Miltenyi Biotec

# Diamond Plasmacytoid Dendritic Cell Isolation Kit II
human

Order no. 130-097-240

## Contents



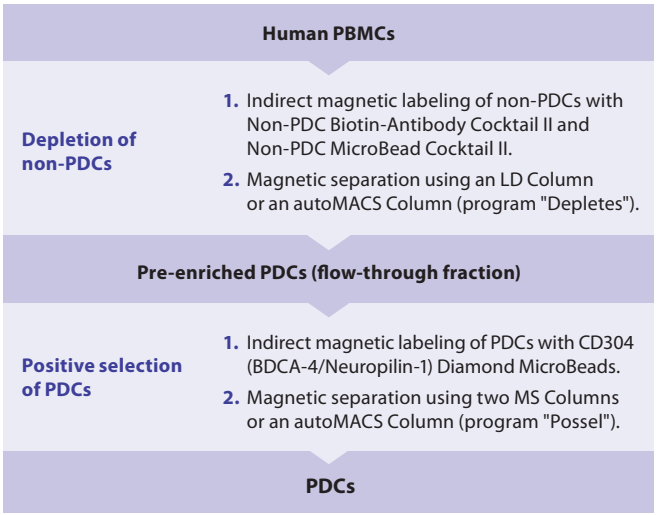
## 1. Description

| | |
|---|---|
| **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). **2 mL CD304 (BDCA-4/Neuropilin-1) Diamond MicroBeads, human:** MicroBeads conjugated to monoclonal anti-CD304 (BDCA-4/Neuropilin-1) antibodies (isotype: mouse IgG1). |
| **Capacity** | For $2\times10^9$ 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 Diamond Plasmacytoid Dendritic Cell Isolation Kit II has been developed for the isolation of extremely pure plasmacytoid dendritic cells (PDCs) from peripheral blood mononuclear cells (PBMCs). The expected purity is almost 100%.

The isolation of PDCs is performed in a two-step procedure. First, the non-PDCs are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens and Anti-Biotin MicroBeads. Additionally, non-PDCs are directly magnetically labeled with a cocktail of MicroBead-conjugated antibodies against antigens that are not expressed on PDCs. The labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator. In the second step, the pre-enriched PDCs are labeled with PDC-specific CD304 (BDCA-4/Neuropilin-1) Diamond MicroBeads and isolated by positive selection fraction 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 PDCs can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the PDCs must be separated over a second 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 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



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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]
Binding of antibodies to CD304 (BDCA-4/Neuropilin-1) does not have a substantial effect on IFN type I production in PDCs after stimulation.[1,9,10]

▲ The BDCA-2 and BDCA-4 (Neuropilin-1) antigens have been assigned to CD303 and CD304, respectively, at the 8th International Workshop in Human Leukocyte Differentiation Antigens in Adelaide, Australia, in 2004.

### 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.

- MACS Columns and MACS Separators: Depletion of non-PDCs can be performed on an LD Column. The subsequent positive selection of PDCs can be performed on two MS Columns. Positive selection and 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 |
|---|---|---|---|
| **Positive selection** | | | |
| MS | $10^7$ | $2\times10^8$ | MiniMACS, OctoMACS, VarioMACS, SuperMACS II |
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS II |
| **Positive selection or depletion** | | | |
| autoMACS | $2\times10^8$ | $4\times10^9$ | 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), CD303 (BDCA-2)-APC (# 130-090-905), 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, 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 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 of non-PDCs

▲ 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 $2\times10^8$ 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 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. Resuspend cell pellet in 400 µL of buffer per $10^8$ total cells.
8. Add 100 µL of Non-PDC MicroBead Cocktail II per $10^8$ total cells.
9. Mix well and incubate for 15 minutes at 2–8 °C.





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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 cells in a final volume of 500 µL per $10^8$ total cells.
12. Proceed to magnetic separation (2.3).

### 2.3 Magnetic separation: Depletion of non-PDCs

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of PDCs. For details refer to 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 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 pre-enriched PDC 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 PDCs.

**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.
4. Proceed to 2.4 for the labeling of PDCs.

**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.
4. Proceed to 2.4 for the labeling of PDCs.

### 2.4 Magnetic labeling of PDCs

▲ 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 cells directly in 100 µL CD304 (BDCA-4/Neuropilin-1) Diamond MicroBeads.
3. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
4. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend up to $10^8$ cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
6. Proceed to magnetic separation (2.5).

### 2.5 Magnetic separation: Positive selection of PDCs

**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 2 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 2×1 mL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   ▲ **Note:** Perform washing steps by adding buffer aliquots only when 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 cells by firmly pushing the plunger into the column.
7. To increase purity of PDCs, 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 or the autoMACS® Separator**

**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:

   **Positive selection: Possel**
   Collect positive fraction in row C of the tube rack. This is the enriched PDC fraction.

**Magnetic separation with the autoMACS® Separator**

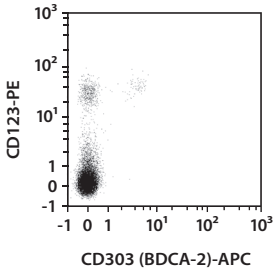
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   **Positive selection: Possel**
   Collect positive fraction from outlet port pos1. This is the enriched PDC fraction.

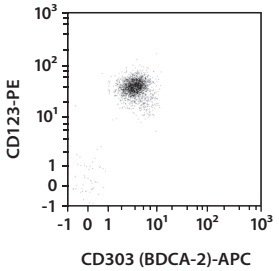
## 3. Example of a separation using the Diamond Plasmacytoid Dendritic Cell Isolation Kit II

PDCs were isolated from human PBMCs by using the Diamond Plasmacytoid Dendritic Cell Isolation Kit II, an LD and two MS Columns, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD123-PE and CD303 (BDCA-2)-APC 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.

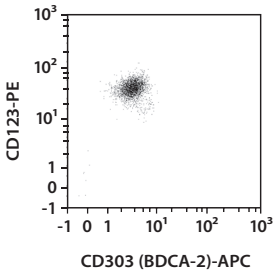
Before separation




Pre-enriched PDCs




Isolated PDCs

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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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