Miltenyi Biotec

Generation of gene-engineered hematopoietic stem cells

CliniMACS Prodigy® Hematopoietic Stem Cell Engineering System

Application

The CliniMACS Prodigy® Hematopoietic Stem Cell Engineering System allows fully automated transduction of human CD34$^+$ cells from patient material for the generation of gene-engineered hematopoietic stem cells (HSCs).

This application sheet gives an overview of the process, the specifications, and materials needed. In addition, it elucidates the setup of the CliniMACS Prodigy Tubing Set (TS) 520 and the performance data.

Specifications

Cellular starting material:	CD34$^+$ cells , e.g., enriched from mobilized leukapheresis
Starting cell number:	at least 2×10^7 cells
Starting sample volume:	40–250 mL
Final product:	gene-engineered CD34$^+$ cells
Final product volume:	100 mL
Process time:	2–3 days

Products	Amount required*
CliniMACS Prodigy® Instrument	1 piece
CliniMACS Prodigy TS 520	1 set
HSC-Brew GMP Medium	2 L
MACS® GMP Recombinant Human Flt3-Ligand (100 µg)	1 vial
MACS GMP Recombinant Human SCF (100 µg)	1 vial
MACS GMP Recombinant Human TPO (50 µg)	1 vial
MACS GMP Recombinant Human IL-3 (25 µg)	1 vial
1 m Tube Extension	1 piece
3-way Tube Adapter	2 pieces

Additional materials	Amount required*
Transfer bag 150 mL	3 bags
Transfer bag 600 mL	1 bag
Luer/Spike Interconnector	4 pieces
Formulation solution (physiological sodium chloride solution with 0.5% (w/v) human serum albumin)	1 L
Lentiviral vector	MOI depends on the used vector

*Amount is given for the one-hit lentiviral transduction of 1×10^8 cells in 100 mL. Please discuss your specific requirements with your Miltenyi Biotec representative.

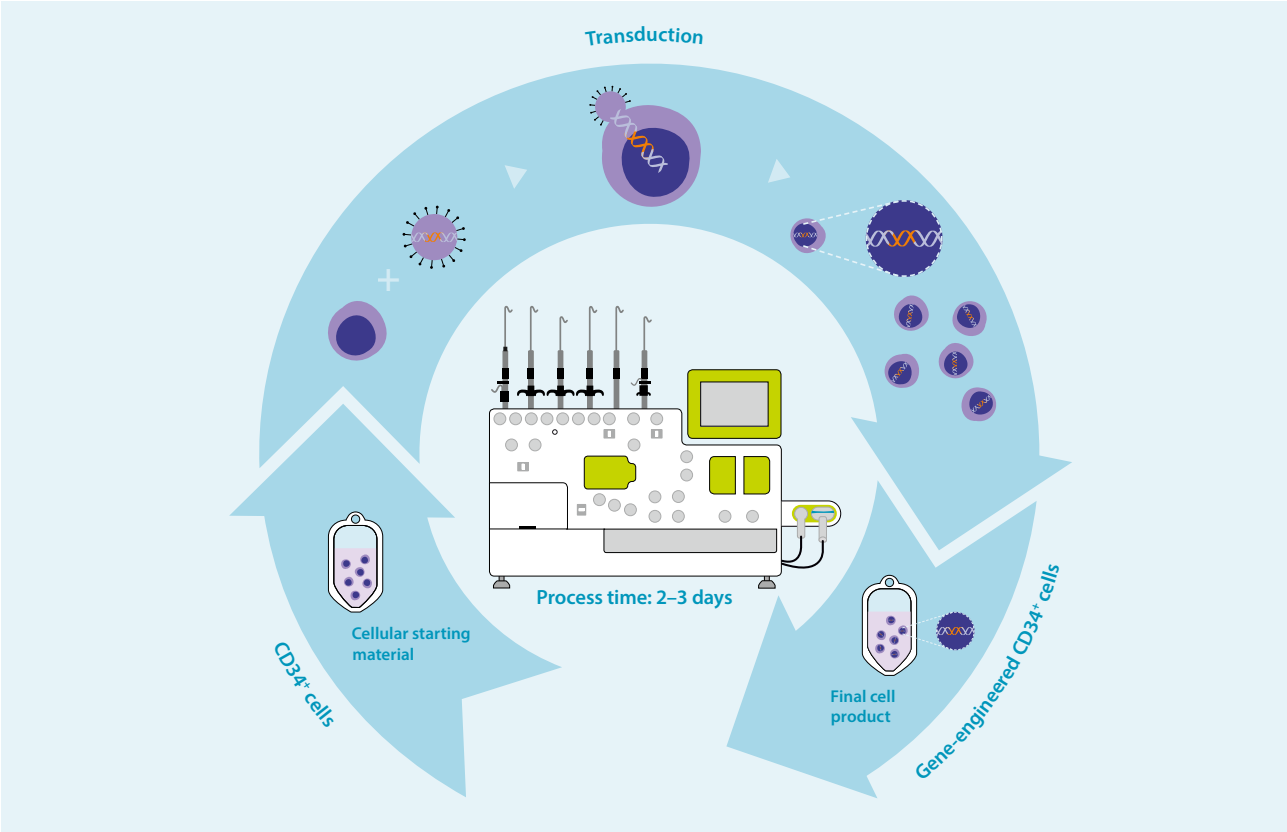
Process overview for HSC engineering

Pre-process	Tubing set installation and priming ▼ Connection of cellular starting material to tubing set ▼ Transfer of $CD34^+$ cells into HSC-Brew GMP Medium with MACS® GMP Recombinant Human Flt3-Ligand, SCF, TPO, and IL-3
Viral transduction	Lentiviral transduction
Cell harvest and formulation	Washing of cells (2×) and harvest in 100 mL formulation solution
Post-process	Tubing set deinstallation

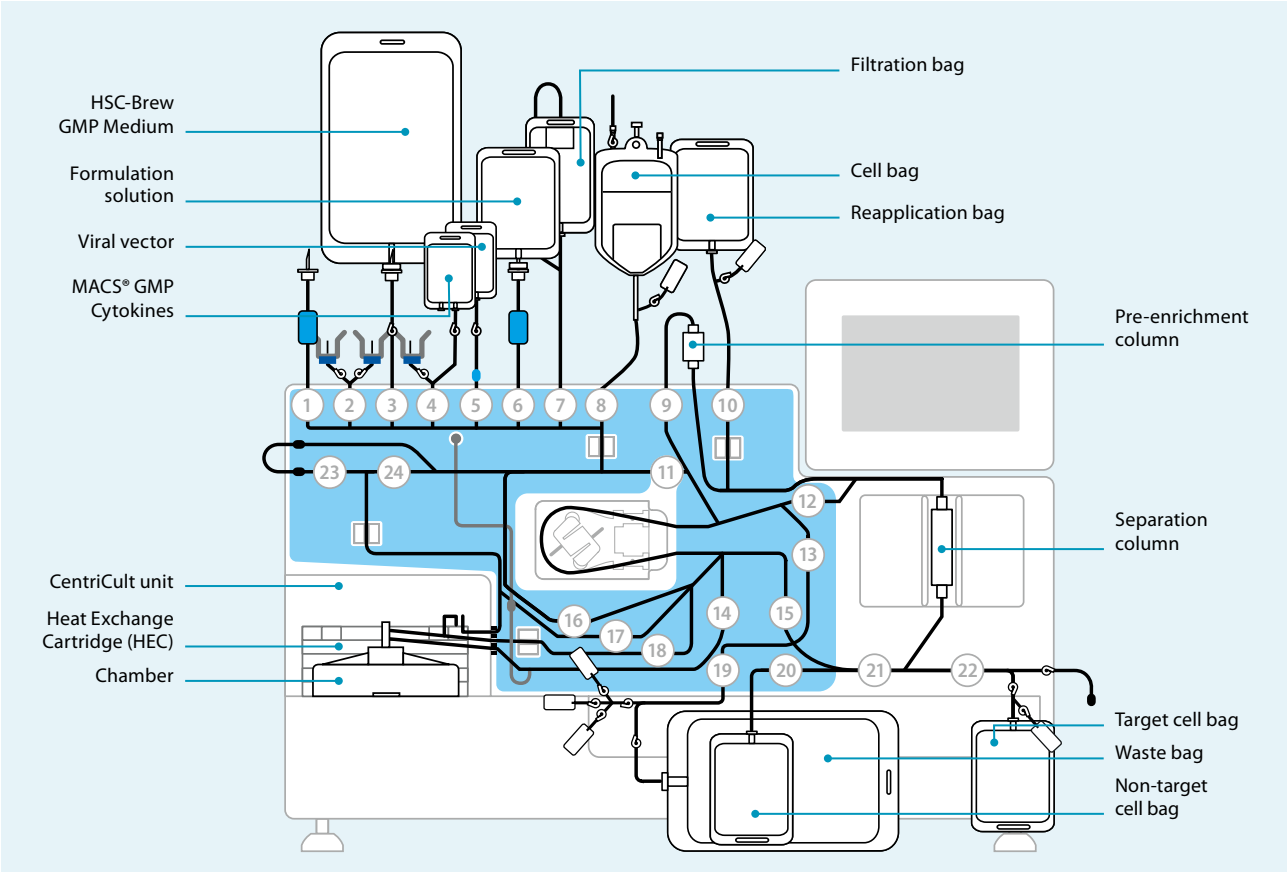
2–3 days for total process*

*The duration of the HSC engineering process depends on the number of transduction cycles

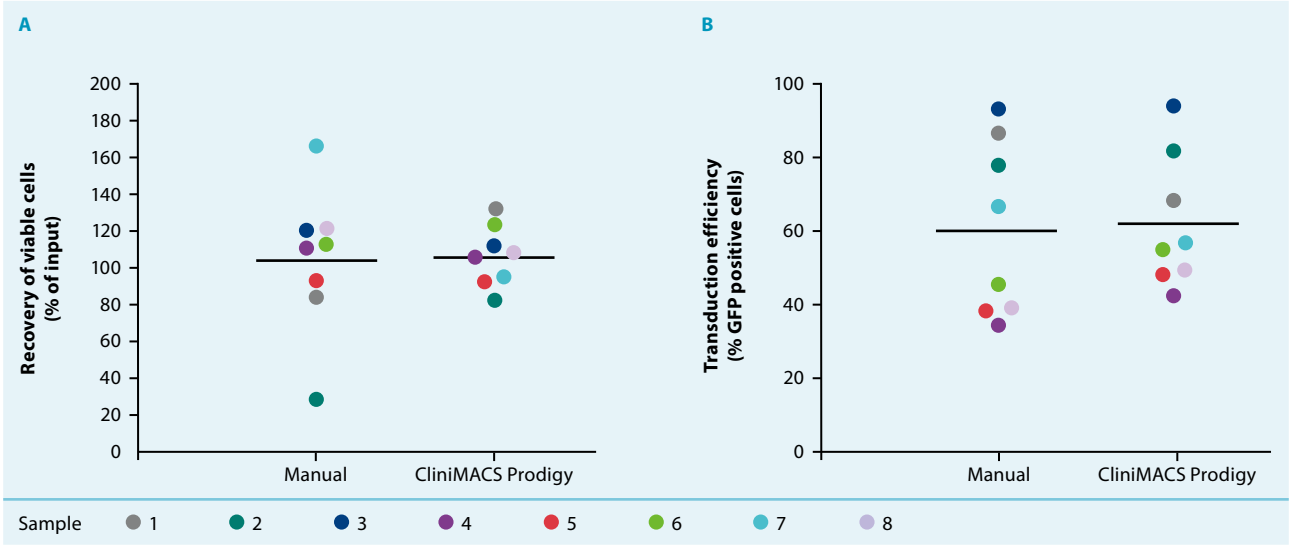
Principle of the HSC engineering process on the CliniMACS Prodigy®



CliniMACS Prodigy TS 520 setup for HSC engineering



Performance data



Human CD34⁺ cells enriched from eight mobilized leukapheresis products were transferred into HSC-Brew GMP Medium supplemented with MACS® GMP Recombinant Human Flt3-Ligand, SCF, TPO, and IL-3 (day 0) and transduced with lentiviral vector encoding GFP (MOI 100) on day 1 using the CliniMACS Prodigy® HSC Engineering System. The same experiment was performed manually following a standard protocol using 24-well plates. (**A**) On day 2, similar cell recovery was detected when using the CliniMACS Prodigy HSC Engineering System and manual handling. (**B**) Harvested gene-engineered CD34⁺ cells were further cultured manually in 96-well plates and transduction efficiency was measured on day 5. Transduction efficiency of CD34⁺ cells processed with the CliniMACS Prodigy was comparable to that of cells processed manually.

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

130-126-605