Miltenyi Biotec

Large-scale manufacturing of transduced T cells

CliniMACS Prodigy® T Cell Transduction – Large Scale

Application

Fully automated labeling, enrichment, activation, transduction, and expansion of human T cells from frozen or fresh leukapheresis for the production of gene-engineered T cells.

This application sheet gives an overview of the specifications and materials required to perform the T Cell Transduction – Large Scale (TCT–LS) Process. Furthermore, it provides an overview of the setup for the tubing set, general workflow, and internal performance data.

Specifications

Process name:	T Cell Transduction – Large Scale
Selection capacity:	Up to 3×10^9 labeled cells within 10×10^9 WBCs
Sample volume for selection:	Fresh: 50–600 mL Frozen: 50–300 mL
TransAct™ Large Scale stimulation capacity:	4×10^8 T cells recommended
Expansion capacity:	3×10^7 T cells/mL in max. 600 mL culture volume
Final product harvest volume:	Four different harvest types available: • Optional rebuffering step • Optional transfer of cells into Target Cell Bag
Process time:	12 days recommended

Products required*

CliniMACS® and MACS® GMP Products	Amount required
CliniMACS Prodigy with T Cell Transduction – Large Scale application software	1
CliniMACS CD4 and CD8 Reagent/GMP MicroBeads	1 piece each
CliniMACS CD62L Reagent/GMP MicroBeads	1 piece
CliniMACS Prodigy TS 620	1 piece
CliniMACS PBS/EDTA Buffer (3 L)	1 piece
TexMACS™ GMP Medium	5×2 L bags
MACS GMP Recombinant Human IL-7 and IL-15	1 vial each per one 2 L medium bag
MACS GMP Recombinant Human IL-2	<1 vial per one 2 L medium bag
MACS GMP T Cell TransAct Large Scale	1 vial

Additional material / equipment*
Viral vector
3-way Tube Adapter (Miltenyi Biotec)
Triple Sampling Adapter (Miltenyi Biotec)
Sterile water, syringes, hypodermic needles
MACS GMP Vectofusin®-1
Formulation solution (e.g. CliniMACS Formulation Solution)
Human serum albumin
Human AB serum
Sterile tube welder
Uninterruptable power supply
CO_2 and compressed air supply
Cell counter and/or flow cytometer

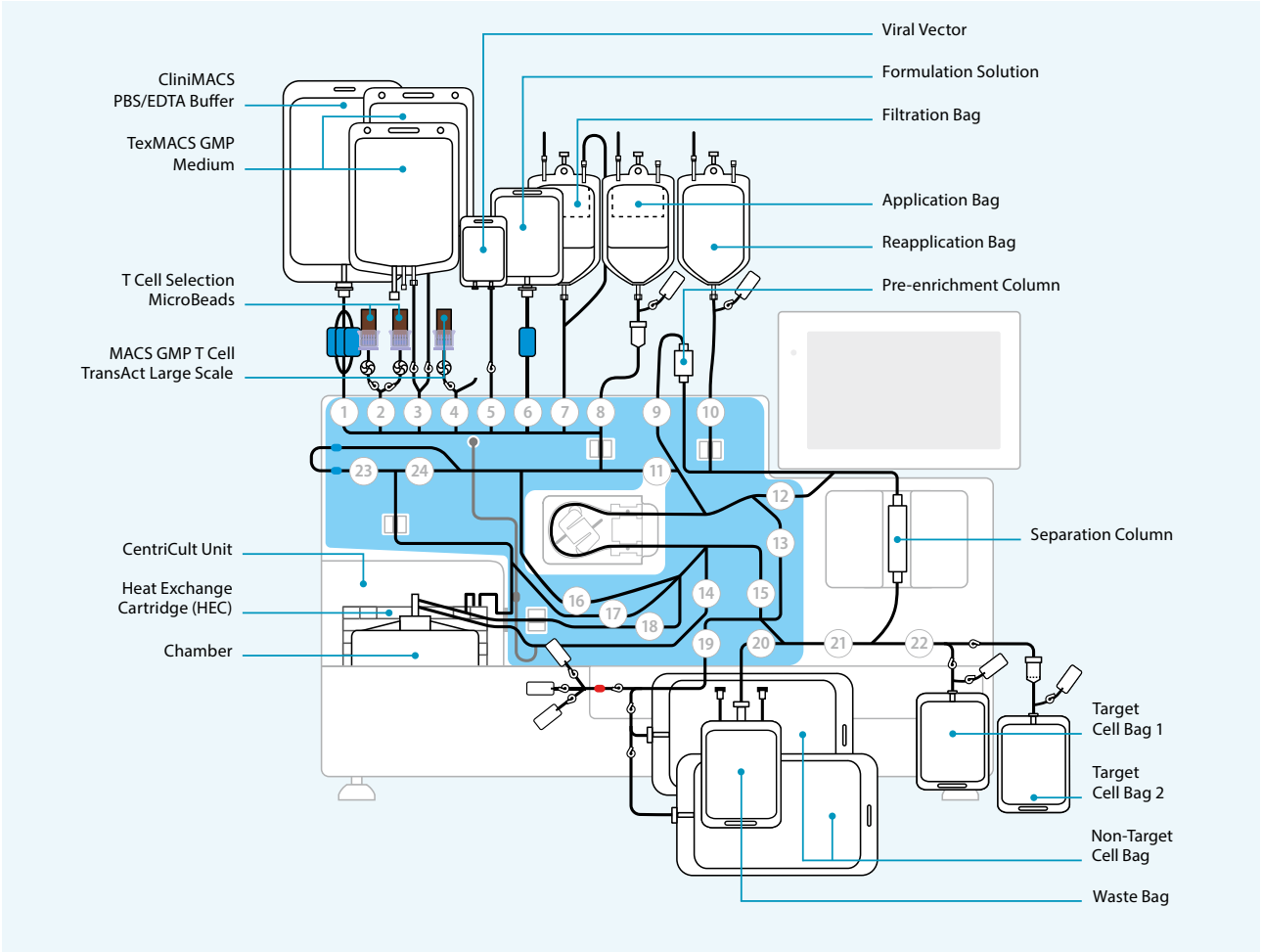
*Depending on the chosen protocol some consumables might not be needed. Contact your Miltenyi Biotec representative for support.

Process overview

Pre-process	Tubing set installation and priming ▼ Connection of fresh or frozen cell product ▼
T cell enrichment	T cell enrichment (CD4/CD8) ▼
Activation	Stimulation of T cells with MACS GMP T Cell TransAct Large Scale ▼
Viral transduction	Lentiviral or retroviral (+Vectofusin-1) ▼
Cell expansion	Expansion in TexMACS Medium with AB serum, IL-7, and IL-15 ▼
Cell harvest and formulation	Four different harvest types available offering optional rebuffering step and optional transfer of cells into Target Cell Bag ▼
Post-process	Tubing set deinstallation ▼
	12 days recommended**

** The duration of the T Cell Transduction – Large Scale Process depends on the required cell numbers.

CliniMACS Prodigy TS 620 setup for T Cell Transduction – Large Scale



Performance data

	Start product	Isolated cells		Final product				
Conditions	CD4⁺ and CD8⁺ T cells (%)	CD4⁺ and CD8⁺ T cells (%)	CD3⁺ cells viability (%)	CD4⁺ and CD8⁺ T cells (%)	CD3⁺ T cells ($\times 10^{10}$)	CAR⁺ T cells (%)	Viability (%)	CAR⁺ T cell number ($\times 10^{9}$)
Untransduced								
Fresh LP, IL-7 and IL-15 (n = 1 separation, n = 2 culture)	57.79	91.00	98.81	96.69±0.08	1.80±0.11	–	95.12±0.39	–
Transduced								
Fresh LP, IL-7 and IL-15 (n = 2)	44.61 ±6.57	86.60 ±3.81	98.38 ±0.56	97.31 ±0.96	1.71 ±0.05	29.55 ±0.79	96.63 ±0.08	5.03 ±0.27
Frozen LP, IL-7 and IL-15 (n = 3)	*48.30 ±4.02	84,76 ±2.25	94,43 ±3.06	96.40 ±1.25	1.93 ±0.15	41.98 ±13.70	94.79 ±1.94	7.67 ±1.87
Fresh LP, IL-2 (n = 1)	36.91	74.92	98.06	90.63	1.62	29.69	91.30	4.81
Frozen LP, IL-2 (n = 1)	*53.98	81.82	90.54	97.11	1.46	58.06	98.08	8.48

*Sample taken after thawing from application bag

Table 1: Internal data showing performance data of several TCT-LS runs. Fresh or frozen leukapheresis from healthy donors was used as a starting product. The culture was performed with media supplementation of IL-2, or a combination of IL-7 and IL-15. Two runs were performed without transduction, and the remaining seven with lentiviral transduction. Following T cell isolation, high T cell purities and viabilities were observed in all conditions. High expansion rates and viabilities were also observed after culture. The different transduction efficiencies between process runs are most likely a donor variability effect. This data demonstrates that the TCT-LS is robust enough to deal with variable conditions and still produce a good result.

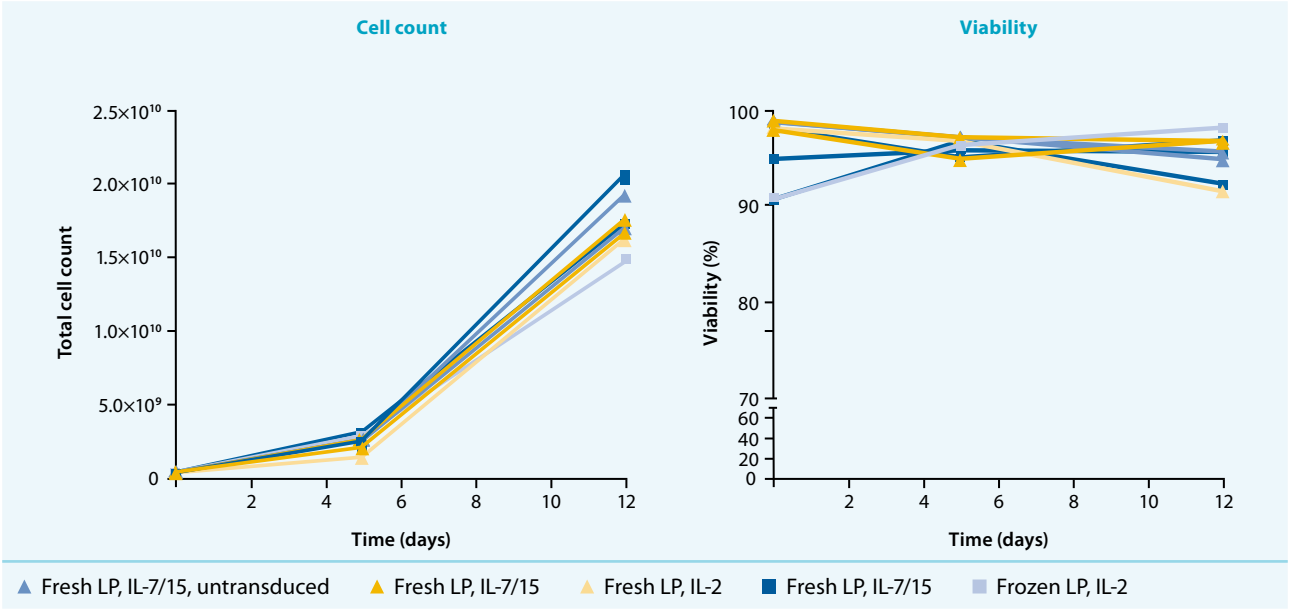


Figure 1: Internal data showing the cell expansion and viability during a complete TCT-LS Process. Different starting products and culture conditions were used, and remarkedly, no huge difference between the variable conditions were observed, demonstrating the robustness of the process. As depicted in the left graph, cell expansion markedly increased after day six, reaching up to 2.05×10^{10} T cells in the end product, whilst viability remained >90% during the whole process.

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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**
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