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:	~ 5 × 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
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

Preprocess	Tubing set installation and priming ▼ Connection of fresh or frozen cell product ▼
T cell enrichment	Enrichment of $CD4^+$/$CD8^+$ or $CD62L^+$ T cells ▼
Activation	Stimulation with MACS GMP T Cell TransAct ▼
Viral transduction	Lentiviral or retroviral (with Vectofusin®-1) ▼
Cell expansion	Expansion in TexMACS Medium with AB serum, supplemented with IL-7 and IL-15 or with IL-2 ▼
Formulation and fill of final product	Automated formulation and fill of the desired dose of the drug product and collection of QC samples ▼
Postprocess	Tubing set de-installation

Flexible process time (12 days for default protocol)*

*Process duration depends on target cell yield.

Performance data

	Start product	Isolated cells		Final product				
Conditions	**$CD4^+$ and $CD8^+$ T cells (%)**	**$CD4^+$ and $CD8^+$ T cells (%)**	**$CD3^+$ cell viability (%)**	**$CD4^+$ and $CD8^+$ T cells (%)**	**$CD3^+$ T cells ($\times 10^9$)**	**CAR^+ T cells (%)**	**$CD3^+$ cell viability (%)**	**CAR^+ T cell number ($\times 10^9$)**
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.

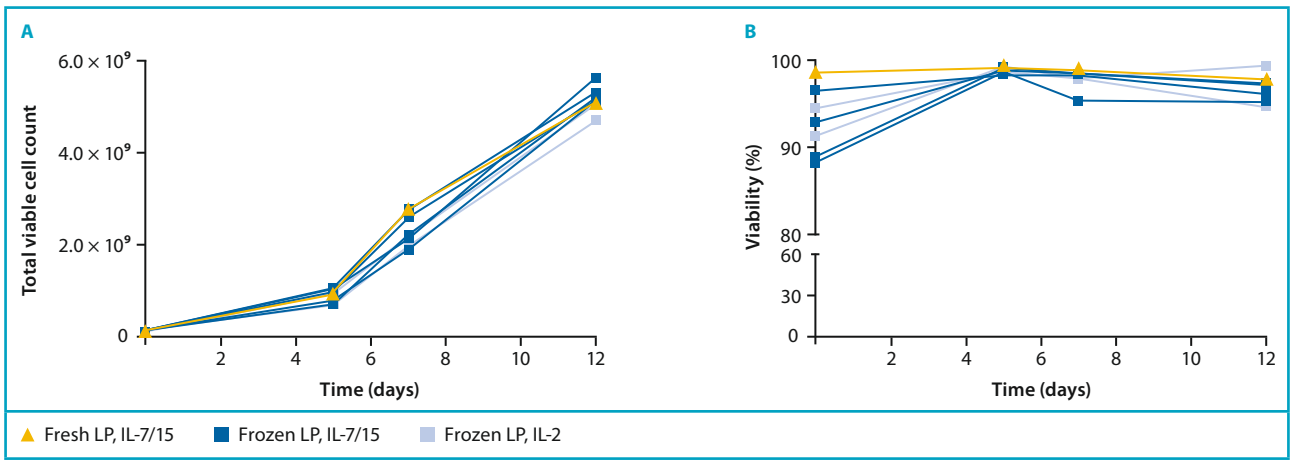


Figure 1: Internal data showing (A) total viable CD3⁺T cell expansion and (B) overall viability over a 12-day TCTf process. Starting material was either fresh-in or frozen-in leukapheresis (LP) from healthy donors. Cultures were supplemented with IL-2 or IL-7/15 in TexMACS Medium. T cell expansion increased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

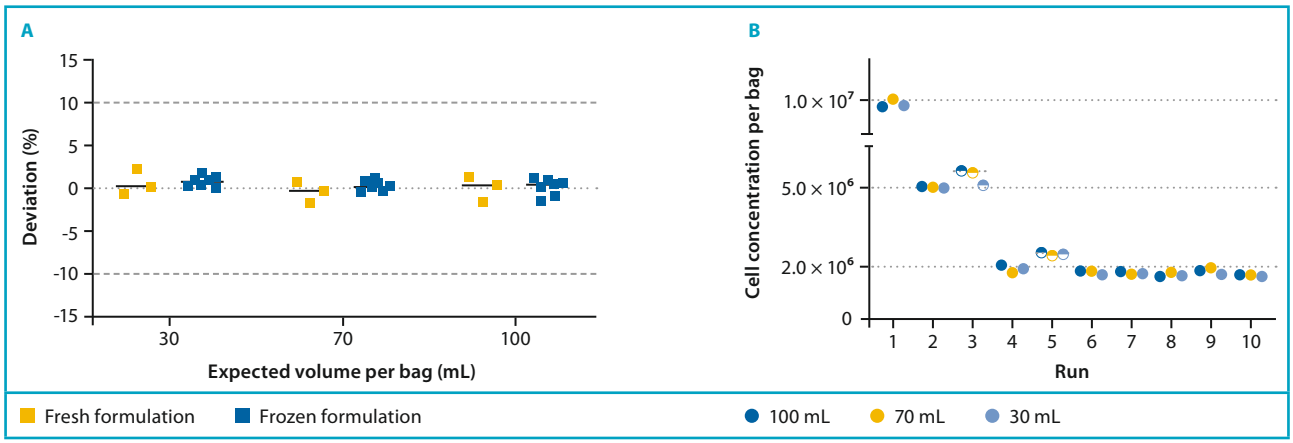


Figure 2: Performance of automated formulation and filling. For each run, three product bags (30 mL, 70 mL, and 100 mL) were filled under fresh or frozen conditions. Fresh formulation was performed in formulation solution, while frozen formulation included cryopreservation solution. (A) Deviation from 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 ±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.

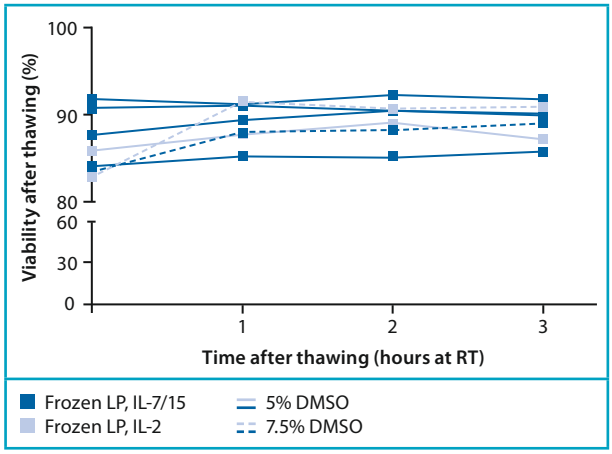
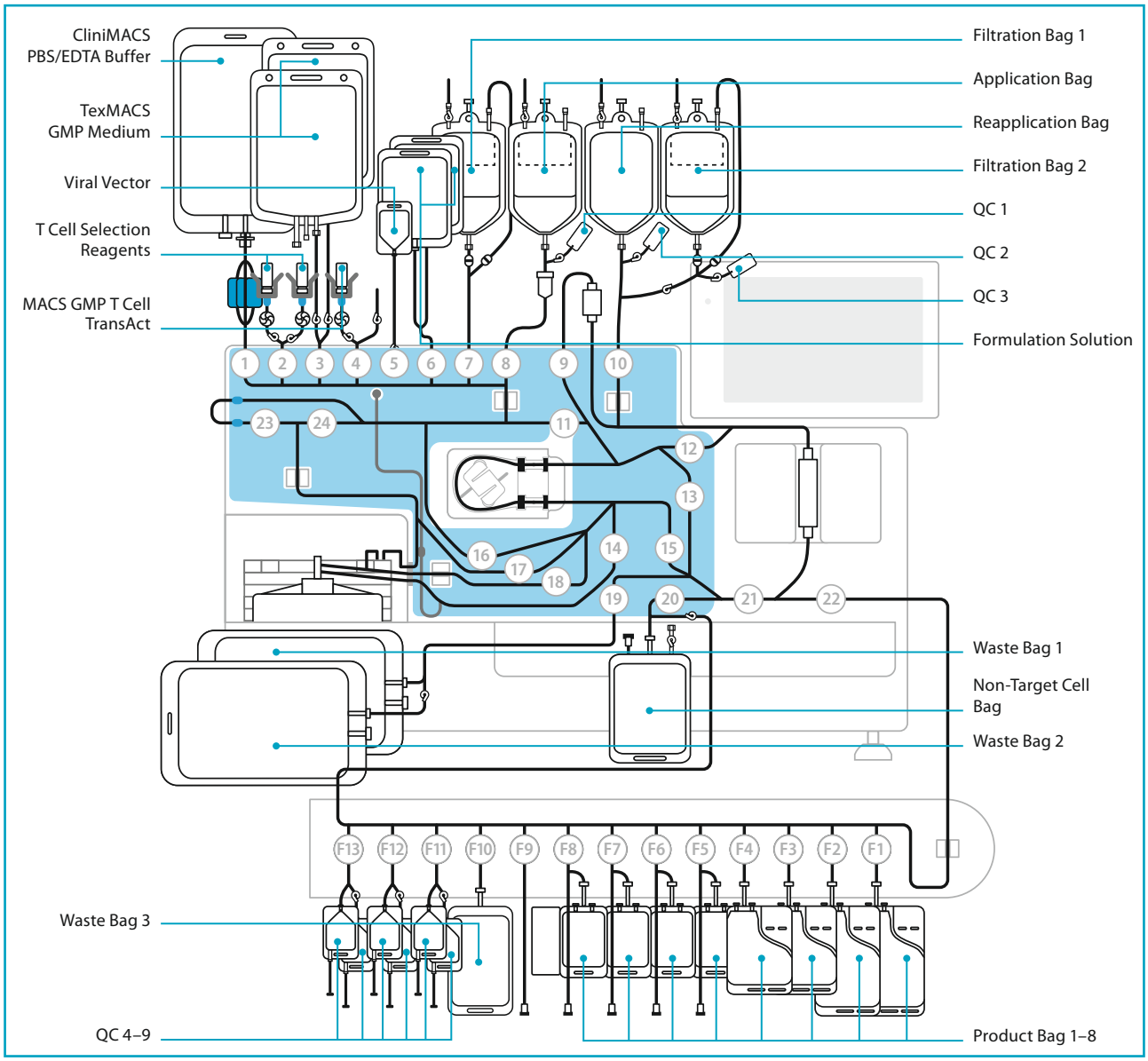


Figure 3: Viability after thawing of drug product bags following frozen formulation. CAR T cell products, previously cultured in the presence of IL-2 or IL-7/15, were thawed in a water bath and held at room temperature (RT) for 1, 2, or 3 hours before viability assessment via 7-AAD staining. Viabilities 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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