



# Large-scale manufacturing of transduced T cells

## CliniMACS Prodigy® T Cell Transduction – Large Scale

### Application

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

<b>Process name:</b>	T Cell Transduction – Large Scale
<b>Selection capacity:</b>	Up to $3 \times 10^9$ labeled cells within $10 \times 10^9$ WBCs
<b>Sample volume for selection:</b>	Fresh: 50–600 mL Frozen: 50–300 mL
<b>TransAct™ Large Scale stimulation capacity:</b>	$4 \times 10^8$ T cells recommended
<b>Expansion capacity:</b>	$3 \times 10^7$ T cells/mL in max. 600 mL culture volume
<b>Final product harvest volume:</b>	Four different harvest types available: <ul style="list-style-type: none"> <li>• Optional rebuffering step</li> <li>• Optional transfer of cells into Target Cell Bag</li> </ul>
<b>Process time:</b>	12 days recommended

### Products required

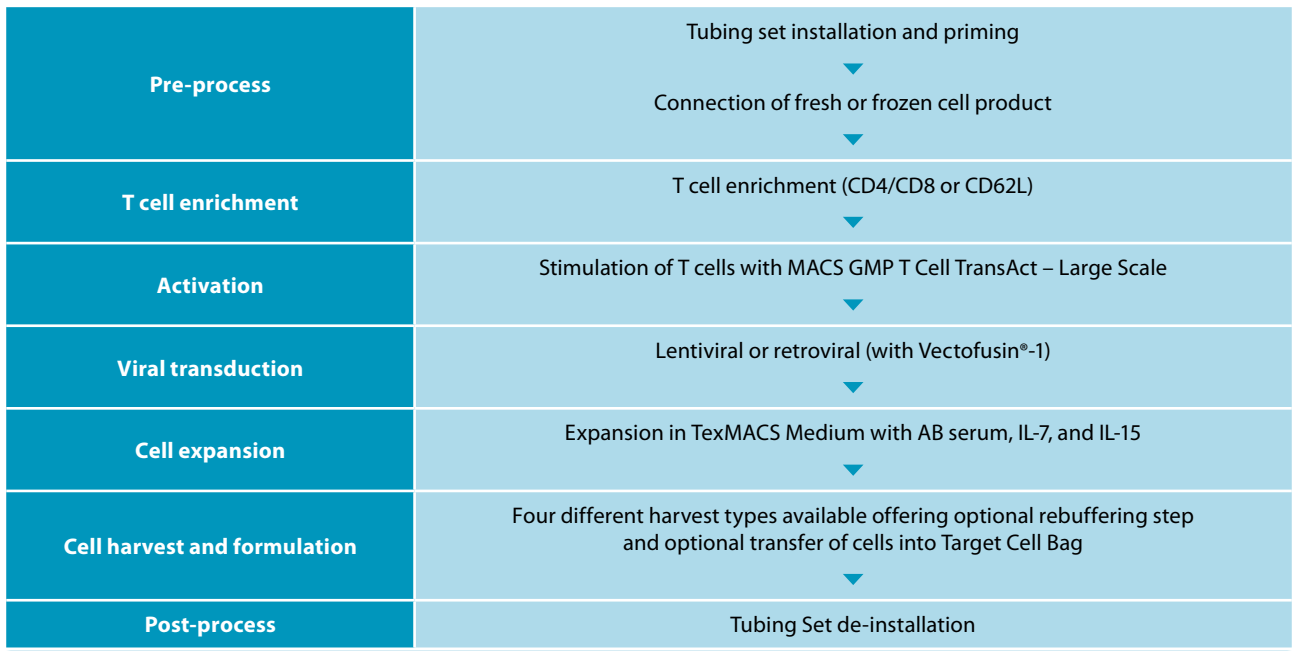
CliniMACS® and MACS® GMP Products	Amount required
Application software for the CliniMACS Prodigy with T Cell Transduction – Large Scale	1
CliniMACS CD4 Reagent CR/GMP*	1 piece
CliniMACS CD8 Reagent CR/GMP*	1 piece
CliniMACS CD62L Reagent CR/GMP	1 piece
CliniMACS Prodigy TS 620	1 piece
CliniMACS PBS/EDTA 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

\*Alternatively, available with CE certification.

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 <sub>2</sub> 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.

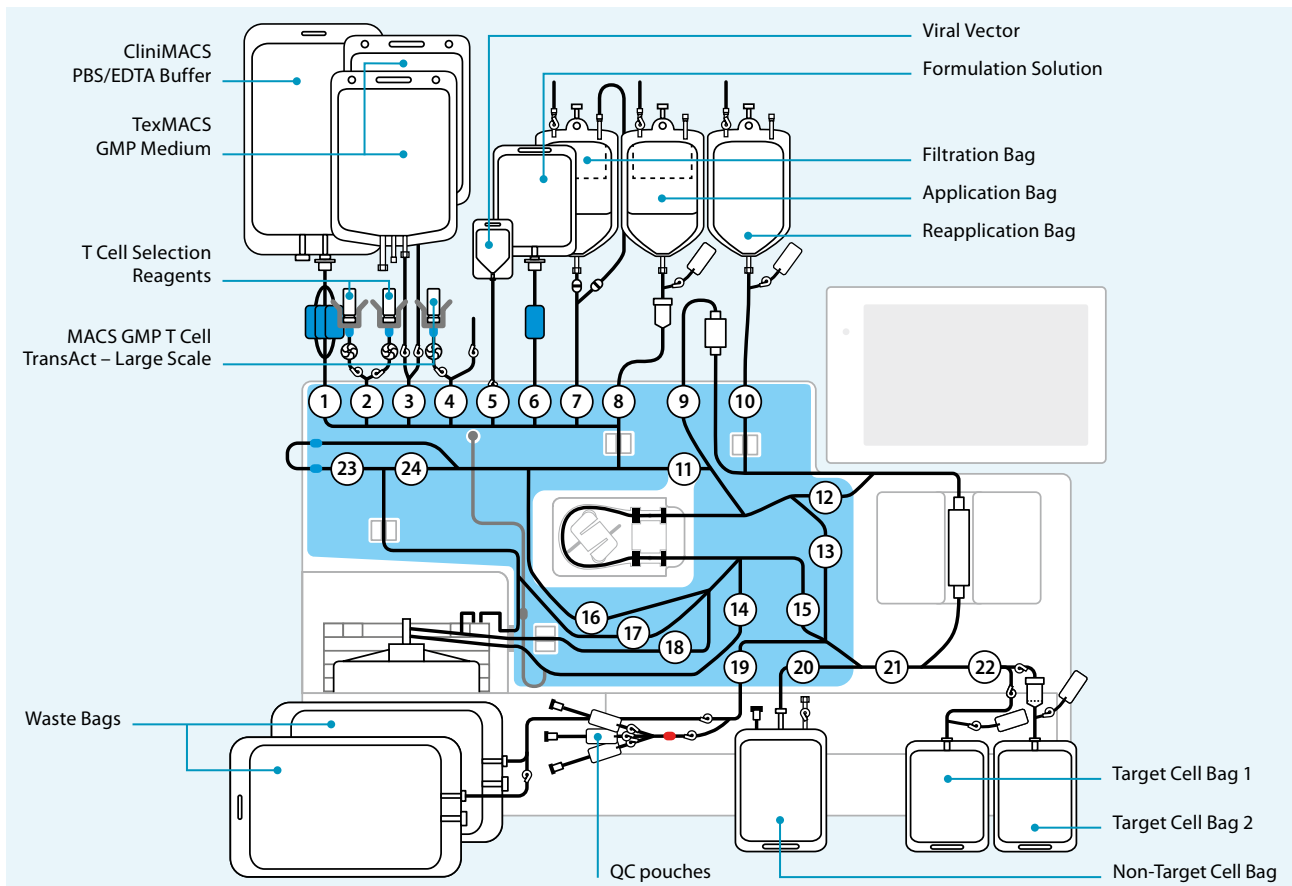
## Process overview



**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

Conditions	Start product	Isolated cells		Final product				
	CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells (%)	CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells (%)	CD3 <sup>+</sup> cells viability (%)	CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells (%)	CD3 <sup>+</sup> T cells ( $\times 10^{10}$ )	CAR <sup>+</sup> T cells (%)	Viability (%)	CAR <sup>+</sup> T cell number ( $\times 10^9$ )

### Untransduced

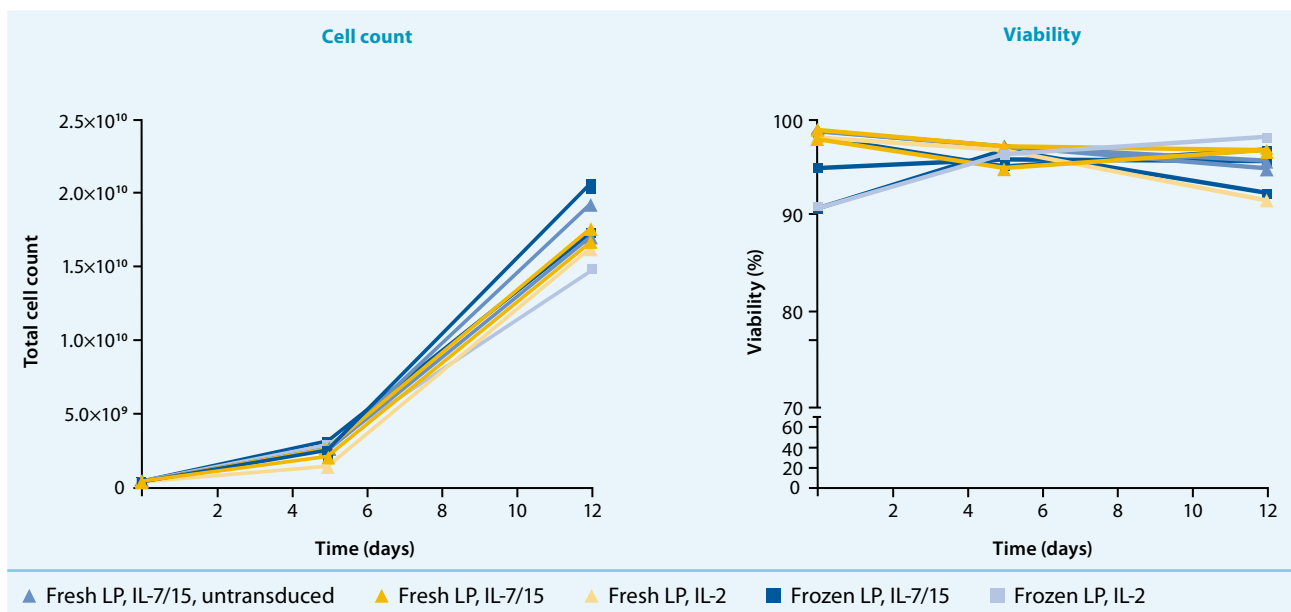
Fresh LP, IL-7 and IL-15 (n = 1 separation, n = 2 culture)	57.79	91.00	98.81	96.69 $\pm$ 0.08	1.80 $\pm$ 0.11	–	95.12 $\pm$ 0.39	–
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### Transduced

Fresh LP, IL-7 and IL-15 (n = 2)	44.61 $\pm$ 6.57	86.60 $\pm$ 3.81	98.38 $\pm$ 0.56	97.31 $\pm$ 0.96	1.71 $\pm$ 0.05	29.55 $\pm$ 0.79	96.63 $\pm$ 0.08	5.03 $\pm$ 0.27
Frozen LP, IL-7 and IL-15 (n = 3)	*48.30 $\pm$ 4.02	84.76 $\pm$ 2.25	94.43 $\pm$ 3.06	96.40 $\pm$ 1.25	1.93 $\pm$ 0.15	41.98 $\pm$ 13.70	94.79 $\pm$ 1.94	7.67 $\pm$ 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 on the performance of several TCT-LS runs. Fresh or frozen leukapheresis from healthy donors was used as the starting product. The culture was performed with media supplemented with 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 difference in transduction efficiencies between process runs is most likely an effect of donor variability. This data demonstrates that the TCT-LS is robust enough to handle 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 remarkably, no major differences between the variable conditions were observed, demonstrating the robustness of the process. As depicted in the left graph, cell expansion increased significantly after day six, reaching up to  $2.05 \times 10^{10}$  T cells in the end product, whilst viability remained  $>90\%$  during the whole process.



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MACS GMP Products are for *ex vivo* cell processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative. MACS GMP Products are designed, manufactured and tested under an ISO 13485 quality management system and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. The manufacturing and testing of MACS GMP Products of biological origin are in compliance with EP chapter 5.2.12 for "Raw materials of biological origin for the production of cell-based and gene therapy medicinal products".

The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485.

In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans, the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"), or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.

In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE) or FDA approval.

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