Miltenyi Biotec

CD11b MicroBeads UltraPure
mouse

Order no. 130-126-725

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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	2 mL CD11b MicroBeads UltraPure, mouse: MicroBeads conjugated to anti-mouse CD11b antibodies (isotype: human recombinant IgG1).
Capacity	For 2×10^9 mouse total cells, up to 200 separations.
Product format	CD11b MicroBeads UltraPure 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 $CD11b^+$ cells are magnetically labeled with CD11b MicroBeads UltraPure. 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 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

CD11b MicroBeads UltraPure, mouse are developed for separation of mouse cells based on expression of the CD11b antigen. In mouse, the CD11b antigen is expressed in monocytes, granulocytes, and macrophages (e.g. Kupffer cells and microglia). To a lower extent, its expressed on NK cells, $CD5^+$ B1 cells, and a subset of dendritic cells.
CD11b antibody reacts with the mouse CD11b antigen (Mac-1 α; integrin αM chain), which is part of the CD11b/CD18 heterodimer (Mac-1 α, Mβ2 integrin), also known as the C3 complement receptor. It functions as a receptor for complement (C3bi), fibrinogen, or clotting factor X.

1.3 Applications

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

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

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

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- MACS® Columns and MACS Separators: Monocytes, granucolytes, and macrophages can be enriched (positive selection) or depleted by using MS, LS, or XS Columns. For efficient depletion of myeloid cells it is recommended to use LD Columns. Positive selection or depletion can also be performed by using the autoMACS® Separator or the MultiMACS™ Cell24 Separator Plus.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II,
	10^8	10^9	MultiMACS Cell24 Separator Plus
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, SuperMACS II, MultiMACS Cell24 Separator Plus
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

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

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) Fluorochrome-conjugated CD11b antibodies for flow cytometric analysis (clone REA592). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 (70 µm) (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ 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

▲ 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 70 µm filter (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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD11b MicroBeads UltraPure per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., CD11b-Vio® Bright FITC (REA592), according to manufacturer's recommendation.
7. Add buffer to a final volume of 500 µL for up to 5×10^7 cells.

   ▲ **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).

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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 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 CD11b$^+$ 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.

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. 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.

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 for highest purity

   or

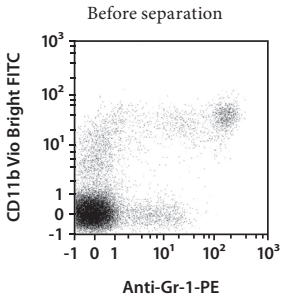
   Possels for highest recovery

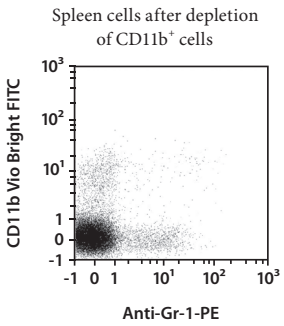
   Collect positive fraction in row C of the tube rack.

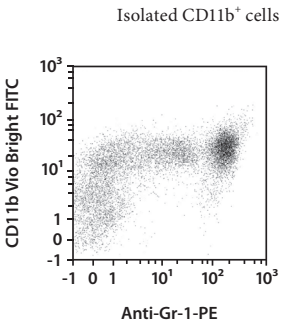
   Depletion: DepleteS
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using CD11b MicroBeads UltraPure

CD11b$^+$ cells were isolated from single-cell suspension of mouse spleen using CD11b MicroBeads UltraPure, one MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD11b-Vio® Bright FITC (REA592) and Anti-Gr-1-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris, doublets, and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.







4. References

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