Miltenyi Biotec

Lineage Cell Depletion Kit
mouse

Order No. 130-090-858

Index

1. Description

Components	**1 mL Biotin-Antibody Cocktail:** Cocktail of biotin-conjugated monoclonal antibodies CD5, CD45R (B220), CD11b, Anti-Gr-1 (Ly-6G/C), 7-4 and Ter-119. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal Anti-Biotin antibody (clone: Bio3-18E7.2; mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	The Biotin-Antibody Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

Using the Lineage Cell Depletion Kit, lineage negative cells are isolated by depletion of cells expressing a panel of so-called "lineage" antigens (negative selection). Lineage$^+$ cells are indirectly magnetically labeled using a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between both labeling steps no washing steps are required. The magnetically labeled lineage cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled lineage negative cells pass through the column.

1.2 Background and product applications

The Lineage Cell Depletion Kit is a magnetic labeling system for the depletion of mature hematopoietic cells, such as T cells, B cells, monocytes/macrophages, granulocytes and erythrocytes and their committed precursors from bone marrow. For depletion, cells are magnetically labeled with a cocktail of biotinylated antibodies against a panel of so-called "lineage" antigens (CD5, CD45R (B220), CD11b, Anti-Gr-1 (Ly-6G/C), 7-4, and Ter-119 antibodies) and Anti-Biotin MicroBeads.
This labeling procedure leaves lineage negative cells untouched, thus allowing further separation of lineage$^-$ cells according to expression of markers such as CD117 or Sca-1.[1–3]

Examples of applications

- Isolation of lineage negative cells from murine bone marrow, e.g. for a subsequent magnetic enrichment of CD117$^+$ cells from lineage$^-$ bone marrow cells.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) 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 (4–8 °C).

  ▲ **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 calf serum. 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 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g. Anti-Biotin-APC (# 130-090-856), CD117-PE (# 130-091-730) or CD117-APC (# 130-091-729).
- (Optional) PI (propidium iodide) or 7-AAD for the flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 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 by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** 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).

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 gentle pipetting them several times.
3. 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.
4. Wash cells by adding buffer, centrifuge at 300×g for 10 minutes at 4–8 °C. Pipette off supernatant completely.
5. Resuspend cell pellet in buffer and take an aliquot for cell counting.

2.2 Magnetic labeling

▲ Work fast, keep cells cold, 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 10^7 total cells. When working with fewer than 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 2×10^7 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.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 minutes at 4–8 °C.
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
8. Mix well and incubate for additional 15 minutes at 4–8 °C.
9. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells.

▲**Note:** When working with bone marrow of normal mice, the number of labeled cells is almost equal to the number of total cells. Be careful and do not exceed the column capacity for labeled cells.

Magnetic separation with MS and LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.

   Allow the cells to pass through and collect effluent as fraction with unlabeled cells, representing the enriched lineage negative cell fraction.
4. Wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL

   Collect the effluent in the same tube as effluent of step 3. This fraction represents the enriched lineage negative cells.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled lineage positive cells.

Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing magnetically labeled cells in the autoMACS Separator. Choose program "Deplete".
3. Collect negative fraction (outlet port "neg1"). This fraction represents the enriched lineage negative cells.
4. (Optional) Collect positive fraction (outlet port "pos1"). This fraction represents the magnetically labeled lineage positive cells.

2.4 (Optional) Evaluation of lineage negative cell purity

The purity of the enriched lineage negative cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with Anti-Biotin-APC (# 130-090-856).

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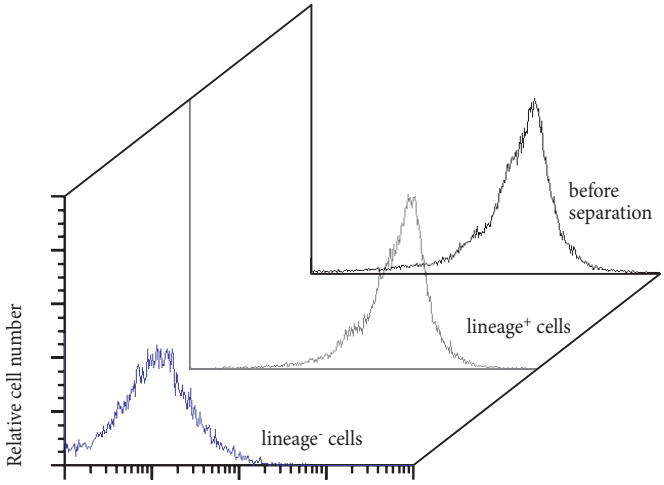
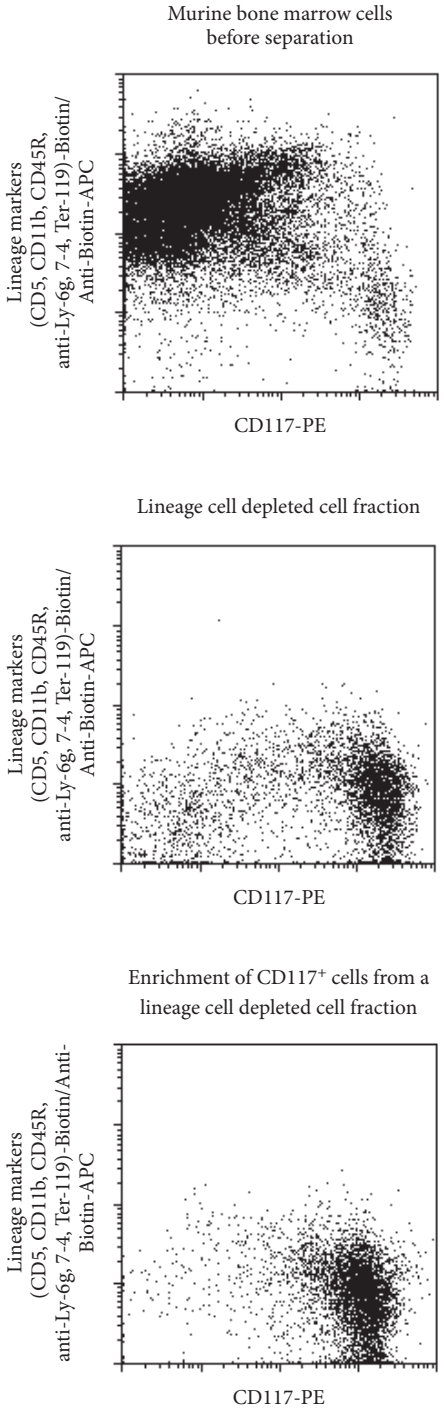
3. Examples of separations using the Lineage Cell Depletion Kit

A. Isolation of untouched lineage negative cells from a mouse bone marrow cell suspension using the Lineage Cell Depletion Kit and a MidiMACS™ Separator with an LS Column. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



B. Isolation of lineage$^-$ / CD117$^+$ cells from a mouse bone marrow cell suspension using the Lineage Cell Depletion Kit, CD117 MicroBeads and a MidiMACS™ Separator with LS Columns. The cells are fluorescently stained with CD117-PE and a panel of biotinylated antibodies against lineage markers and Anti-Biotin-APC. Cell debris and dead cells were excluded from the analysis.



4. References

1. Orlic (2002) Int J Hematol 76 Suppl. 1: 144–145.
2. Orlic *et al.* (2001) PNAS 98: 10344–10349.
3. Lagasse *et al.* (2000) Nat. Med. 6: 1229–1234.

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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