Miltenyi Biotec

CD11b (Microglia) MicroBeads
mouse/human

Order no. 130-093-634

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1. Description

Components	2 mL CD11b (Microglia) MicroBeads, mouse/human: MicroBeads conjugated to monoclonal anti-mouse/human CD11b (Mac-1α) antibodies (isotype: rat IgG2b).
Capacity	For 2×10^9 cells, up to 200 separations with **mouse** cells. For 10^9 cells, up to 100 separations with **human** cells.
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. 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.

1.2 Background information

Microglia, often called brain macrophages, are the resident immune-effector cells in the 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[1-3]. CD11b (Microglia) MicroBeads have been optimized to isolate $CD11b^+$ cells from single-cell suspensions of brain tissue[1].

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, 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/human serum albumin, mouse/human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- 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 or the autoMACS Pro 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

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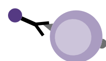
- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628)
- (Optional) Neural Tissue Dissociation Kit (T) (# 130-093-231)
- (Optional) gentleMACS™ Dissociator (# 130-093-235)
- (Optional) Fluorochrome-conjugated CD11b antibody for flow cytometric analysis, e.g., CD11b-FITC (# 130-081-201), CD11b-PE (# 130-091-240), or CD11b-APC (# 130-091-241). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) 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 (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation of neural cells

When working with neural mouse tissues, prepare a single-cell suspension by using the Neural Tissue Dissociation Kit (P) (# 130-092-628) or the Neural Tissue Dissociation Kit (T) (# 130-093-231), which can also be used in combination with the gentleMACS Dissociator. For details please refer to 5. Appendix or see the respective data sheets. General Protocols are also available 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).



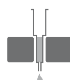
2.2 Magnetic labeling of neural cells

▲ 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).

▲ 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. When working with **mouse** cells, resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
   When working with **human** cells, resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
4. When working with **mouse** cells, add 10 µL of CD11b (Microglia) MicroBeads per 10^7 total cells.
   When working with **human** cells, add 20 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 see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see 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.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL   LS: 3×3 mL
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

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see 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 effluent; 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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Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

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

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

▲ Buffers used for operating the autoMACS Separator or 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.

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.

   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

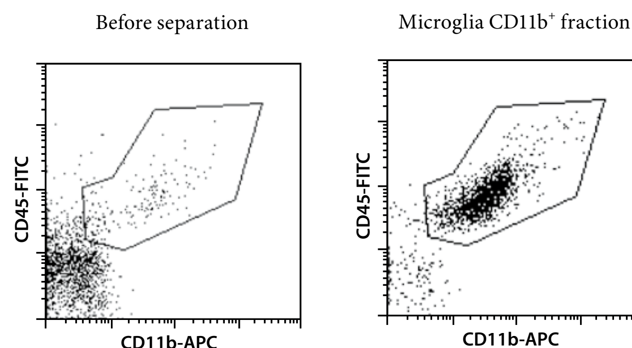
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

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

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

3. Example of a separation using the 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 (# 130-091-241) and CD45-FITC (# 130-091-609). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Ndhlovu, L. C. *et al.* (2001) Critical Involvement of OX40 Ligand Signals in the T Cell Priming Events During Experimental Autoimmune Encephalomyelitis. J. Immunol. 167: 2991–2999.
2. Li, H. *et al.* (2006) Different Neurotropic Pathogens Elicit Neurotoxic CCR9- or Neurosupportive CXCR3-Expressing Microglia. J. Immunol. 177: 3644–3656.
3. Boddaert, J. *et al.* (2007) Evidence of a Role for Lactadherin in Alzheimer's Disease. Am. J. Pathol. 170: 921–929.

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

5. Appendix: Dissociation of mouse brain tissue using the Neural Tissue Dissociation Kit (P) or the Neural Tissue Dissociation Kit (T)

Reagent and instrument requirements

- Neural Tissue Dissociation Kit (P) (# 130-092-628)
- Neural Tissue Dissociation Kit (T) (# 130-093-231)
- Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} or Mg^{2+} (Sigma-Aldrich # H4891), in the following referred to as HBSS (w/o)
- HBSS with Ca^{2+} or Mg^{2+} (Sigma-Aldrich # H1387), in the following referred to as HBSS (w)
- beta-mercaptoethanol (e.g. Sigma # 63689)
- 30 µm cell strainer (e.g. Pre-Separation Filters (# 130-041-407)
- Sterile scalpel
- 35 mm diameter sterile petri dish
- Sterile glass pipettes
- (Optional) Sterile filters (e.g. Millipore # SLGP R25 LS)
- (Optional) 0.9 M sucrose solution in HBSS, pH 7–7.5
- MACSmix™ Tube Rotator (# 130-090-753) or water bath at 37°C

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Reagent and instrument preparation

▲ For cell culture experiments subsequent to tissue dissociation, Solution 4 of the Neural Tissue Dissociation Kit (P) or the Neural Tissue Dissociation Kit (T) should be sterile filtered.

▲ Volumes given below are for up to 400 mg of starting tissue material. When working with less than 400 mg, use the same volumes as indicated. When working with more than 400 mg, scale up all reagent volumes and total volumes accordingly. Tissue quantities of 400 mg and less can be processed in a single 2 mL reaction tube, while tissue quantities of greater than 400 mg can be pooled and processed in an appropriate-sized conical tube.

▲ **Note:** The weight of a mouse brain (C57/BL6, postnatal day 6) corresponds to approximately 200 mg).

1. Add beta-mercaptoethanol to Solution 2 to a final concentration of 0.067 mM. For example, add 13.5 µL of 50 mM beta-mercaptoethanol to 10 mL of Solution 2.

   ▲ **Note:** This solution will then be stable for 1 month at 4 °C.
2. Resuspend the lyophilized powder in the vial labeled Solution 4 with 1 mL of Storage Buffer for Solution 4. Do **not** vortex. This solution should then be sterile filtered in the case of cell culture applications, aliquoted and stored at –20 °C for later use.

Table 1:

	Enzyme mix 1		Enzyme mix 2	
NTDK (P)	Solution 1 50 µL	Solution 2 1900 µL	Solution 3 20 µL	Solution 4 10 µL
NTDK (T)	Solution 1 200 µL	Solution 2 1750 µL	Solution 3 20 µL	Solution 4 10 µL

Neural tissue dissociation protocol

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ This protocol describes the dissociation of mouse brain tissue, though, in principle, it is transferable to other neural tissue types.

1. Fire polish three glass pipettes so that decreasing tip diameters are achieved.
2. Prepare 1950 µL enzyme mix 1 per 400 mg tissue by adding Solution 1 to Solution 2 (see table 1) and vortex. Preheat the mixture at 37 °C for 10–20 min before use.
3. Remove the mouse brain. Determine the weight of tissue in 1 mL of cold HBSS (w/o) to make sure the 400 mg limit per digestion is not exceeded.
4. Place the brain on the lid of a 35 mm diameter petri dish, remove the meninges (optional), and cut brain into small pieces using a scalpel.

   ▲ **Note:** For certain applications such as cultivation of neuronal cells, meninges should be removed.
5. Using a 1 mL pipette tip, add 1 mL of cold HBSS (w/o) and pipette pieces back into an appropriate-sized tube (see below). Rinse with HBSS (w/o).

   ▲ **Note:** When using < 200 mg of brain tissue, return pieces to a 2 mL reaction tube. For tissue quantities > 200 mg, pipette pieces into a 15 mL conical tube.

   ▲ **Note:** When working with mice > P10, cut 2–5 mm off the end of the pipette tip to facilitate pipetting.
6. Centrifuge at 300×g for 2 min at 4 °C and aspirate the supernatant carefully.
7. Add 1950 µL of pre-heated enzyme mix 1 (Solutions 1 and 2) per up to 400 mg tissue. Incubate in closed tubes for 20 minutes at 37 °C under slow, continuous rotation using a MACSmix Tube Rotator. Alternatively incubate in a water bath, inverting the tube several times every 5 minutes to resuspend settled cells.
8. Prepare 30 µL enzyme mix 2 per 400 mg tissue by adding 20 µL of Solution 3 to 10 µL of Solution 4 (see table 1). Then add to sample.
9. Invert gently to mix. Do **not** vortex.
10. Dissociate tissue mechanically using the wide-tipped, fire-polished Pasteur pipette by pipetting up and down 10 times slowly. Avoid forming air bubbles.
11. Incubate at 37 °C for 15 min using a MACSmix Tube Rotator or in a water bath, agitating as above.
12. Dissociate tissue mechanically using the other two fire-polished pipettes in decreasing diameter. Pipette slowly up and down 10 times with each, or as long as tissue pieces are still observable. Care to avoid the formation of air bubbles.
13. Apply the single-cell suspension to a 30 µm cell strainer and wash with 10 mL of HBSS (w). Discard cell strainer and centrifuge at 300×g for 10 min at room temperature.

    ▲ **Note:** Cells with a diameter > 30 µm, such as Purkinje cells or motor neurons, may be lost. To obtain these cells within the flow through, use a cell strainer with an appropriate diameter.
14. Repeat washing and centrifugation step.

    ▲ **Note:** If problems with the formation of a compact pellet occur after either washing step, add 20 µL of Solution 3 and 10 µL of Solution 4 per mL of cell suspension, mix gently and incubate for a minimum of 5 minutes at 37 °C in a water bath, followed by washing as above.
15. (Optional, instead of step 14) For myelin removal, discard supernatant and resuspend cell pellet in 10 mL of cold sucrose solution. Centrifuge at 850×g, 4 °C for 10 min, then discard the supernatant carefully. Finally, resuspend in 10 mL of HBSS (w) and centrifuge at 300×g for 10 min at room temperature.

    ▲ **Note:** Though removal of myelin will increase purities during subsequent flow cytometric analyses and cell separation using MACS Technology, the overall yield of cells will decrease.
16. Cells should be processed immediately for further applications.

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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