CD11c MicroBeads UltraPure, mouse (92-01-0264)

[Components]

2 mL CD11c MicroBeads UltraPure, mouse: UltraPure MicroBeads conjugated to monoclonal anti-mouse CD11c antibodies (isotype: human recombinant IgG1).

[Size] For $2x10^9$ 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 labels.

[Principle]

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.

[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, Peyer´s patches, colonic lamina propria, bone marrow, epidermis, lung, liver, or brain.

● Pre-enrichment of $CD11c^+$ cells for the isolation of rare cell subsets, such as DCs and macrophages..

[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 . 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.

● (Optional) Spleen Dissociation Kit, mouse

● (Optional) Fluorochrome-conjugated CD11c antibodies for flow cytometric analysis

● (Optional) Propidium Iodide Solution or 7-AAD Staining Solution for flow cytometric exclusion of dead cells.

● (Optional) Dead Cell Removal Kit for the depletion of dead cells.

● (Optional) Pre-Separation Filters (30 µm) to remove cell clumps.

[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.

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

▲ Dead cells may bind non-specifically to MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit.

[2. Magnetic labeling]

▲ Work fast, keep the 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) 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 (3).

[3. Magnetic separation]

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD11c^+$ cells.

▲ 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.

2. Prepare column by rinsing with 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 flowthrough from step 3.

MS: 3×500 µL LS: 3×3 mL

▲ **Note: Perform washing steps by adding buffer aliquots only when the column reservoir is empty.**

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 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 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.