Plasmacytoid Dendritic Cell Isolation Kit
human

Magnetic cell sorting

Order no. 130-092-207

Index

1. Description

Components	**2 mL PDC Biotin-Antibody Cocktail, human**: Cocktail of monoclonal biotin-conjugated antibodies against antigens that are not expressed by plasmacytoid dendritic cells. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).
Size	For 2×10^9 total cells; up to 20 separations.
Product format	The PDC Biotin-Antibody Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration dates are indicated on the vial labels.

1.1 Principle of MACS® separation

Using the Plasmacytoid Dendritic Cell Isolation Kit, human plasmacytoid dendritic cells (PDCs) are isolated by depletion of non-PDCs (negative selection). Non-PDCs 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-PDCs are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled PDCs pass through the column.

1.2 Background and product applications

PDCs are one of two 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 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 CD32, CD64, or FcεRI.[9]
The Plasmacytoid Dendritic Cell Isolation Kit is developed for the isolation of untouched PDCs from PBMCs. Therefore, non-PDCs, i.e. T cells, B cells, NK cells, myeoid dendritic cells, monocytes, granulocytes, and erythroid cells, are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. Upon subsequent magnetic separation the labeled cells are retained on the column, while the unlabeled PDCs are collected in the flow-through.

Examples of applications

- Functional studies on PDCs, in which effects due to antibody-crosslinking of cell surface proteins should be avoided, like for example studies on PDC activation or signal transduction.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) 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 (4–8 °C).

  ▲ **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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

140-001-515.02

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

- MACS Columns and MACS Separators:

Column	max. number of labeled cells	max. number of total cells	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

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

▲ **Note:** Separation can be performed using an LS or an LD Column. If the recovery of PDCs is most important, the use of an LS Column is recommended. If the purity of the enriched PDCs is most important, the use of an LD Column is recommended.

- 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), or CD45-APC (# 130-091-230).
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation (e.g. Ficoll-Paque®, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

▲ **Note:** 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 non-plasmacytoid dendritic cells

▲ Work fast, keep the 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of the PDC Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 10 minutes at 4–8 °C.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
7. Repeat step 6.
8. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
9. Add 100 µL of Anti-Biotin MicroBeads per 10^8 total cells.
10. Mix well and incubate for 15 minutes at 4–8 °C.

    ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
11. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
12. Resuspend cells in a final volume of 500 µL per 10^8 total cells:

    ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
13. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

Depletion with LS Column

1. Place LS Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with 3 mL of buffer:
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 3×3 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled enriched plasmacytoid dendritic cell fraction.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-plasmacytoid dendritic cells.

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.

4. Collect unlabeled cells which pass through and wash column with 2×1 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled enriched plasmacytoid dendritic cell fraction.

5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-plasmacytoid dendritic cells.

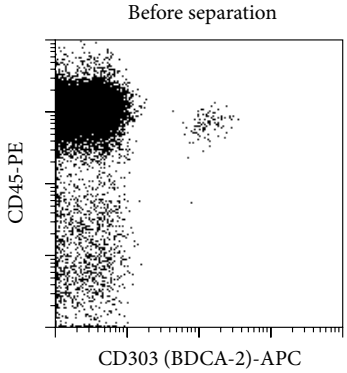
Depletion with autoMACS™ Separator

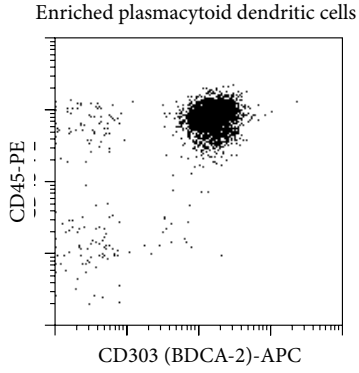
▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Depletes".
3. Collect the unlabeled fraction (outlet port "neg1"). This is the enriched plasmacytoid dendritic cell fraction.
4. (Optional) Collect positive fraction (outlet port “pos1”). This fraction represents the magnetically labeled non-plasmacytoid dendritic cells.

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

Isolation of human PDCs from PBMCs by using the Plasmacytoid Dendritic Cell Isolation Kit, MidiMACS™ Separator, and an LD Column. Cells were stained with CD303 (BDCA-2)-APC and CD45-PE.





4. References

1. Dzionek, A. *et al.* (2002) Plasmacytoid dendritic cells: from specific surface markers to specific cellular functions. Hum. Immunol. 63: 1133–1148. [2423]
2. McKenna, K. *et al.* (2005) Plasmacytoid dendritic cells: linking innate and adaptive immunity. J. Virol. 79: 17–27. [6933]
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. [1265]
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. [6936]
5. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-4 and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. 165: 6037–6046. [898]
6. Grabbe, S. *et al.* (2000) Dendritic cells: multi-lineal and multi-functional. Immunol. Today 21: 431–433. [899]
7. Penna, G. *et al.* (2001) Cutting edge: selective usage of chemokine receptors by plasmacytoid dendritic cells. J. Immunol. 167: 1862–1866. [1069]
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. [1215]
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. [1264]

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.

140-001-515.02