

## Contents

1. Description
  - 1.1 Principle of the MACS® Separation
  - 1.2 Background information
  - 1.3 Applications
  - 1.4 Reagent and instrument requirements
2. Protocol
  - 2.1 Sample preparation
  - 2.2 Magnetic labeling
  - 2.3 Magnetic separation
  - 2.4 Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator
3. Example of a separation using the Pan-DC Enrichment Kit
4. References

## 1. Description

<b>Components</b>	<p><b>2 mL Pan-DC Biotin-Antibody Cocktail, human:</b> Cocktail of biotin-conjugated monoclonal antibodies against antigens that are not expressed by dendritic cells.</p> <p><b>2 mL Pan-DC MicroBead-Antibody Cocktail, human:</b> Cocktail of monoclonal MicroBead-conjugated antibodies against antigens that are not expressed by dendritic cells and MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).</p> <p><b>1 mL FcR Blocking Reagent, human:</b> Human Ig.</p>
<b>Capacity</b>	For $2 \times 10^9$ total cells, up to 20 separations.
<b>Product format</b>	The Pan DC Biotin-Antibody Cocktail and the Pan DC MicroBead-Antibody Cocktail are supplied in a solution containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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

Using the Pan-DC Enrichment Kit, human dendritic cells (MDC1s, MDC2s, and PDCs) are isolated by depletion of non-target cells (negative selection). Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-Biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. Additionally, non-target cells are directly magnetically labeled with

a cocktail of MicroBead-conjugated antibodies against antigens that are not expressed by dendritic cells. The magnetically labeled non-target cells are depleted by retaining them within a MACS Column in the magnetic field of a MACS Separator while the unlabeled DCs run through.

### 1.2 Background information

Dendritic cells are most effective antigen-presenting cells and play a key role in initiating and directing immune responses. In blood, there are at least three different subsets: plasmacytoid dendritic cells (PDCs) and two distinct subsets of myeloid dendritic cells (MDC1s and MDC2s).<sup>1,2</sup> The subsets show phenotypic and functional differences.<sup>2-5</sup> The Pan-DC Enrichment Kit allows the concurrent isolation of untouched PDCs, MDC1s, and MDC2s by depletion of non-DCs, i.e., T cells, B cells, NK cells, monocytes, granulocytes, and erythroid cells.

In healthy donors, dendritic cells represent about 1% of all PBMCs, of which approximately 0.37% are PDCs, 0.6% are MDC1s and 0.03% are MDC2s.

### 1.3 Applications

- Analysis of gene expression in blood dendritic cells
- Elucidation of specific signal transduction pathways
- Analysis of antigen uptake, processing, and presentation
- Stimulation of primary T cell responses by antigen-pulsed blood dendritic cells

### 1.4 Reagent and instrument requirement

- **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 human serum albumin, human serum, or fetal bovine serum. Buffers or media containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  are not recommended for use.
- **MACS Columns and MACS Separators:** Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	$10^8$	$2 \times 10^9$	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	$10^9$	$2 \times 10^{10}$	SuperMACS II
autoMACS	$2 \times 10^8$	$4 \times 10^9$	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric analysis, for example, CD1c (BDCA-1)-PE (# 130-090-508), CD303 (BDCA-2)-FITC (# 130-090-510), CD141 (BDCA-3)-APC (# 130-090-907). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters, 30  $\mu$ m (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

## 2. Protocol

### 2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 $\times$ g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the protocols section at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

▲ 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, 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<sup>8</sup> total cells. When working with fewer than 10<sup>8</sup> cells, use same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2 $\times$ 10<sup>8</sup> 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  $\mu$ m nylon mesh (Pre-Separation Filters, 30  $\mu$ m # 130-041-407) to remove cell clumps which may clog the column. Moistent filter with buffer before use.

▲ The recommended incubation temperature is 20–22 °C. Nevertheless, use for all steps pre-cooled buffer (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 $\times$ g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 350  $\mu$ L of buffer per 10<sup>8</sup> total cells.
4. Add 50  $\mu$ L of FcR Blocking Reagent per 10<sup>8</sup> total cells.
5. Add 100  $\mu$ L of Pan DC Biotin-Antibody Cocktail per 10<sup>8</sup> total cells.

6. Mix well and incubate for 5 minutes at room temperature.
7. Add 400  $\mu$ L of buffer per 10<sup>8</sup> total cells.
8. Add 100  $\mu$ L of Pan DC MicroBead-Antibody Cocktail per 10<sup>8</sup> total cells.
9. Mix well and incubate for 5 minutes at room temperature.
10. Wash cells by adding 10 mL of buffer per 10<sup>8</sup> cells and centrifuge at 300 $\times$ g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10<sup>8</sup> cells in 500  $\mu$ L of buffer.
12. Proceed to magnetic separation (2.3).



### 2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total 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 LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
  2. Prepare column by rinsing with 3 mL of buffer.
  3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched dendritic cell fraction.
  4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched dendritic cell fraction, and combine with the effluent from step 3.
- ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-target cells by firmly pushing the plunger into the column.

#### Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of  $\geq$  10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

#### Magnetic separation with the autoMACS® Pro Separator

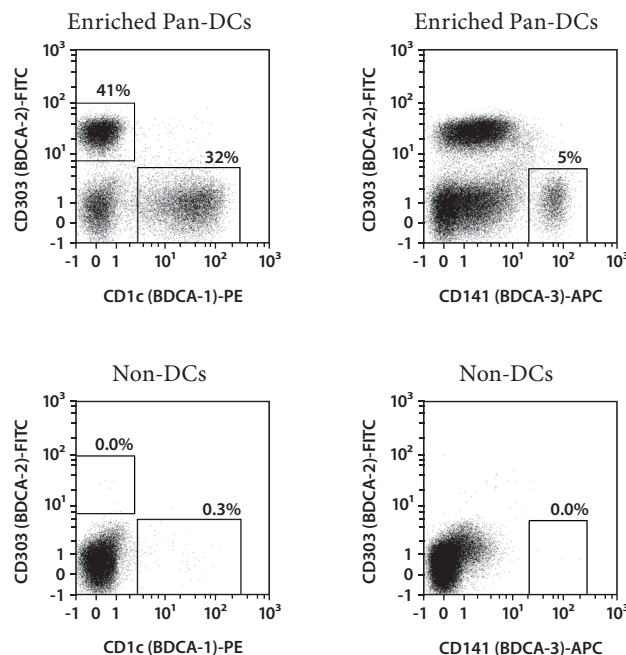
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.

- For a standard separation choose the following program:

#### Depletion: Depletes

Collect negative fraction in row B of the tube rack. This fraction represents the enriched dendritic cell fraction.

- (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-target cells.



#### Magnetic separation with the autoMACS® Separator

- Prepare and prime the instrument.
- Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
- For a standard separation choose the following program:

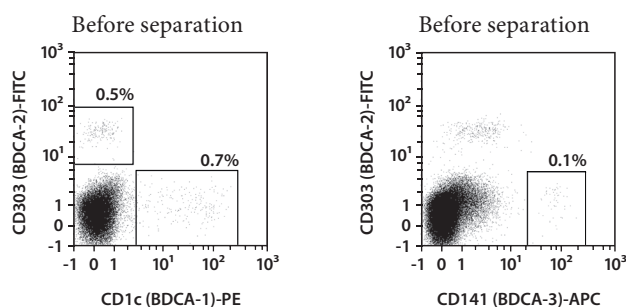
#### Depletion: Depletes

Collect negative fraction from outlet port neg1. This fraction represents the enriched dendritic cell fraction.

- (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-target cells.

### 3. Example of a separation using the Pan-DC Enrichment Kit

Isolation of untouched Pan-DCs from human PBMCs using the Pan-DC Enrichment Kit, human, a MidiMACS Separator and an LS Column. The cells were fluorescently stained with CD303 (BDCA-2)-FITC, CD141 (BDCA-3)-APC, CD1c (BDCA-1)-PE, and CD20-PerCP and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris, dead cells, and B cells were excluded from the analysis based on scatter signals, propidium iodide fluorescence, and CD20 expression.



### 4. References

- O'Doherty, U. *et al.* (1994) Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* 82: 487–493.
- Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, BDCA-4: Three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165: 6037–6046.
- Penna, G. *et al.* (2001) Cutting Edge: Selective usage of chemokine receptors by plasmacytoid dendritic cells. *J. Immunol.* 167: 1862–1866.
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- Krug, A. *et al.* (2001) Toll-like receptor reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur. J. Immunol.* 31: 3026–3037.

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit [www.miltenyibiotec.com/local](http://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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