Miltenyi Biotec

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

Order no. 130-097-149

Contents

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	**1 vial 304 (BDCA-4/Neuropilin-1) MicroBeads, human – lyophilized**: MicroBeads conjugated to monoclonal anti-human CD304 (BDCA-4/Neuropilin-1) antibodies (isotype: mouse IgG1).
	2 mL FcR Blocking Reagent, human: Human IgG.
	2 mL Reconstitution Buffer
Capacity	For 2×10^9 total cells.
Product format	Lyophilized MicroBeads.
	Reconstitution Buffer contains 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. For information about reconstitution of the lyophilized MicroBeads and storage after reconstitution refer to chapter 2.1.

1.1 Principle of the 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 PDCs in human blood[1–5], bone marrow[6], and cord blood[11]. Exclusive expression of CD304 (BDCA-4/Neuropilin-1) on PDCs allows their direct isolation. In blood and bone marrow, CD304 (BDCA-4/Neuropilin-1)$^+$ PDCs are 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 neither express myeloid markers such as CD13 and CD33, nor Fc receptors such as CD32, CD64, or FceRI. Their lymphoid origin is indicated by their plasmacytoid morphology and the expression of the pre-T cell receptor α-chain. Freshly isolated CD1c (BDCA-1)$^+$ or CD141 (BDCA-3)$^{++}$ myeloid blood dendritic cells and monocytes do not express CD304 (BDCA-4/Neuropilin-1), but expression of CD304 (BDCA-4/Neuropilin-1) is induced on myeloid blood dendritic cells and monocytes upon culturing.[1, 6] In inflamed tonsils, CD304 (BDCA-4/Neuropilin-1) expression is, apart from PDCs, also detected on some other cells, primarily follicular B helper memory T cells.[6]

CD304 (BDCA-4) was shown to be identical to neuropilin-1 (NP-1).[6] Neuropilin-1 has formerly been discovered to be expressed on numerous nonhematopoietic cell types including neurons, endothelial and tumor cells.

Unlike binding of antibodies to CD303 (BDCA-2), binding of antibodies to CD304 (BDCA-4/Neuropilin-1) does not have a substantial effect on IFN type I production in PDCs after induction by, e.g., influenza virus.[5, 6, 14]

1.3 Applications

- Isolated CD304 (BDCA-4/Neuropilin-1)$^+$ PDCs were used, for example, to examine expression of Toll-like receptors[4, 7, 8, 12], chemokine receptors[3, 8, 10], or new antigens, e.g., EMR[2,9]and for studies on dendritic cell activation[4], migration[3], cytokine production[4, 6, 8], and T cell polarization[4, 6, 13]. Functional and phenotypical analysis was performed, e.g., of PDCs isolated from PMBCs and synovial fluid from psoriatic arthritis and rheumotoid arthritis patients[15].

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)

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140-003-512.03

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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD304 (BDCA-4/Neuropilin-1)$^+$ cells can be enriched by using MS, LS, or XS Columns. Positive selection can also be performed by using the autoMACS Pro or 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 II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Positive selection			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, 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 refer to www.miltenyibiotec.com/antibodies.

▲ **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 Solution (# 130-093-233) 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, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Reconstitution of MicroBeads

Reconstitute the lyophilized MicroBeads by adding all Reconstitution Buffer to the vial. Mix by pipetting up and down until resuspended. After reconstitution the MicroBeads are stable for 6 months at 2–8 °C. Write the new expiration date after reconstitution on the vial label.

2.2 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™.

▲ **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).

2.3 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 10^7 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 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).

2.4 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 refer to the table in section 1.4.

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

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 refer to 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 Pro Separator or the autoMACS Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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 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: Posseld
   Collect positive fraction in row C of the tube rack.

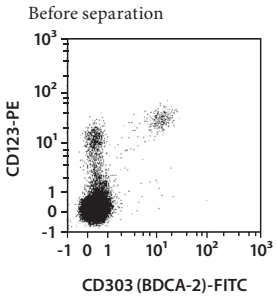
Magnetic separation with the autoMACS Separator

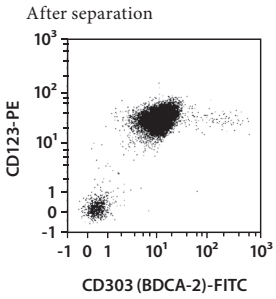
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:

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

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

CD304 (BDCA-4/Neuropilin-1) MicroBeads were reconstituted as described in 2.1. PDCs were isolated from PBMCs using the CD304 (BDCA-4/Neuropilin-1) MicroBead Kit, MS Columns, and a MiniMACS™ Separator. Cells were stained with CD303 (BDCA-2)-FITC and CD123-PE.
Like CD304 (BDCA-4/Neuropilin-1), the CD303 (BDCA-2) antigen is specifically expressed on plasmacytoid dendritic cells in blood, and allows their direct identification. Apart from plasmacytoid dendritic cells, CD123 is also expressed at high levels on basophils and at low levels on monocytes and myeloid dendritic cells.





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