093-231)
- (Optional) Brain Tumor Dissociation Kit (P) (# 130-095-942)
- (Optional) Brain Tumor Dissociation Kit (T) (# 130-095-939)
- (Optional) Adult Brain Dissociation Kit (# 130-107-677) for rodents older than P7.
- (Optional) Myelin Removal Beads II (# 130-096-733, # 130-096-433)
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Fluorochrome-conjugated CD11b antibody for flow cytometric analysis, e.g., CD11b-FITC, CD11b-PE, or CD11b-APC. For more information about other fluorochrome conjugates refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) MACS® SmartStrainers (70 µm) (# 130-098-462) or MACS SmartStrainers (30 µm) (# 130-098-458)
- (Optional) Pre-Separation Filters (70 µm) (# 130-095-823)

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from your tissue of interest by using the Neural Tissue Dissociation Kit (P) (# 130-092-628) or (T) (# 130-093-231) (p≤7) or the Adult Brain Dissociation Kit (# 130-107-677) (p>7), or the Brain Tumor Dissociation Kit (P) (# 130-095-942) or (T) (# 130-095-939), which can also be used in combination with the gentleMACS Dissociators. For details please refer to the respective data sheets.

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 a nylon mesh (MACS SmartStrainers (70 µm), # 130-098-462 or MACS SmartStrainers (30 µm), # 130-098-458) 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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD11b (Microglia) MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. 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.
8. 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 $CD11b^+$ 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.

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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. To increase the purity of microglia cells, it is recommended to enrich the positive fraction over a second MS or LS Column. Repeat the manetic 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.

Magnetic separation with the MultiMACS™ Cell24 Separator

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

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.

▲ 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.

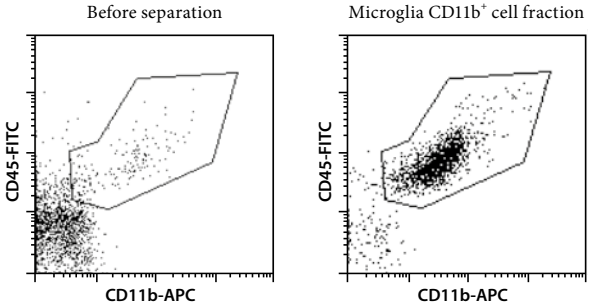
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

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

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

3. Example of a separation using CD11b (Microglia) MicroBeads

$CD11b^+$ cells were isolated from mouse neural cell suspension using the CD11b (Microglia) MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with CD11b-APC and CD45-FITC. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

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

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Warranty

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