Miltenyi Biotec

CD11b/c (Microglia) MicroBeads

rat

1 mL	130-105-634
50 µL	130-105-643

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/c (Microglia) MicroBeads, rat** or **50 µL CD11b/c (Microglia) MicroBeads, rat – small size:** MicroBeads conjugated to monoclonal anti-rat CD11b/c antibodies (isotype: human IgG1).
Capacity	1 mL for 5×10^8 total cells or 50 µL for 2.5×10^7 total cells.
Product format	CD11b/c (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, the CD11b/c$^+$ cells are magnetically labeled with CD11b/c (Microglia) 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 CD11b/c$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD11b/c$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD11b/c$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD11b/c$^+$ cells must be separated over a second column.

1.2 Background information

CD11b/c (Microglia) MicroBeads have been developed for the isolation of rat cells based on the expression of the CD11b and CD11c antigens. The CD11b/c (Microglia) antibody appears to recognize a common epitope shared between CD11b and CD11c.
CD11b, also known as integrin alpha M (ITGAM) or Mac-1, is a component of the complement factor 3 (CR3), whereas CD11c, also called as integrin alpha X (ITGAX) , is a component of complement receptor 4 (CR4).
CD11b and CD11c are expressed on micoglia, macrophages, monocytes, granulocytes, NK cells, and dendritic cells.
For optimal results, the Neural Tissue Dissociation Kit (P) (# 130-092-628) is required for the generation of a single-cell suspension.
Microglia isolated with the CD11b/c (Microglia) MicroBeads can be cultivated using DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptavidin, and 2 mM L-glutamine.
For flow cytometrical analysis, co-staining with CD11b/c and CD45 fluorochromes is recommended.

1.3 Applications

- Positive selection or depletion of cells expressing rat CD11b/c antigen.
- Identification and enumeration of CD11b/c$^+$ cells by flow cytometry.

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 rat serum albumin, rat serum, or fetal bovine serum (FBS).

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-673.04

- MACS Columns and MACS Separators: CD11b/c$^+$ 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
LS	2×10^7	4×10^7	MidiMACS, QuadroMACS
Depletion			
LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS
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.

- Pre-Separation Filters (70 µm) (# 130-095-823)
- (Optional) MACS SmartStrainers (70 µm) (# 130-098-462) or MACS SmartStrainers (30 µm) (# 130-098-458)
- (Optional) Neural Tissue Dissociation Kit (P) (# 130-092-628) for the generation of single-cell suspension from rat brain tissue younger than P7.
- (Optional) Adult Brain Dissociation Kit, mouse and rat (# 130-107-677) are highly recommended for the generation of single-cell suspension from rat brain tissue older than P7.
- (Optional) Brain Tumor Dissociation Kit (P) (# 130-095-942)
- (Optional) Fluorochrome-conjugated CD11b/c antibodies for flow cytometric analysis, e.g., CD11b/c-FITC and CD45. 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.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) 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 suspension from neural tissues younger than P7 refer to the data sheet of Neural Tissue Dissociation Kit (P) (# 130-092-628), which can be used in combination with the gentleMACS Dissociators. For preparation of single-cell suspension from neural tissues older than P7, refer to the data sheet of Adult Brain Dissociation Kit, mouse and rat (# 130-107-677), used in combination with gentleMACS Octo Dissociator with Heaters.

For details refer to www.gentlemacs.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 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 is is important to obtain an single-cell suspension using the Neural Tissue Dissociation Kit (P) (P≤7), or the Adult Brain Dissociation Kit (P>7). Subsequently, 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. Moister 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 CD11b/c (Microglia) MicroBeads per 10^7 total cells.
5. Mix well. Do not vortex. Incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies after 5 minutes of the total incubation time, e.g., 10 µL of CD11b/c-FITC and additional CD45 fluorochromes, and incubate for the remaining 10 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 10^7 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
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 CD11b/c$^+$ 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 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. To increase the purity of microglia cells, the eluted fraction can be enriched over another 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.

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. 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:

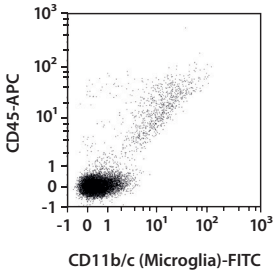
   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

   Depletion: Depl05
   Collect negative fraction in row B of the tube rack.

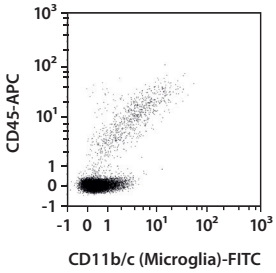
3. Example of a separation using CD11b/c MicroBeads

CD11b/c$^+$ cells were isolated from day P3 postnatal rat brain tissue using the Neural Tissue Dissociation Kit (P), the gentleMACS Dissociator, CD11b/c (Microglia) MicroBeads, a MiniMACS™ Separator, and two MS Columns. Cells were fluorecently stained with CD11b/c-FITC in combination with CD45-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.

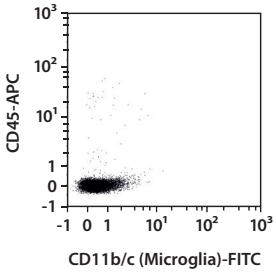
Before MicroBead labeling



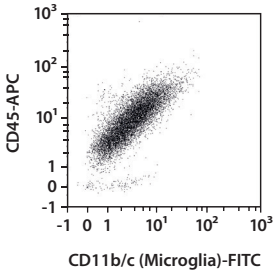
Before isolation



Negative fraction



Isolated CD11b/c$^+$ cells



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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