Miltenyi Biotec

Monocyte Isolation Kit (BM)

mouse

Order no. 130-100-629

Contents

1. Description

Components	**1 mL Monocyte Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal antibodies. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1). **1 mL FcR Blocking Reagent, mouse**
Capacity	For 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 Monocyte Isolation Kit (BM), mouse, monocytes 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 anti-biotin monoclonal antibodies conjugated to 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 monocytes pass through the column.

1.2 Background information

The Monocyte Cell Isolation Kit has been developed for the isolation of monocytes from suspensions of mouse bone marrow. Isolation of highly pure monocytes is achieved by depletion of magnetically labeled non-target cells, i.e., T cells, B cells, NK cells, dendritic cells, erythroid cells, and granulocytes.

1.3 Applications

- Isolation of mouse monocytes from mouse bone marrow for *in vitro* analysis, differentiation studies, co-culture or adoptive transfer experiments.

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: Choose the appropriate MACS Separator and MACS Columns 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
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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD115-PE (# 130-096-308), CD11b-VioBlue (# 130-097-336) or CD3ε-PE (# 130-092-976), CD45R (B220) (# 130-091-828), Anti-NK1.1-PE (# 130-095-867), Anti-Ly-6G-PE (# 130-093-139), CD49b-PE (# 130-091-816, Anti-Ly-6C-FITC (#130-093-134), and Anti-Siglec-F-APC (# 130-098-452). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (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.

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2. Protocol

2.1 Sample preparation

When working with tissues, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

For details see the protocols section at www.miltenyibiotec.com/protocols.

Preparation of bone marrow cells

▲ All steps should be performed on ice.

1. Collect murine bone marrow cells from femur (and tibias) by flushing the shaft with buffer using a syringe and a 26G needle.
2. Disaggregate cells by gently pipetting them several times.
3. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.
4. Wash cells by adding buffer, centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
5. Resuspend cell pellet in buffer and take an aliquot for cell counting.

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 5×10^7 total cells. When working with fewer than 5×10^7 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 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, # 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 175 µL of buffer per 5×10^7 total cells.
4. Add 25 µL of FcR Blocking Reagent. Mix well.
5. Add 50 µL of Monocyte Biotin-Antibody Cocktail per 5×10^7 total cells.
6. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 10 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 400 µL of buffer per 5×10^7 cells
9. Add 100 µL of Anti-Biotin MicroBeads per 5×10^7 total 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 magnetically labeled non-target cells. For details see table in section 1.4.

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

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched monocytes.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched monocytes, and combine with the effluent from step 3.

   ▲ **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 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-monocytes by firmly pushing the plunger into the column.

Magnetic separation 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. This fraction represents the enriched monocytes.w
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-monocytes.

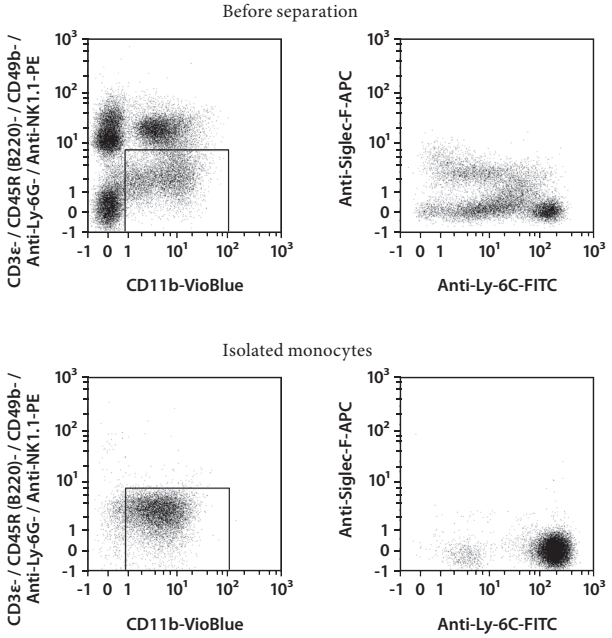
Magnetic separation with the autoMACS® Separator

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. This fraction represents the enriched monocytes.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-monocytes.

3. Example of a separation using the Monocyte Isolation Kit (BM)

Monocytes were isolated from C57BL/6 bone marrow cells by using the Monocyte Isolation Kit (BM), mouse, an LS Column and a QuadroMACS Separator. The cells were fluorescently stained with CD11b-VioBlue, Lin cocktail (CD3ε-PE, CD45R (B220)-PE, Anti-NK1.1-PE, Anti-Ly-6G-PE, CD49b-PE), Anti-Ly-6C-FITC and Anti-Siglec-F-APC and analyzed by flow cytometry using the MACSQuant Analyser. 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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