



Miltenyi Biotec



Automated T cell manufacturing from start to fill

CliniMACS Prodigy® T Cell Transduction and Automated Formulation

Application

This application enables automated manufacturing of gene-engineered T cells, covering all steps from processing various input materials to formulation and fill of the final drug product. It supports flexible formulation in different volumes, concentrations, and doses.

This application sheet provides an overview of the CliniMACS Prodigy T Cell Transduction and Automated Formulation (TCTf) Process, including specifications, required materials, workflow, internal performance data, and tubing set setup.

Specifications

Process name:	T Cell Transduction and Automated Formulation
Sample volume for selection:	50–300 mL
Selection capacity:	Up to 3×10^9 labeled cells within 20×10^9 WBCs
TransAct™ stimulation capacity:	Recommended 1×10^8 T cells
Expansion capacity:	$\sim 5 \times 10^9$ T cells in 12 days
Final product filling volume:	Up to 8 product bags
bags 1–2:	Fresh 10–300 mL / Frozen 55–100 mL
bags 3–4:	Fresh 10–250 mL / Frozen 30–70 mL
bags 5–8:	Fresh 10–50 mL / Frozen 10–30 mL
Process time:	Flexible (default: 12 days)

Products required

CliniMACS® and MACS® GMP Products	Amount required
CliniMACS Prodigy	1 unit
CliniMACS Formulation Unit	1 unit
Application software for the CliniMACS Prodigy T Cell Transduction and Automated Formulation	1
CliniMACS Prodigy TS 521	1 set
CliniMACS CD62L Reagent CR/GMP or CliniMACS CD4 and CD8 Reagents CR/GMP	1 vial each
CliniMACS PBS/EDTA Buffer (3 L)	1 piece
CliniMACS Formulation Solution	2 × 1 L bags
TexMACS™ GMP Medium	3 × 2 L bags
MACS GMP Recombinant Human IL-2 or IL-7 and IL-15	3 vials each
MACS GMP T Cell TransAct	1 vial

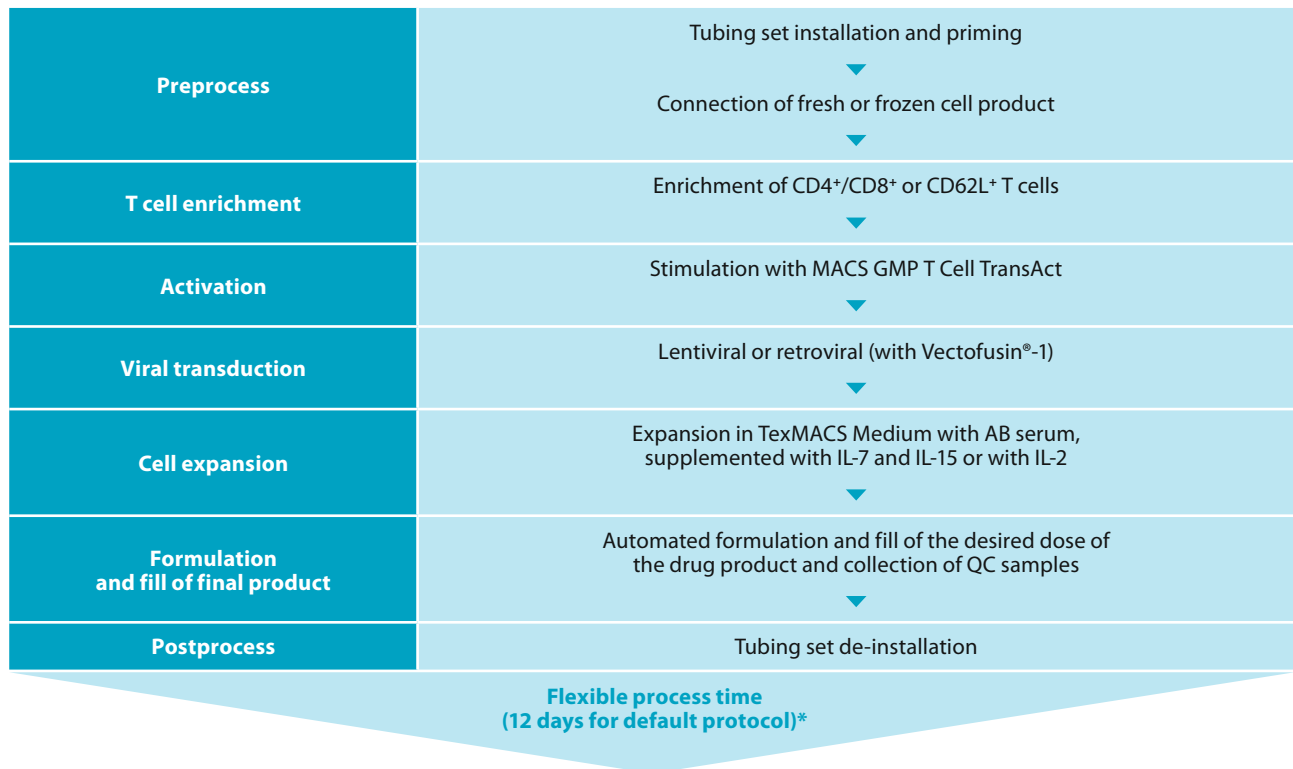
Additional material/equipment*

- Viral vector
- Human serum albumin and human AB serum
- MACS GMP Vectofusin®-1
- Cryopreservation solution: e.g., CliniMACS Cryo Supplement
- Sterile water, syringes, hypodermic needles
- Sterile tube welder
- Uninterrupted power supply
- CO₂ 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



*Process duration depends on target cell yield.

Performance data

Conditions	Start product	Isolated cells			Final product			
	CD4 ⁺ and CD8 ⁺ T cells (%)	CD4 ⁺ and CD8 ⁺ T cells (%)	CD3 ⁺ cell viability (%)	CD4 ⁺ and CD8 ⁺ T cells (%)	CD3 ⁺ T cells (× 10 ⁹)	CAR ⁺ T cells (%)	CD3 ⁺ cell viability (%)	CAR ⁺ T cell number (× 10 ⁹)
Fresh LP, IL-7/IL-15 (n = 1)	45.07	79.25	98.62	95.40	4.92	44.95	98.06	2.21
Frozen LP, IL-7/IL-15 (n = 4)	43.07 ±8.65	75.82 ±2.83	91.46 ±2.96	97.87 ±0.54	5.09 ±0.31	39.24 ±4.12	96.43 ±0.76	1.99 ±0.23
Frozen LP, IL-2 (n = 2)	33.45 ±4.29	75.89 ±5.71	92.92 ±1.63	95.36 ±1.46	4.47 ±0.39	47.46 ±5.62	98.21 ±0.89	2.14 ±0.44

Table 1: Internal data on the performance of several TCTf process runs. Fresh or frozen leukapheresis products from healthy donors were used as the starting material. Cultures were supplemented with IL-2 or a combination of IL-7 and IL-15 (IL-7/15) in TexMACS Medium. 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 TCTf process is robust enough to handle both fresh and frozen starting materials and enables successful T cell expansion under different cytokine conditions, yielding clinically relevant CAR T cell doses with high viability.

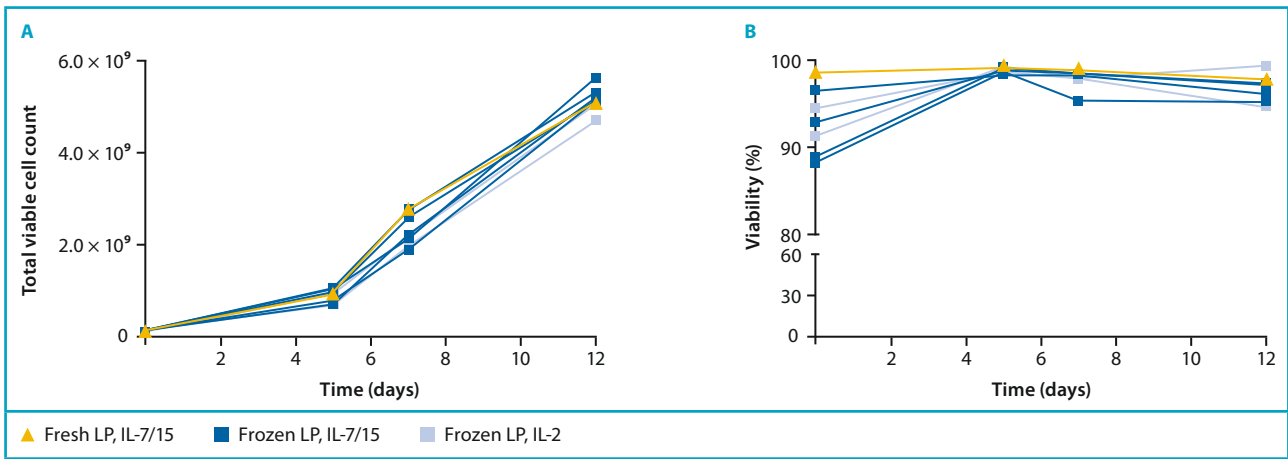


Figure 1: Internal data showing (A) total viable CD3⁺ T cell expansion and (B) overall viability over a 12-day TCTf process. Starting material was either fresh-in or frozen-in leukapheresis (LP) from healthy donors. Cultures were supplemented with IL-2 or IL-7/15 in TexMACS Medium. T cell expansion increased significantly after day 5, reaching up to 5.7×10^9 viable cells, with viabilities remaining above 95% across all conditions.

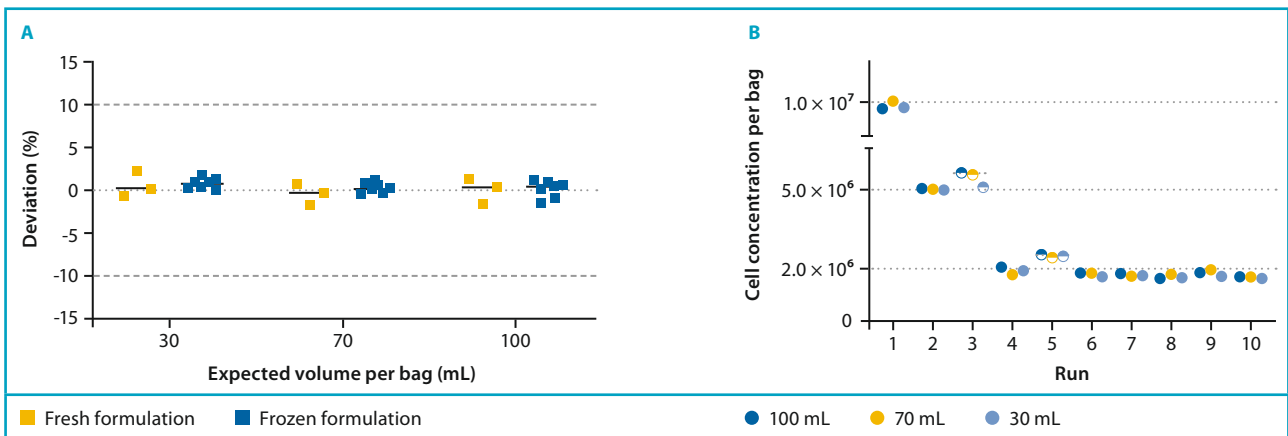


Figure 2: Performance of automated formulation and filling. For each run, three product bags (30 mL, 70 mL, and 100 mL) were filled under fresh or frozen conditions. Fresh formulation was performed in formulation solution, while frozen formulation included cryopreservation solution. (A) Deviation from expected fill volumes for fresh and frozen formulation conditions. Volume deviation was calculated as the difference between the expected and measured fill volumes, and remained within $\pm 3\%$ for all tested conditions. (B) CAR T cell concentration per bag from each run. Dotted lines represent target concentrations for drug product. Mean absolute deviation (MAD) from the target concentration ranged from 0.5% to 16% across all three bags. Half-filled dots indicate the target concentration with an additional 10% overage.

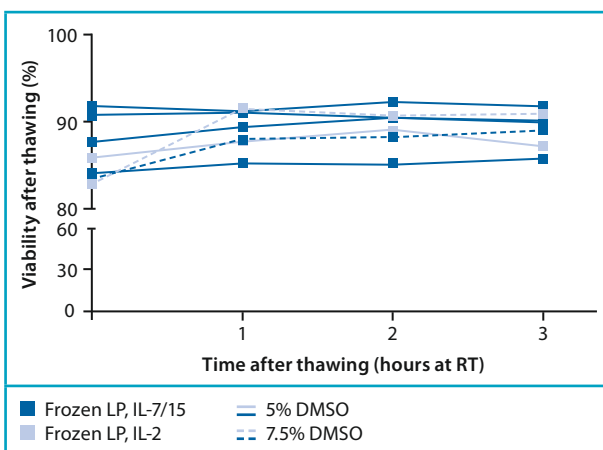
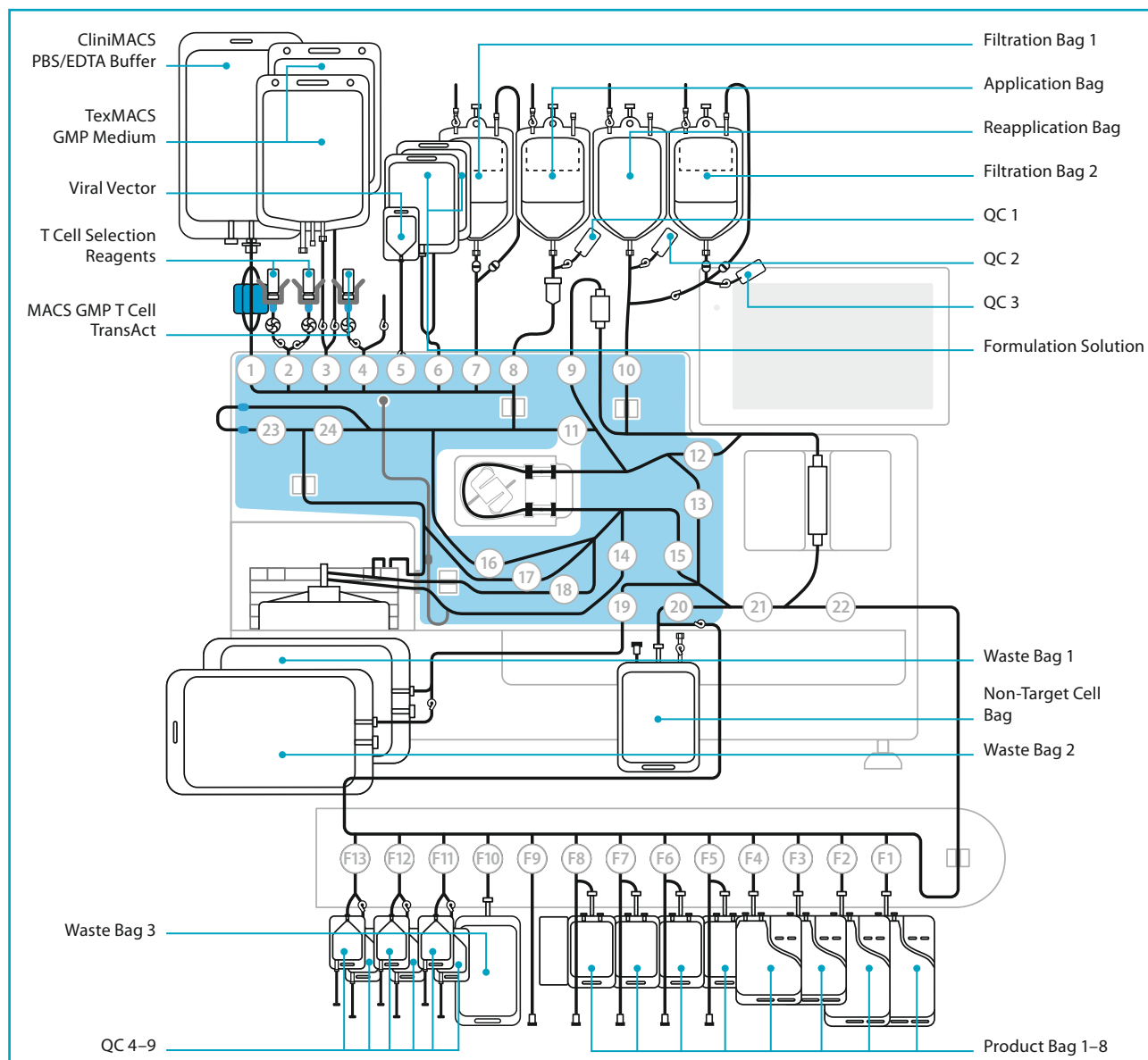


Figure 3: Viability after thawing of drug product bags following frozen formulation. CAR T cell products, previously cultured in the presence of IL-2 or IL-7/15, were thawed in a water bath and held at room temperature (RT) for 1, 2, or 3 hours before viability assessment via 7-AAD staining. Viabilities remained high under all tested conditions, with values consistently above 80%. Cryopreservation was performed using two DMSO concentrations: 5% and 7.5% in the final drug product.

CliniMACS Prodigy TS 521 setup for T Cell Transduction and Automated Formulation



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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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