



Miltenyi Biotec

## Antibodies

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### 1. Description

<b>Clone</b>	JF05-1C2.4.1 (isotype: rat IgG2b).
<b>Product format</b>	1 mL Anti-mPDCA-1 antibodies, mouse: monoclonal Anti-mPDCA-1 antibodies conjugated either to fluorescein-isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), biotin (Biotin), or as unconjugated antibody (pure). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. Antibodies are supplied in a solution containing 0.1% gelatine and 0.05% sodium azide.
<b>Product size</b>	For 10 <sup>9</sup> nucleated cells, up to 100 stainings.
<b>Storage</b>	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

#### 1.1 Background and product applications

The mouse plasmacytoid dendritic cell antigen 1 (mPDCA-1) is specifically expressed on murine plasmacytoid dendritic cells (PDCs), a subset of dendritic cells detected at low frequency in all lymphoid tissues, peripheral blood and some non-lymphoid tissues.<sup>1</sup> Anti-mPDCA-1 antibodies allow a single-color identification of CD11c<sup>+</sup>CD45R(B220)<sup>+</sup>Ly-6C<sup>+</sup> PDCs in different tissues (e.g. spleen, thymus, bone-marrow, lymph nodes and liver).

#### Product applications

- Direct identification of mPDCA-1<sup>+</sup> PDCs in different tissues by flow cytometry, fluorescence microscopy or immunohistochemistry.
  - Evaluation of MACS<sup>®</sup> separations by flow cytometry or fluorescence microscopy, for example:
    - The isolation of dendritic cells by using CD11c MicroBeads, mouse (# 130-052-001);
    - The isolation of PDCs by using the Plasmacytoid Dendritic Cell Isolation Kit, mouse (# 130-091-263) or Anti-mPDCA-1 MicroBeads (130-091-965).
- ▲ **Note:** For *in vivo* depletion experiments a special product format of Anti-mPDCA-1 pure, mouse - functional grade is available (#130-091-978).

## Anti-mPDCA-1 antibodies mouse

Anti-mPDCA-1-FITC	130-091-961
Anti-mPDCA-1-PE	130-091-962
Anti-mPDCA-1-APC	130-091-963
Anti-mPDCA-1-Biotin	130-091-964
Anti-mPDCA-1 pure	130-091-979

### 1.2 Examples of staining concentrations

Anti-mPDCA-1-conjugate	FITC	PE	APC	Biotin
<b>Recommended antibody dilution</b>				
<b>Flow cytometry<sup>a</sup></b>				
- in general	1:11	1:11	1:11	1:11
- formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11	1:11
- Anti-mPDCA-1 MicroBead-labeled cells	1:11	1:11	1:11	1:11
<b>Immunohistochemistry<sup>c</sup></b>				

- a) Given antibody dilutions are for a cell concentration of up to 1×10<sup>8</sup> cells/mL buffer.  
 b) For optimal results, cells have to be stained prior to fixation.  
 c) For immunohistochemical staining, the optimal antibody dilution has to be tested.

### 1.3 Reagent requirements

- **Buffer:** Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 in autoMACS<sup>™</sup> 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 mouse serum albumin, mouse serum or fetal calf serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (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.
- (Optional) PI (propidium iodide) or 7-AAD for flow- cytometric exclusion of dead cells. For cell fixation and flow- cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.
- (Optional) Fc receptor blocking reagent (CD16/32 monoclonal antibody) to avoid Fc receptor-mediated fluorescent staining.

### 2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10<sup>7</sup> total cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend 10<sup>7</sup> cells in 100 µL of buffer.
  - ▲ **Note:** For optimal staining results, blocking of Fc receptor-mediated staining using an Fc receptor blocking reagent (CD16/32 mAb) is recommended.
2. Add 10 µL of Anti-mPDCA-1 antibody.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
  - ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

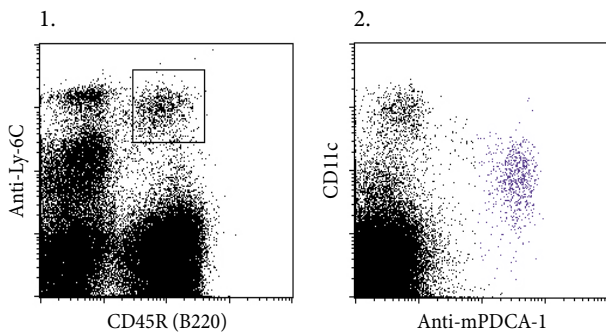
4. Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at  $300\times g$  for 10 minutes. Pipette off supernatant completely.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

### 3. Example of immunofluorescent staining with Anti-mPDCA-1 antibodies

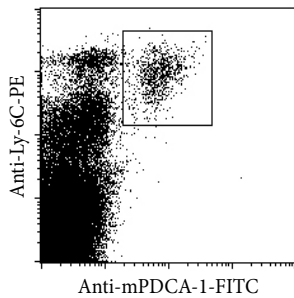
The identification of murine PDCs in blood or tissue is commonly based on their expression of Ly-6C, CD45R (B220) and CD11c. A) shows the detection of PDCs in murine spleen by multi-color staining with Anti-Ly-6C, CD45R (B220), CD11c and Anti-mPDCA-1. Note that all  $CD11c^+ CD45R(B220)^+ Ly-6C^+$  PDCs express mPDCA-1 and that mPDCA-1 is only expressed on  $CD11c^+ CD45R(B220)^+ Ly-6C^+$  PDCs. B-E) shows stainings of spleen cells with Anti-mPDCA-1-FITC (B) Anti-mPDCA-1-PE (C) Anti-mPDCA-1-APC (D) Anti-mPDCA-1-Biotin and Anti-Biotin-APC (E), and Anti-Ly-6C respectively.

#### A. Identification of $CD11c^+ CD45R (B220)^+ Ly-6C^+$ PDCs

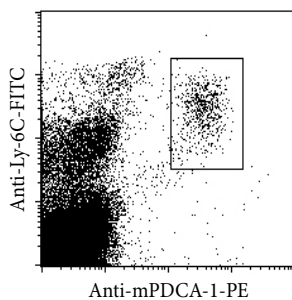
1. Cells are gated on  $CD45R (B220)^+ Ly-6C^+$  cells
2. Cells as gated in 1) are shown in purple



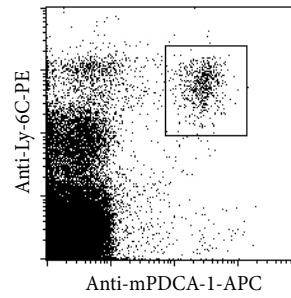
#### B. Direct identification of PDCs with Anti-mPDCA-1-FITC



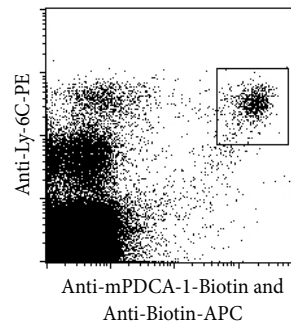
#### C. Direct identification of PDCs with Anti-mPDCA-1-PE



#### D. Direct identification of PDCs with Anti-mPDCA-1-APC



#### E. Direct identification of PDCs with Anti-mPDCA-1-Biotin and Anti-Biotin-APC



## 4. Reference

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

### 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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