CD117 MicroBead Kit
human

Contents

1. Description

Components	**2 mL CD117 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD117 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent:** human IgG
Capacity	For 2×10^9 total cells, up to 20 separations.
Product format	All reagents 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

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

The CD117 MicroBead Kit is developed for the separation of human cells based on the expression of the CD117 antigen. CD117 (also known as *c-kit,* steel factor receptor or SCF receptor) is a 145 kDa cell surface glycoprotein with tyrosine kinase activity. This molecule is suggested to be involved in signaling, activation and proliferation of cells. The CD117 antigen is expressed on about 1-3% of mononuclear cells from peripheral blood (PBMCs) and cord blood, and on up to 10% of bone marrow cells. Around 25% of CD117^{+} cells were found to express CD133 and CD34. CD117 is further expressed on basophils, myeloid dendritic cells, TCRαβ^{+} T cells, CD19^{+} pro–B cells, and CD56^{+} NK cells[1], as well as on mast cells, melanocytes, and AML (acute myeloid leukemia) blasts[2]. The CD117-specific monoclonal antibody (mAb) conjugated to the MicroBeads does not interfere with stem cell factor (SCF)-binding.

1.3 Applications

- Positive selection of cells expressing human CD117 antigen.
- Isolation of hematopoietic stem and progenitor cells and marrow stromal cells (MSCs) from human bone marrow.
- Isolation of stem cells from amniotic fluid.[3]
- Isolation of mast cells.[4]

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 human serum albumin, human 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
Positive selection			
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 CD117 antibodies for flow-cytometric or fluorescence microscopic control of CD117^{+} stem and progenitor cell purification, e.g. CD117 (A3C6E2)-PE (# 130-091-734) or CD117 (A3C6E3)-APC (# 130-091-733). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

- (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 Sample preparation

Preparation of cells from leukapheresis material

1. Pass cells through 30 μm nylon mesh or Pre-Separation Filters (# 130-041-407) in order to remove clumps. Wash cells once with buffer and resuspend cell pellet in a final volume of 300 μL of buffer per 10^8 cells. For fewer than 10^8 total cells use 300 μL. Proceed to magnetic labeling.

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

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).

2.2 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 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. 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 300 μL of buffer per 10^8 total cells.
4. Add 100 μL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 μL of CD117 MicroBeads per 10^8 total cells.

6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. (Optional) Add staining antibodies, e.g., 10 μL of CD117 (A3C6E2)-PE (# 130-091-734), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** CD117 antibodies recognizing a CD117-epitope similar to clone AC126, e.g., 104D2, are not recommended for use.

8. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. 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.

10. 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 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.

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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® 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 one of the following programs:

   For positive selection of CD117⁺ cells from bone marrow or chord blood choose:
   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

   For positive selection of CD117⁺ cells from PBMCs choose:
   Positive selection: Posseld2
   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].

3. For a standard separation choose one of the following programs:

   For positive selection of CD117⁺ cells from bone marrow or chord blood choose:
   Positive selection: Possel
   Collect positive fraction from outlet port pos1.

   For positive selection of CD117⁺ cells from PBMCs choose:
   Positive selection: Posseld2
   Collect positive fraction from outlet port pos2.

3. Example of a separation using the CD117 MicroBead Kit

Separation of CD117⁺ cells from bone marrow using CD117 MicroBeads and a MiniMACS™ Separator with an MS Column. The cells are fluorescently stained with CD117 (A3C6E2)-PE (# 130-091-734) and CD133/2 (293C3)-APC (#130-090-854). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.
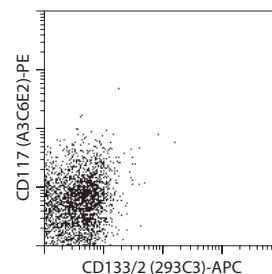

Before separation
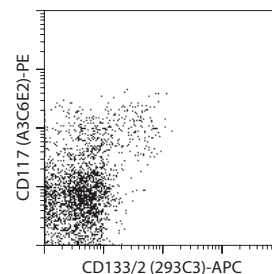
CD117⁺ cells
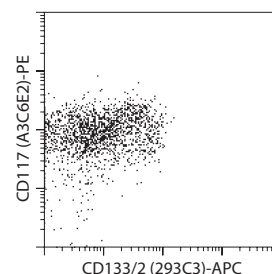
CD117⁻ cells

4. References

1. Guth, S. *et al.* (1995) Immunomagnetic isolation and surface phenotypiing of human c-kit receptor-expressing cells from peripheral blood. (Abstract) 9th International Congress of Immunology.

2. Buhring, H. J. *et al.* (1991) The product of the proto-oncogene c-kit (P145c-kit) is a human bone marrow surface antigen of hematopoietic precursor cells which is expressed on a subset of acute non-lymphoblastic leukemic cells. Leukemia 5: 854–860.

3. De Coppi, P. *et al.* (2007) Isolation of amniotic stem cell lines with potential for therapy. Nat. Biotechnol. 25(1): 100–106.

4. Gebhardt, T. *et al.* (2005) Growth, phenotype, and function of human intestinal mast cells are tightly regulated by transforming growth factor beta1. Gut 54(7): 928–934.

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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