Miltenyi Biotec

Generation of tumor-reactive T cells

CliniMACS Prodigy® Tumor Reactive T Cell Process

Application

Fully automated stimulation, labelling, enrichment, activation, transduction, and expansion of human T cells from patient material (either derived from tumor or via leukapheresis) for the production of tumor-reactive T cells.

This application sheet gives an overview of the specifications and materials required to perform the Tumor Reactive T Cell (TRT) Process on the CliniMACS Prodigy. In addition, it illustrates the process workflow and tubing set configuration, and provides performance data of our own in-house results.

Specifications

Process name:	Tumor Reactive T Cell Process
Starting cell number for stimulation/ selection:	Tumor digest: up to 2–4×10^8 Leukapheresis: up to 1×10^9
Starting cell number for expansion:	Up to 1×10^7 (recommended 1×10^6)
Sample volume for selection:	20–200 mL
Final cell product volume:	100 mL
Process time:	12–15 days

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Material required

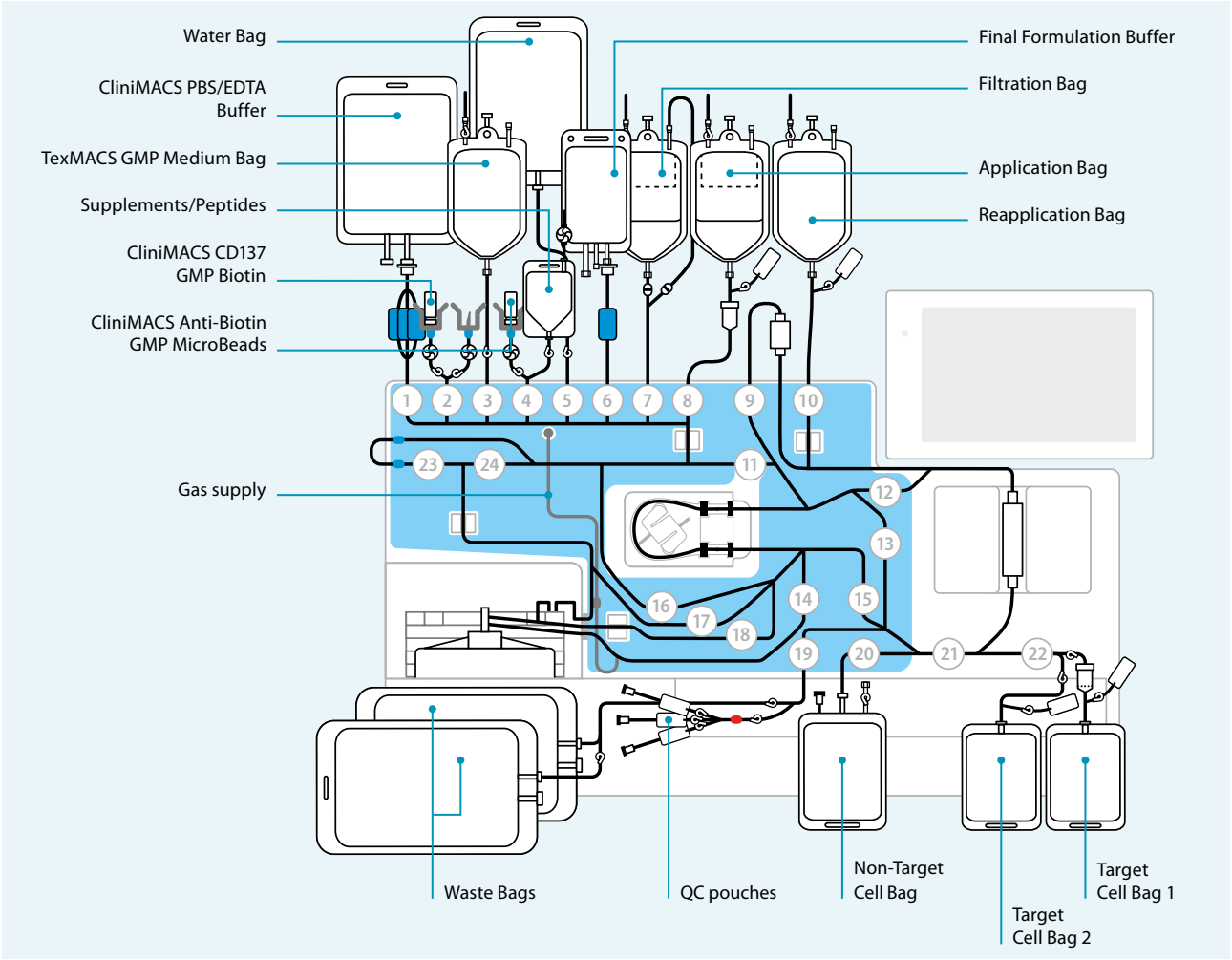
CliniMACS® Materials	Amount required
CliniMACS Prodigy	1 unit
CliniMACS Prodigy TS 520	1 piece
CliniMACS PBS/EDTA Buffer	1×3 L
CliniMACS PBS/EDTA Buffer	1×1 L
CliniMACS CD137 GMP Biotin	1 vial
CliniMACS Anti-Biotin GMP MicroBeads	1 vial
TexMACS™ GMP Medium (2 L bag)	~3×2 L bag
MACS® GMP CD3 pure	1 vial
MACS GMP Recombinant Human IL-2 (500 µg)	~4–8 vials
MACS GMP Vectofusin®-1	Variable

Additional materials	Amount required
Luer/Spike Interconnector	Variable
Triple sampling adapter	Variable
20 mL Reagent Bag	1–2 pieces
Human serum albumin (HSA)	Variable
Human AB serum	Variable
Sterile water for injection	Variable
Viral vector	Variable
Formulation solution	Variable
150 mL Transfer Bags	Variable
Syringes	Variable
Uninterruptable power supply	Presumed on site
CO_2 and compressed air supply	Presumed on site
Sterile tubing welder	Presumed on site
Device to measure glucose and lactate levels	Presumed on site
Flow cytometer, e.g., MACSQuant® Analyzer 10 / Cell counter	Presumed on site
X-ray device for preparation of feeder cells	Presumed on site

Process overview

Pre-process	Tubing set installation and priming ▼ Connection of starting material to tubing set ▼
Stimulation	LP: Peptides (+ cytokines) Tumor: Tumor digest (+ peptides/cytokines) ▼
T cell enrichment	$CD137^+$ T cell enrichment ▼
Activation	Feeder cells (Irradiated LP or PBMCs) addition MACS GMP CD3 pure ▼
Optional transduction	Lentiviral transduction (+ MACS GMP Vectofusin®-1) ▼
Cell expansion	Expansion in TexMACS Medium with IL-2 ▼
Cell harvest and final formulation	Cells wash and harvest in 100 mL of buffer ▼
Post-process	Tubing Set de-installation
	12–15 days

CliniMACS Prodigy TS 520 setup for the Tumor Reactive T Cell Process



Performance data

Starting product	Starting product CD137⁺ labeled cells (%)	Isolated CD137⁺ cells (%)	Recovery of CD137⁺ cells (%)	CD3⁺ T cells (%)	CD4⁺/CD8⁺ T cells (%)	Viability (%)	Fold expansion	T cell number (×10⁶)
MEL 1	20.1	95.3	58.1	98.4	25.1 / 73.1	98.8	80,556	1,450
MEL 2	23.8	97.1	41.3	73.8	40.0 / 16.3	96.8	131	2.63
LP 3	4.9	89.2	22.2	97.2	37.8 / 56.3	99.3	5,300	5,300
LP 4	7.6	88.7	36.7	98.2	18.9 / 74.7	98.8	3,769	5,676

Table 1: Isolation of tumor-reactive T cells derived from patient material (frozen melanoma digests, MEL) and healthy donor leukapheresis material stimulated with peptides (LP). In-house data.

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