Miltenyi Biotec

# Anti-mPDCA-1 MicroBeads
mouse

Order no. 130-091-965

## Index



## 1. Description

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|---|---|
| **Components** | 2 mL Anti-mPDCA-1 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse mPDCA-1 (isotype: rat IgG1) antibody. |
| **Size** | For $2\times10^9$ nucleated cells. |
| **Product format** | Anti-mPDCA-1 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, mPDCA-1$^+$ plasmacytoid dendritic cells (PDCs) are magnetically labeled with Anti-mPDCA-1 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 mPDCA-1$^+$ PDCs are retained on the column. The unlabeled cells run through and this cell fraction is depleted of mPDCA-1$^+$ PDCs. After removal of the column from the magnetic field, the magnetically retained mPDCA-1$^+$ PDCs can be eluted as the positively selected cell fraction.

### 1.2 Background and product applications

Anti-mPDCA-1 MicroBeads were developed for the enrichment of murine PDCs from different organs. The murine plasmacytoid dendritic cell antigen-1 (mPDCA-1) is specifically expressed on PDCs, a subset of CD11c$^+$ dendritic cells, detected at low frecquency in all lymphoid tissues, peripheral blood and some non-lymphoid tissues.[1] In murine spleen, bone-marrow and lymph nodes, mPDCA-1 is exclusively expressed on cells which are CD11c$^+$ CD45R (B220)$^+$ Ly-6C$^+$, i.e. on cells with the phenotype of murine PDCs. Multi-color staining of spleen cells clearly revealed, that all CD11c$^+$ CD45R (B220)$^+$ Ly-6C$^+$ PDCs are mPDCA-1$^+$ and that mPDCA-1 expression is restricted to PDCs.

**Examples of applications**

- Isolation or depletion of mPDCA-1$^+$ PDCs from different tissues.
- Enrichment of PDCs from single-cell suspensions of lymphoid and non-lymphoid tissues for the analysis of:
  - antigen-uptake and antigen-processing;
  - T cell activation or T cell tolerance induction;
  - cross-priming of cytotoxic T cells;
  - T helper cell polarization by PDCs.

### 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) 1:20 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 serum or fetal calf serum. Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- MACS Columns and MACS Separators: mPDCA-1$^+$ PDCs can be enriched by using MS, LS or XS Columns (positive selection). Anti-mPDCA-1 MicroBeads can be used for depletion of mPDCA-1$^+$ PDCs on 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.

- 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 $MgCl_2$, 1.8 mM $CaCl_2$.


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- Cell strainer (70 µm).
- (Optional) Fluorochrome- or Biotin-conjugated Anti-mPDCA-1 antibodies, e.g. Anti-mPDCA-1-FITC (# 130-091-961), Anti-mPDCA-1-PE (# 130-091-962), Anti-mPDCA-1-APC (# 130-091-963), or Anti-mPDCA-1-Biotin (# 130-091-964).
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-mPDCA-1-Biotin (# 130-091-964).
- (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

For highest recovery and purity of plasmacytoid dendritic cells from murine spleen or lymph nodes, single-cell suspensions need 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 µL of Collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue into small pieces by using sharp scissors.
3. Incubate the spleen pieces in Collagenase D solution for 30 minutes at 37 °C.
4. Pass the entire material, i.e. remaining fragments and Collagenase D-released cells, gently through a 70 µm cell strainer using a plunger.
5. Collect all cells in a 15 mL tube and dilute 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 the Dead Cell Removal Kit (# 130-090-101) is recommended.
6. Proceed to magnetic labeling (2.2).

### 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 $2\times10^8$ 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 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 400 µL buffer per $10^8$ total cells.
4. Add 100 µL of Anti-mPDCA-1 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.
7. Wash cells by adding 10-20× labeling volume of buffer per $10^8$ 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.
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 mPDCA-$1^+$ PDCs (see table in section 1.3).

**Magnetic separation with MS or LS Columns**

▲ To achieve highest purities, perform two consecutive column runs.

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.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a 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.
7. 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 (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"

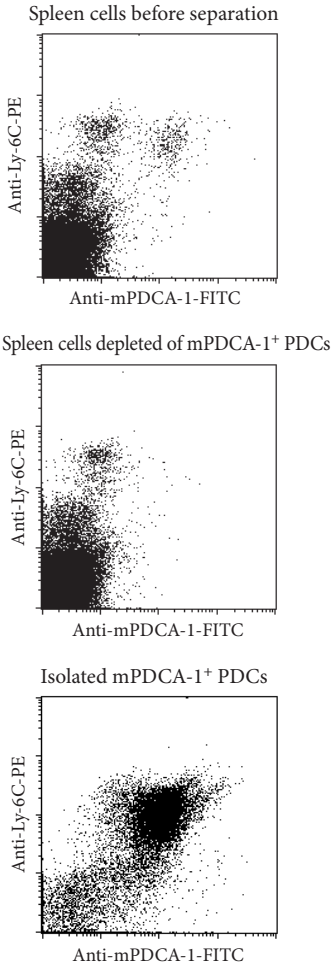
   Depletion: "Depletes"

   ▲ **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 enriched mPDCA-1$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the mPDCA-1$^-$ cell fraction.

## 3. Example of a separation using Anti-mPDCA-1 MicroBeads

The mPDCA-1$^+$ PDCs were isolated from a mouse spleen cell suspension using Anti-mPDCA-1 MicroBeads, two MS Columns and a MiniMACS™ Separator. The cells are fluorescently stained with Anti-mPDCA-1-FITC (# 130-091-961) and Anti-Ly-6C-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.




## 4. Reference

1. Fischer, J. *et al.*, manuscript in preparation.

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.

### 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.





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