Miltenyi Biotec

CliniMACS Prodigy® Adherent Cell Culture System

Differentiation of human pluripotent stem cells into mesencephalic dopaminergic progenitor cells

Application

The CliniMACS Prodigy® Adherent Cell Culture System facilitates highly specific and efficient differentiation of human pluripotent stem cells (PSCs) into mesencephalic dopaminergic (mesDA) progenitor cells in large scale. 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.

Products

Consumables	Amount required
CliniMACS Prodigy® Instrument	1 piece
CliniMACS Prodigy TS 730	1 set
iPS-Brew GMP Medium	500 mL
MACS GMP Recombinant Human TGF-β1 (5 µg)	1 vial
CliniMACS® PBS/EDTA Buffer (2×3 L)	3 L
1 m Tube Extension	1 piece
3-way Tube Adapter	1 piece

Differentiation media[1,2]	Amount required
Neural induction medium (NIM) Containing: MACS® NeuroBrew®-21 w/o Vitamin A, N-2 Supplement, SB431542, human Noggin, human SHH (C24II), CHIR99021, Purmorphamine	1.5 L
Neural proliferation medium (NPM) Containing: MACS NeuroBrew-21 w/o Vitamin A, N-2 Supplement, SB431542, human Noggin, human SHH (C24II), CHIR99021, Purmorphamine	4 L
Neural differentiation medium (NDM) Containing: MACS NeuroBrew-21 w/o Vitamin A, Human FGF-8b, Human BDNF, L-Ascorbic acid 2-phosphate	3 L

Specifications

Process capacity:	scalable
Number of PSCs for initial expansion:	1×10^6 cells
Number of PSCs for differentiation:	approx. 5×10^7 cells
Number of final mesDA progenitor cells:	approx. 3.8×10^9 cells
Total process time:	21 days (5 days of expansion and 16 days of differentiation)
Total hands-on time:	approx. 12 h

Additional materials	Amount required
Corning® CellSTACK® accessories, fill cap, 3.2 mm I.D. tubing, female Luer Lock with male Luer plug	3 pieces
Corning CellSTACK 5 Chamber	1 piece
Corning CellSTACK 2 Chamber	2 pieces
Corning 1000 mL Easy Grip Polystyrene Storage Bottles with Dip Tube, with 0.2 µm MLL/FLL Filter*	10 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	3 pieces
CTS™ TrypLE™ Select Enzyme, 100 mL, Thermo Fisher	500 mL
Defined Trypsin Inhibitor, 100 mL, Thermo Fisher	300 mL
Biolaminin 521 LN (LN521), 100 µg, BioLamina	2 vials
Biolaminin 111 LN (LN111), 500 µg, BioLamina	12 vials

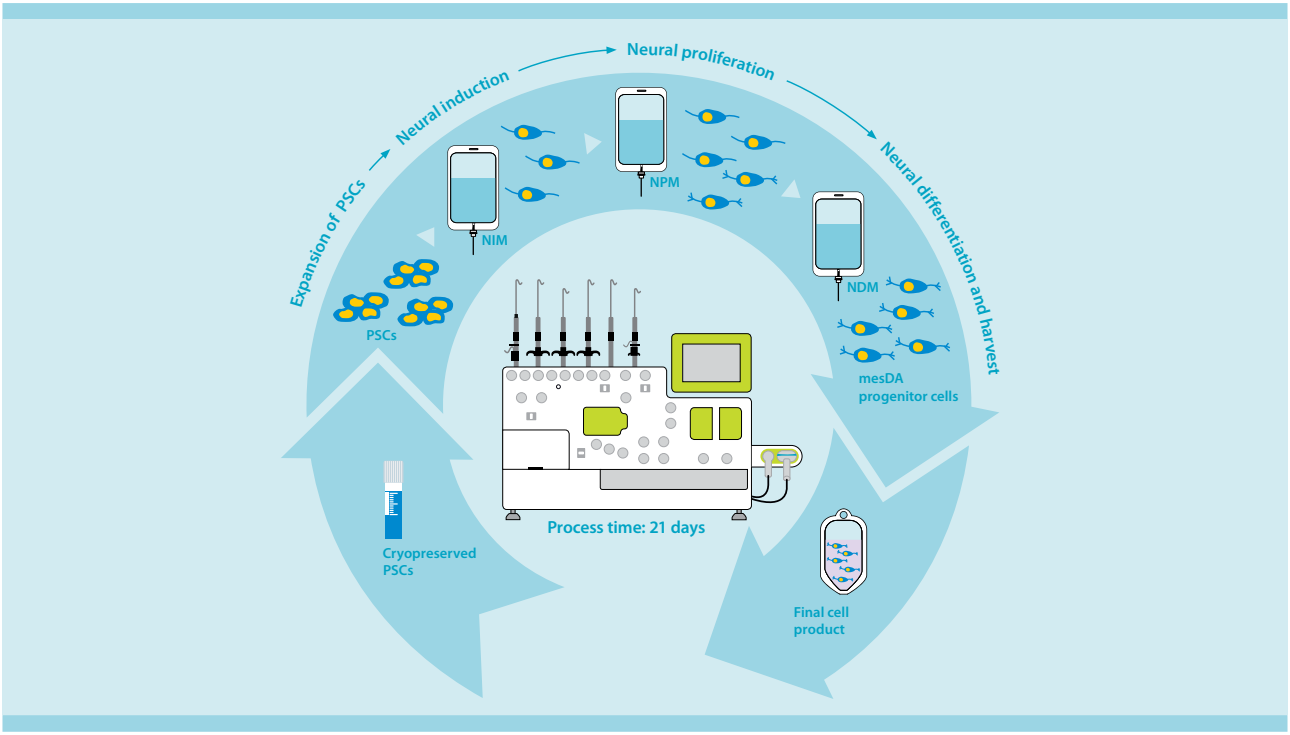
*Used as alternative vessels for iPS-Brew GMP Medium and differentiation medium

Process overview for mesDA progenitor cell differentiation

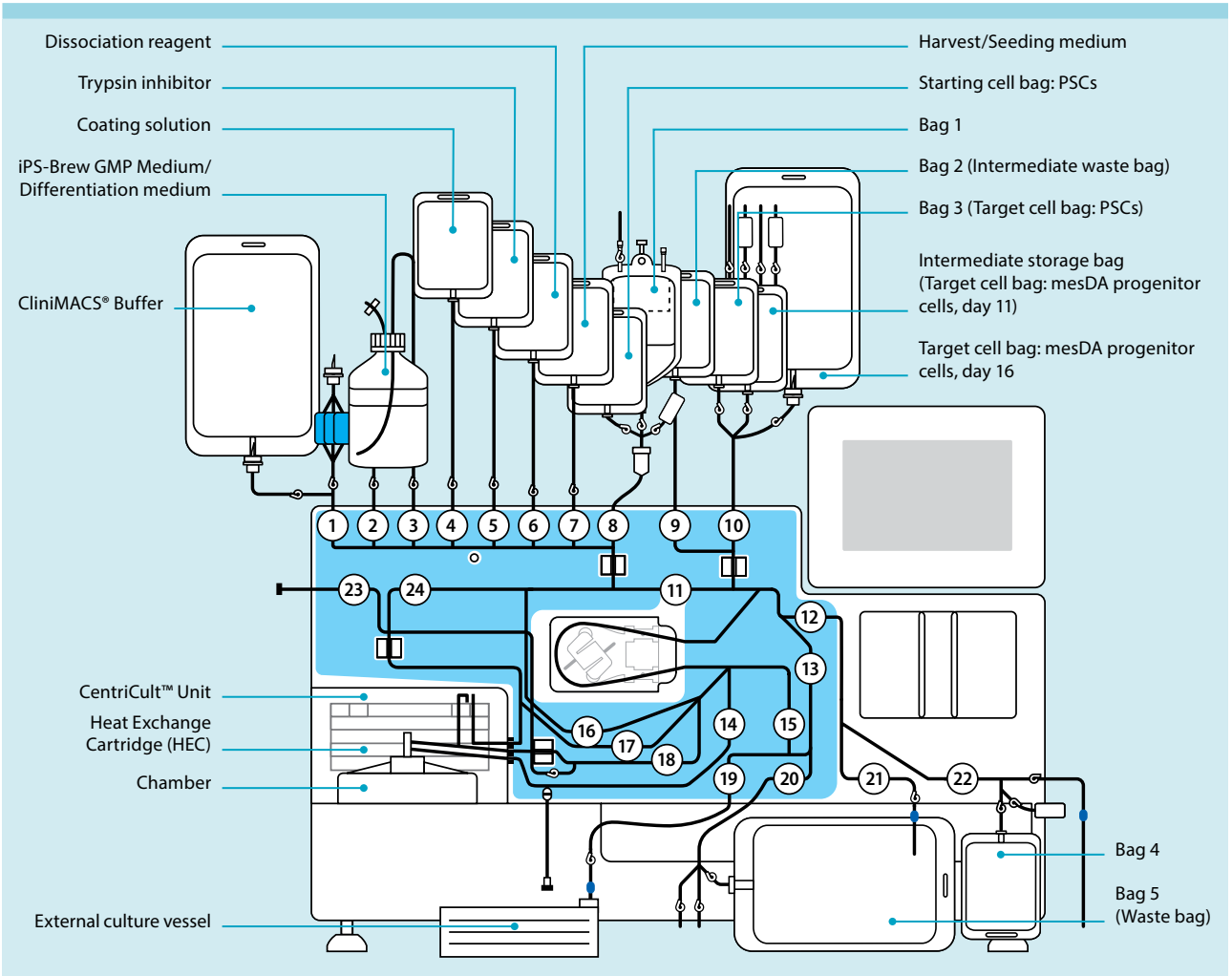
Pre-process (day –6)	Tubing set installation and priming ▼ Blocking of the tubing set with culture medium ▼ Coating of the CliniMACS Prodigy® chamber with LN521
Inoculation (day –5)	Inoculation of PSCs in the chamber
Cultivation and medium change (day –3, –2, –1)	Medium change with iPS-Brew GMP Medium
Coating (day –1)	Coating of two CellSTACK® 2 Chambers with LN111
Harvest and inoculation (day 0)	Automated harvest of PSCs ▼ Sample collection for QC and cell counting ▼ Inoculation of PSCs in two CellSTACK 2 Chambers with NIM
Cultivation and medium change (day 2, 4, 6, 8, 10)	Medium change with NIM (day 2) ▼ Medium change with NPM (day 4, 6, 8, 10)
Coating (day 10)	Coating of one CellSTACK 5 Chamber with LN111
Harvest and re-seeding (day 11)	Semi-automated harvest of mesDA progenitor cells ▼ Sample collection for QC and cell counting ▼ Inoculation of cells in one CellSTACK 5 Chamber with NDM
Cultivation and medium change (day 14)	Medium change with NDM
Harvest and final formulation (day 16)	Semi-automated harvest of mesDA progenitor cells ▼ Sample collection for QC and cell counting ▼ Storage of cells in the target cell bag
Post-process (day 16)	Tubing set deinstallation
Quality control (>day 16)	Flow cytometry–based mesDA progenitor cell characterization

21 days for total process

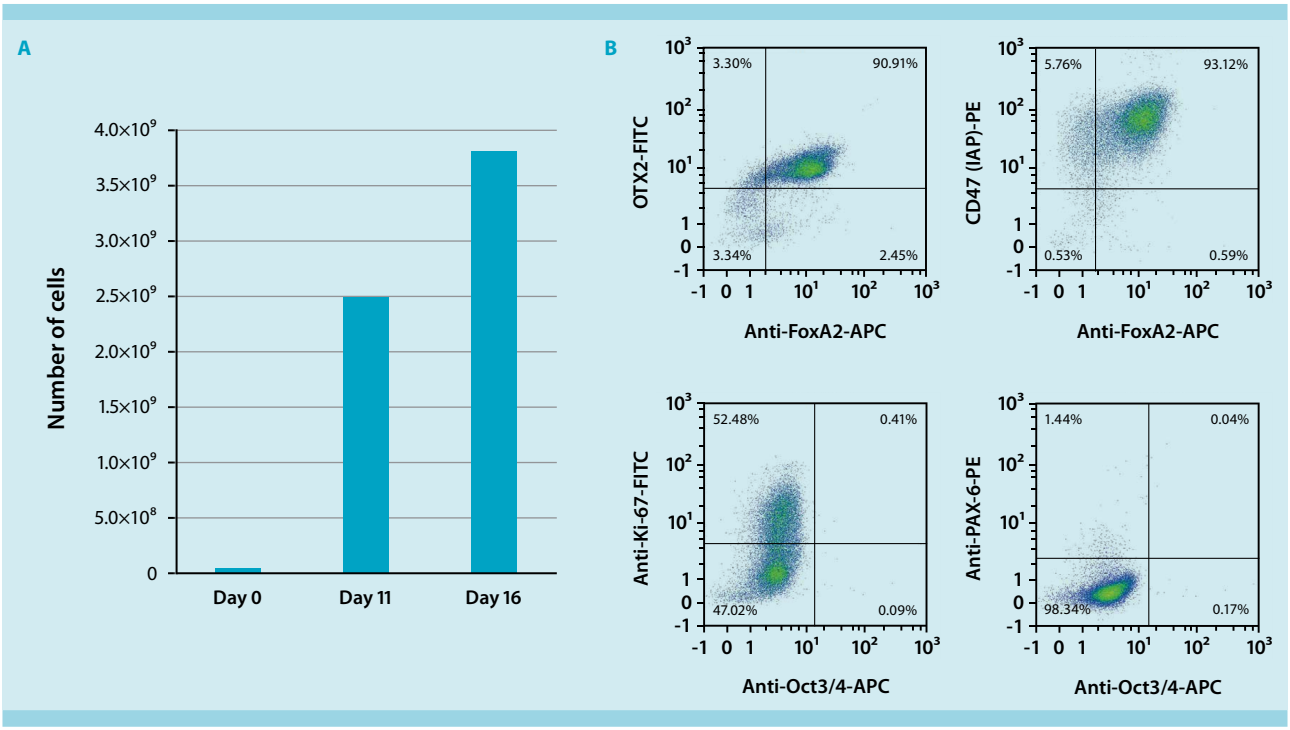
Principle of the mesDA progenitor cell differentiation process using the CliniMACS Prodigy®



CliniMACS Prodigy TS 730 setup for mesDA progenitor cell differentiation



Performance data



1×10^6 human PSCs were expanded in the CliniMACS Prodigy® chamber for 5 days in iPS-Brew GMP Medium. Differentiation into mesDA progenitor cells took place in two Corning® CellSTACK® 2 Chambers from day 0 to day 11 (in NIM from day 0 to day 4, and in NPM from day 4 to day 11), and in one Corning CellSTACK 5 Chamber in NDM from day 11 to day 16 using the CliniMACS Prodigy Adherent Cell Culture System. (A) Approx. 5×10^7 PSCs were used for the differentiation into mesDA progenitor cells (day 0). After 16 days of differentiation, approx. 3.8×10^9 mesDA progenitor cells could be harvested. (B) Flow cytometry–based quality control analysis demonstrated that over 90% of the processed cells expressed markers specific for mesDA progenitor cells (FoxA2, OTX2, and CD47). In contrast, cells positive for the dorsal brain marker PAX-6 and the PSC marker Oct3/4 were lacking. The number of cells expressing the proliferation marker Ki-67 was also reduced.

References

1. Kirkeby, A. *et al.* (2017) Predictive Markers Guide Differentiation to Improve Graft Outcome in Clinical Translation of hESC-Based Therapy for Parkinson's Disease. Cell Stem Cell 20: 135–148.
2. Lehnen, D. *et al.* (2017) IAP-Based Cell Sorting Results in Homogeneous Transplantable Dopaminergic Precursor Cells Derived from Human Pluripotent Stem Cells. Stem Cell Reports 9: 1207–1220.

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