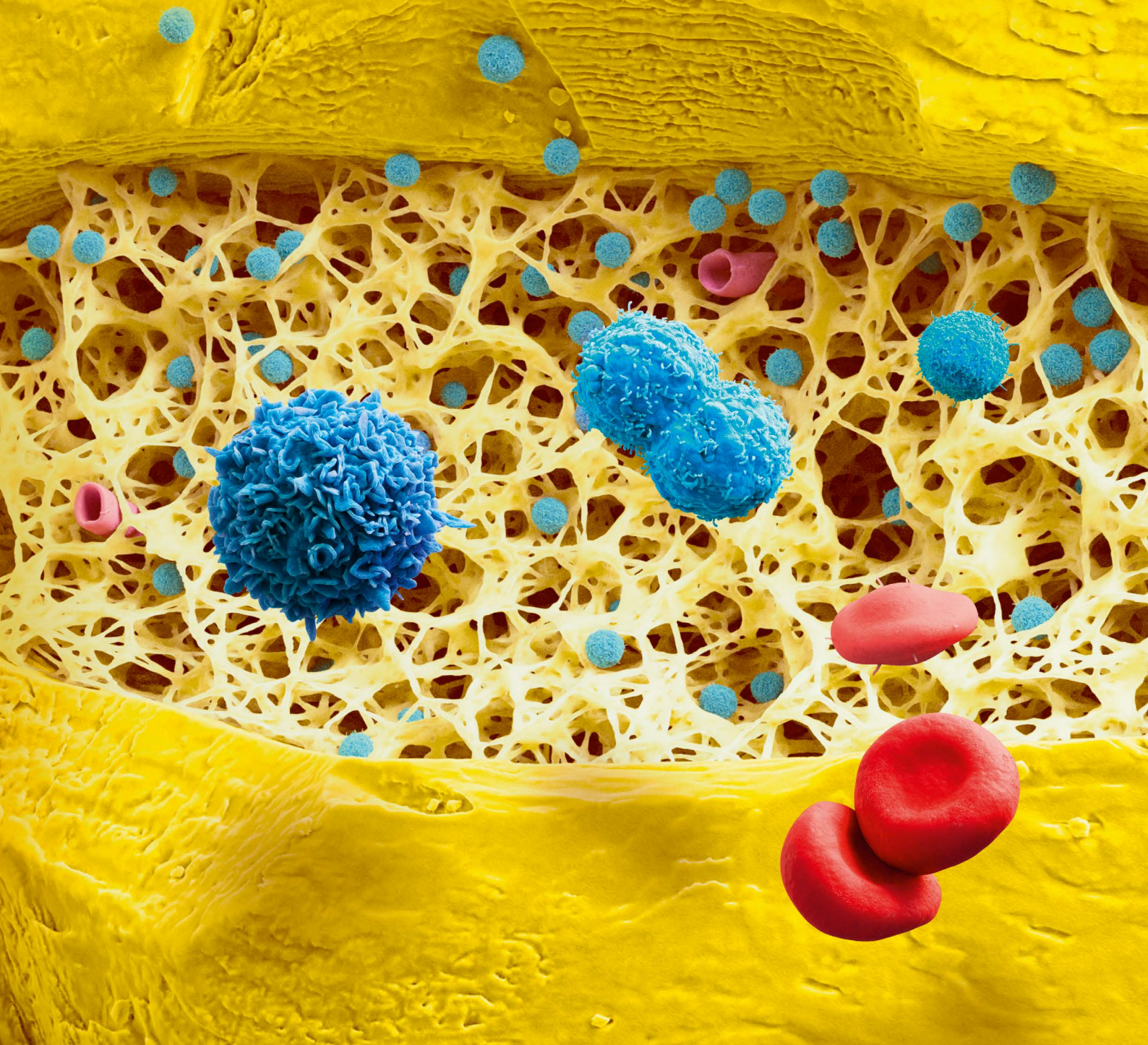
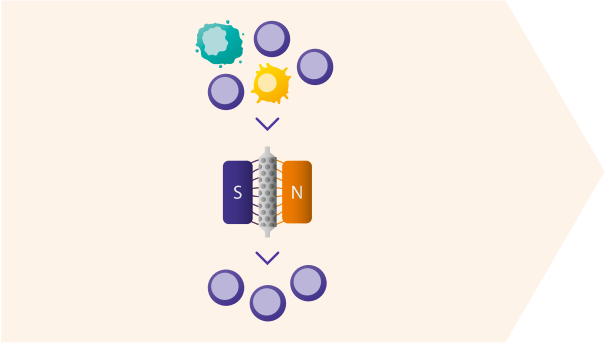
Miltenyi Biotec

Complete solutions for human hematopoietic stem cell research



HSC enrichment



Isolate hematopoietic stem cells (HSCs) from various starting materials with MACS® Technology and achieve highly pure samples by choosing the right kit for your downstream applications.

- CD34 MicroBead Kit UltraPure, human, for debris-rich samples (fig. 1).
- CD34$^+$CD38$^-$ Cell Isolation Kit, human, for primitive HSC enrichment.
- Diamond CD34 Isolation Kit, human, combines lineage depletion and CD34$^+$ enrichment.
- CD133 MicroBead Kit Hematopoietic Tissue, human, for CD133 enrichment of primitive and early HSCs.

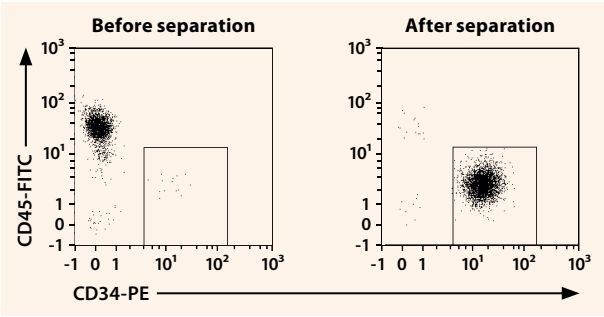
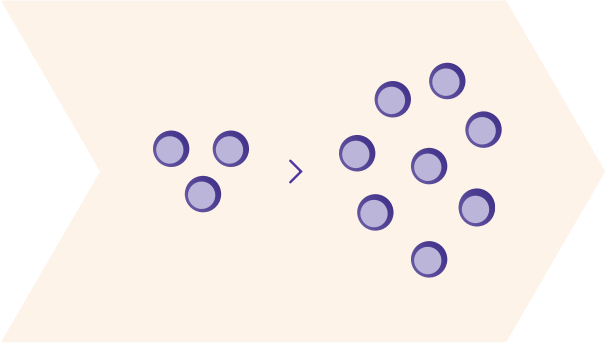


Figure 1: Isolation of CD34$^+$ cells from a PBMC sample using the CD34 MicroBead Kit UltraPure. Flow cytometric analysis shows high purity after enrichment.

Products	Order no.
CD34 MicroBead Kit, human ● ●	130-046-702
CD34 MicroBead Kit UltraPure, human ● ●	130-100-453
CD34$^+$CD38$^-$ Cell Isolation Kit, human ●	130-114-822
Diamond CD34 Isolation Kit, human ●	130-094-531
CD133 MicroBead Kit – Hematopoietic Tissue, human ●	130-100-830

N.B. Indicated for isolation out of ● umbilical cord blood, bone marrow, apheresis product and ● peripheral blood, and differentiated ES and iPS cells.

Expansion



StemMACS™ HSC Expansion Media XF, supplemented with StemMACS HSC Expansion Cocktail, ensures robust expansion of human HSCs.

- Xeno- and serum-free basic medium.
- Maintains high levels of CD34$^+$ cells.
- Preserves characteristic surface phenotype of early HSCs.

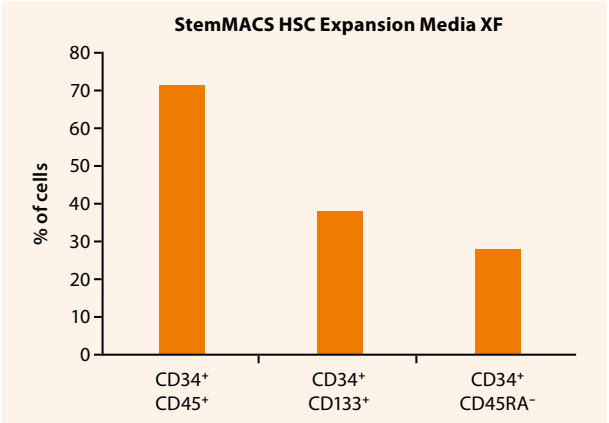
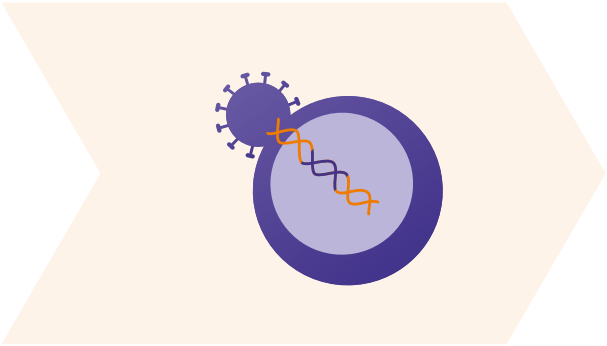


Figure 2: CD34$^+$ cord blood cells were expanded for seven days in StemMACS HSC Expansion Media XF supplemented with StemMACS HSC Expansion Cocktail. The cell surface phenotype was assessed by flow cytometry.

Products	Order no.
StemMACS HSC Expansion Media XF, human, 100 mL	130-100-473
StemMACS HSC Expansion Media XF, human, 500 mL	130-100-463
StemMACS HSC Expansion Cocktail, human	130-100-843

Transduction



The transduction enhancer Vectofusin®-1 streamlines the lentiviral and retroviral transduction of HSCs (fig. 3).

Key benefits include:

- Water-soluble synthetic peptide.
- No pre-coating required (unlike recombinant fibronectin).
- High cell viability.

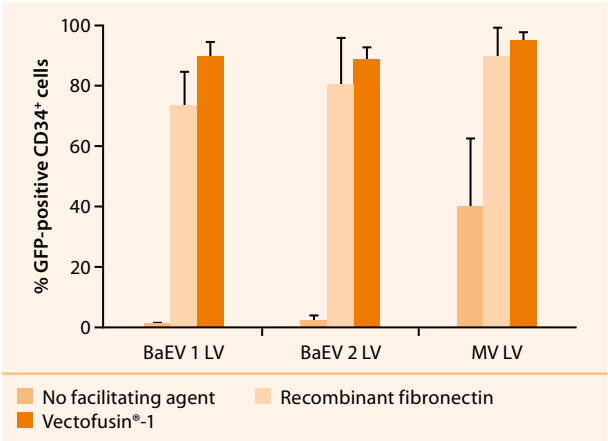
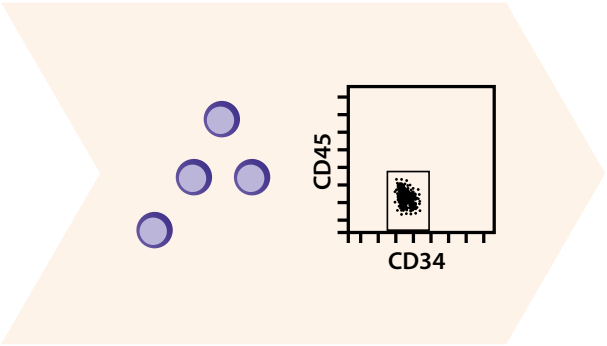


Figure 3: Magnetically isolated CD34+ cells were transduced with lentiviral vectors (LVs) encoding GFP at a multiplicity of infection (MOI) of 10, either without a facilitating agent, in the presence of Vectofusin®-1, or on recombinant fibronectin–coated plates. LVs displayed baboon envelope (BaEV) or measle virus (MV) pseudo types. Two different BaEV constructs were used: BaEV 1 and BaEV 2. Vectofusin®-1 does not require pre-coating, and enhances the transduction similar to recombinant fibronectin.

Product	Order no.
Vectofusin®-1	130-111-163

Flow cytometry



Analyze HSCs with our constantly growing portfolio of antibodies for the detection of HSC markers, such as CD34, CD38, CD133, and CD117.

- REAfinity™ Recombinant Antibodies eliminate the need for Fcγ receptor blocking and require only one isotype control, saving time and effort.
- VioDye® Fluorochromes enable multicolor flow cytometry with high fluorescence intensity and minimal spillover.
- REAlease® Fluorochrome Technology allows label removal after cell sorting for downstream applications.

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Discover the future of human HSC research and gene engineering with our advanced tools here:

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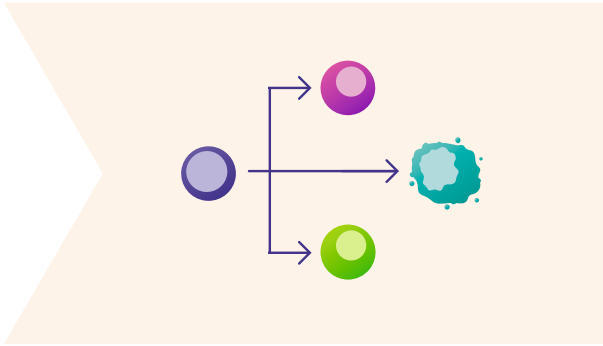
Functional analysis



Colony-forming unit (CFU) assays help to examine the ability of HSCs to proliferate and differentiate into colonies in response to cytokine stimulation. Analysis can be performed visually with a microscope or by flow cytometric analysis.

- StemMACS HSC-CFU Media are semi-solid media and can be used for standard CFU assays for visual scoring.
- The StemMACS HSC-CFU Assay Kit, human, combines differentiation in methylcellulose-free cell culture with a flow cytometric readout for high-throughput and user-independent analysis (fig. 4).

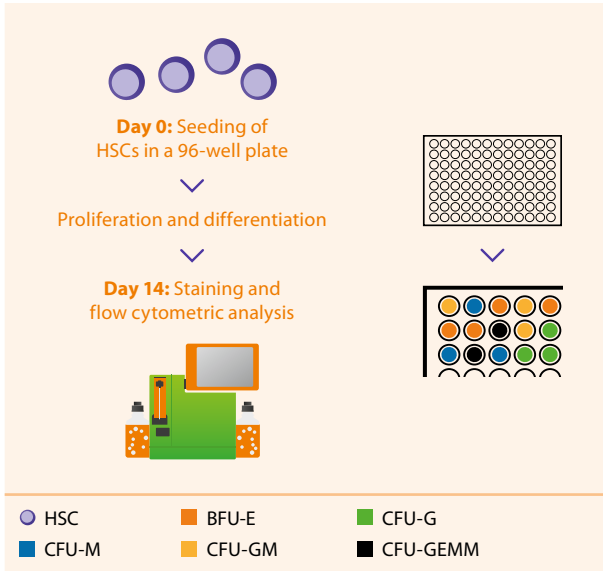


Figure 4: Workflow of the CFU assay using the StemMACS HSC-CFU Assay Kit, human, with flow cytometric analysis. Cells, diluted in StemMACS HSC-CFU Assay Media, were plated in 96-well plates to proliferate and differentiate for 14 days. After the incubation period, cells were stained with the StemMACS HSC-CFU Assay Cocktail and analyzed by flow cytometry. BFU-E: burst-forming unit-erythroid, CFU-G: CFU-granulocyte, CFU-M: CFU-macrophage, CFU-GM: CFU-granulocyte-macrophage, CFU-GEMM: CFU-granulocyte/erythrocyte/macrophage/megakaryocyte.

Irrespective of which analysis you choose, the results of our HSC-CFU assays are similar.

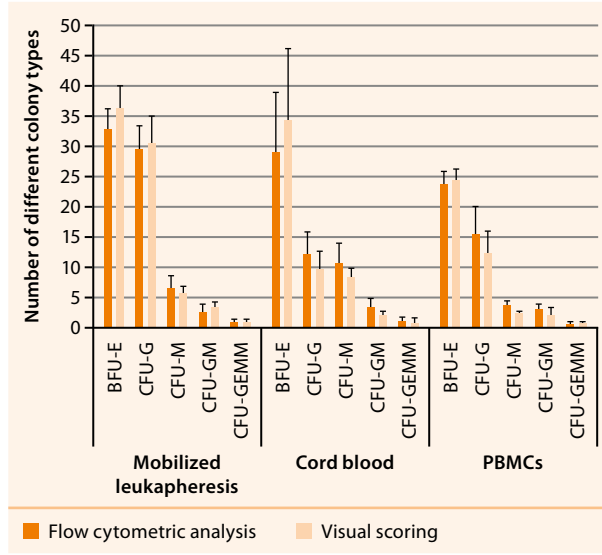


Figure 5: CFU assay results by flow cytometric analysis and visual scoring. CD34⁺ cells from three different sources (mobilized leukapheresis, cord blood, peripheral blood mononuclear cells [PBMCs]) were seeded with media from the StemMACS HSC-CFU Assay Kit, human, (250 HSCs per 96-well plate) for flow cytometric analysis, and the StemMACS HSC-CFU Media (250 HSCs per 35 mm cell culture dish) for visual scoring. Cells were incubated for 14 days and analyzed with the MACSQuant® Analyzer (flow cytometric analysis) or with a microscope (visual scoring). The distribution of the different colony types was highly comparable between both methods.

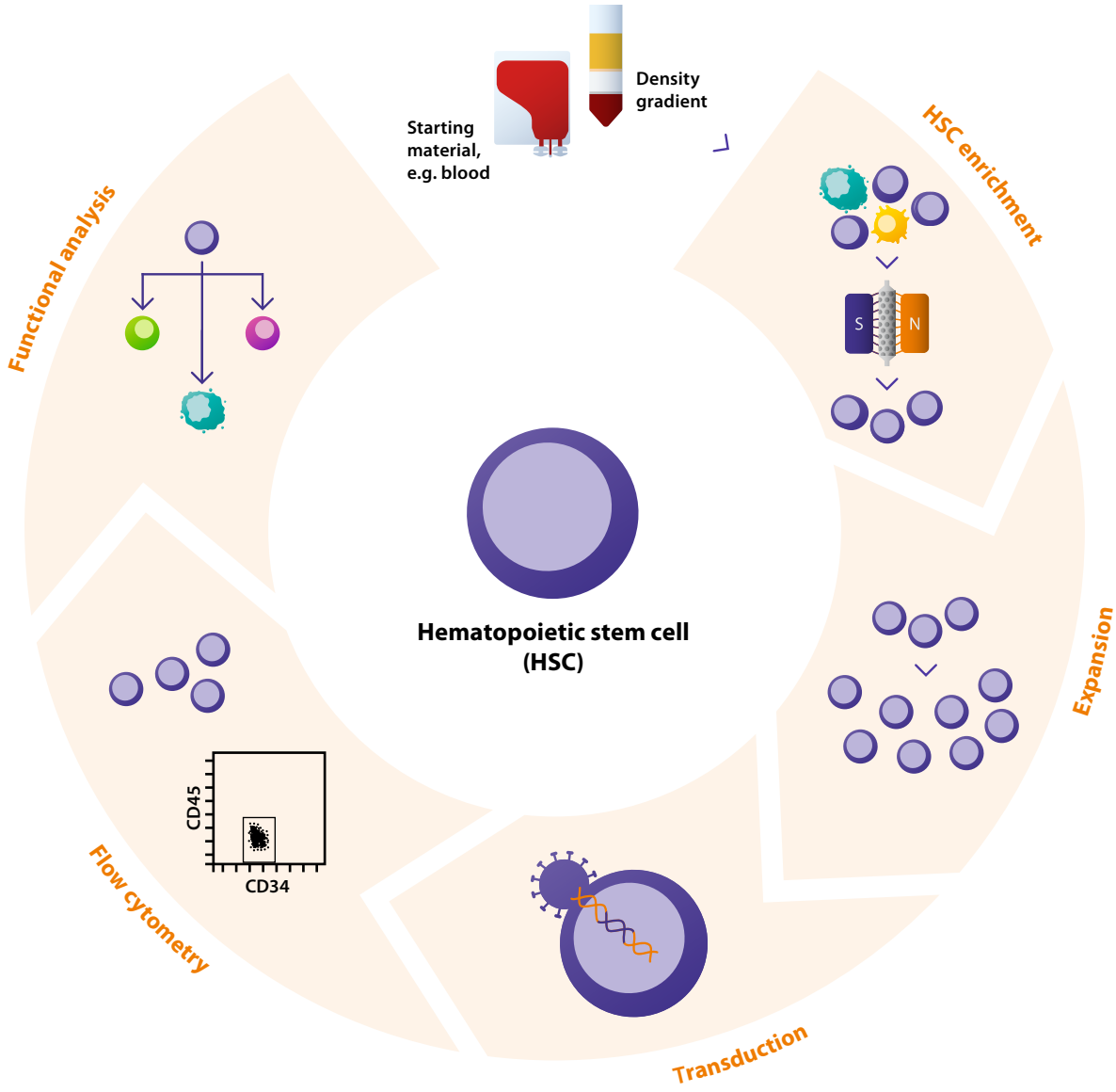
Products	Order no.
StemMACS HSC-CFU complete with Epo, human	130-091-280
StemMACS HSC-CFU lite with Epo, human	130-091-281
StemMACS HSC-CFU Assay Kit, human	130-125-042

HSC workflow – Overview

Hematopoietic stem cells (HSCs) are tissue-specific adult stem cells that can differentiate into all blood cell types, maintaining blood homeostasis throughout life.

HSCs are widely used as a model to study the regulation of hematopoiesis and to uncover mechanisms that govern stem cell self-renewal or directed differentiation into specific blood cell types.

Clinically, HSCs or HSC-enriched fractions are used to treat blood related diseases, like leukemia, sickle cell disease, β-thalassemia or immune defect severe combined immunodeficiency (SCID). HSCs can be isolated from various sources such as umbilical cord blood, bone marrow, apheresis product, peripheral blood, or differentiated embryonic and induced pluripotent stem (ES/iPS) cells. Depending on the starting material, HSC content varies from 0.1 to 2.5%.



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