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1. Description

Components	2 mL Pan DC MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD11c (N418) antibodies (isotype: hamster IgG) and Anti-mPDCA-1 antibodies (isotype rat IgG1).
Size	For 2×10^9 nucleated cells.
Product format	Pan DC 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 dendritic cells (DCs) are magnetically labeled with Pan DC 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 DCs are retained on the column. The unlabeled cells run through and this cell fraction is depleted of DCs. After removing the column from the magnetic field, the magnetically retained DCs can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Pan DC MicroBeads consist of CD11c and Anti-mPDCA-1 MicroBeads. CD11c is expressed at high levels on classical DCs and at low to intermediate levels on plasmacytoid dendritic cells (PDCs). The mouse plasmacytoid dendritic cell antigen-1 (mPDCA-1) is specifically expressed on PDCs with the phenotype CD11c^{low}, CD45R(B220)⁺, and Ly-6C⁺ in lymphoid and non-lymphoid organs. Due to the low expression of CD11c on PDCs, using CD11c expression might not lead to enrichment of PDCs to the same extent as classical DCs. Therefore, Pan DC MicroBeads were developed to isolate both, classical and plasmacytoid, DC subsets with highest recovery.

Examples of applications

- Isolation of total 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 highly pure DCs from lymphoid and non-lymphoid organs.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate-buffered saline) pH 7.2, 0.5% 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 (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 serum, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- MACS Columns and MACS Separators: Total DCs can be enriched by using MS, LS, or XS Columns (positive selection). Pan DC MicroBeads can be used for depletion of DCs on LD, CS, or D Columns. Cells which strongly express the CD11c and mPDCA-1 antigens can also be depleted using MS, LS, or XS 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	2×10 ⁸	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10 ⁸	2×10 ⁹	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10 ⁹	2×10 ¹⁰	SuperMACS
Depletion			
LD	10 ⁸	5×10 ⁸	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10 ⁸		VarioMACS, SuperMACS
D	10 ⁹		SuperMACS
Positive selection or depletion			
autoMACS	2×10 ⁸	4×10 ⁹	autoMACS

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

- Collagenase D: 2 mg/mL (Collagenase D >0.15 U/mg, e.g. from Roche Diagnostics, Germany) in 10 mM Hepes-NaOH pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂.

- (Optional) Fluorochrome-conjugated CD4, CD8, CD11c, or mPDCA-1 antibodies, respectively. For more information on these MACS Antibodies please visit www.miltenyibiotec.com.
- (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.
- (Optional) FcR blocking reagent (CD16/32 monoclonal antibody) to avoid Fc receptor-mediated fluorescent staining.

2. Protocol

2.1 Sample preparation

For highest recovery and purity of total DCs from mouse spleen, single-cell suspensions have to be prepared by enzymatic disaggregation with Collagenase D. Protocols which rely only on mechanical disruption are not recommended.

1. Place isolated spleen in a 6 cm petri-dish with sufficient Collagenase D solution to completely cover the bottom of the dish (5 mL/spleen).
2. Inject mouse spleen with 500 μ L of Collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue into smaller pieces using sharp scissors.
3. Incubate the spleen pieces in Collagenase D solution for 30 minutes at 37 °C.
4. Pass the whole material, i.e. remaining fragments and Collagenase D-released cells, gently through a 70 μ m cell strainer using a plunger.
5. Collect all cells in a 15 mL tube and wash the cells by adding buffer to a final volume of 14 mL.
▲ **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 by using the Dead Cell Removal Kit (# 130-090-101) is recommended.
6. Proceed to magnetic labeling (2.2).



2.2 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 separation.** Pass cells through 30 μ m 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 $300 \times g$ for 10 minutes. Pipette off supernatant completely.

3. Resuspend cell pellet in 400 μ L of buffer per 10^8 total cells.
▲ **Note:** To obtain high purities of DCs ($\geq 90\%$), Fc receptor-mediated magnetic labeling should be blocked by adding Fc receptor blocking reagent (CD16/32 antibody) or mouse immunoglobulin (1 mg per 500 μ L labeling volume) to the cell suspension before adding Pan DC MicroBeads.
4. Add 100 μ L of Pan DC MicroBeads per 10^8 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 staining antibodies at the titer recommended by the manufacturer and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at $300 \times g$ for 10 minutes. Pipette off supernatant completely.
8. Resuspend up to 10^8 cells in 500 μ L of buffer.
▲ **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 μ L of buffer.
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 total DCs (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 μ L 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: $3 \times 500 \mu$ L 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:** For a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
7. To increase the purity of the enriched DCs, repeat the magnetic separation procedure as described in steps 1–6 by using 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: "Posseld2"

Depletion: "Depletes"

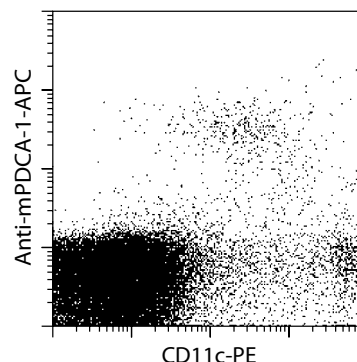
▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".

3. When using the program "Posseld2", collect positive fraction (outlet port "pos2"). This is the purified total DC fraction.
When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the non-DC fraction.

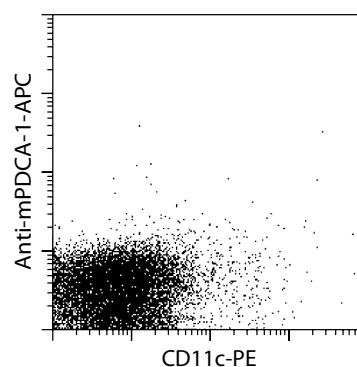
3. Example of a separation using Pan DC MicroBeads

Total DCs were isolated from a mouse spleen cell suspension using Pan DC MicroBeads, a MiniMACS™ Separator, and two MS Columns. The cells are fluorescently stained with CD11c-PE (# 130-091-830) and Anti-mPDCA-1-APC (# 130-091-963). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

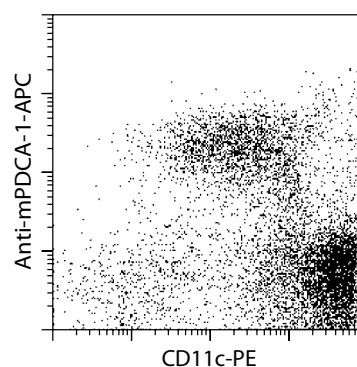
Spleen cells before separation



Spleen cells depleted of DCs



Isolated total DCs



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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