Miltenyi Biotec

Plasmacytoid Dendritic Cell Isolation Kit II
human

Order no. 130-097-415

Contents

1. Description

Components	**2 mL Non-PDC Biotin-Antibody Cocktail II, human:** Cocktail of biotin-conjugated monoclonal antibodies against antigens that are not expressed by plasmacytoid dendritic cells. **2 mL Non-PDC MicroBead Cocktail II, human:** Cocktail of MicroBead-conjugated monoclonal antibodies against antigens that are not expressed by plasmacytoid dendritic cells and MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).
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

Using the Plasmacytoid Dendritic Cell Isolation Kit II, human plasmacytoid dendritic cells (PDCs) are isolated by depletion of non-target cells. Non-target cells 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.

Additionally, non-PDCs are directly magnetically labeled with a cocktail of MicroBead-conjugated antibodies against antigens that are not expressed on PDCs. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled PDCs run through the column.

1.2 Background information

PDCs are one of three subsets of dendritic cells originally identified in human peripheral blood. They are also known as plasmacytoid T cells, plasmacytoid monocytes, lymphoid dendritic cells, IFNα/β-producing cells (IPCs), or type 2 pre-dendritic cells (pDC2).[1,2] Upon viral infection, they produce high amounts of type I interferons, which block viral replication and stimulate innate and adaptive immune responses. In culture, they mature into potent antigen-presenting cells, after exposure to IL-3 alone or in combination with an appropriate stimulus. In healthy donors, PDCs represent about 0.4% of total peripheral blood mononuclear cells (PBMCs). Apart from blood, immature PDCs have been found in human lymphoid tissue and in inflammatory sites, e.g., skin of systemic lupus erythematosus (SLE)[3] or *psoriasis vulgaris* patients[4]. In blood and bone marrow, PDCs are identified as being CD303 (BDCA-2)$^+$, CD304 (BDCA-4/Neuropilin-1)$^+$, CD123$^+$, CD11c$^-$. Unlike CD123, CD303 (BDCA-2) and CD304 (BDCA-4/Neuropilin-1) are exclusively expressed on PDCs.[5-8] Further, they are CD4$^+$, CD45RA$^+$, CD141 (BDCA-3)^{dim}, CD1c (BDCA-1)$^-$, CD2$^-$, lack expression of lineage markers (CD3, CD14, CD16, CD19, CD20, CD56), and express neither myeloid markers, e.g., CD13 and CD33, nor Fc receptors such as CD16, CD64, or FcεRI.[9]

1.3 Applications

- Isolation of PDCs from human PBMCs for phenotypic, functional or molecular analyses, e.g., studies on expression of Toll-like receptors[8,11-13], chemokine receptors[7,12,14], or new antigens, and on dendritic cell activation[8], migration[7], cytokine production[1,8,12], and T cell polarization[1,8,15].

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) 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.

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- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **Note:** For optimal purity and recovery performance, the use of LD Columns is recommended. LS Columns can be used for increasing recovery while purity might be reduced.

▲ **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, e.g., CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), CD303 (BDCA-2)-APC (# 130-090-905), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), CD45-APC (# 130-091-230), CD123-FITC (# 130-090-897), CD123-PE (# 130-090-899), or CD123-APC (# 130-090-901). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, 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 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, 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. 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of the Non-PDC Biotin-Antibody Cocktail II per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add 400 µL of buffer per 10^8 cells.
8. Add 100 µL of the Non-PDC MicroBead Cocktail II per 10^8 cells.
9. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of PDCs. 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

▲ For optimal purity and recovery performance, the use of LD Columns is recommended.

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the 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 enriched plasmacytoid dendritic cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with LS Columns

▲ LS Columns can be used for increasing recovery while purity might be reduced.

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 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched plasmacytoid dendritic cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched plasmacytoid dendritic cells and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-plasmacytoid dendritic cells by firmly pushing the plunger into the column.

Depletion 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 the following program:

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® Separator

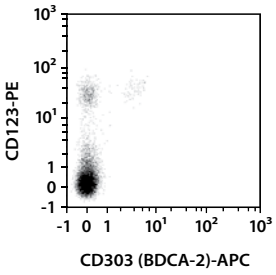
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

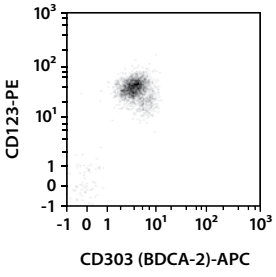
3. Example of a separation using the Plasmacytoid Dendritic Cell Isolation Kit II

PDCs were isolated from PBMCs by using the Plasmacytoid Dendritic Cell Isolation Kit II, a MidiMACS™ Separator, and an LD Column. The cells were fluorescently stained with CD303 (BDCA-2)-APC and CD123-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation



Enriched PDCs



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4. References

1. Dzionek, A. *et al.* (2002) Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions. Hum. Immunol. 63: 1133–1148.
2. McKenna, K. *et al.* (2005) Plasmacytoid dendritic cells: linking innate and adaptive immunity. J. Virol. 79: 17–27.
3. Rönnblom, L. and Alm, G.V. (2001) A pivotal role for the natural interferon α-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. J. Exp. Med. 194: F59–F63.
4. Gilliet, M. *et al.* (2004) Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch. Dermatol. 140: 1490–1495.
5. 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.
6. Grabbe, S. *et al.* (2000) Dendritic cells: multi-lineal and multi-functional. Immunol. Today 21: 431–433.
7. Penna, G. *et al.* (2001) Cutting edge: selective usage of chemokine receptors by plasmacytoid dendritic cells. J. Immunol. 167: 1862–1866.
8. Krug, A. *et al.* (2001) Toll-like receptor reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur. J. Immunol. 31: 3026–3037.
9. Dzionek, A. *et al.* (2001) BDCA-2, a novel plasmacytoid dendritic cell–specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon α/β induction. J. Exp. Med. 194: 1823–1834.
10. Colonna, M. *et al.* (2004) Plasmacytoid dendritic cells in immunity. Nature Immunol. 5: 1219–1226.
11. Hornung, V. *et al.* (2002) Quantitative expression of Toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J. Immunol. 168: 4531–4537.
12. Jarrossay, D. *et al.* (2001) Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. Eur. J. Immunol. 31: 3388–3393.
13. Matsumoto, M. *et al.* (2003) Subcellular localization of Toll-like receptor 3 in human dendritic cells. J. Immunol. 171: 3154–3162.
14. Penna, G. *et al.* (2002) Cutting edge: differential chemokine production by myeloid and pasmacytoid dendritic dells. J. Immunol. 169: 6673–6676.
15. Kaser, A. *et al.* (2004) Interleukin-18 attracts plasmacytoid dendritic cells (DC2s) and promotes Th1 induction by DC2s through IL-18 receptor expression. Blood 103: 648–655.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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