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1. Description

Components	2 mL FcR Blocking Reagent, human: Human IgG
	2 mL CD141 (BDCA-3) MicroBeads, human: MicroBeads conjugated to monoclonal CD141 (BDCA-3) antibodies (isotype: mouse IgG1).
Size	For 2×10^9 total cells, up to 20 separations.
Product format	Products 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 CD141 (BDCA-3)⁺ cells are magnetically labeled with CD141 (BDCA-3) 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 CD141 (BDCA-3)⁺ cells are retained on the column, while the unlabeled CD141 (BDCA-3)^{-dim} cells are collected in the flow-through. After removing the column from the magnetic field, the retained CD141 (BDCA-3)⁺ cells are eluted and once again separated over a new column, to achieve highest purities.

1.2 Background and product applications

▲ Recently, BDCA-3 was detected to be identical to CD141 (Thrombomodulin). Accordingly the formerly called BDCA-3 Cell Isolation Kit was renamed CD141 (BDCA-3) MicroBead Kit.

CD141 (BDCA-3)¹ is expressed at high levels only on a minor subpopulation of myeloid dendritic cells in human blood (about 0.04% of peripheral blood mononuclear cells (PBMCs)). CD141 (BDCA-3)⁺⁺ myeloid dendritic cells are CD11c^{dim}, CD123⁻, CD1c (BDCA-1)⁻, CD4⁺, and lack expression of lineage markers (CD3, CD14, CD16, CD19, CD20, and CD56). They express myeloid markers such as CD13 and CD33 and are monocytoïd in appearance (fig. 1). In contrast to CD1c (BDCA-1)⁺ myeloid blood dendritic cells, CD141 (BDCA-3)⁺⁺ myeloid blood dendritic cells do not express CD2 and Fc receptors such as CD32, CD64, and FcεRI. Further, CD141 (BDCA-3)⁺⁺ myeloid dendritic cells differ from CD1c (BDCA-1)⁺ myeloid dendritic cells in terms of Toll-like receptor expression, cytokine production, and T helper cell polarization.³ In order to discriminate the CD141 (BDCA-3)⁺⁺CD1c (BDCA-1)⁻ from CD1c (BDCA-1)⁺ myeloid dendritic cells (MDC1s), they have been designated type-2 myeloid dendritic cells (MDC2s).

Apart from CD141 (BDCA-3)⁺⁺ myeloid dendritic cells, the CD141 (BDCA-3) antigen is also expressed at a much lower level on CD1c (BDCA-1)⁺ myeloid dendritic cells, CD303 (BDCA-2)⁺ CD304 (BDCA-4/Neuropilin-1)⁺ plasmacytoid dendritic cells, CD14⁺ monocytes, and granulocytes in blood.

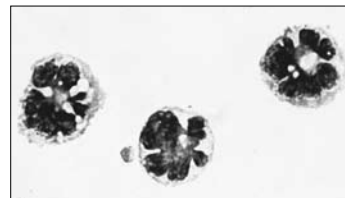


Fig. 1: CD141 (BDCA-3)⁺⁺ blood dendritic cells were isolated by MACS Technology using the CD141 (BDCA-3) MicroBead Kit and stained by May-Grünwald/Giemsa staining.

Examples of applications

- Isolation of CD141 (BDCA-3)⁺ myeloid dendritic cells from human blood for phenotypic and functional characterization,^{1,3} for biochemical and molecular analysis, or for expression analyses, e.g. of newly defined antigens on different dendritic cell subsets⁴.
- Isolation of human CD141 (BDCA-3)⁺⁺ myeloid dendritic cells from mice engrafted with human CD34⁺ hematopoietic progenitors.⁵

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 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 human serum albumin, human serum, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD141 (BDCA-3)⁺ cells can be enriched by using MS, LS or XS Columns (positive selection). 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
Positive selection			
autoMACS	2 × 10 ⁸	4 × 10 ⁹	autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for evaluation of MACS separation by flow cytometry or fluorescence microscopy, for example CD141 (BDCA-3)-FITC (# 130-090-513), CD141 (BDCA-3)-PE (# 130-090-514), or CD141 (BDCA-3)-APC (# 130-090-907) for detection of isolated CD141 (BDCA-3)⁺ myeloid dendritic cells. CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), or CD303 (BDCA-2)-APC (# 130-090-905) for detection of co-isolated plasmacytoid dendritic cells. CD1c (BDCA-1)-FITC (# 130-090-507) or CD1c (BDCA-1)-PE (# 130-090-508) for detection of co-isolated CD1c (BDCA-1)⁺ myeloid dendritic cells. CD14-FITC (# 130-080-701), CD14-PE (# 130-091-242), or CD14-APC (# 130-091-243) for detection of co-isolated monocytes.

▲ **Note:** Fluorescence staining of CD141 (BDCA-3) by CD141 (BDCA-3)-FITC (# 130-090-513), CD141 (BDCA-3)-PE (# 130-090-514), or CD141 (BDCA-3)-APC (# 130-090-907) can be carried out simultaneously with magnetic labeling of the cells, due to the fact that the antibody clone (AD5-14H12) used for fluorochrome conjugates recognizes a different CD141 (BDCA-3) epitope than the antibody clone used for MicroBeads. Fluorescence staining of markers other than CD141 (BDCA-3), can also be done simultaneously with magnetic labeling.

- (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

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation (e.g. Ficoll-Paque™, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** 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, 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 10⁸ total cells. When working with fewer than 10⁸ 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 2×10⁸ 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 300 µL of buffer per 10⁸ total cells.
4. Add 100 µL of FcR Blocking Reagent per 10⁸ total cells.
5. Add 100 µL of CD141 (BDCA-3) MicroBeads per 10⁸ total cells.
6. 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. (Optional) Add staining antibodies after 10 minutes of incubation, e.g. add 50 µL of CD141 (BDCA-3)-PE (# 130-090-514), and incubate for 5 minutes at 4–8 °C.
 8. Wash cells by adding 5–10 mL buffer per 10⁸ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
 9. Resuspend up to 10⁸ cells in 500 µL of buffer.
- ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. 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 CD69⁺ cells (see table in section 1.3).

Positive selection 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 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 μ L LS: 3 \times 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 the fraction with 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".

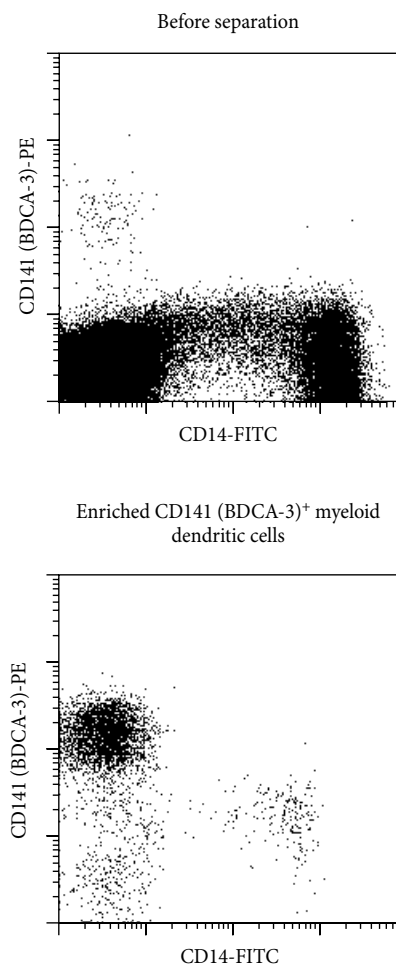
Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Apply magnetically labeled cells to the autoMACS. Choose the separation program "Posseld".
▲ **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 "Posseld", collect the positive fraction (outlet port "pos2"). This is the purified CD141 (BDCA-3)⁺ cell fraction.

3. Example of a separation using the CD141 (BDCA-3) MicroBead Kit

Isolation of CD141 (BDCA-3)⁺ myeloid dendritic cells from PBMCs using the CD141 (BDCA-3) MicroBead Kit, MiniMACS™ Separator, and two MS Columns. Cells were stained with CD141 (BDCA-3)-PE and CD14-FITC. Note that some CD1c (BDCA-1)⁺ myeloid dendritic cells, CD303 (BDCA-2)⁺ plasmacytoid dendritic cells, and CD14⁺ monocytes are co-enriched, although they express CD141 (BDCA-3) at low levels.



4. References

1. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3 and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165: 6037–6046. [898]
2. Grabbe, S. *et al.* (2000) Dendritic cells: multi-lineal and multi-functional. *Immunol. Today* 21: 431–433. [899]
3. Lebre, C. *et al.* (2003) BDCA-3^{hi} dendritic cells: a novel subset with distinct phenotypical characteristics. Doctoral dissertation, University of Amsterdam, Netherlands.
4. Kwakkenbos, M. J. *et al.* (2002) The human EGF-TM7 family member EMR2 is a heterodimeric receptor expressed on myeloid cells. *J. Leukoc. Biol.* 71: 854–862. [2437]
5. Palucka, A. K. *et al.* (2003) Human dendritic cell subsets in NOD/SCID mice engrafted with CD34⁺ hematopoietic progenitors. *Blood* 102: 3302–3310. [4227]

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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