



Magnetic cell sorting

CD1c (BDCA-1) Dendritic Cell Isolation Kit non-human primate

Order No. 130-091-878

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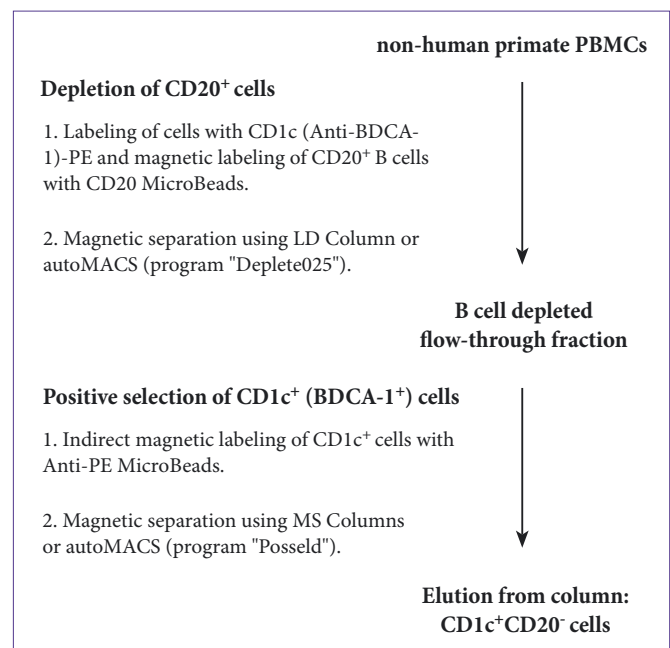
1. Description

Components	<p>2 mL CD20 MicroBeads, non-human primate: MicroBeads conjugated to a monoclonal CD20 antibody (isotype: mouse IgG1).</p> <p>2x1 mL CD1c (Anti-BDCA-1)-PE Antibody: monoclonal CD1c (Anti-BDCA-1) antibody conjugated to R-phycoerythrin (isotype: mouse IgG2a; clone: AD5-8E7).</p> <p>2 mL Anti-PE MicroBeads: MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).</p> <p>2 mL FcR Blocking Reagent: human IgG.</p>
Product size	For 1x10 ⁹ total cells, up to 10 separations
Product format	CD20 MicroBeads are supplied as a suspension containing 0.1% gelatine and 0.05% sodium azide. CD1c (Anti-BDCA-1)-PE Antibody is supplied in a solution containing 0.1% gelatine and 0.05% sodium azide. Anti-PE MicroBeads are supplied as a suspension containing 0.05% sodium azide. FcR Blocking Reagent is supplied in a solution containing 0.1% gelatine 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.

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 MACS® separation

The isolation of CD1c⁺ blood dendritic cells from peripheral blood mononuclear cells (PBMCs) is performed by two magnetic separation steps. In the first step, 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. Upon separation, the labeled CD1c⁺ blood dendritic cells are retained on the column and are eluted after removing the column from the magnetic field.



1.2 Background and product applications

Human as well as rhesus monkey dendritic cells represent a heterogeneous 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⁻ 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 monocytoïd 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.

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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.

Examples of applications

- Isolation of 1c (BDCA-1)⁺ myeloid dendritic cells.
- Isolation for studies on dendritic cell activation, migration, cytokine production, and T cell polarization.

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 gelatine, HSA, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- MACS Columns and MACS Separators: Depletion of CD20⁺ cells is performed on an LD Column. The subsequent positive selection of CD1c (BDCA-1)⁺ blood dendritic cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS™ 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
Positive selection or depletion			
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 flow cytometric analysis, e.g. CD14-FITC (# 130-080-701), CD14-APC (# 130-091-243), or CD20-FITC (# 130-091-108).
- (Optional) Propidium iodide (PI) 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 rhesus monkey anticoagulated peripheral blood or buffy coat, PBMCs can be isolated by density gradient centrifugation, e.g. Ficoll-Paque™, as with human samples (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.



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 10⁸ 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 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 cells at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 120 μL of buffer per 10⁸ cells.
4. Add 80 μL of **FcR Blocking Reagent** per 10⁸ cells.
5. Add 200 μL of **CD1c (Anti-BDCA-1)-PE** per 10⁸ total cells.
6. Mix well and incubate for 5 minutes in the dark at 4–8 °C.
7. Add 400 μL of buffer per 10⁸ cells.
8. Add 200 μL of **CD20 MicroBeads** per 10⁸ total cells.
9. Mix well and incubate for 15 minutes at 4–8 °C.
10. Wash cells by adding 10–20 mL of buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Pipette off supernatant completely.
11. Resuspend cell pellet in buffer:
 - Depletion with LD Column: 500 μL for up to 1.25×10⁸ cells
 - Depletion with autoMACS: 500 μL for up to 1×10⁸ cells
 - ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation: Depletion of CD20⁺ cells

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 2×1 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect the entire flow-through as B cell depleted fraction.

Depletion 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. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Deplete025".
3. Collect unlabeled fraction (outlet port "neg1"). This is the B cell depleted fraction.
4. Proceed to 2.4 for the enrichment 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 10⁸ cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cells at 300×g for 10 minutes. Pipette off 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 15 minutes at 4–8 °C.
5. Wash cells by adding 10–20 mL of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
6. Resuspend up to 10⁸ cells in 500 µL of buffer.
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 (see "Column data sheets").
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times once 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 fraction with magnetically labeled cells (CD1c (BDCA-1)⁺ cells) by firmly applying the plunger supplied with the column.
7. Isolation of CD1c (BDCA-1)⁺ 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 column.

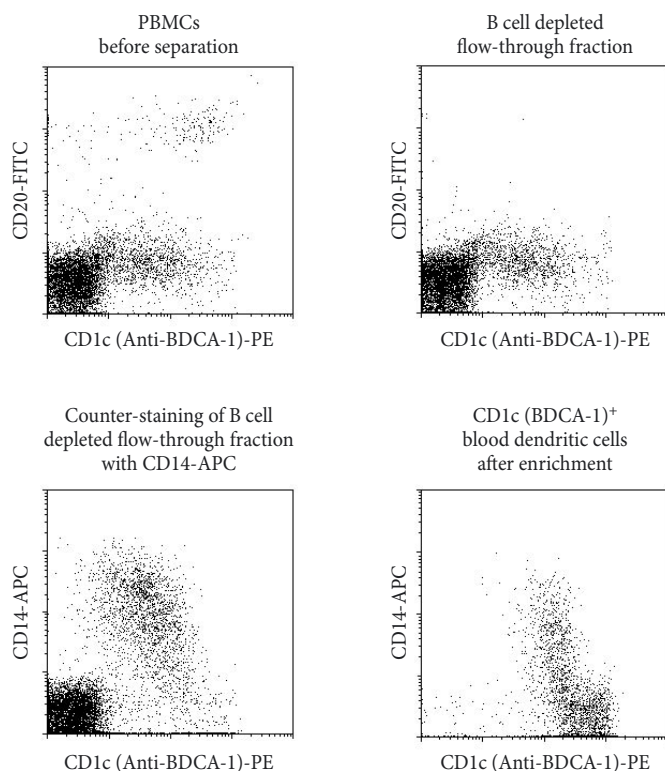
Positive selection with the autoMACS™ Separator

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

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Posseld".
3. Collect positive fraction (outlet port "pos2"). This is the enriched CD1c (BDCA-1)⁺ 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 (Anti-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 PI fluorescence.



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. [898]
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 monkeys and their mobilization with Flt3 ligand. *Blood* 102: 2513-2521. [3099]

Warning

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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