Miltenyi Biotec

CD11c MicroBeads UltraPure
mouse

Order no. 130-125-835

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	2 mL CD11c MicroBeads UltraPure, mouse: UltraPure MicroBeads conjugated to monoclonal anti-mouse CD11c antibodies (isotype: human recombinant IgG1).
Capacity	For 2×10^9 total cells.
Product format	CD11c 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 $CD11c^+$ cells are magnetically labeled with CD11c 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 $CD11c^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD11c^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD11c^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD11c MicroBeads UltraPure have been developed for the isolation of mouse $CD11c^+$ cells, such as dendritic cells (DCs), from single-cell suspensions of lymphoid and non-lymphoid tissues. Unlike in humans, CD11c is expressed in mice on all defined DC subsets and on some populations of tissue macrophages. With CD11c MicroBeads UltraPure, complicated procedures for the isolation of DCs are replaced by a fast and simple positive selection strategy. This product has been optimized by implementation of our recombinant antibody technology (REAfinity) to virtually abolish non-specific binding through Fc-receptors.

1.3 Applications

- Isolation of DCs for analysis of their phenotypical and functional properties or studies on T cell activation, polarization, and tolerance induction in different experimental mouse models.
- Isolation of DCs from spleen and lymph nodes[1], Peyer´s patches[2], colonic lamina propria[3], bone marrow[4], epidermis[5], lung[6], liver[7], or brain[8].
- Pre-enrichment of $CD11c^+$ cells for the isolation of rare cell subsets, such as DCs and macrophages.

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.

- MACS Columns and MACS Separators: $CD11c^+$ cells can be enriched by using MS or LS Columns. Positive selection 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	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

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

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140-005-703.02

- (Optional) Spleen Dissociation Kit, mouse (# 130-095-926)
- (Optional) gentleMACS™ Octo Dissociator with Heaters (# 130-096-427)
- (Optional) Fluorochrome-conjugated CD11c antibodies for flow cytometric analysis, e.g., CD11c-VioBlue®. 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 (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

For highest recovery and purity of $CD11c^+$ DCs from mouse spleen, it is recommended to perform enzymatic digestion using the Spleen Dissociation Kit, mouse (# 130-095-926).

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of CD11c MicroBeads UltraPure per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 10 mL of buffer per 10^8 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.
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 $CD11c^+$ 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 $CD11c^+$ 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 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.

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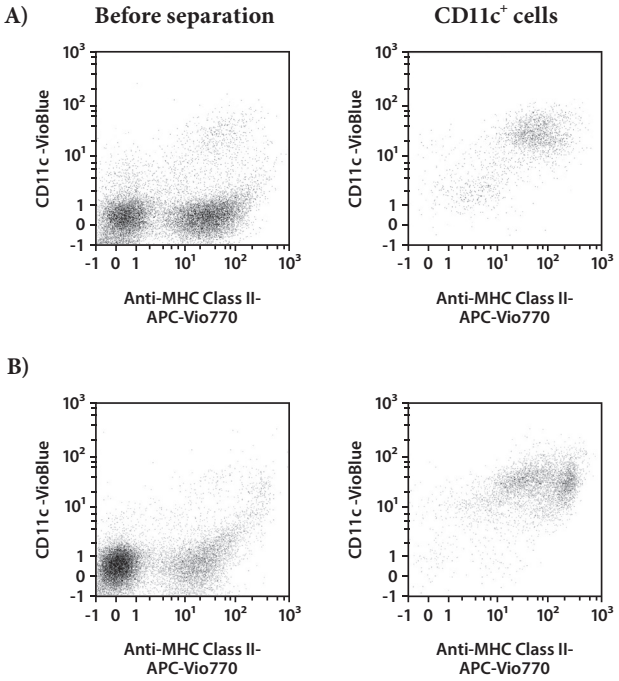
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.

3. For a standard separation choose one of the following programs:

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

3. Example of a separation using CD11c MicroBeads UltraPure

CD11c$^+$ cells were isolated from single-cell suspension of mouse spleen (generated with the Spleen Dissociation Kit and a gentleMACS™ Octo Dissociator with Heaters) (A) or lymph nodes (B) using CD11c MicroBeads UltraPure, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD11c-VioBlue® (# 130-102-413) and Anti-MHC Class II-APC (#130-123-785) 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.



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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

140-005-703.02

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