Miltenyi Biotec

Generation of gene-engineered T cells via electroporation and/or transduction

CliniMACS Prodigy® T Cell Engineering

Application

Fully automated cell labeling, enrichment, activation, genetic engineering via electroporation and/or transduction and expansion of human T cells from patient material for production of gene-engineered T cells. The flexible combination of electroporation and transduction within one closed manufacturing process enables complex gene-editing applications.

This application sheet gives an overview of the specifications and materials needed to perform T cell engineering (TCE) on the CliniMACS Prodigy in combination with the CliniMACS® Electroporator. Furthermore, it illustrates the process workflow, as well as the configuration of the CliniMACS Prodigy Tubing Set, and provides performance data.

Specifications

Process name:	T Cell Engineering		
Selection capacity:	Up to 3×10^9 cells		
Sample volume for selection:	50–280 mL		
TransAct™ stimulation capacity:	$0.2–2\times10^8$ cells (2×10^8 recommended for electroporation)		
Electroporation pulses:		**1st pulse**	**2nd pulse**
	Voltage (V)	50–1000	50–500
	Length (µs)	5–100,000	0–100,000
	Mode	Square, burst	Square, burst
	Polarity	unipolar, bipolar	unipolar
	Burst length (µs)	5–100,000	5–100,000
Cell volume for electroporation:	20–157 mL		
Cell density for electroporation:	Recommended 1×10^7 cells/mL (1×10^6 to 5×10^7 cells/mL)		
Final product harvest volume:	Harvest type 1: 100 mL Harvest type 2: up to 900 mL		
Process time:	10–14 days		

Products required*

Devices	Comment
CliniMACS Prodigy with T Cell Engineering	
CliniMACS Electroporator	For electroporation

CliniMACS and MACS GMP products	Comment
CliniMACS CD62L Reagent / GMP MicroBeads or CliniMACS CD4 and CD8 Reagent / GMP MicroBeads	For T cell isolation
CliniMACS Prodigy TS 520	
CliniMACS PBS / EDTA Buffer	For T cell isolation and optional cell wash
CliniMACS Electroporation Buffer	For electroporation
TexMACS® GMP Medium	For cell cultivation
MACS® GMP Recombinant Human IL-7 / IL-15 or IL-2	For cell cultivation
MACS GMP T Cell TransAct	For T cell activation

Additional material / equipment	Comment
Viral vector and / or nucleic acid or nucleoprotein complex to be electroporated	Depending on whether transduction and/ or electroporation is desired
Triple Sampling Adapter (Miltenyi Biotec)	For additional sampling
Sterile water, syringes, hypodermic needles or Cytokine Vial Adapter (Miltenyi Biotec)	For cytokine reconstitution or For cytokine reconstitution in a closed system
MACS GMP Vectofusin-1 (Miltenyi Biotec)	For transduction, optional
Formulation solution (e.g. CliniMACS Formulation Solution)	For formulation of cells during harvest
Human Serum Albumin	For CliniMACS PBS/EDTA Buffer supplementation
Human AB serum	For addition to medium, optional
Sterile tube welder	For sterile tube connection
Uninterruptable power supply	As safety measurement
CO_2 and compressed air supply	For cell cultivation
Cell counter and / or flow cytometer	For IPC and QC

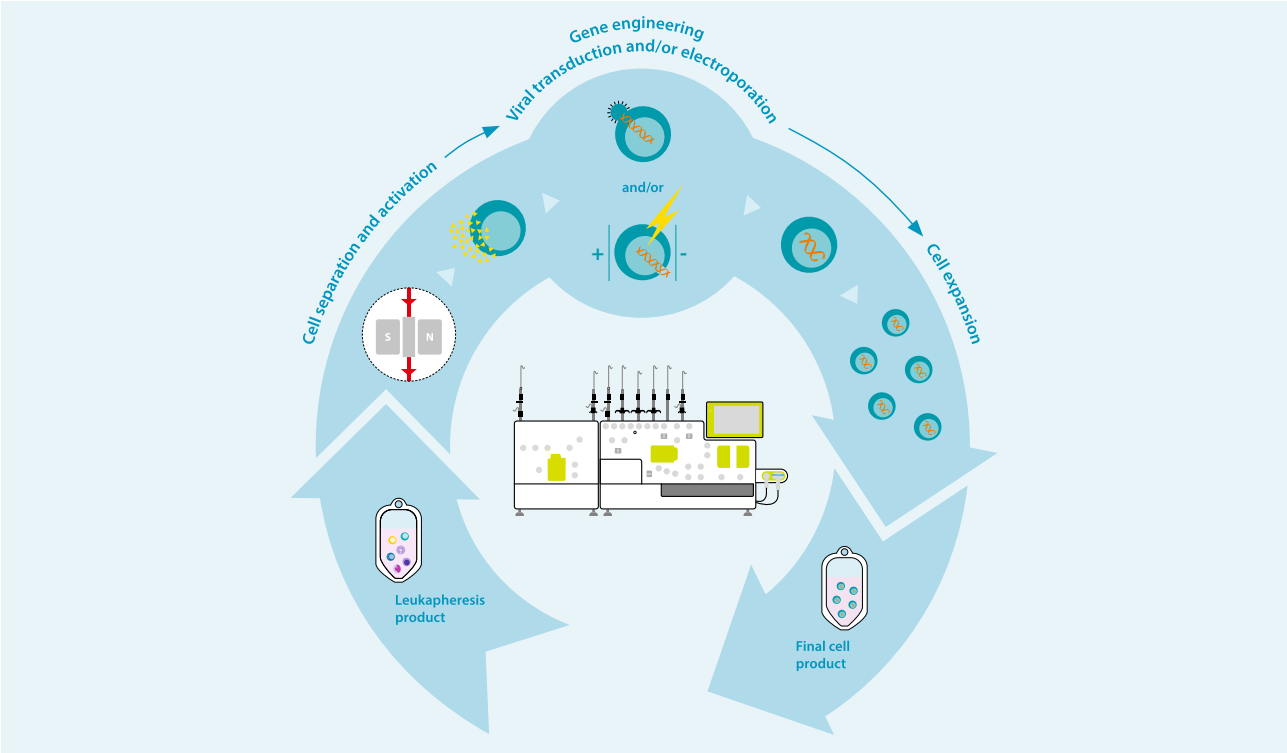
*Depending on the chosen protocol some consumables might not be needed at all or in various amounts. Please contact your responsible Miltenyi Biotec representative for support.

Process overview

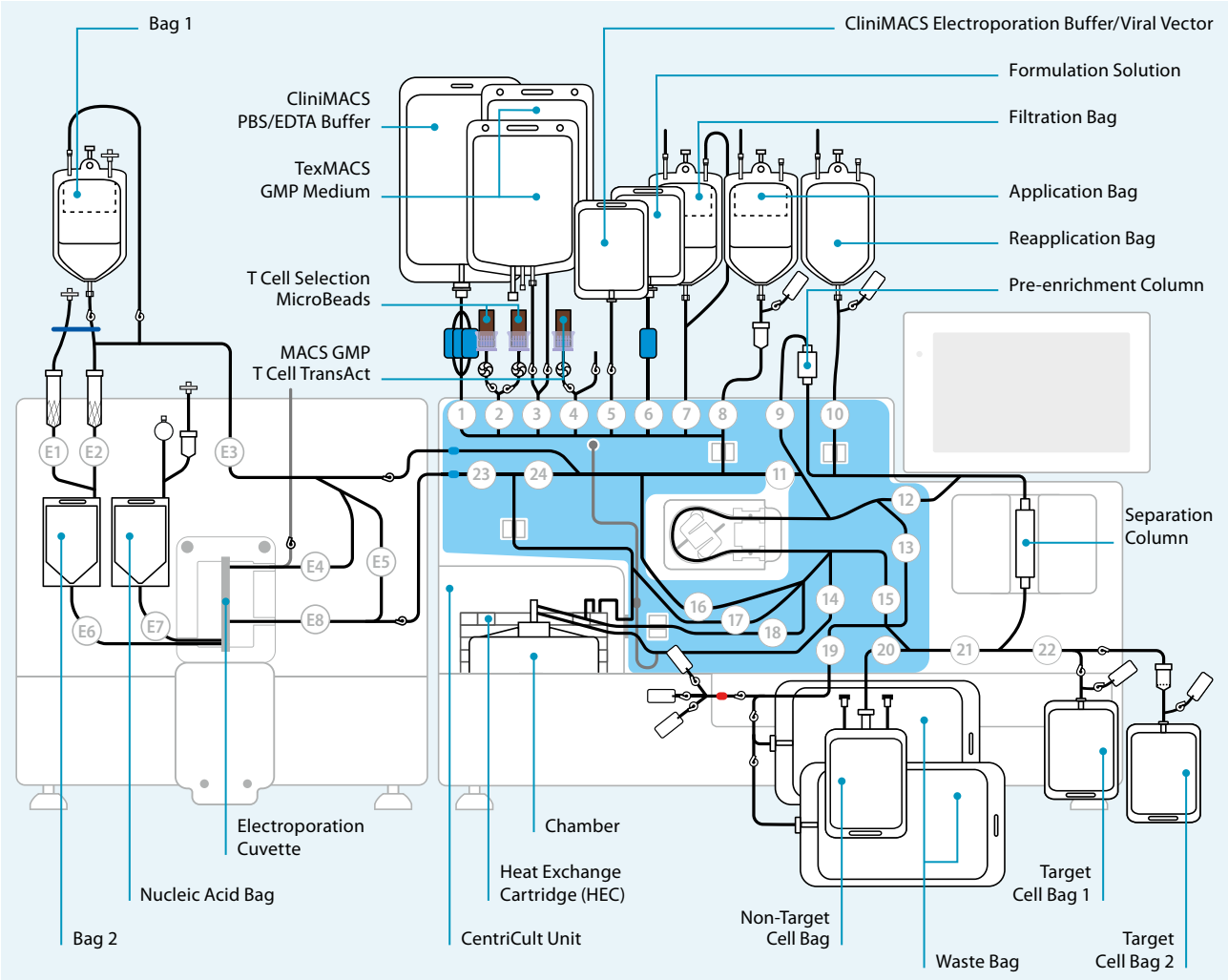
Pre-process	Tubing set installation ▼ Tubing set priming (buffer and medium) ▼
Enrichment	Enrichment preparation ▼ Enrichment CD4 / CD8 or CD62L ▼
Culture preparation	Connection of culture materials and entry of cultivation parameters ▼
Cultivation	Culture setup (rinsing with medium, cell seeding, activation) ▼ Cultivation with flexible activities (e.g. electroporation, feeding, media exchange, sampling, etc.) ▼
Post-process	Formulation and harvest ▼
Process time (approx.)	10–14 days

Table 1: Overview of the full T cell engineering process. When choosing the full process option, T cell enrichment, culture preparation and cultivation are performed. Other process options (so called cases) are available for flexible protocol adaption, e.g., option to start the process without T cell enrichment and process resume cases (not shown).

Principle of CliniMACS Prodigy T Cell Engineering



CliniMACS Prodigy TS 520 setup for T cell engineering



Performance data

N=3	Starting material	Isolated cells	
		Purity (%)	Viability (%)
Healthy donor	39.5% ± 9.9%	83.0% ± 10.8%	98% ± 0%

Table 2: Cell separation performance data for CD4⁺/CD8⁺ T cells. (Data from Alzubi, J. & Lock, D. *et al.* (2020))

N=3	Start of cultivation	Final cell product			
	Number of seeded CD4⁺/CD8⁺ T cells	Number of harvested CD4⁺/CD8⁺ T cells	CAR⁺ cells (%)	TCR ko cells (%)	Viability (%)
Healthy donor	$2.1\times10^8 \pm 0.47\times10^9$	$2.24\times10^9 \pm 1.46\times10^9$	52.5% ± 17.6%	34.4% ± 12.8%	93.3% ± 4.16%

Table 3: Cultivation and gene-editing performance data after viral transduction with the CAR and electroporation to knock out the endogenous TCR (Data from Alzubi, J. & Lock, D. *et al.* (2020)).

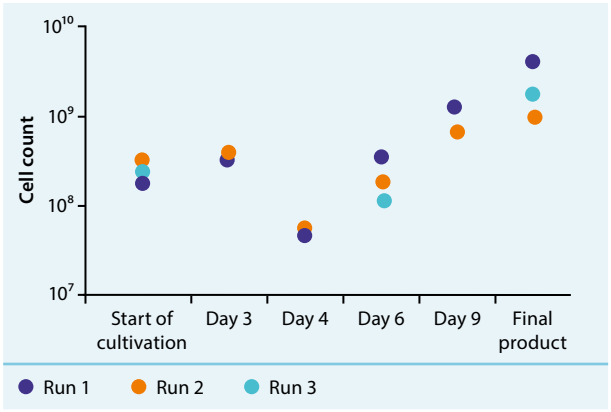


Figure 1: Total cell count over the whole manufacturing process (n=3). Cell numbers are dropping significantly after electroporation-induced stress on day three. However, cell numbers recover quickly afterwards (Data from Alzubi, J. & Lock, D. *et al.* (2020)).

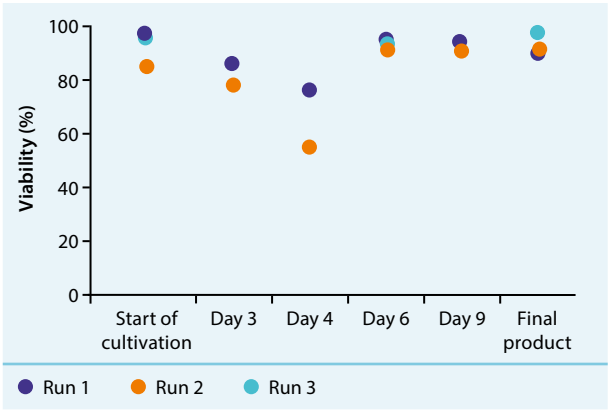


Figure 2: Viability of T cells after successful gene-editing using the CliniMACS Electroporator. After the electroporation step at day three cell viability is slightly decreased. At day six viability is already over 95% again (Data from Alzubi, J. & Lock, D. *et al.* (2020)).

References

Alzubi, J. & Lock, D. *et al.* (2020) Automated generation of gene-edited CAR T cells at clinical scale. Molecular Therapy – Methods & Clinical Development 20: 379–388.

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