Miltenyi Biotec

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

human

Order no. 130-119-475

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.

Description

This product is for research use only.

Components

2 mL CD19 MicroBeads, human:
MicroBeads conjugated to a monoclonal CD19 antibody (isotype: mouse IgG1).

2 mL CD14 MicroBeads, human:
MicroBeads conjugated to a monoclonal CD14 antibody (isotype: mouse IgG2a).

2 mL CD1c (BDCA-1)-Biotin, 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 in a two-step procedure. First, the CD1c (BDCA-1)–expressing B cells are magnetically labeled with CD19 MicroBeads and optionally CD14$^+$ myeloid cells with CD14 MicroBeads, whereas CD1c$^+$ cells are labeled with CD1c (BDCA-1)-Biotin. The magnetically labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator. The flow-through fraction is depleted of CD19$^+$ and optionally of CD14$^+$ cells.
In the second step, the CD1c (BDCA-1)$^+$ myeloid dendritic cells in the flow-through fraction are magnetically labeled with Anti-Biotin MicroBeads and isolated by positive selection 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 (BDCA-1)$^+$ myeloid dendritic cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD1c (BDCA-1)$^+$ cells must be separated over a second column.

Human PBMCs:
Depletion of CD19$^+$ B cells and optionally CD14$^+$ myeloid cells

1. Magnetic labeling of CD19$^+$ B cells with CD19 MicroBeads. (Optional) Magnetic labeling of CD14$^+$ myeloid cells.
2. Biotin-labeling of myeloid dendritic cells with CD1c (BDCA-1)-Biotin.
3. Magnetic separation using an LD Column or an autoMACS Column (program "Depletes").

Flow-through fraction:
Positive selection of CD1c (BDCA1$^+$) cells

1. Indirect magnetic labeling of biotin-labeled CD1c (BDCA-1)$^+$ myeloid dendritic cells with Anti-Biotin MicroBeads.
2. Magnetic separation using two MS Columns or autoMACS Columns (program "Posseld2").

CD1c (BDCA-1)$^+$ myeloid dendritic cells (eluted fraction)

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. CD1c (BDCA-1)$^+$

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-005-368.02

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)$^-$, $CD2^+$, $CD45RO^+$, CD141 (BDCA-3)^{low}, CD303 (BDCA-2)$^-$, and CD304 (BDCA-4/Neuropilin-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. Recently, a functionally different population of $CD14^+CD1c^+$ myeloid cells has been reported. To avoid the co-enrichment of such population, an optional removal reagent for $CD14^+$ myeloid cells is included. In blood, apart from myeloid dendritic cells, a subset of 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.

1.3 Applications

- Isolation of CD1c (BDCA-1)$^+$ myeloid dendritic cells to examine expression of Toll-like receptors, chemokine receptors, or new antigens, e.g., DCAL-1 and EMR2.
- Isolation for studies on dendritic cell activation, migration, cytokine production, and T cell polarization, particularly in comparison with monocyte-derived dendritic cells.
- Isolation of $CD1c^+$ DCs for studies evaluating new checkpoint inhibitors or dissecting their mechanisms of actions in tumor settings.

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 (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

- MACS Columns and MACS Separators: Depletion of $CD19^+$ B cells and optionally of $CD14^+$ myeloid cells can be performed on an LD Column. The subsequent positive selection of CD1c (BDCA-1)$^+$ myeloid 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, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	$2×10^8$	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
Depletion or positive selection			
autoMACS	$2×10^8$	$4×10^9$	autoMACS Pro

▲ **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., CD1c (BDCA-1)-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

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

- (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.

1. 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™.

▲ **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.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.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 $CD19^+$ and optionally of $CD14^+$ 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 100 µL of buffer per 10^8 total cells.

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-005-368.02

4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. If optional depletion of $CD14^+$ cells is required, add 100 µL of CD14 MicroBeads per 10^8 total cells; otherwise use 100 µL of buffer instead.
6. Add 100 µL of CD19 MicroBeads per 10^8 total cells.
7. Add 100 µL of CD1c (BDCA-1)-Biotin per 10^8 total cells.
8. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
9. Wash cells by adding 10–20× labeling volume and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of $CD19^+$ and optionally of $CD14^+$ cells

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 cell 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 $(BDCA\text{-}1)^+$ myeloid 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: Depletes
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the labeling of CD1c $(BDCA\text{-}1)^+$ myeloid dendritic cells.

2.4 Magnetic labeling of CD1c $(BDCA\text{-}1)^+$ myeloid 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 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).

2.5 Magnetic separation: Positive selection of CD1c $(BDCA\text{-}1)^+$ 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 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 $(BDCA\text{-}1)^+$ myeloid dendritic cells by firmly pushing the plunger into the column.
7. To increase purity of CD1c $(BDCA\text{-}1)^+$ myeloid 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

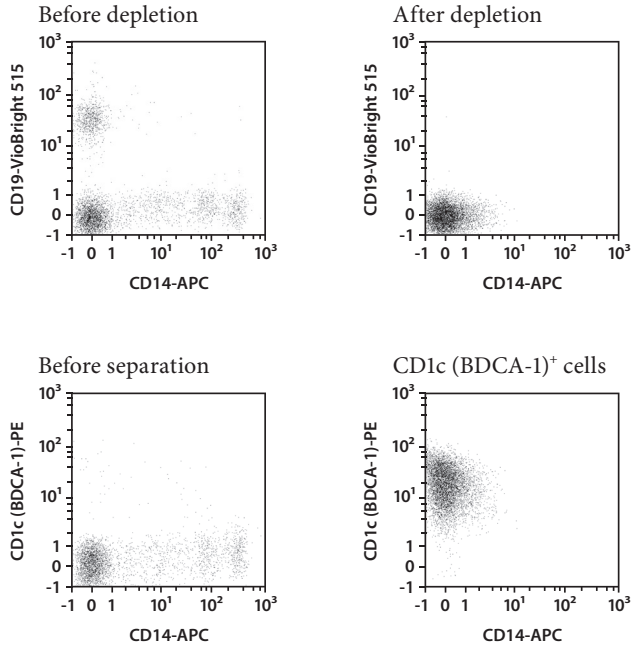
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
3. For a standard separation choose the following program:
   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack. This is the enriched CD1c $(BDCA\text{-}1)^+$ myeloid dendritic cell fraction.

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-005-368.02

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 were fluorescently stained with CD1c (BDCA-1)-PE, CD14-APC, or CD19-VioBright™ 515. Cell debris and dead cells were excluded from the analysis based on scatter signals and prodipium iodide fluorescence and erythrocytes based on CD45-negativity.



4. References

1. Dzionek, A. *et al.* (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. Mathan, T. S. M. *et al.* (2017) Harnessing RNA sequencing for global, unbiased evaluation of two new adjuvants for dendritic-cell immunotherapy. Oncotarget. 8(12): 19879–19893.
3. Khasawneh, A. *et al.* (2017) Myeloid but not plasmacytoid blood DCs possess Th1 polarizing and Th1/Th17 recruiting capacity in psoriasis. Immunol. Lett. 189: 109–113.
4. Karyampudi, L. *et al.* (2016) PD-1 Blunts the Function of Ovarian Tumor-Infiltrating Dendritic Cells by Inactivating NF-κB. Cancer Res. 76(2): 239–250.
5. van Beek, J. J. *et al.* (2016) Human blood myeloid and plasmacytoid dendritic cells cross activate each other and synergize in inducing NK cell cytotoxicity. Oncoimmunology 5(10): e1227902.
6. Su, S. *et al.* (2017) Blocking the recruitment of naive CD4$^+$ T cells reverses immunosuppression in breast cancer. Cell Res. 27(4): 461–482.

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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MidiMACS, the Miltenyi Biotec logo, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, VarioMACS, and VioBright are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.

Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.