Miltenyi Biotec

Manufacturing gene-engineered NK cells via viral transduction

CliniMACS Prodigy® NK Cell Transduction

Application

Fully automated manufacturing of genetically modified NK cells via viral transduction from fresh, unmobilized leukapheresis with the CliniMACS Prodigy NK Cell Transduction protocol comprises two parts, enabled by two different processes. Part 1 consists of fully automated depletion of T cells with the CliniMACS Prodigy CD3/CD56 System. Part 2 consists of subsequent enrichment, activation, transduction, expansion, and harvest of human NK cells with the CliniMACS Prodigy CD56 Engineering Process.

This process outline sheet gives an overview of the specifications and materials used for the CliniMACS Prodigy NK Cell Transduction. Furthermore, it illustrates the configuration of the required CliniMACS Prodigy Tubing Sets and provides performance data.

Specifications

Process names:	LP-3-56 Separation and PD-56 Engineering
Depletion capacity (LP-3-56 Separation):	Up to 9.6×10^9 $CD3^+$ cells within 40×10^9 white blood cells (WBCs) in 50–600 mL
Enrichment capacity (PD-56 Engineering):	Up to 5×10^9 $CD56^+$ cells within 20×10^9 WBCs in 50–650 mL
Starting cell number for culture:	Recommended $1–1.5\times10^8$ cells
Expansion capacity:	Up to 1.2×10^7 cells/mL in max. 250 mL culture volume
Final product harvest volume:	Formulation in 100 mL
Process time:	14 days recommended (up to 21 days enabled)

Products required*

CliniMACS Prodigy CD3/CD56 System

CliniMACS® and MACS® GMP products	Amount	Comment
CliniMACS Prodigy with LP-3-56 Separation software	1 instrument	
CliniMACS Prodigy TS 320	1 piece	For T cell depletion
CliniMACS CD3 Reagent (different regulatory variants available)	1 piece	For T cell depletion
CliniMACS PBS/EDTA Buffer 3 L	2 bags	For T cell depletion

* The amounts of some materials may differ depending on the chosen protocol.

CliniMACS Prodigy CD56 Engineering

CliniMACS and MACS GMP Products	Amount	Comment
CliniMACS Prodigy with PD-56 Engineering Software	1 instrument	
CliniMACS Prodigy TS 520	1 piece	For NK cell enrichment
CliniMACS CD56 Reagent (Different regulatory variants available)	1 vial	For NK cell enrichment
CliniMACS PBS/ EDTA Buffer 3 L	1 bag	For NK cell enrichment
NK MACS GMP Medium	2×2 L bags	For NK cell activation and expansion
MACS GMP Recombinant Human IL-2	2 vials (500 µg/ vial)**	For NK cell activation and expansion
MACS GMP Recombinant Human IL-15	2 vials (25 µg/vial)**	For NK cell activation and expansion
MACS GMP Recombinant Human IL-1β	1 vial (25 µg)	For initial NK cell activation only (optional)
CliniMACS Formulation Solution	1 L	For cell harvesting
MACS GMP Vectofusin®-1	1 vial	For transduction, will be premixed with viral vector (optional depending on vector type)

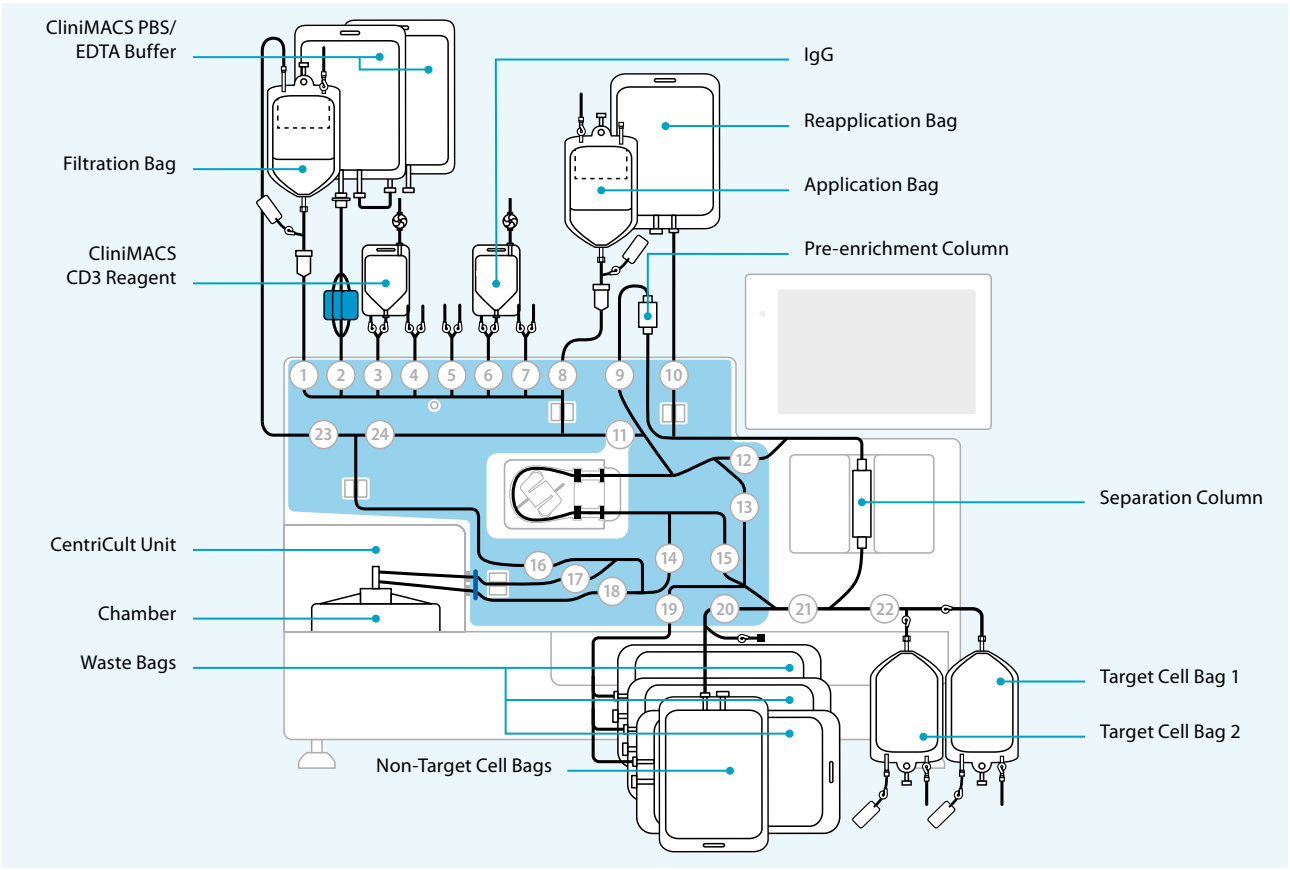
Additional material / equipment	Comment
IgG solution	For T cell depletion (10 mL of 5%)
Human Serum Albumin (HSA)	Pharmaceutical grade HSA, to supplement buffer (0.5%)
Transfer Set Coupler/ Coupler (Miltenyi Biotec)	Interconnection of CliniMACS PBS/EDTA Buffer bags
Luer/Spike Interconnector (Miltenyi Biotec) and 150 mL transfer bags	For connection of IgG to TS 320 For connection of IL-1β to TS 520
Viral vector (e.g. BaEV lentivirus)	For transduction
Triple Sampling Adapter (Miltenyi Biotec)	For every additional three samplings during cultivation
Sterile water, syringes, hypodermic needles or Cytokine Vial Adaptor (Miltenyi Biotec)	For cytokine reconstitution For cytokine reconstitution in a closed system
Human AB serum	Pharmaceutical grade, to supplement medium (5%)
Sterile tube welder	For sterile tube connection
Uninterruptable power supply	As safety precaution
CO_2 and compressed air supply	If cultivation is required
Cell counter and/or flow cytometer	For IPC and QC

** Number of vials may differ according to lot-specific activity. Different filling amounts are available. Please discuss your specific requirements with your Miltenyi Biotec representative.

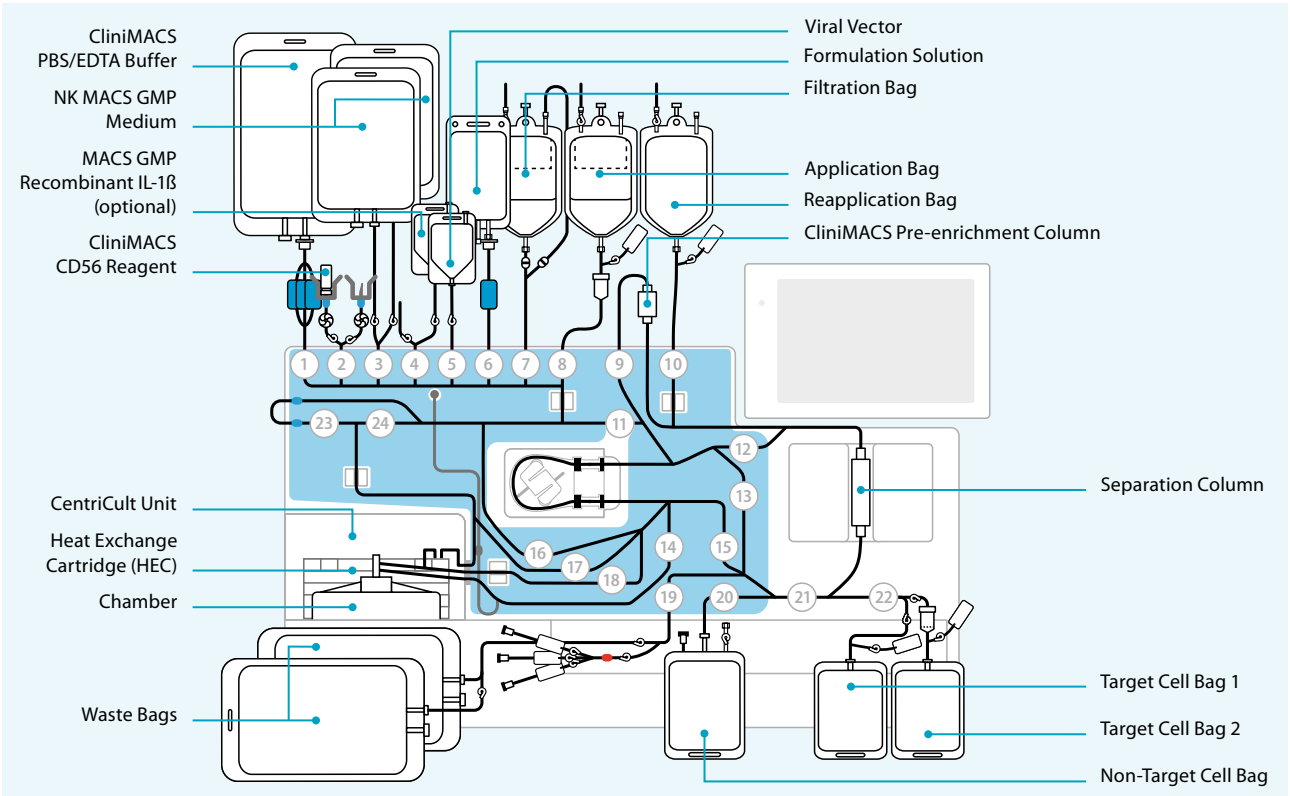
Process overview

	Part 1: LP-3-56 Separation (Case 2)	Part 2: PD-56 Engineering (Case 1)
Pre-process	Tubing set (TS) installation and priming ▼ Connection of starting materials to TS 320 ▼	Tubing set installation and priming ▼ Connection of starting materials to TS 520 ▼
T cell depletion	T cell depletion via CD3 ▼	–
NK cell enrichment	–	NK cell enrichment via CD56 ▼
Activation	–	Activation in NK MACS Medium containing IL-2 and IL-15 (and optional: IL-1β) ▼
Viral transduction	–	Viral vector (e.g. BaEV LV + MACS GMP Vectofusin-1) ▼
Cell expansion	– –	Culture wash ▼ Expansion in NK MACS Medium with IL-2 and IL-15 ▼
Cell harvest and formulation	–	Cells washed and harvested in 100 mL of formulation buffer ▼
Post-process	TS deinstallation	TS deinstallation
Process time	**3.5–5 h**	**14 days**

CliniMACS Prodigy TS 320 setup for CD3+ T cell depletion



CliniMACS Prodigy TS 520 setup for NK cell transduction



Performance data

Depletion and enrichment

	Starting material	Enriched cells				
	CD3⁻CD56⁺ NK cells (%)	Enriched CD3⁻CD56⁺ NK cell purity (%)	Enriched CD3⁻CD56⁺ NK cell viability (%)	Recovery of CD3⁻CD56⁺ NK cells (%)	T cell log depletion	B cell log depletion
Healthy donor (n = 7)	8.21 ±0.91	93.96 ±2.42	96.43 ±1.80	38.77 ±7.61	4.53 ±0.20	2.80 ±0.28

Table 1: Internal data showing performance of depletion of CD3⁺ cells and enrichment of CD56⁺ cells. NK cells were isolated by depleting CD3⁺ cells first with the CliniMACS Prodigy CD3/CD56 System and subsequent enrichment of CD56⁺ cells with the CliniMACS Prodigy CD56 Engineering Process. This data summarizes the results of seven independent manufacturing runs with fresh, unmobilized leukapheresis as starting material. A high T cell depletion performance was generally achieved, while the NK cell recovery may have differed due to donor variability. NK cell purity and viability was generally very high.

Transduction and expansion

	Start of cultivation	Final cell product				
	Number of seeded CD3⁻CD56⁺ NK cells	Number of harvested CAR⁺ NK cells	Number of harvested CD3⁻CD56⁺ NK cells	NK cell purity (%)	CAR⁺ NK cell frequency (%)	Viability (%)
Healthy donor (n = 7)	1.08×10^8 $\pm0.31\times10^8$	9.69×10^8 $\pm4.40\times10^8$	1.71×10^9 $\pm0.92\times10^9$	99.01 ±0.70	62.49 ±17.34	87.61 ±4.34

Table 2: Internal data showing performance of NK cell transduction and expansion. Enriched NK cells were transduced and expanded with the CliniMACS Prodigy CD56 Engineering Process. Cells were seeded on day zero, transduced on day two, and washed on day three. Regular feeding and media exchange steps were performed until the harvest on day 14. From day seven on, cells were kept shaking during the remaining cultivation phase. While IL-1β was only added in the initial phase, IL-2 and IL-15 were added to the medium for the whole cultivation phase. The final cell product showed a high NK cell purity, viability, and transduction efficiency.

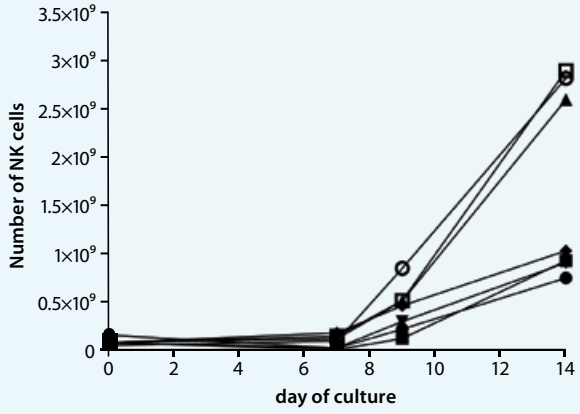


Figure 1: Internal data showing the expansion curve of transduced NK cells during 14 days in culture. (n = 7). The NK cell count remains steady for the first week and strongly increases in the second week of cultivation. Donor to donor variability is expected for NK cell expansion. In this particular data set, final number of total NK cells after 14 days leads to distinguish two groups of donors.

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