Miltenyi Biotec

Plasmacytoid Dendritic Cell Isolation Kit

mouse

Order no. 130-107-093

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.

1. Description

This product is for research use only.

Components	**2 mL Plasmacytoid Dendritic Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against antigens not expressed by plasmacytoid dendritic cells. **4 mL Anti-Biotin MicroBeads UltraPure:** UltraPure MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **1 mL FcR Blocking Reagent, mouse**
Capacity	For 2×10^9 total cells.
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, mouse, 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 an anti-biotin monoclonal antibody conjugated to UltraPure MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. 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

The Plasmacytoid Dendritic Cell Isolation Kit has been designed for the isolation of untouched PDCs from single-cell suspensions of lymphoid organs. Freshly isolated PDCs express low levels of MHC class II and costimulatory molecules and are poor stimulators of T helper cells. Upon stimulation with bacterial DNA containing particular unmethylated CpG motifs (CpG-DNA) or upon viral challenge they produce large amounts of type I IFN and acquire antigen-presenting capacity.[1,2] PDCs can be directly identified in lymphoid and non-lymphoid organs by the specific expression of mPDCA-1 (murine plasmacytoid dendritic cell antigen-1).[3] Recently, mPDCA-1 has been identified as bone marrow stromal antigen 2 (BST2)[4].

1.3 Applications

Isolation of untouched plasmacytoid dendritic cells from single-cell suspensions of spleen and lymph nodes for the analysis of:

- antigen-uptake and antigen-processing;
- T cell activation or T cell tolerance induction;
- cross-priming of cytotoxic T cells;
- T helper cell polarization by plasmacytoid dendritic cells;
- PDC function where effects due to antibody-crosslinking on cell surface proteins should be avoided.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

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

140-004-771.02

- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Column 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
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
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. Anti-mPDCA-1-FITC (# 130-102-229) and Anti-Siglec-H-PE (# 130-102-261). For more information about other fluorochrome conjugates 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

For highest recovery and purity of PDCs from mouse spleen or lymph nodes, prepare a single-cell suspensions using manual methods or the gentleMACS™ Dissociators in combination with the Spleen Dissociation Kit, mouse (# 130-095-926).

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

▲ 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 separation. 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 350 µL of buffer per 10^8 total cells.
4. Add 50 µL of FcR Blocking Reagent, mouse per 10^8 total cells.
5. Add 100 µL of Plasmacytoid Dendritic Cell Biotin Cocktail per 10^8 cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Add 300 µL of buffer per 10^8 cells.
8. Add 200 µL of Anti-Biotin MicroBeads UltraPure per 10^8 cells.
10. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
11. 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 labeled cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

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 the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched plasmacytoid dendritic cell fraction.
4. Wash column with appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched plasmacytoid dendritic cells, and combine with the flow-through from step 3.

   MS: 500 µL LS: 3 mL

   ▲ **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 the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled non-plasmacytoid dendritic cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL

140-004-771.02

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

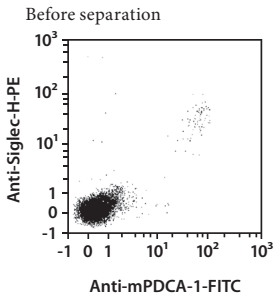
▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

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 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.
   This fraction represents the enriched plasmacytoid dendritic cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-plasmacytoid dendritic cells.

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

PDCs were isolated from a mouse spleen cell suspension by using the Plasmacytoid Dendritic Cell Isolation Kit, a MidiMACS™ Separator, and an LS Column. Cells were fluorescently stained with Anti-mPDCA-1-FITC and Anti-Siglec-H-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.



4. References

1. Shortman, K. and Liu, Y. J. (2002) Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. 2(3): 151–161.
2. Nakano, H. *et al.* (2001) $CD11c^{+}B220^{+}Gr\text{-}1^{+}$ cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. J. Exp. Med. 194(8): 1171–1178.
3. MACS&more (2004) mPDCA-1: A presumably novel antigen exclusively expressed by murine plasmacytoid dendritic cells. Vol. 8-1: 13.
4. Blasius, A. L. *et al.* (2006) Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in naive mouse, but a promiscuous cell surface antigen following IFN stimulation. J. Immunol. 177: 3260–3265.

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