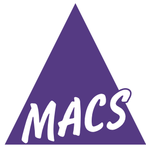

CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit
human

Order no. 130-090-506

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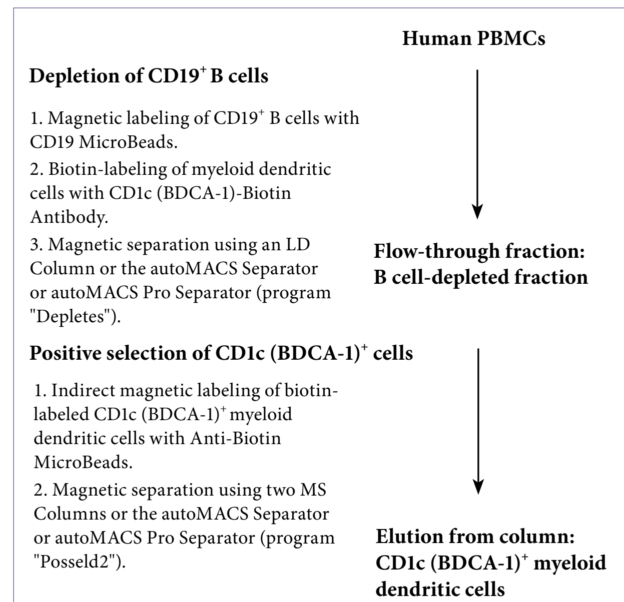
1. Description

Components	**2 mL CD19 MicroBeads, human:** MicroBeads conjugated to a monoclonal CD19 antibody (isotype: mouse IgG1). **2 mL CD1c (BDCA-1)-Biotin Antibody, human:** monoclonal CD1c (BDCA-1) antibody conjugated to biotin (clone: AD5-8E7, isotype: mouse IgG2a). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** human IgG.
Capacity	For 2×10^9 total cells, up to 20 separations.
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	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

The isolation of CD1c (BDCA-1)$^+$ myeloid dendritic cells is performed by two magnetic separation steps. In the first step, CD1c (BDCA-1)–expressing B cells are magnetically labeled with CD19 MicroBeads and, subsequently, depleted by separation over a MACS® Column which is placed in the magnetic field of a MACS Separator. In the second step, CD1c (BDCA-1)$^+$ myeloid dendritic cells in the B cell–depleted flow-through fraction are indirectly magnetically labeled with CD1c (BDCA-1)-Biotin and Anti-Biotin MicroBeads. Upon separation, the labeled CD1c (BDCA-1)$^+$ myeloid dendritic cells are retained within the column and are eluted after removing the column from the magnetic field. To increase the purity, the positively selected cell fraction containing the CD1c (BDCA-1)$^+$ myeloid dendritic cells is separated over a second column.



1.2 Background information

The CD1c (BDCA-1) antigen is specifically expressed on dendritic cells, which are CD11c^{high} CD123^{low} and represent the major subset of myeloid dendritic cells in human blood.[1–6] CD1c (BDCA-1)$^+$ dendritic cells show a monocytoid morphology (fig. 1) and express myeloid markers such as CD13 and CD33 as well as Fc receptors such as CD32, CD64, and FcεRI. Furthermore, they were determined to be CD4$^+$, Lin (CD3, CD16, CD19, CD20, CD56)$^-$, CD2$^+$, CD45RO$^+$, CD141 (BDCA-3)^{low}, CD303 (BDCA-2)$^-$, and CD304 (BDCA-4/ Neuropilin-1)$^-$.[1] A minor proportion of CD1c (BDCA-1)$^+$ myeloid dendritic cells expresses CD14 and CD11b. CD1c (BDCA-1) is also found on CD1a$^+$ dendritic cells generated *ex vivo* from monocytes or hematopoietic precursor cells, and on CD1a$^+$ Langerhans cells in skin. In blood, apart from myeloid dendritic cells, a subset of small resting B cells expresses CD1c (BDCA-1). For this reason, the CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit includes CD19 MicroBeads for depletion of B cells prior to the enrichment of CD1c (BDCA-1)$^+$ myeloid dendritic cells. In order to discriminate the CD1c (BDCA-1)$^+$ from CD1c (BDCA-1)$^-$ CD141 (BDCA-3)$^+$ myeloid dendritic cells, they have been designated type 1 myeloid dendritic cells (MDC1s).

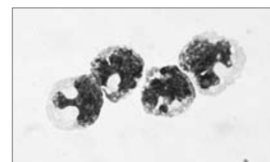


Fig. 1: CD1c (BDCA-1)$^+$ blood dendritic cells were isolated by MACS Technology using CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit and stained by May-Grünwald/Giemsa staining.

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1.3 Applications

- Isolation of CD1c (BDCA-1)$^+$ myeloid dendritic cells to examine expression of Toll-like receptors,[3,4,10] chemokine receptors,[2,4] or new antigens, e.g., DCAL-1[5] and EMR2[6].
- Isolation for studies on dendritic cell activation,[3] migration,[2] cytokine production,[3,4,8] and T cell polarization,[3] particularly in comparison with monocyte-derived dendritic cells[7].
- Isolation of myeloid dendritic cells from mice engrafted with human CD34$^+$ hematopoietic progenitor cells[9], or, as an alternative to CD1a MicroBeads, for the isolation of epidermal Langerhans cells[11].

1.4 Reagent and instrument requirements

- 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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of CD19$^+$ B cells is performed on an LD Column. The subsequent positive selection of CD1c (BDCA-1)$^+$ myeloid dendritic cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS™ or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Depletion and positive selection			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

- (Optional) Fluorochrome-conjugated antibodies for evaluation of MACS separation, for example, for identification of CD1c (BDCA-1)$^+$ dendritic cells, e.g., CD1c (BDCA-1)-PE (# 130-090-508), CD1c (BDCA-1)-APC (# 130-090-903); or for analysis of B cell depletion, e.g., CD19-FITC (# 130-091-328), CD19-PE (# 130-091-247), or CD19-APC (# 130-091-248); or for detection of CD14 expression on a minor population of CD1c (BDCA-1)$^+$ dendritic cells, e.g., CD14-FITC (# 130-080-701), CD14-PE (# 130-091-242), CD14-APC (# 130-091-243). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

  ▲ **Note:** Fluorescence labeling has to be performed **after** magnetic separation.

  ▲ **Note:** CD1c (BDCA-1)-FITC (clone AD5-8E7, # 130-090-507) is **not recommended** for staining in combination with the CD1c (BDCA-1) Dendritic Cell Isolation Kit.

- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion 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, for example, using Ficoll-Paque™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

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

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at 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, 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 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 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. Wet filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 200 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 µL of CD19 MicroBeads per 10^8 total cells.

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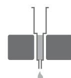
6. Add 100 µL of CD1c (BDCA-1)-Biotin per 10^8 total cells.
7. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
8. Wash cells by adding 10−20× labeling volume and centrifuge at 300×g for 10 minutes at 2−8 °C. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: depletion of CD19$^+$ B cells

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details 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 that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Magnetic separation with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

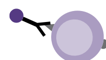
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Depletion with the autoMACS™ 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 at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. Choose the following program:
   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

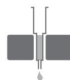
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose the following program:
   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack.



2.4 Magnetic labeling of CD1c (BDCA-1)$^+$ myeloid dendritic cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer.
3. Add 100 µL of Anti-Biotin MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
5. Wash cells by adding 10−20× labeling volume of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).



2.3 Magnetic separation: positive selection of CD1c (BDCA-1)$^+$ myeloid dendritic cells

Positive selection with MS Columns

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

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled CD1c (BDCA-1)$^+$ myeloid dendritic cells by firmly pushing the plunger into the column.
7. To increase purity of CD1c (BDCA-1)$^+$ myeloid dendritic cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new MS Column.

Positive selection with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

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▲ 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.

Positive selection with the autoMACS™ 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 at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. Choose the following program:
   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos2.

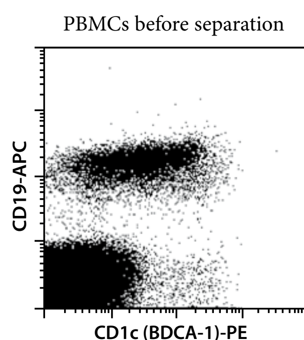
Positive selection with the autoMACS™ Pro Separator

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.
3. For a standard separation choose one of the following programs:
   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

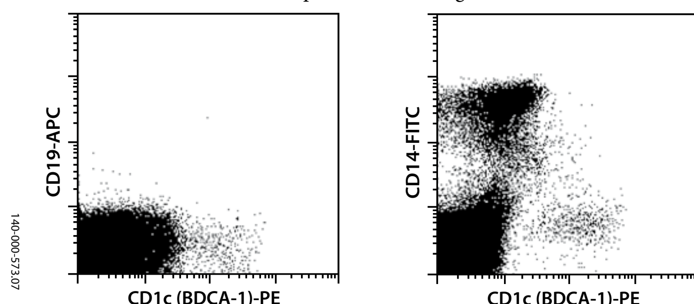
3. Example of a separation using the CD1c (BDCA-1)+ Dendritic Cell Isolation Kit

CD1c (BDCA-1)+ myeloid dendritic cells were isolated from human PBMCs using the CD1c (BDCA-1)+ Dendritic Cell Isolation Kit, an LD Column, two MS Columns, a MidiMACS™ Separator and a MiniMACS™ Separator. Cells are fluorescently stained with CD1c (BDCA-1)-PE (# 130-090-508) and CD19-APC (# 130-091-248) or CD14-FITC (# 130-080-701), respectively. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

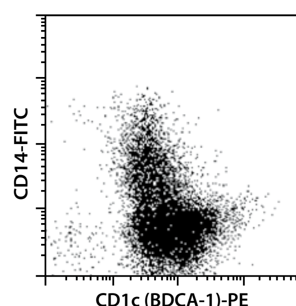
PBMCs before separation



B cell depleted flow-through fraction



Isolated CD1c (BDCA-1)+ myeloid dendritic cells



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All protocols and data sheets are available at www.miltenyibiotec.com.

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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