Miltenyi Biotec

Myeloid Dendritic Cell Isolation Kit
human

Order no. 130-094-487

Contents

1. Description

Components	**2 mL Non-Myeloid Dendritic Cell Antibody-Biotin Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens not expressed by myeloid dendritic cells. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal antibodies against biotin (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Isotype: 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 Myeloid Dendritic Cell Isolation Kit has been developed for the concurrent isolation of CD1c (BDCA-1)$^+$ and CD141 (BDCA-3/Thrombomodulin)$^+$ dendritic cells from peripheral blood mononuclear cells (PBMCs). Human myeloid dendritic cells (MDCs) are isolated by depletion of non-MDCs (negative selection) by using the Myeloid Dendritic Cell Isolation Kit. Non-MDCs are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies as primary labeling reagent, and anti-biotin-monoclonal antibodies conjugated to MicroBeads as secondary labeling reagent. The magnetically labeled non-MDCs are depleted by retaining these cells onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The unlabeled MDCs pass through the column.

1.2 Background information

Dendritic cells (DCs) are the main antigen presenting cells, capable of initiating primary immune responses.[1,2] The human DC compartment can broadly be subdivided in myeloid and plasmacytoid DCs. Two subsets of myeloid dendritic cells (MDCs) have been characterized according to the expression of CD1c (BDCA-1) and CD141 (BDCA-3), respectively.[3,4] CD1c (BDCA-1)$^+$ MDCs are CD11c^{high}, CD32$^+$, CD64$^+$, FcεR1$^+$, and CD123^{low} and have an average frequency of 0.6% in peripheral blood. Some CD1c (BDCA-1)$^+$ MDCs express CD14 and CD11b. CD1c (BDCA-1) is also expressed on a subset of small resting B cells.

CD141 (BDCA-3/Thrombomodulin)^{high} MDCs are a minor subset with a frequency of about 0.04% in peripheral blood. CD141 (BDCA-3)^{high} MDCs are CD11c^{dim}, CD123$^-$, and CD4$^+$.[3] The CD141 (BDCA-3) antigen is also expressed at a much lower level on CD1c (BDCA-1)$^+$ MDCs, plasmacytoid DCs, monocytes, and granulocytes.

1.3 Applications

- Functional studies of MDCs in which they should not be magnetically labeled, e.g., if subsets are to be separated according to IL-12 secretion patterns using IL-12 Secretion Assay – Cell Enrichment and Detection Kit (# 130-092-122).

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Non-MDCs can be depleted with the use of LD Columns. Depletion 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS,
Depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD141 (BDCA-3)-FITC (# 130-090-513), CD19-PE (# 130-091-247), CD14-VioBlue™ (# 130-094-364), or CD1c (BDCA-1)-APC (# 130-090-903). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (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 (# 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™.

▲ **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 see 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

▲ 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, # 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. 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.
5. Add 100 µL of Non-Myeloid Dendritic Cell Antibody-Biotin Cocktail per 10^8 total cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Repeat step 7.
9. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
10. Add 100 µL of Anti-Biotin MicroBeads per 10^8 total cells.
11. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
12. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
13. Resuspend cells in a final volume of 500 µL per 10^8 total cells .
14. 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 MDCs. For details see 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 see 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.

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. Program recommendations below refer to separation of human PBMCs.

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.

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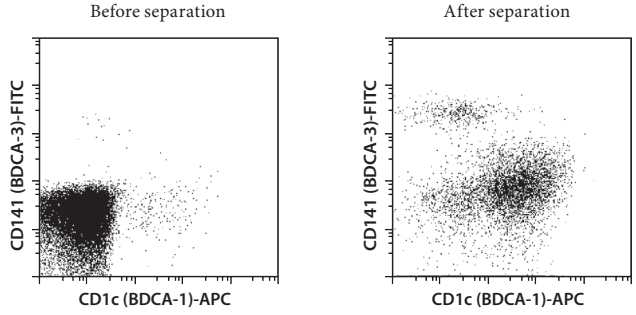
3. For a standard separation choose the following program:
   Depletion: "Depl025"
   Collect negative fraction in row B of the tube rack.

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 pos1.
3. For a standard separation choose the following program:
   Depletion: "Depl025"
   Collect negative fraction from outlet port neg1.

3. Example of a separation using the Myeloid Dendritic Cell Isolation Kit

MDCs were isolated from human PBMCs using the Myeloid Dendritic Cell Isolation Kit, an LD Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD141 (BDCA-3)-FITC (# 130-090-513), CD19-PE (# 130-091-247), CD14-VioBlue (# 130-094-364), and CD1c (BDCA-1)-APC (# 130-090-903) and analyzed by flow cytometry. For the enumeration of MDCs before isolation, exclusion of $CD1c^+CD19^+$ B cells and $CD1c^+CD14^+$ monocytes was performed. Shown are CD1c (BDCA-1) and CD141 (BDCA-3) stained aliquotes of total cells before (with exclusion gate of B cells and monocytes) and after separation (without exclusion gate).



4. References

1. Inaba, K. *et al.* (1998) Efficient Presentation of Phagocytosed Cellular Fragments on the Major Histocompatibility Complex Class II Products of Dendritic Cells. J. Exp. Med. 188: 2163–2173.
2. Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. Nature 392: 245–252.
3. 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.
4. Grabbe, S.*et al.* (2000) Dendritic cells: multi-lineal and multi-functional. Immunol. Today 21: 431–433.

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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