# CD11b MicroBeads
mouse/human

## Magnetic cell sorting

Order No. 130-049-601

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## 1. Description

| | |
|---|---|
| **Components** | 2 mL CD11b MicroBeads, mouse/human: MicroBeads conjugated to monoclonal rat anti-mouse/human CD11b (Mac-1α) antibodies (isotype: rat IgG2b; clone: M1/70.15.11.5). |
| **Size** | For $1\times10^9$ human total cells, up to 100 separations;<br>for $2\times10^9$ mouse total cells, up to 200 separations. |
| **Product format** | CD11b MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide. |
| **Storage** | Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label. |

### 1.1 Principle of MACS® separation

First the CD11b$^+$ cells are magnetically labeled with CD11b 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$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD11b$^+$ cells. After removal of the column from the magnetic field, the magnetically retained CD11b$^+$ cells can be eluted as the positively selected cell fraction.

### 1.2 Background and product applications

CD11b MicroBeads are developed for separation of human and mouse cells based on expression of the CD11b antigen. In humans, CD11b is strongly expressed on myeloid cells, and weakly expressed on NK cells and some activated lymphocytes. In mouse, the CD11b antigen is expressed on monocytes/macrophages, and to a lower extent on granulocytes, NK cells, CD5$^+$ B1 cells and a subset of dendritic cells.
The CD11b (Mac-1 α; integrin $\alpha_M$ chain) antibody reacts with the 170 kDa $\alpha_M$ subunit of CD11b/CD18 heterodimer (Mac-1, $\alpha_M\beta_2$ intergrin). It functions as a receptor for complement (C3bi), fibrinogen or clotting factor X.

**Examples of applications**

- Positive selection or depletion of human monocytes/macrophages and granulocytes from peripheral blood or lymphoid tissue.
- Positive selection or depletion of myeloid cells from human and mouse bone marrow.
- Positive selection or depletion of mouse macrophages from lymphoid tissue.

### 1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) in autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).

  ▲ **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 gelatine, mouse/human serum or fetal calf serum. Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- MACS Columns and MACS Separators: Monocytes and macrophages can be enriched (positive selection) or depleted by using MS, LS or XS Columns. For efficient depletion of myeloid cells from bone marrow, and depletion of granulocytes and NK cells we recommend using LD, CS or D Columns. Positive selection or depletion can also be performed by using the autoMACS Separator.

| Column | max. number of labeled cells | max. number of total cells | Separator |
|---|---|---|---|
| **Positive selection** | | | |
| MS | $10^7$ | $2\times10^8$ | MiniMACS, OctoMACS, VarioMACS, SuperMACS |
| LS | $10^8$ | $2\times10^9$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| XS | $10^9$ | $2\times10^{10}$ | SuperMACS |
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| CS | $2\times10^8$ | | VarioMACS, SuperMACS |
| D | $10^9$ | | SuperMACS |
| **Positive selection or depletion** | | | |
| autoMACS | $2\times10^8$ | $4\times10^9$ | autoMACS |

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

- (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).

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Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

- (Optional) PI (propidium iodide) or 7-AAD for flow-cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

## 2. Protocol

### 2.1 Sample preparation

**Sample preparation of human PBMCs**

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

**Sample preparation of mouse tissue**

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101) is recommended.

### 2.2 Magnetic labeling of human PBMCs

▲ 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 $2\times10^7$ 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per $10^7$ total cells.
4. Add 20 µL of CD11b MicroBeads per $10^7$ total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add a fluorochrome conjugated antibody, e.g. add 10 µL of CD11b-FITC (# 130-081-201), and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. 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 $1.25\times10^8$ cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

### 2.3 Magnetic labeling of mouse 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 $2\times10^7$ 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 90 µL of buffer per $10^7$ total cells.
4. Add 10 µL of CD11b MicroBeads per $10^7$ total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. (Optional) Add a fluorochrome conjugated antibody, e.g. add 10 µL of CD11b-FITC (# 130-081-201), and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. 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 $1.25\times10^8$ cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

### 2.4 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD11b^+$ cells (see table in section 1.3).

**Magnetic separation with MS or LS Columns**

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL.

3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL.
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared column.

### Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

### Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator (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 which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

### Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (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 (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which 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

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Possel"

   Depletion: "Depletes"

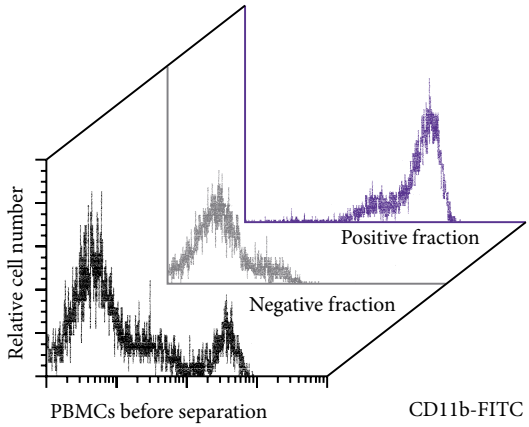
   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CD11b$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD11b$^-$ cell fraction.

## 3. Example of a separation using CD11b MicroBeads

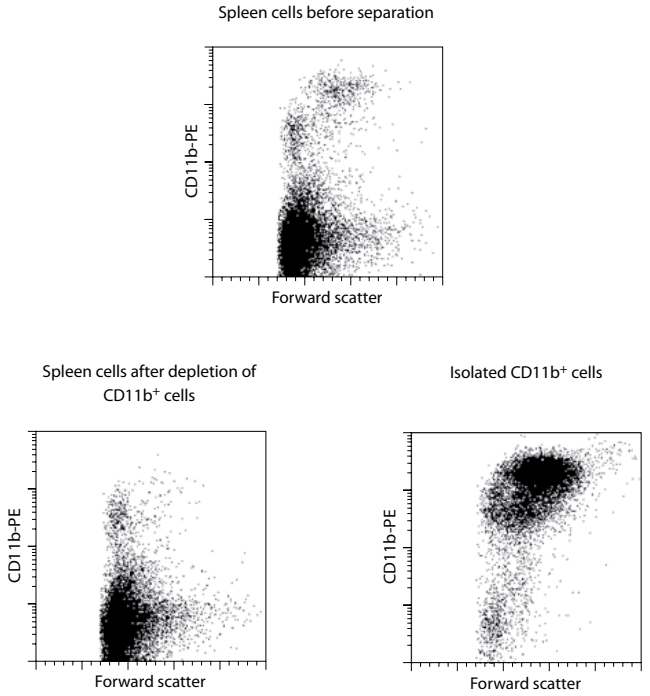
### A: Separation of human PBMCs

Separation of human PBMCs using CD11b MicroBeads. Cells are stained with CD11b-FITC (# 130-081-201). Monocytes can be identified as CD11b$^{bright}$ cells and NK cells as CD11b$^{dim}$ cells.




### B: Separation of CD11b$^+$ cells from a mouse spleen cell suspension

Positive selection of CD11b$^+$ cells from a mouse spleen cell suspension using CD11b MicroBeads, an MS Column and a MidiMACS™ Separator.

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## 4. References

1. Ehlich, A; Martin, VM; Müller, W; Rajewsky, K (1994) Analysis of the B Cell Progenitor Compartment at the Level of Single Cells. Current Biology 4: 573-583. [33]

### 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.

### Warranty

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