Miltenyi Biotec

CD117 MicroBeads
mouse – lyophilized

Order no. 130-097-146

Contents

1. Description

Components	**1 vial CD117 MicroBeads, mouse – lyophilized**: MicroBeads conjugated to monoclonal anti-mouse CD117 antibodies (isotype: rat IgG2b). **2 mL Reconstitution Buffer**
Capacity	For 10^9 total cells.
Product format	Lyophilized MicroBeads. Reconstitution Buffer contains 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. For information about reconstitution of the lyophilized MicroBeads and storage after reconstitution refer to chapter 2.1.

1.1 Principle of the 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 information

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.

1.3 Applications

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

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: CD117$^+$ cells can be enriched by using MS, LS, or XS Columns. Positive selection 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
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	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™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD117-APC or CD117-PE, Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613), and Biotin Antibody-APC or Biotin Antibody-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

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- (Optional) Lineage Cell Depletion Kit, mouse (# 130-090-858).
- (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.

2. Protocol

2.1 Reconstitution of MicroBeads

Reconstitute the lyophilized MicroBeads by adding all Reconstitution Buffer to the vial. Mix by pipetting up and down until resuspended. After reconstitution the MicroBeads are stable for 9 months at 2–8 °C. Write the new expiration date after reconstitution on the vial label.

2.2 Sample preparation

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

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ 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).

2.3 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 10^7 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 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 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD117 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of CD117-APC, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
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).

2.4 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 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 only when 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 CD117$^+$ cells, 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.

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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:

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

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:

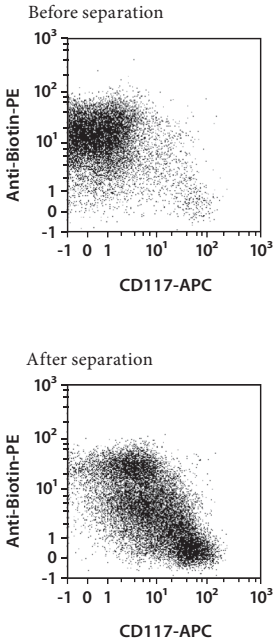
   Positive selection: Possel
   Collect positive fraction from outlet port pos1.

2.5 (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, CD117-APC), Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613) and Biotin Antibody-APC or Biotin Antibody-PE.

3. Example of a separation using CD117 MicroBeads

CD117 MicroBeads were reconstituted as described in 2.1. CD117$^+$ cells were isolated from mouse bone marrow using CD117 MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD117-APC, Lineage Cell Detection Cocktail-Biotin, and Anti-Biotin-PE 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.



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, D. *et al.* (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. PNAS 98: 10344–10349.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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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