Miltenyi Biotec

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

non-human primate

Order no. 130-091-878

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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.

1. Description

This product is for research use only.

Components

2 mL CD20 MicroBeads, non-human primate: MicroBeads conjugated to a monoclonal CD20 antibody (isotype: mouse IgG1).

2×0.2 mL CD1c (BDCA-1)-PE: monoclonal CD1c (BDCA-1) antibody conjugated to R-phycoerythrin (isotype: mouse IgG2a; clone: AD5-8E7).

2 mL Anti-PE MicroBeads: MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).

2 mL FcR Blocking Reagent: human IgG.

Capacity For 10^9 total cells, up to 10 separations.

Product format Anti-PE MicroBeads are supplied in buffer containing 0.05% sodium azide. All other 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.

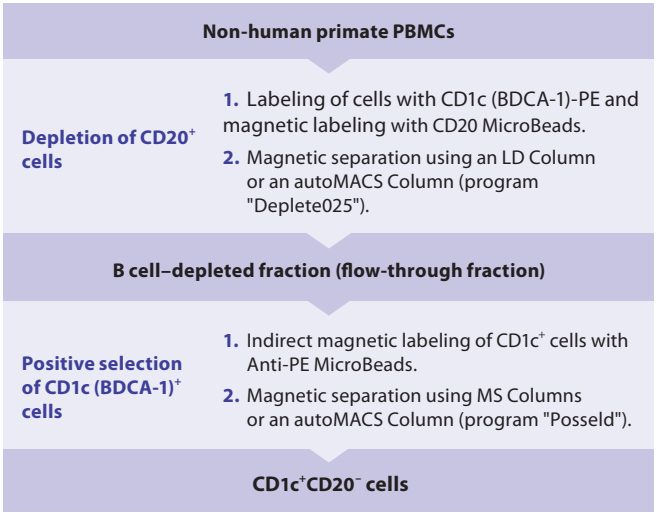
This product is applicable for the separation of cells from rhesus monkey (*Macaca mulatta*). The CD20 and CD1c antibodies have been tested to cross-react with cynomolgus monkey (*Macaca fascicularis*). Cross-reactivity with other non-human primates has not been tested.

1.1 Principle of the MACS® Separation

The isolation of CD1c$^+$blood dendritic cells from peripheral blood mononuclear cells (PBMCs) is performed in a two-step procedure. First, cells are labeled with CD1c (Anti-BDCA-1)-PE. Subsequently, CD1c expressing B cells are magnetically labeled with CD20 MicroBeads and thereafter depleted by separation over an LD Column, which is placed in the magnetic field of a MACS Separator. In the second step, CD1c$^+$ blood dendritic cells in the B cell depleted flow-through fraction are magnetically labeled with Anti-PE MicroBeads.

In the second step, the CD1c$^+$ blood dendritic cells in the B cell–depleted flow-through fraction are labeled with Anti-PE MicroBeads and isolated by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.

After removing the column from the magnetic field, the magnetically retained CD1c$^+$ blood dendritic cells can be eluted as the positively selected cell fraction.



1.2 Background information

Human as well as rhesus monkey dendritic cells represent a heterogenous population composed of several subsets. Myeloid and plasmacytoid DCs, differentiated by their expression of CD11c and CD123, respectively, were further characterized by means of new markers against human blood dendritic cell antigens (BDCA). Two human myeloid dendritic cell populations have been described: CD11c^{high} CD123^{low} CD1c (BDCA-1)$^+$ DCs and CD11c^{dim} CD123$^-$

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BDCA-3$^+$ DCs, whereas plasmacytoid CD11c$^-$ CD123$^+$ dendritic cells are characterized by the expression of BDCA-2 or BDCA-4. Human CD1c (BDCA-1)$^+$ dendritic cells show a monocytoid morphology 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$^+$, BDCA-3^{low}, BDCA-2$^-$ and BDCA-4$^-$.1 A minor proportion of human CD1c (BDCA-1)$^+$ myeloid dendritic cells expresses CD14 and CD11b.
Recently, rhesus monkey myeloid CD11c$^+$CD123$^-$ dendritic cells were reported to express CD1c (BDCA-1).[2]
In blood, apart from myeloid dendritic cells, a subset of B cells also expresses CD1c (BDCA-1). For this reason, the CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit includes CD20 MicroBeads for depletion of B cells prior to the enrichment of CD1c (BDCA-1)$^+$ blood dendritic cells.

1.3 Applications

- Isolation of CD1c$^+$ myeloid dendritic cells.
- Isolation for studies on dendritic cell activation, migration, cytokine production, and T cell polarization.

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 serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

- MACS Columns and MACS Separators: Depletion of CD20$^+$ cells can be performed on an LD Column. The subsequent positive selection of CD1c (BDCA-1)$^+$ blood dendritic cells can be performed on two MS Columns. Positive selection and depletion can also be performed by using 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, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD14-FITC, CD14-APC, or CD20-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with rhesus monkey anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™, as with human samples.

▲ **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 refer to the protocols section 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 of CD20$^+$ cells

▲ 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 2×10^8 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 labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 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×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 280 µL of buffer per 10^8 total cells.
4. Add 80 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 40 µL of CD1c (BDCA-1)-PE per 10^8 total cells.
6. Mix well and incubate for 5 minutes in the refrigerator in the dark (2–8 °C).
7. Add 400 µL of buffer per 10^8 cells.
8. Add 200 µL of CD20 MicroBeads per 10^8 total cells.

9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 10–20 mL of buffer and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
11. Resuspend cell pellet in buffer:
    Depletion with LD Column: 500 µL for up to 1.25×10^8 cells
    Depletion with autoMACS: 500 µL for up to 1×10^8 cells
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of CD20$^+$ cells

▲ Choose an LS Column and MACS Separator according to the number of total cells and the number of CD20$^+$ cells. For details refer to table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to 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 pre-enriched B cell–depleted fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the labeling of CD1c (BDCA-1)$^+$ blood dendritic cells.

Depletion with the autoMACS® Pro Separator

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

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

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 and collection tubes into the Chill Rack.
3. For a standard separation choose the following program:

   Depletion: Deplete025

   Collect unlabelled cells in row B of the tube rack. This fraction is depleted from B cells.
4. Proceed to 2.4 for the labeling of CD1c (BDCA-1)$^+$ blood dendritic cells.



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

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

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 800 µL of buffer.
3. Add 200 µL of Anti-PE MicroBeads.
4. Mix well and incubate for 105 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 10–20 mL 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.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of CD1c (BDCA-1)$^+$ blood 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 refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as 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)$^+$ blood dendritic cells by firmly pushing the plunger into the column.
7. To increase purity of CD1c (BDCA-1)$^+$ blood dendritic cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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 and collection tubes into the Chill Rack.

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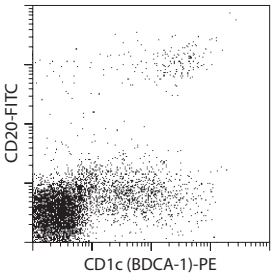
3. For a standard separation choose the following program:

   Positive selection: Posseld
   Collect positive fraction in row C of the tube rack. This is the enriched CD1c (BDCA-1)$^+$ blood dendritic cell fraction.

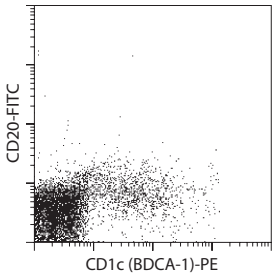
3. Example of a separation using the CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit

Isolation of CD1c (BDCA-1)$^+$ dendritic cells from rhesus monkey PBMCs using CD1c (BDCA-1)$^+$ Dendritic Cell Isolation Kit. Cells were incubated with CD1c (BDCA-1)-PE and subsequently magnetically labeled with CD20 MicroBeads. B cells were depleted using an LD Column. The B cell–depleted fraction was magnetically labeled with Anti-PE MicroBeads and separated over an MS Column. Cell samples were stained with CD20-FITC and CD14-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

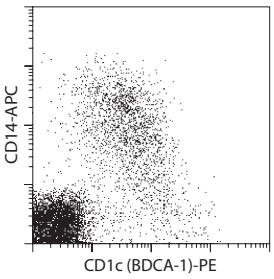
PBMCs before separation



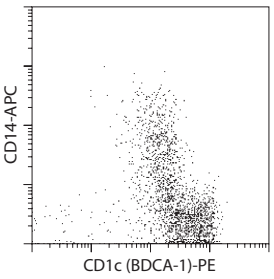
B cell–depleted flow through fraction



Counter staining of B cell–depleted flow through fraction with CD14-APC



CD1c (BDCA-1)$^+$ blood dendritic cells after enrichment



4. References

1. Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, DW., Schmitz, J. (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.
2. Coates, P.T.H., Barratt-Boyes, S. M., Zhang, L., Donnenberg, V.S., O´Connell, P.J., Logar, A.J., Duncan, J., Murphey-Corb, M., Donnenberg, A.D. Morelli, A. E., Maliszewski, C.R., Thomson, A.W. (2003) Dendritic cell subsets in blood and lymphoid tissue of rhesus monkes and their mobilization with Flt3 ligand. Blood 102: 2513-2521.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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