Miltenyi Biotec

CliniMACS Prodigy® Adherent Cell Culture System

GMP-compliant human pluripotent stem cell expansion process

Application

The CliniMACS Prodigy® Adherent Cell Culture System allows GMP-compliant and scalable human pluripotent stem cell (PSC) cultivation.
This application sheet gives an overview of the entire process and quality control assays, and provides information about the required materials. In addition, it elucidates the setup of the tubing set CliniMACS Prodigy TS 730 and the performance data.

Specifications

Process capacity:	scalable (up to 5×10^7 PSCs in the CliniMACS Prodigy chamber; up to 2×10^8 PSCs in one Corning® CellSTACK® 1 Chamber; up to 1×10^9 PSCs in one Corning CellSTACK 5 Chamber)
Starting cell number:	1×10^6 cells
Final cell number:	approx. 5×10^8 cells
Total process time:	10 days
Total hands-on time:	approx. 5 h

Products

Consumables	Amount required
CliniMACS Prodigy® Instrument	1 piece
CliniMACS Prodigy TS 730	1 set
iPS-Brew GMP Medium	3 L
CliniMACS® PBS/EDTA Buffer (2×3 L)	3 L
MACS GMP Recombinant Human TGF-β1 (25 µg)	1 vial
1 m Tube Extension	2 pieces

Additional materials	Amount required
Corning® CellSTACK® accessories, fill cap, 3.2 mm I.D. tubing, female Luer Lock with male luer plug	1 piece
Corning CellSTACK 5 Chamber	1 piece
Corning 1000 mL Easy Grip Polystyrene Storage Bottles with Dip Tube, with 0.2 µm MLL/FLL Filter*	2 pieces
Flexboy® Bag 50 mL, Inlet: Luer Lock male + cap, Outlet: Luer Lock female + cap, Sartorius	3 pieces
Flexboy Bag 500 mL, Inlet: Luer Lock male + cap, Outlet: Luer Lock female + cap, Sartorius	2 pieces
CTS™ TrypLE™ Select Enzyme, 100 mL, Thermo Fisher	300 mL
Defined Trypsin Inhibitor, 100 mL, Thermo Fisher	100 mL
Biolaminin 521 LN (LN521), 500 µg, BioLamina	4 vials

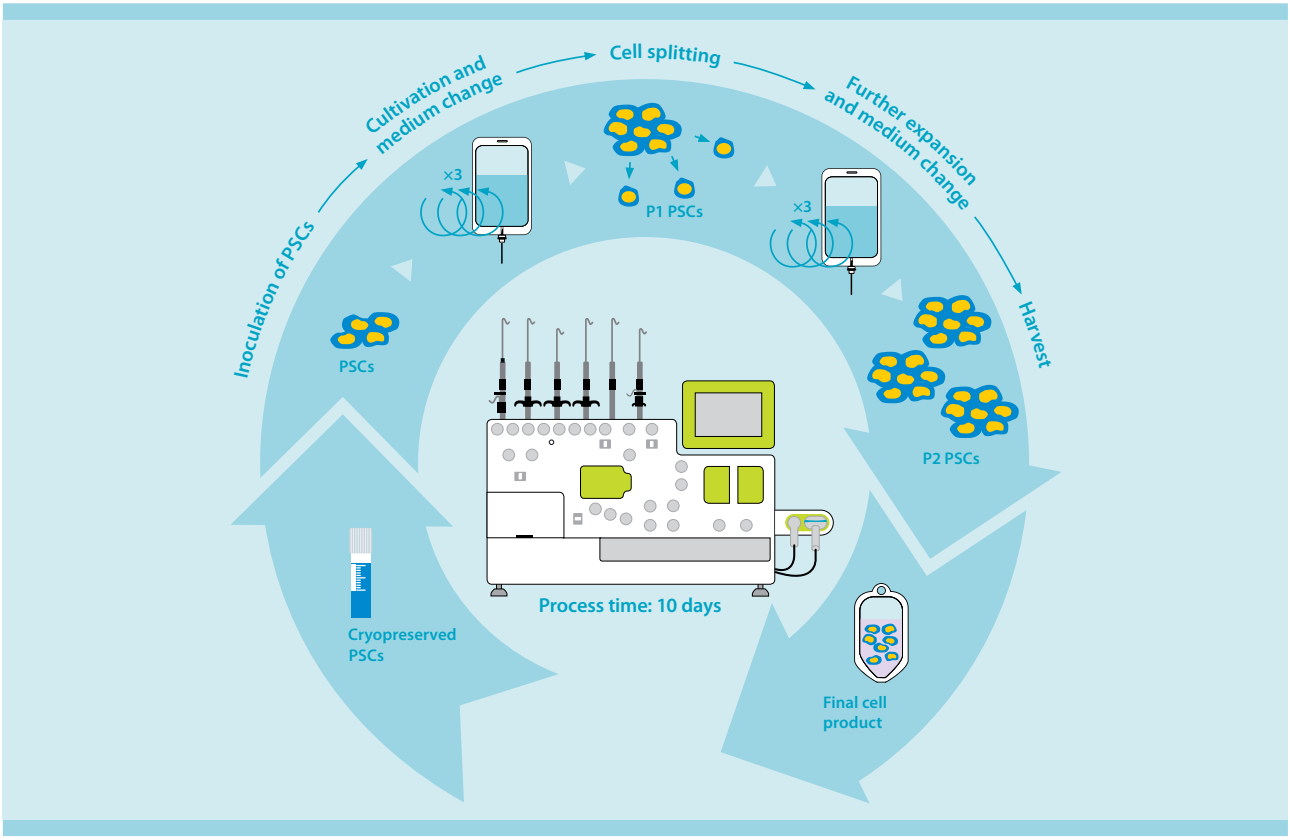
*Used as alternative vessels for iPS-Brew GMP Medium

Process overview for PSC expansion

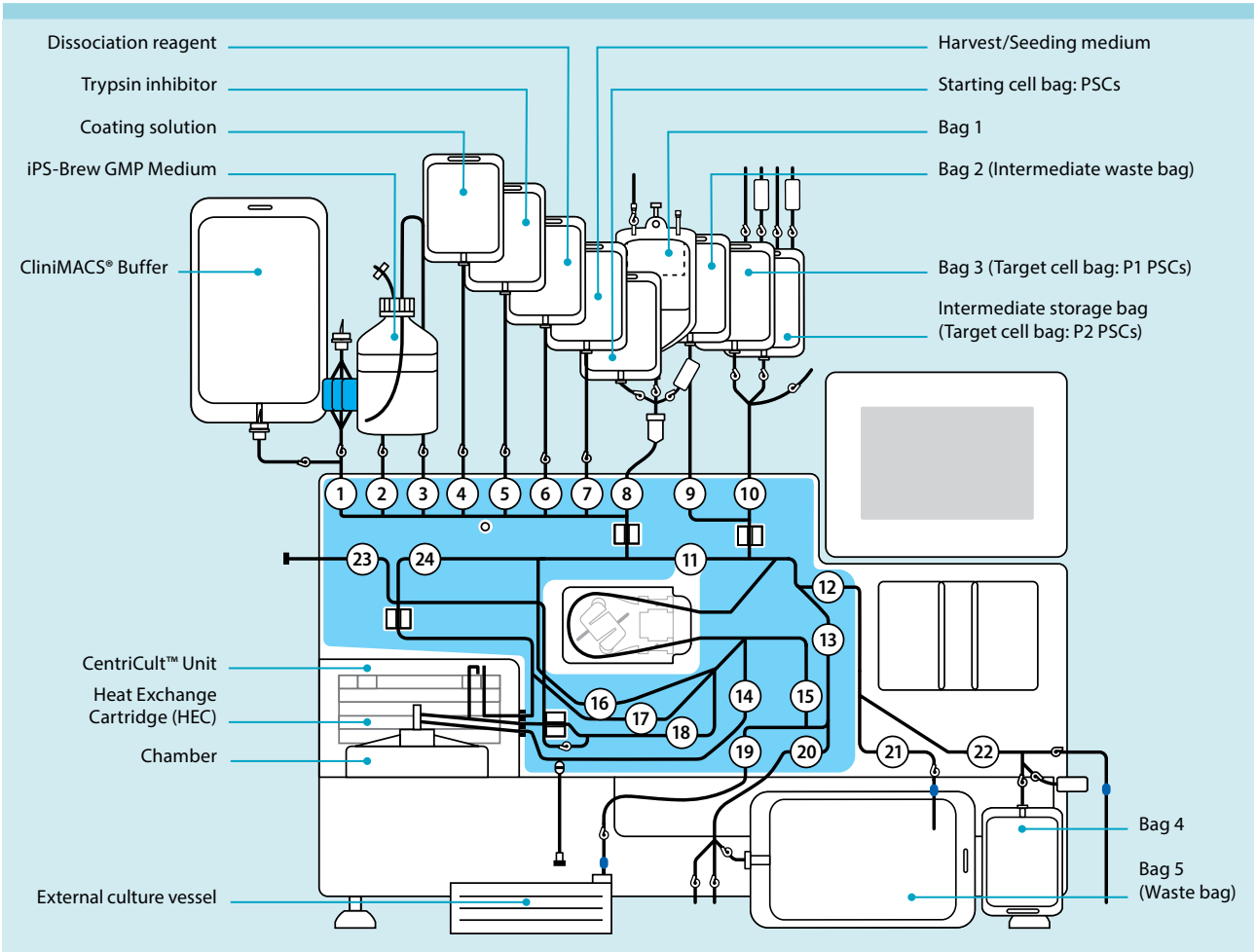
Pre-process (day –1)	Tubing set installation and priming ▼ Blocking of the tubing set with culture medium ▼ Coating of the CliniMACS Prodigy® chamber with LN521
Inoculation (day 0)	Inoculation of PSCs in the CliniMACS Prodigy chamber
Cultivation and medium change (day 2, 3, 4)	Medium change ▼ Coating of one CellSTACK 5 Chamber with LN521 (day 4)
Harvest and inoculation (day 5)	Automated harvest of P1 PSCs ▼ Sample collection for QC and cell counting ▼ Inoculation of P1 PSCs in one CellSTACK 5 Chamber
Cultivation and medium change (day 7, 8, 9)	Medium change
Harvest and final formulation (day 10)	Semi-automated harvest of P2 PSCs ▼ Sample collection for QC and cell counting ▼ Storage of cells in the target cell bag
Post-process (day 10)	Tubing set deinstallation
Quality control (> day 10)	PSC characterization (e.g. App note: Multicolor flow cytometry analysis of human pluripotent stem cell cultures[1]) Trilineage differentiation potential of PSCs (e.g. StemMACS™ Trilineage Differentiation Kit, human[2])

10 days for total process

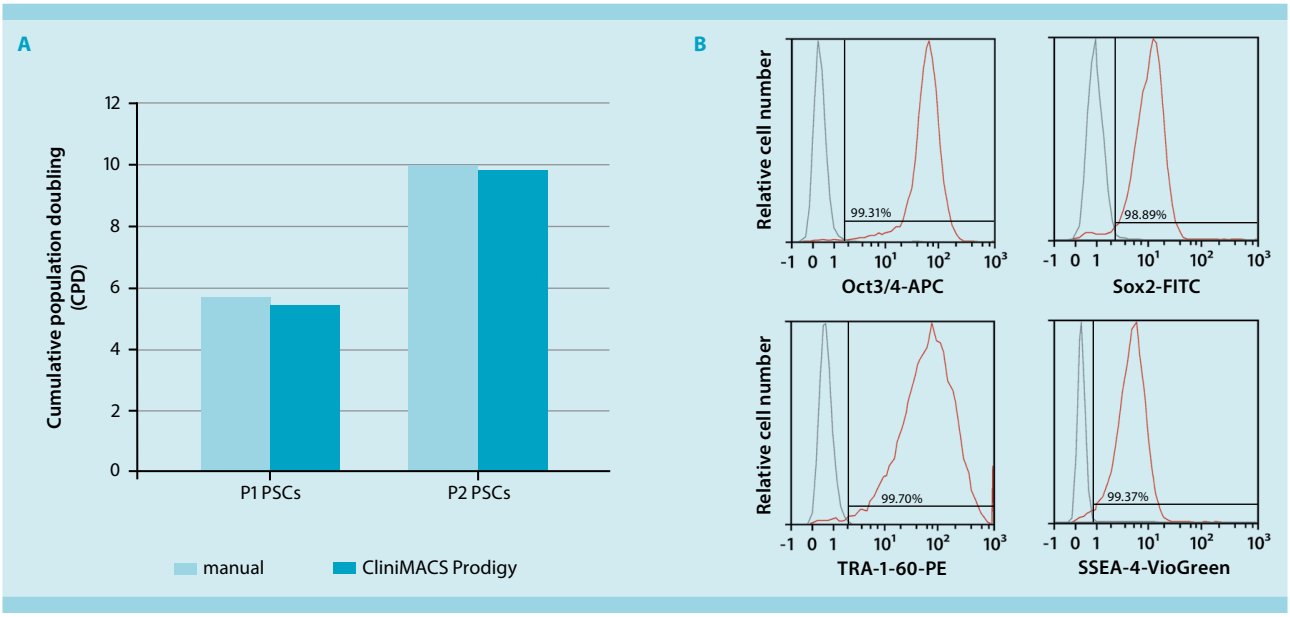
Principle of the PSC expansion process using the CliniMACS Prodigy®



CliniMACS Prodigy TS 730 setup for PSC expansion



Performance data



1×10^6 cryopreserved human PSCs were initially seeded and cultured in the CliniMACS Prodigy® chamber until day 5 and further expanded in one Corning® CellSTACK® 5 Chamber from day 5 to day 10 in iPS-Brew GMP Medium using the CliniMACS Prodigy Adherent Cell Culture System. As a control, cells were cultured with manual laboratory standard using 6-well plates and T75 flasks. (A) After 10 days of expansion, a clinically relevant number of P2 PSCs (approx. 5×10^8 cells) was harvested using the CliniMACS Prodigy Adherent Cell Culture System. The cumulative population doubling rate was comparable to the one obtained with manual laboratory standard. (B) Flow cytometry analysis confirmed the quality of PSCs processed with the CliniMACS Prodigy Adherent Cell Culture System. Harvested PSCs showed high expression levels of the key pluripotency markers Oct3/4, Sox2, TRA-1-60, and SSEA-4.

References

1. Miltenyi Biotec (2016) Multicolor flow cytometry analysis of human pluripotent stem cell cultures. **www.miltenyibiotec.com/flow-analysis-PSC**
2. Miltenyi Biotec (2017) StemMACS™ Trilineage Differentiation Kit – Protocol for flow analysis. **www.miltenyibiotec.com/Tri-Dif-Kit**

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Miltenyi Biotec Inc. | Phone 800 FOR MACS | Phone +1 530 888 8871 | Fax +1 877 591 1060 | macsus@miltenyi.com | **www.miltenyibiotec.com**

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