Miltenyi Biotec

Pan Dendritic Cell Isolation Kit
mouse

Order no. 130-100-875

Contents

1. Description

Components	**2 mL Pan Dendritic Cell Biotin-Antibody Cocktail, mouse:** Cocktail of monoclonal biotin-conjugated antibodies against antigens that are not expressed by dendritic cells. **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **1 mL FcR Blocking Reagent, mouse**
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 Pan Dendritic Cell Isolation Kit, mouse conventional dendritic cells (DCs) and plasmacytoid dendritic cells (PDCs) are isolated by depletion of non-target cells (negative selection). Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and Anti-Biotin MicroBeads, as secondary labeling reagent. 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 DCs run through the column.

1.2 Background information

Dendritic cells (DCs) are the most effective antigen-presenting cells and play a key role in initiating and directing immune responses. In the spleen, there are four major subsets, which can be generally divided into IFN-alpha producing plasmacytoid DC (characterized by the expression of $CD11b^-Ly\text{-}6C^+B220^+mPDCA\text{-}1^+Siglec\text{-}H^+$) and three conventional DC subsets ($CD11b^+CD4^+CD8^-$, $CD11b^+CD4^-CD8^-$, and $CD11b^-CD8^+CD4^-$). In addition, all DCs are commonly characterized by the expression of the DC marker CD11c.

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

- MACS Columns and MACS Separators: Depletion of non-DCs is performed on an LS Column. 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see 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) gentleMACS Dissociator (# 130-093-235) or gentleMACS Octo Dissociator (# 130-095-937) and gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Fluorochrome-conjugated CD4, CD8, CD11b, CD11c, Siglec-H, or mPDCA-1 antibodies for flow cytometric analysis. For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

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- (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 (# 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.

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.
   ▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or by using the Dead Cell Removal Kit (# 130-090-101) is recommended.
6. Proceed to magnetic labeling (2.2).

▲ Alternatively, mouse spleen can be dissociated using the gentleMACS™ Dissociator. For further details refer to Spleen Dissociation Kit, mouse (# 130-095-926) data sheet.

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, # 130-041-407) to remove cell clumps which may clog the column. Wet 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 350 µL of buffer per 10^8 total cells.
4. Add 50 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 µL of Pan Dendritic Cell Biotin-Antibody Cocktail per 10^8 cells. The final labeling volume is 500 µL per 10^8 cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 800 µL of buffer.
9. Add 200 µL of Anti-Biotin MicroBeads 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 see table in section 1.4.

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
   Allow the cells to pass through and collect effluent as fraction with unlabeled cells, representing the enriched dendritic cell fraction.
4. Wash column with 2×3 mL of buffer. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
   Collect entire effluent in the same tube as effluent of step 3. This fraction represents the enriched dendritic cells.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-dendritic cells.

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Magnetic separation with the autoMACS® Separator or the autoMACS® Pro 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.
   ▲ **Note:** If recovery is priority "Deplete" could be used instead.

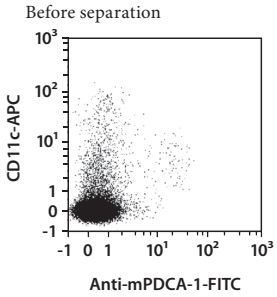
Magnetic separation with the autoMACS® Separator

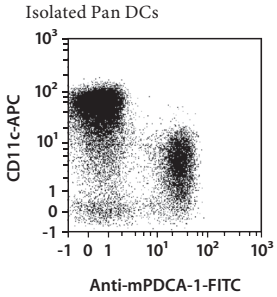
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.
   ▲ **Note:** If recovery is priority "Deplete" could be used instead.

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

Pan DCs were isolated from mouse spleen cell suspension using the Pan Dendritic Cell Isolation Kit, mouse, an LS Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD11c-APC (# 130-091-844) and mPDCA-1-FITC (# 130-091-961) 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.





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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