Isolation of CD34$^+$ HLA-DR$^-$ cells

CD34$^+$HLA-DR$^-$ cells from peripheral blood, cord blood or bone marrow can be isolated with the CD34 MultiSort Kit. CD34$^+$ hematopoietic progenitor cells are magnetically labeled using CD34 MultiSort MicroBeads. The magnetically labeled cells are enriched on MACS® Columns in the magnetic field of the MACS Separator. Then, the CD34$^+$ cells are incubated with the MACS MultiSort Release Reagent which enzymatically removes the magnetic particle from the CD34 antibody. In the next step the CD34$^+$ cells are magnetically labeled with HLA-DR MicroBeads. The CD34$^+$HLA-DR$^+$ cells are depleted on MACS® Columns in the magnetic field of the MACS Separator. The non-magnetic fraction contains the CD34$^+$HLA-DR$^-$ cells.
The following protocol includes fluorescent labeling of the cells for subsequent flow cytometric analysis.

Instrument and reagent requirements

- MiniMACS™ Separator, VarioMACS™ Separator or SuperMACS™ Separator; MS Columns; MS Column Adapter in combination with VarioMACS Separator or SuperMACS Separator; Column Adapter for MS, LS and LD Columns in combination with SuperMACS™ II Separator.
- CD34 MultiSort Kit (# 130-056-701) containing:
  MACS MultiSort MicroBeads conjugated to monoclonal anti-human CD34 antibody. Isotype: mouse IgG1. Clone: QBEND/10.
  MultiSort Release Reagent for enzymatic release of MultiSort MicroBeads bound to the cell surface.
  MultiSort Stop Reagent to inhibit the release reaction for further separations.
- HLA-DR MicroBeads (# 130-046-101).
- Buffer: phosphate buffered saline (PBS) supplemented with 0.5% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.2.
- Fluorochrome conjugated antibodies: e.g. MACS CD34-PE (# 130-081-002) and HLA-DR-FITC.

Magnetic labeling of 2×10^8 total cells in suspension

- Isolate mononuclear cells from anti-coagulated peripheral blood, bone marrow or cord blood. To remove clumps, pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407). Dead cells can be removed by density gradient centrifugation (e.g. Ficoll-Paque™).
- Resuspend 2×10^8 cells with buffer in a final volume of 600 µL.
- Add 200 µL FcR Blocking Reagent to the cell suspension to inhibit unspecific or Fc-receptor binding of CD34 MultiSort MicroBeads to non-target cells.
- Label cells by adding 200 µL CD34 MultiSort MicroBeads, mix well and incubate for 30 minutes at 4–8 °C.
- Add HLA-DR-FITC according to manufacturer´s recommendation and 100 µL CD34-PE. Incubate for 10 minutes in the dark at 4–8 °C.
- Wash cells by adding 1 mL of buffer, centrifuge at 300×g for 10 minutes, remove supernatant completely, resuspend in 500 µL of buffer. Remove a small sample for flow cytometric analysis.

Magnetic separation

- Place an MS Column (combined with the appropriate Column Adapter) in the magnetic field of the MACS Separator.
- Prepare the column by washing with 500 µL of buffer.
- Apply cell suspension on top of the column. Let the unlabeled cells pass through. Rinse with 3×500 µL of buffer.
- Remove column from separator, place column on a suitable collection tube, pipette 1 mL of buffer onto the column and flush out magnetically labeled fraction using the plunger supplied with the column.
- To achieve a higher purity, apply magnetically labeled fraction onto a new, freshly prepared column. Let the unlabeled cells pass through. Rinse with 3×500 µL of buffer.
- Elute magnetically labeled fraction as described above.
- With magnetically labeled fraction subsequently proceed to "Removal of MACS MultiSort MicroBeads".

Removal of MACS® MultiSort MicroBeads using MACS MultiSort Release Reagent

- Incubate magnetically labeled cells with 20 µL MACS® MultiSort Release Reagent per mL of cell suspension for 10 minutes at 4–8 °C.
- Separate cells over a new MS Column to remove any remaining magnetically labeled cells. Prepare the column by washing with 500 µL of buffer.
- Apply cell suspension on top of the column. Let the unlabeled cells pass through. Rinse with 3×500 µL of buffer. Collect effluent. Remove a small sample for flow cytometric analysis.
- Wash cells from released fraction, remove supernatant completely and resuspend cell pellet in buffer in a final volume of 40 µL.
- Add 60 µL MACS MultiSort Stop Reagent per 40 µL cell suspension and mix well.
- Add 100 µL HLA-DR MicroBeads and incubate for 15 minutes at 4–8 °C.
- Cells can now be separated according to HLA-DR expression using an MS Column.

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This MACS® product is for in vitro research use only
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