Miltenyi Biotec

CD304 (BDCA-4/ Neuropilin-1) MicroBead Kit human

Order no. 130-090-532

Contents

1. Description

Components	**2 mL CD304 (BDCA-4/Neuropilin-1) MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD304 (BDCA-4/ Neuropilin-1) antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Human IgG.
Capacity	For 2×10^9 total cells, up to 20 separations.
Product format	The products 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 MACS® Separation

First, the CD304 (BDCA-4/Neuropilin-1)$^+$ cells are magnetically labeled with CD304 (BDCA-4/Neuropilin-1) 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 CD304 (BDCA-4/Neuropilin-1)$^+$ cells are retained within the column. The unlabeled cells run through; this fraction is thus depleted of CD304 (BDCA-4/Neuropilin-1)$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD304 (BDCA-4/Neuropilin-1)$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD304 (BDCA-4/Neuropilin-1)$^+$ cells is separated over a second column.

1.2 Background information

CD304 (BDCA-4/Neuropilin-1)[1] is specifically expressed by plasmacytoid dendritic cells (PDCs) in human peripheral blood[1–5], cord blood[12], and bone marrow[6]. Exclusive expression of CD304 (BDCA-4/Neuropilin-1) on plasmacytoid dendritic cells allows their direct isolation by positive selection. In blood and bone marrow, CD304 (BDCA-4/Neuropilin-1)$^+$ plasmacytoid dendritic cells are characterized as being CD11c$^-$, CD123^{high}, CD4$^+$, CD45RA$^+$, CD303 (BDCA-2)$^+$, CD141 (BDCA-3)^{dim}, CD1c (BDCA-1)$^-$, and CD2$^-$. They lack expression of lineage markers (CD3, CD14, CD16, CD19, CD20, CD56), and express neither myeloid markers, like CD13 and CD33, nor Fc receptors, such as CD32, CD64, or FcεRI. Freshly isolated CD1c (BDCA-1)$^+$ or CD141 (BDCA-3)$^{++}$ blood dendritic cells and monocytes do not express CD304 (BDCA-4/Neuropilin-1) but expression of CD304 (BDCA-4/Neuropilin-1) is induced in culture.[1,6] In inflamed tonsils, CD304 (BDCA-4/Neuropilin-1) expression is, apart from plasmacytoid dendritic cells, also detected on some other cells, primarily follicular B helper memory T cells.[6] Additionally, Neuropilin-1 is known to be expressed on numerous non-hematopoietic cell types, e.g. neurons, endothelial and tumor cells.

In contrast to CD304 (BDCA-4/Neuropilin-1), binding of antibody to CD303 (BDCA-2) has been shown to inhibit the production of type I interferon in influenza virus-stimulated plasmacytoid dendritic cells.[5]

1.3 Applications

Isolation of CD304 (BDCA-4/Neuropilin-1)$^+$ plasmacytoid dendritic cells from peripheral blood, cord blood, or bone marrow, for example,

- to examine expression of Toll-like receptors,[4,7,8,11] chemokine receptors,[3,8,10] or new antigens[2,9] or
- for studies on dendritic cell activation,[4] migration,[3] cytokine production,[4,6,8] and T cell polarization[4,6,13].

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.

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- MACS Columns and MACS Separators: CD304 (BDCA-4/Neuropilin-1)$^+$ cells can be enriched by using MS, LS, or XS Columns (positive selection). 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
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, 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 flow cytometric or fluorescence microscopic evaluation of MACS separation, e.g. CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), or CD303 (BDCA-2)-APC (# 130-090-905) for identification of plasmacytoid dendritic cells; or CD123-FITC (# 130-090-897), CD123-PE (# 130-090-899), or CD123-APC (# 130-090-901) for control. For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

  ▲ **Note:** CD304 (BDCA-4/Neuropilin-1) antibodies are not recommended for staining in combination with the CD304 (BDCA-4/Neuropilin-1) MicroBead 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 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 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.

▲ 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 300 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 CD304 (BDCA-4/Neuropilin-1) MicroBeads per 10^8 total cells.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. (Optional) Add staining antibodies after 10 minutes of incubation, e.g. add 50 µL of CD303 (BDCA-2)-FITC (# 130-090-510), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
8. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. 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

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD304 (BDCA-4/Neuropilin-1)$^+$ cells. For details 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. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL

3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times once the column reservoir is empty. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
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 appropiate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. To increase purity of CD304 (BDCA-4/Neuropilin-1)$^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. 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 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.

Magnetic separation 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. For a standard separation choose the following program:

   Positive selection: "Posseld"
   Collect positive fraction from outlet port pos2.

Magnetic separation with the autoMACS™ Pro Separator

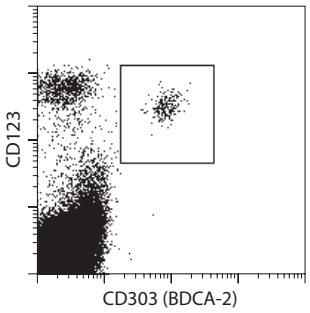
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Positive selection: "Posseld"
   Collect positive fraction in row C of the tube rack.

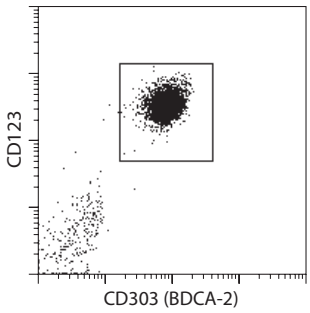
3. Example of a separation using the CD304 (BDCA-4/Neuropilin-1) MicroBead Kit

Isolation of CD304 (BDCA-4/Neuropilin-1)$^+$ plasmacytoid dendritic cells from PBMCs using the CD304 (BDCA-4/Neuropilin-1) MicroBead Kit, two MS Columns, and a MiniMACS™ Separator. Cells were stained for CD303 (BDCA-2) and CD123. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Before separation



Isolated CD304 (BDCA-4/ Neuropilin-1)$^+$ plasmacytoid dendritic cells



4. References

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