



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

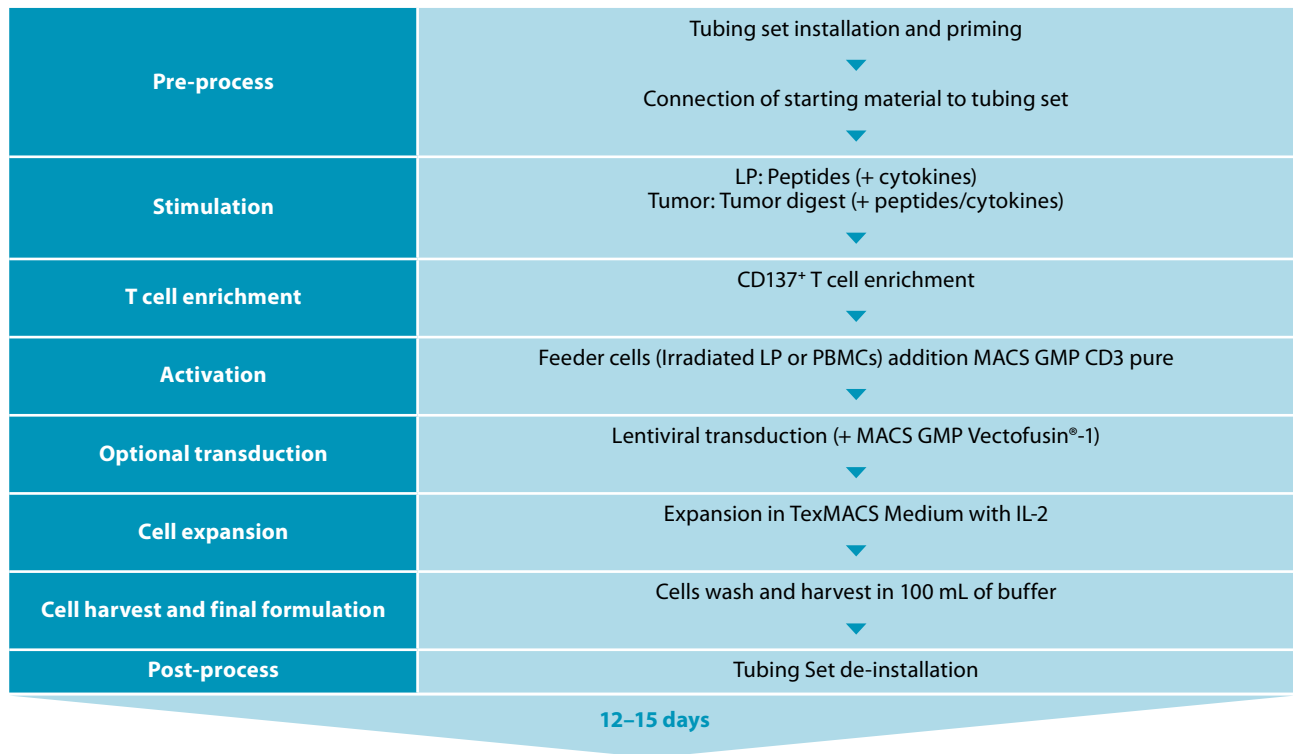
| | |
|--|---|
| Process name: | Tumor Reactive T Cell Process |
| Starting cell number for stimulation/selection: | Tumor digest: up to $2-4 \times 10^8$ Leukapheresis: up to 1×10^9 |
| Starting cell number for expansion: | Up to 1×10^7 (recommended 1×10^6) |
| Sample volume for selection: | 20–200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

Material required

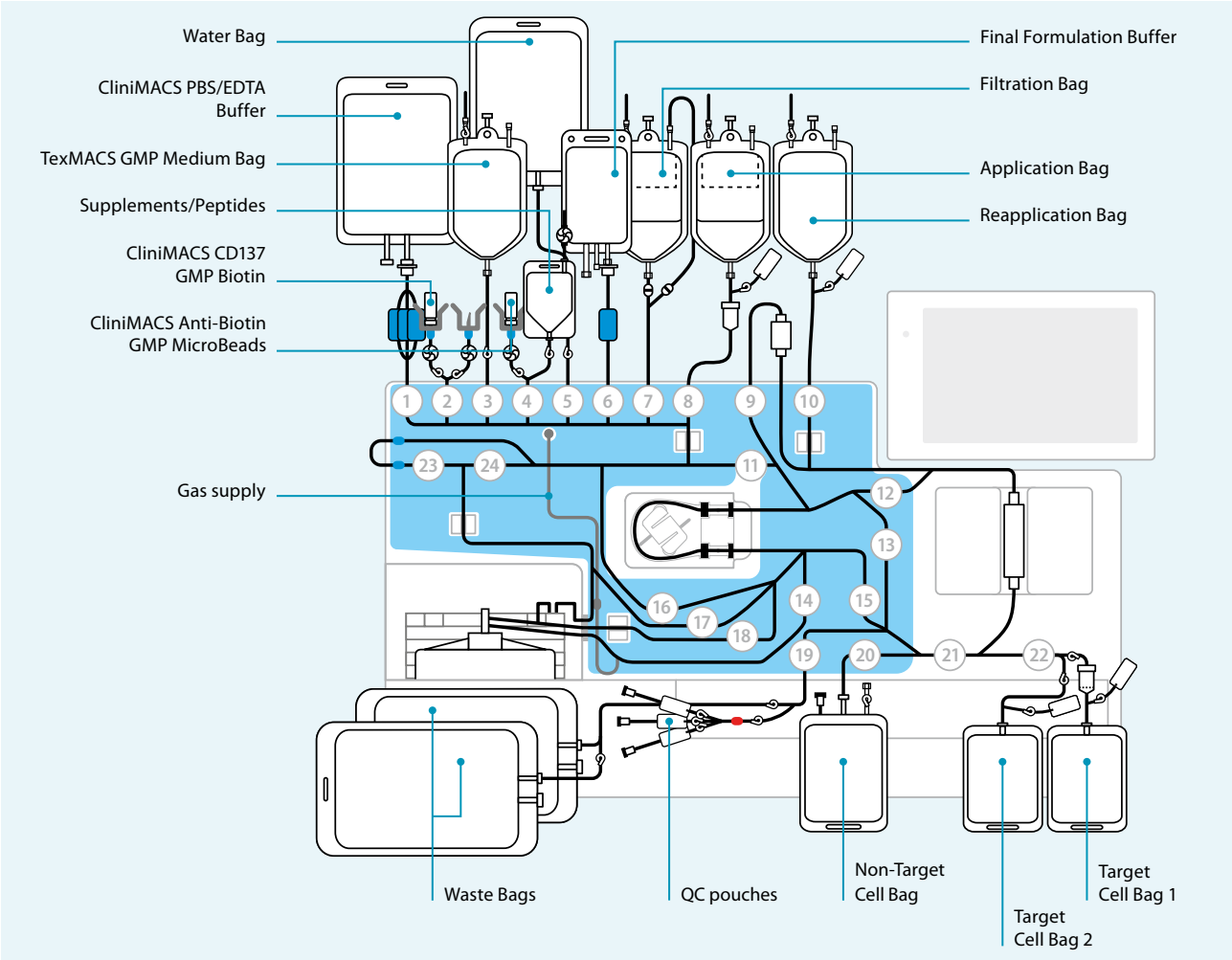
| CliniMACS® Materials | Amount required |
|--|-----------------|
| CliniMACS Prodigy | 1 unit |
| CliniMACS Prodigy TS 520 | 1 piece |
| CliniMACS PBS/EDTA Buffer | 1×3 L |
| CliniMACS PBS/EDTA Buffer | 1×1 L |
| CliniMACS CD137 GMP Biotin | 1 vial |
| CliniMACS Anti-Biotin GMP MicroBeads | 1 vial |
| 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



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 (×10 ⁶) |
|------------------|---|---------------------------------------|--|------------------------------|--|---------------|----------------|-----------------------------------|
| 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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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.

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