Miltenyi Biotec

Pan DC MicroBeads
mouse

Order no. 130-092-465

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 Pan DC MicroBeads, mouse: MicroBeads conjugated to monoclonal recombinant engineered anti-mouse CD11c (REA754) antibodies (isotype: human IgG1) and Anti-mPDCA-1 antibodies (isotype: rat IgG1).
Capacity	For 2×10^9 nucleated cells.
Product format	Pan DC MicroBeads 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

First, the dendritic cells (DCs) are magnetically labeled with Pan DC MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled DCs are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of DCs. After removing the column from the magnetic field, the magnetically retained DCs can be eluted as the positively selected cell fraction.

1.2 Background information

Pan DC MicroBeads consist of CD11c and Anti-mPDCA-1 MicroBeads. CD11c is expressed at high levels on classical DCs and at low to intermediate levels on plasmacytoid dendritic cells (PDCs). The mouse plasmacytoid dendritic cell antigen-1 (mPDCA-1) is specifically expressed on PDCs with the phenotype $CD11c^{low}$, $CD45R(B220)^+$, and $Ly\text{-}6C^+$ in lymphoid and non-lymphoid organs. Due to the low expression of CD11c on PDCs, using CD11c expression might not lead to enrichment of PDCs to the same extent as classical DCs. Therefore, Pan DC MicroBeads were developed to isolate both, classical and plasmacytoid, DC subsets with highest recovery.

1.3 Applications

- Isolation of total DCs for analysis of their phenotypical and functional properties or studies on T cell activation, polarization, and tolerance induction in different experimental mouse models.
- Isolation of highly pure DCs from lymphoid and non-lymphoid organs.

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

- MACS Columns and MACS Separators: Total DCs can be enriched by using MS, LS, or XS Columns or depleted with the use of LD or D Columns. Cells which strongly express the CD11c and mPDCA-1 antigens can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, SuperMACS II
D	10^9		SuperMACS II

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

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Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

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

- Collagenase D: 2 mg/mL (Collagenase D >0.15 U/mg, e.g. from Roche Diagnostics, Germany) in 10 mM Hepes-NaOH pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$.
- (Optional) Fluorochrome-conjugated CD4, CD8, CD11c, or Anti-mPDCA-1 antibodies for flow cytometric analysis, respectively. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) FcR Blocking Reagent (# 130-092-575) to avoid Fc receptor-mediated fluorescent staining.
- (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 total DCs from mouse spleen, single-cell suspensions have to be prepared by enzymatic disaggregation with Collagenase D. Protocols which rely only on mechanical disruption are not recommended.

▲ 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).

1. Place isolated spleen in a 6 cm petri-dish with sufficient Collagenase D solution to completely cover the bottom of the dish (5 mL/spleen).
2. Inject mouse spleen with 500 µL of Collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue into smaller pieces using sharp scissors.
3. Incubate the spleen pieces in Collagenase D solution for 30 minutes at 37 °C.
4. Pass the whole material, i.e. remaining fragments and Collagenase D-released cells, gently through a 70 µm cell strainer using a plunger.
5. Collect all cells in a 15 mL tube and wash the cells by adding buffer to a final volume of 14 mL.
6. Proceed to magnetic labeling (2.2).

2.2 Magnetic labeling

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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. 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 400 µL of buffer per 10^8 total cells.

   ▲ **Note:** To obtain high purities of DCs (≥ 90 %), Fc receptor-mediated magnetic labeling should be blocked by adding FcR Blocking Reagent (# 130-092-575) or mouse immunoglobulin (1 mg per 500 µL labeling volume) to the cell suspension before adding Pan DC MicroBeads.
4. Add 100 µL of Pan DC MicroBeads per 10^8 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendation.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
9. 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 total DCs. 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.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of DCs, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

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 flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

2.4 Cell separation with the autoMACS® Pro Separator

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

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

2.4.2 Magnetic separation using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose the following program:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes

   Collect negative fraction in row B of the tube rack.

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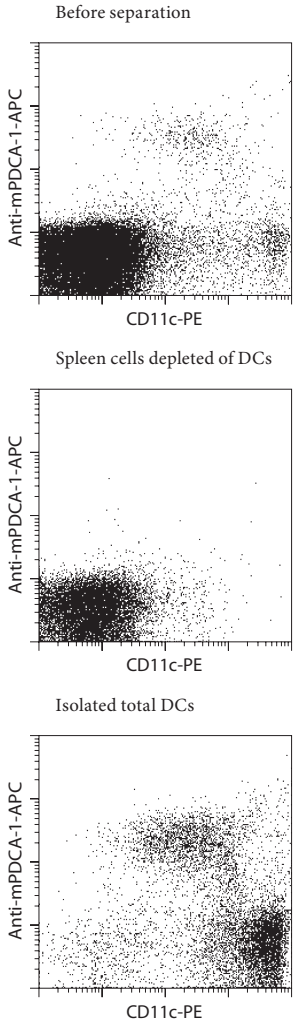
3. Example of a separation using Pan DC MicroBeads

Total DCs were isolated from a mouse spleen cell suspension using Pan DC MicroBeads, a MiniMACS™ Separator, and two MS Columns. The cells are fluorescently stained with CD11c-PE (# 130-091-830) and Anti-mPDCA-1-APC (# 130-091-963). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



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.

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