



CD117 MicroBeads

mouse

Order no. 130-091-224

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1. Description

Components	2 mL CD117 MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD117 antibodies (isotype: rat IgG2b)
Size	For 10 ⁹ total cells, up to 100 separations.
Product format	CD117 MicroBeads are supplied as a suspension 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 MACS® Separation

First, the CD117⁺ cells are magnetically labeled with CD117 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 CD117⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD117⁺ cells. After removing the column from the magnetic field, the magnetically retained CD117⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD117 MicroBeads are developed for the isolation of murine progenitor cells. CD117, also known as c-kit, steel factor receptor and stem cell factor receptor, encodes a 145 kD cell surface glycoprotein belonging to the class III receptor tyrosine kinase family. It is expressed on the majority of hematopoietic progenitor cells, including multipotent hematopoietic stem cells as well as committed myeloid, erythroid and lymphoid precursor cells. In addition to the hematopoietic cell differentiation potential, CD117⁺ stem cells from murine bone marrow were reported to be capable of differentiation into smooth muscle cells, myocytes, and endothelial cells *in vivo*.^{1,2} CD117 is also expressed on a few mature hematopoietic cells, for example, mast cells.

Example applications

- Positive selection or depletion of cells expressing the mouse CD117 antigen.
- Isolation of CD117 positive cells from murine bone marrow after depletion of so-called lineage-positive cells using the Lineage Cell Depletion Kit, mouse (# 130-090-858).

1.3 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 (4–8 °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 human serum albumin, human serum, or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- **MACS Columns and MACS Separators:** CD117⁺ cells can be enriched by using MS, LS, or XS Columns (positive selection). Positive selection can also be performed by using the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
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™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.
- (Optional) CD117-APC (# 130-091-729) or CD117-PE (# 130-091-730), Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613), and Anti-Biotin-APC (# 130-090-856) or Anti-Biotin-PE (# 130-090-756) for flow cytometric analysis.
- (Optional) Lineage Cell Depletion Kit, mouse (# 130-090-858).
- (Optional) Propidium iodide (PI) 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. For details see section 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 Filter, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.
4. Wash cells by adding buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Aspirate supernatant.
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 10⁷ total cells. When working with less than 10⁷ 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 2×10⁷ 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.

1. Determine cell number.
2. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10⁷ total cells.
4. Add 20 µL of CD117 MicroBeads per 10⁷ total cells.
5. Mix well and refrigerate for 15 minutes at 4–8 °C.

▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10⁸ cells in 500 µL of buffer.

▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. 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 CD117⁺ cells. For details see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
MS: 3×500 µL LS: 3×3 mL
Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an 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

▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells over a new, freshly prepared column.

Magnetic separation with XS Columns

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

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 the magnetically labeled cells in the autoMACS Separator. For a standard positive selection, choose program “Possel”.
3. Collect positive fraction from outlet port pos1. This is the purified CD117⁺ cell fraction.

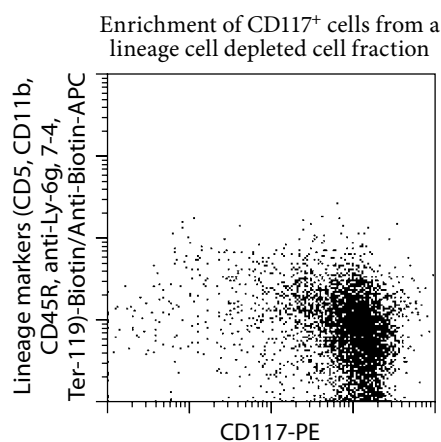
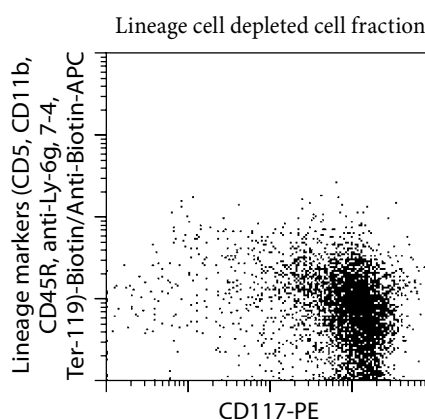
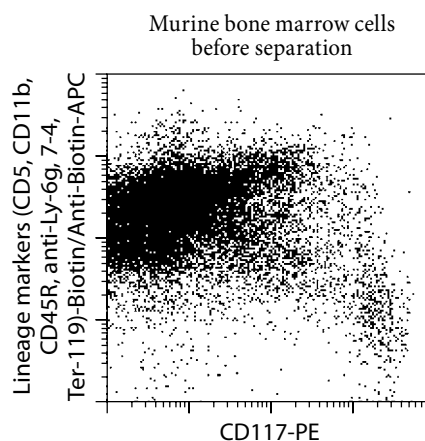
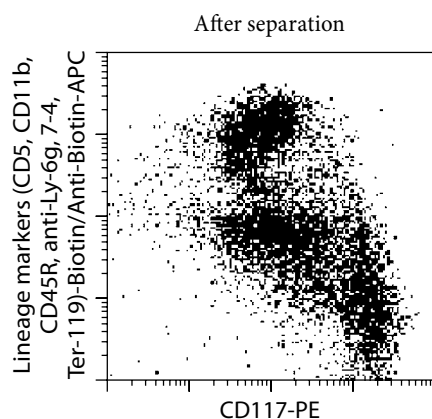
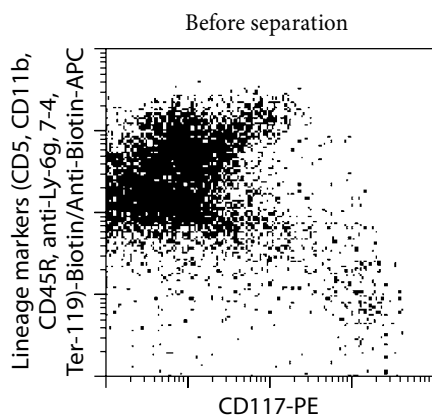
2.4 (Optional) Evaluation of CD117⁺ cell purity

The purity of the CD117⁺ cells can be evaluated by flow cytometry or fluorescence microscopy. Stain cells with fluorochrome conjugated CD117 antibody (e.g. CD117-PE, mouse # 130-091-730, CD117-APC, mouse # 130-091-729), Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613) and Anti-Biotin-APC (# 130-090-856) or Anti-Biotin-PE (# 130-090-756).

3. Examples of a separations using CD117 MicroBeads

A. Separation of bone marrow using CD117 MicroBeads and a MiniMACS™ with an MS Column. The cells are fluorescently stained with CD117-PE (# 130-091-730) and a panel of biotinylated antibodies against lineage markers and Anti-Biotin-APC. 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 MidiMACS™ with LS Columns. The cells are fluorescently stained with CD117-PE and by Anti-Biotin-APC against the panel of biotinylated antibodies used for the Lineage Cell Depletion Kit, mouse (# 130-090-858). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Orlic, D. (2002) Stem cell repair in ischemic heart disease: an experimental model. *Int. J. Hematol.* 76 Suppl. 1: 144–145.
2. Orlic *et al.* (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *PNAS* 98: 10344–10349.

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.

Warranty

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