Direct CD34 Progenitor Cell Isolation Kit

2 ml CD34 MicroBeads
2 ml FcR Blocking Reagent
For 2 x 10^9 total cells

Order No. 130-046-702

Cell Isolation Kits

Overview

The Direct CD34 Progenitor Cell Isolation Kit contains MicroBeads directly conjugated to CD34 antibodies for magnetic labeling of CD34 expressing hematopoietic progenitor cells from peripheral blood, cord blood, bone marrow or apheresis harvest. Hematopoietic progenitor cells, present at a frequency of about 0.05–0.2 % in peripheral blood, 0.1–0.5 % in cord blood and 0.5–3 % in bone marrow, can be rapidly and efficiently enriched to a purity of about 85–98 %.

Research Applications of CD34 Progenitor Cells

- Characterization of hematopoietic progenitor cells and their developmental pathways.
- Studies on stimulation of proliferation and maturation by cytokines.

Isolation Strategy

The isolation of hematopoietic progenitor cells is performed by positive selection of CD34 expressing cells. Mononuclear cells from peripheral blood (PBMC), cord blood, bone marrow or apheresis harvest are obtained by density gradient centrifugation over Ficoll Paque®. Apheresis harvest can also be used as sample without density gradient centrifugation. For MACS separation, $CD34^+$ hematopoietic progenitor cells are magnetically labeled using MACS CD34 MicroBeads. The magnetically labeled cells are enriched on positive selection columns in the magnetic field of the MiniMACS, MidiMACS, VarioMACS or SuperMACS.

Components

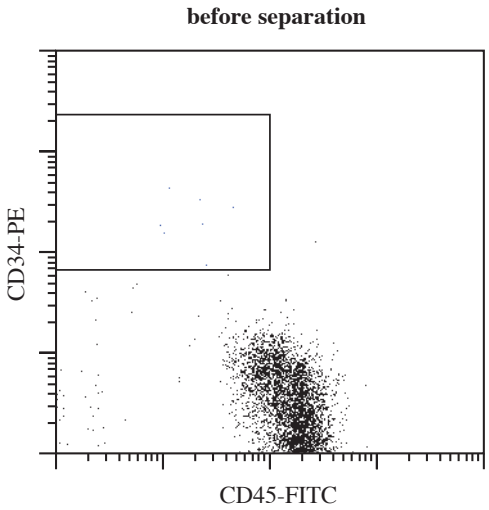
- 2 ml MACS CD34 MicroBeads: Colloidal super-paramagnetic MicroBeads conjugated to monoclonal mouse anti-human CD34 antibody (isotype: mouse IgG1, clone: QBEND/10).
- 2 ml FcR Blocking Reagent. Human IgG.

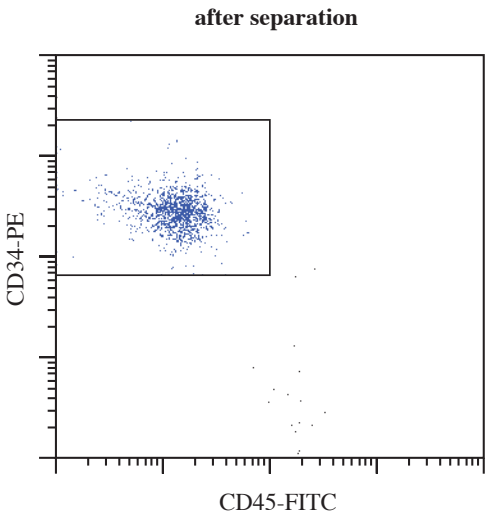
Storage

Store protected from light at 4°C. Do not freeze.

Flow Cytometric Data from Typical CD34 Progenitor Cell Isolations

Isolation of $CD34^+$ cells using Direct CD34 Progenitor Cell Isolation Kit and MiniMACS. Cells were stained with CD34-PE (Order No. 130-081-002) and CD45-FITC (Order No. 130-080-202).





Additional Reagents Required

- Ficoll-Paque® and phosphate buffered saline (PBS) supplemented with 2 mM EDTA or 0.6 % anticoagulants citrate dextrose-formula A (6 % ACD-A: 22.3 g/l glucose, 22 g/l sodium citrate and 8 g/l citric acid in H_2O; see "Important Notes").
- Buffer: PBS pH 7.2, supplemented with 0.5 % bovine serum albumin and 2 mM EDTA or 0.6 % ACD-A. Degas buffer by applying vacuum.

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- (Optional) Fluorochrome conjugated CD34 antibody (e.g. CD34-PE, Order No. 130-081-002) and fluorochrome conjugated CD45 antibody (e.g. CD45-FITC, Order No. 130-080-202) for control of CD34 progenitor cell isolation.

Equipment Required

- Magnetic cell separator MiniMACS, MidiMACS, VarioMACS or SuperMACS.
- MACS column(s) type MS$^+$/RS$^+$, LS$^+$/VS$^+$ or XS$^+$ (plus RS$^+$, VS$^+$ or XS$^+$ column adapter).
- Pre-Separation Filters (Order No. 130-041-407).

Preparation of Peripheral Blood Mononuclear Cells

- Start with fresh human blood treated with an anticoagulant, e.g. heparin, citrate, ACD-A or citrate phosphate dextrose (CPD) or leukocyte-rich buffy coat not older than 8 hours.
- Dilute cells with 2–4 volumes of PBS containing 2 mM EDTA or 0.6 % ACD-A.
- Carefully layer 35 ml of diluted cell suspension over 15 ml Ficoll Paque® (1.077 density) in a 50 ml conical tube and centrifuge at 400xg for 30–40 minutes at 20°C in a swinging-bucket rotor without brake.
- Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
- Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube.
- Fill the conical tube with PBS containing 2 mM EDTA or 0.6 % ACD-A, mix and centrifuge at 300xg for 10 minutes at 20°C. Carefully remove the supernatant completely.
- Resuspend the cell pellet in 50 ml of PBS containing 2 mM EDTA or 0.6 % ACD-A and centrifuge at 200xg for 10–15 minutes at 20°C. Carefully remove the supernatant completely.
- Resuspend the cell pellet in 50 ml of buffer and centrifuge at 200xg for 10–15 minutes at 20°C. Carefully remove the supernatant completely.
- Resuspend cell pellet in a final volume of 300 µl per 10^8 total cells (PBMC of about 100 ml blood). For less than 10^8 total cells, use 300 µl. Proceed to magnetic labeling.

Notes

- The peripheral blood or buffy coat should not be older than 8 hours and supplemented with anticoagulants.
- PBMC may be stored in refrigerator overnight in PBS containing 0.5 % BSA supplemented with autologous serum after the last washing step.

Preparation of Cord Blood Cells

- Dilute anticoagulated cord blood 1:4 with PBS containing 2 mM EDTA or 0.6 % ACD-A and carefully layer 35 ml of diluted cell suspension over 15 ml of Ficoll-Paque®.
- Centrifuge for 35 minutes at 400xg at 20°C in a swinging-bucket rotor (without brake).
- Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
- Carefully collect interphase cells and wash twice in PBS containing 2 mM EDTA or 0.6 % ACD-A. Centrifuge for 10 minutes at 200xg at 20°C.
- Resuspend cell pellet in a final volume of 300 µl of buffer per 10^8 total cells. For less than 10^8 total cells, use 300 µl. Proceed to magnetic labeling.

Notes

- Do not use cord blood older than 4 hours.
- The cord blood should be drawn directly into a 50 ml tube containing 5 ml of PBS supplemented with 2 mM EDTA or 0.6 % ACD-A or 200 U/ml heparin.
- The cord blood should be stored at 4°C prior to separation.

Preparation of Bone Marrow Cells

- Collect bone marrow in 50 ml tubes containing 5 ml PBS containing 2 mM EDTA or 0.6 % ACD-A or 200 U/ml heparin and store at 4°C if the cells cannot be processed immediately.
- For release of the cells, dilute in 10 x excess of RPMI 1640 containing 0.02 % collagenase B and 100 U/ml DNAse and shake gently at room temperature for 45 minutes.
- Pass cells through 30 µm nylon mesh or filter (Order No.: 130-041-407). Wet filter with buffer before use.
- Carefully layer 35 ml of diluted cell suspension over 15 ml of Ficoll-Paque®.
- Centrifuge for 35 minutes at 400xg at 20°C in a swinging-bucket rotor (without brake).
- Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
- Carefully collect interphase cells and wash twice in PBS containing 2 mM EDTA or 0.6 % ACD-A. Centrifuge for 10 minutes at 300xg at 20°C.
- Resuspend cell pellet in a final volume of 300 µl of buffer per 10^8 total cells. For less than 10^8 total cells, use 300 µl. Proceed to magnetic labeling.

Notes

- If cells cannot be separated on the day of harvest, store cells at 4°C.
- Remove all cell clumps by passing cells through 30 µm nylon mesh or Pre-Separation Filter (Order No. 130-041-407) during the cell preparation. Wet filter with buffer before use.

Preparation of Cells from Leukapheresis Material

- Filter apheresis harvest through 30 µm nylon mesh, or Pre-Separation Filter (Order No. 130-041-407 or e.g. “Cell Strainers” from Becton Dickinson), in order to remove clumps, wash cells once with buffer and resuspend in a final volume of 300 µl of buffer per 10^8 cells. For less than 10^8 total cells, use 300 µl. Proceed to magnetic labeling.

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Magnetic Labeling of CD34+ Progenitor Cells

- Add 100 µl FcR Blocking Reagent per 10^8 total cells to the cell suspension to inhibit unspecific or Fc-receptor mediated binding of CD34 MicroBeads to non-target cells.
- Label cells by adding 100 µl CD34 MicroBeads per 10^8 **total** cells, mix well and incubate for 30 minutes in the refrigerator at 6°–12°C.
- (Optional) Add fluorochrome conjugated CD34 antibody recognizing another epitope than QBEND/10 (e.g. CD34-PE, Clone: AC136, Order No. 130-081-002) and fluorochrome conjugated CD45 antibody (e.g. CD45-FITC, Order No. 130-080-202) at the titer recommended by manufacturer and incubate for further 10 minutes in the refrigerator at 6°–12°C.
- Wash cells carefully and resuspend in appropriate amount of buffer (MS+/RS+ column: 500–1000 µl; LS+/VS+ column: 1–10 ml, max. 2×10^8 cells per ml).
- Proceed to magnetic separation.

Magnetic Separation of $< 2 \times 10^9$ Mononuclear Cells

- Choose a column type (MS+/RS+ or LS+/VS+) according to the number of total unseparated cells and place it (with column adapter) in the magnetic field of the MACS separator. Fill and rinse with buffer (MS+/RS+: 500 µl; LS+/VS+: 3 ml; for details, see "Column and Adapter Data Sheets").
- Pass cells through 30 µm nylon mesh or Pre-Separation Filter (Order No. 130-041-407) to remove clumps. Wet filter with buffer before use.
- Apply cells to the column, allow cells to pass through the column and wash with buffer (MS+/RS+: 3 x 500 µl; LS+/VS+: 3 x 3 ml).
- Remove column from separator, place column on a suitable tube and pipette buffer on top of column (MS+/RS+: 1 ml; LS+/VS+: 5 ml). Firmly flush out retained cells with pressure using the plunger supplied with the column.
- Repeat magnetic separation step: apply the eluted cells to a new prefilled positive selection column (for $< 10^7$ CD34+ cells: MS+/RS+; for $< 10^8$ CD34+ cells: LS+/VS+), wash, and elute retained cells in buffer (MS+/RS+: 500 µl; LS+/VS+: 2.5 ml).

Magnetic Separation of 2×10^9–2×10^{10} Mononuclear Cells

- Assemble XS+ column and place it in the column holder of the SuperMACS using XS+ column adapter (for details, see "XS+ Selection Column and SuperMACS Data Sheets").
- Turn 3-way-stopcock to position "fill".
- Fill the column from the bottom with buffer from the syringe until the buffer reaches the syringe cylinder.
- Turn the 3-way-stopcock to position "run" and rinse column by filling from the top with buffer. Allow buffer to run into the column. Then, add more buffer. Rinse with 50 ml of buffer.
- Close 3-way-stopcock; leave the syringe attached during separation, except when refilling with buffer.
- Move column in the magnetic field of the SuperMACS by turning the handle.
- Pass cells through 30 µm nylon mesh or filter to remove cell clumps.
- Apply cells into the syringe cylinder that is set up on the XS+ column and turn 3-way-stopcock to position "run". Allow the cells to pass through the column.
- Remove flow resistor and wash with 4 x 30 ml buffer.
- Close 3-way-stopcock and remove column out of the magnetic field of the SuperMACS by turning the XS+ adapter handle backward (see "Super MACS Starting Kit Data Sheet").
- Detach syringe from the 3-way-stopcock, fill with buffer and attach to port A of the XS+ column.
- Elute retained cells with 20 ml buffer using the syringe.
- Repeat magnetic separation step: apply the eluted cells to a new prefilled XS+ column or VS+ column, wash, and elute retained cells in buffer.

Evaluation of Hematopoietic Progenitor Cell Purity (optional)

The purity of the isolated hematopoietic progenitor cells can be evaluated by flow cytometry or fluorescence microscopy. Fluorescent staining of CD34+ cells can be accomplished by direct immunofluorescent staining using an antibody recognizing an epitope different from that recognized by the CD34 monoclonal antibody QBEND/10 (e.g. CD34-PE, Clone: AC136, Order No. 130-081-002).
For optimal discrimination of CD34+ cells from other leukocytes, counterstain cells with an antibody against CD45 (e.g. CD45-FITC, Order No. 130-080-202). CD34+ cells express CD45 at a lower level as compared to lymphocytes.
Use the antibodies in appropriate concentrations recommended by the manufacturers. Typically, staining for 5 minutes at 6°–12°C should be sufficient. After fluorescence staining, cells should be washed and resuspended in buffer.

Important Notes

- ▲ Avoid capping of antibodies on the cell surface during labeling by working fast, and keeping cells cold. Use cold solutions only. **Attention:** Working on ice requires increased incubation times.
- ▲ Increased temperature and prolonged incubation time for labeling may lead to unspecific cell labeling.
- ▲ If progenitor cells are taken into culture, EDTA in the buffer may have a slightly negative effect on cell proliferation. EDTA can be replaced by other supplements such as 0.6 % ACD-A or citrate phosphate dextrose (CPD).
- ▲ Use degassed buffer only! Excess of gas in buffer will form bubbles in the matrix of the column during separation. This may lead to clogging of the column and decreases the quality of separation.
- ▲ Contamination of the cell preparation with excessive number of thrombocytes can result in low purities and can also cause cell clumping which may clog the column. Additional washes after density gradient centrifugation over Ficoll Paque® at 200 x g for 10 minutes will reduce the number of thrombocytes in the cell preparation.

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References

1. Kögler, G; Callejas, J; Sorg, RV; Fischer, J; Migliaccio, A; Wernet, P (1998) The effect of different thawing methods, growth factor combinations and media on ex vivo expansion of umbilical cord blood primitive and committed progenitors.Bone Marrow Transplant. 21: 233-241. [430]

2. de Wynter, EA; Buck, D; Hart, C; Heywood, R; Coutinho, LH; Cyalton, A; Rafferty, JA; Burt, D; Guenechea, G; Bueren, JA; Gagaen, D; Fairbairn, LJ; Lord, BI; Testa, NG (1998) $CD34^+AC133^+$ Cells Isolated from Cord Blood are Highly Enriched in Long-Term Culture-Initiating Cells, NOD/SCID-Repopulating Cells and Dendritic Cell Progenitors. Stem Cells 16: 387-396. [503]

Warning

Reagents contain sodium azide. Sodium azide yields hydrazoic acid under acid conditions, which is extremely toxic. Azide compounds should be diluted with running water before discarded. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. MILTENYI BIOTEC GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the Technical Specifications of the products. MILTENYI BIOTEC GmbH´s liability is limited to either replacement of the products or refund of the purchase price. MILTENYI BIOTEC GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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