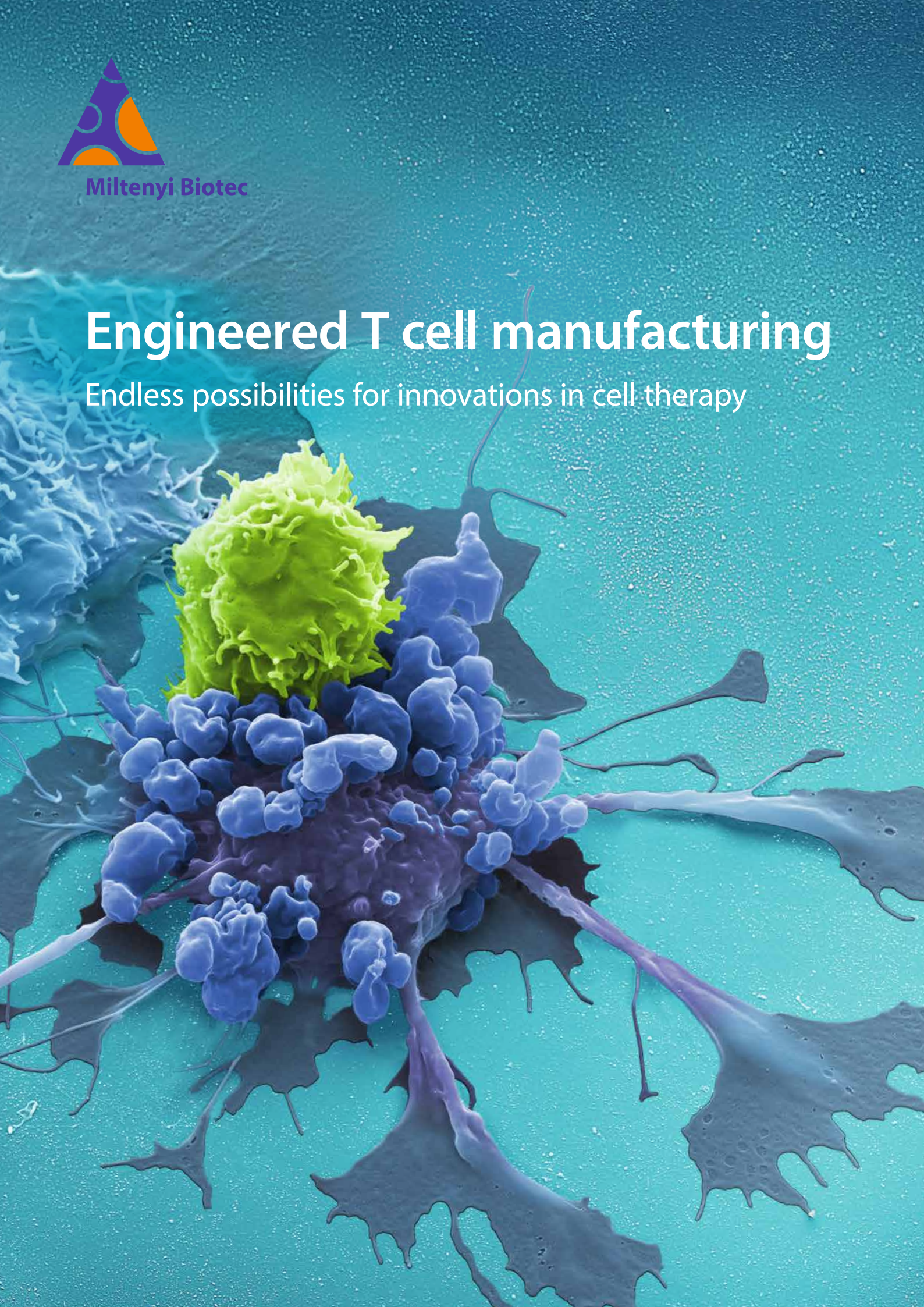
Miltenyi Biotec

# Engineered T cell manufacturing

## Endless possibilities for innovations in cell therapy



# Flexible and customizable options for T cell therapy

## Experience and success in cell manufacturing

For decades, Miltenyi Biotec has been a driving force in the development of cell manufacturing processes. In a series of instrument, reagent, and software innovations, our products and services have sparked a transition in cell processing. Time-consuming and variable manual handling is now highly standardized state-of-the-art automation.

The CliniMACS Prodigy® Platform catalyzes progress especially in T cell engineering. The instrument fosters the seamless transition of novel therapeutic strategies to clinical deployment. At the same time, it maintains a focus on consistently generating high-quality cell products.

## A spectrum of options in T cell engineering

The CliniMACS Prodigy is the manufacturing platform of choice for numerous next-generation T cell therapies, including bispecific[1], switchable[2], and armored[3] CAR constructs, plus non-viral[4] and TCR engineering[5].

Three production processes underlie that diversity. CliniMACS Prodigy T Cell Transduction (TCT) automates efficient T cell isolation and viral transduction from a range of starting materials (table 1). Larger volumes are managed with its large-scale counterpart, TCT–LS. Interested in electroporation for your workflow? CliniMACS Prodigy T Cell Engineering (TCTe) offers that option. Finally, our Customized Application Services can develop a tailored formulation and filling with the CliniMACS® Formulation Unit.

| | CliniMACS Prodigy T Cell Transduction (TCT) | CliniMACS Prodigy T Cell Transduction Large Scale (TCT-LS) | CliniMACS Prodigy T Cell Engineering (TCTe) |
|---|---|---|---|
| Main manufacturing application | CAR T cells | TCR T cells and CAR T cells for solid tumors | Complex engineered T cells |
| **Starting material** | | | |
| Whole blood[6] | ● | | ● |
| Buffy coat | ● | | ● |
| Fresh leukapheresis | ● | ● | ● |
| Frozen leukapheresis | | ● | |
| **Gene engineering method** | | | |
| Lentivirus/retrovirus transduction | ● | ● | ● |
| Electroporation | | | ● |
| Culture capacity | 250 mL | 600 mL | 250 mL |
| **Number of harvested cells** | | | |
| Without electroporation | $5\times10^9$ | $2\times10^{10}$ | $5\times10^9$ |
| With electroporation | | | $2\times10^9$ |

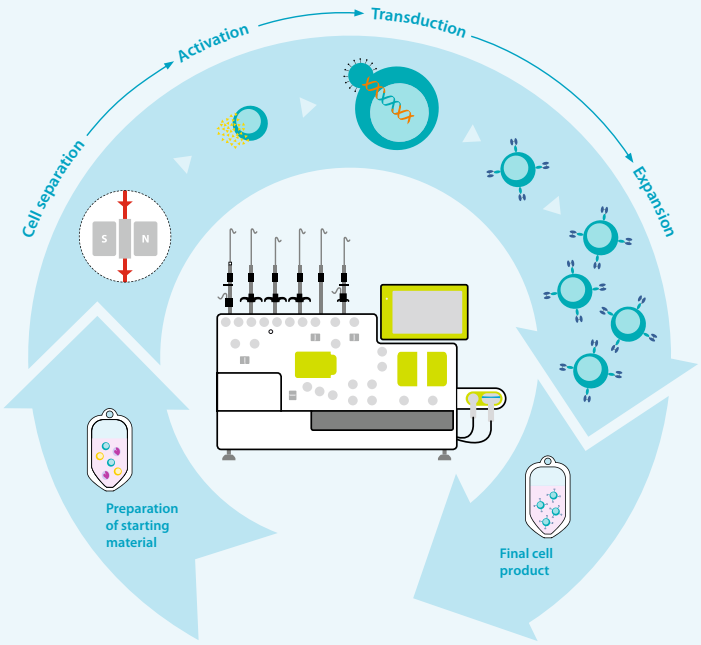
**Table 1:** Overview of the three flexible and customizable processes for T cell therapy manufacturing available on the CliniMACS Prodigy Platform.

LEARN MORE

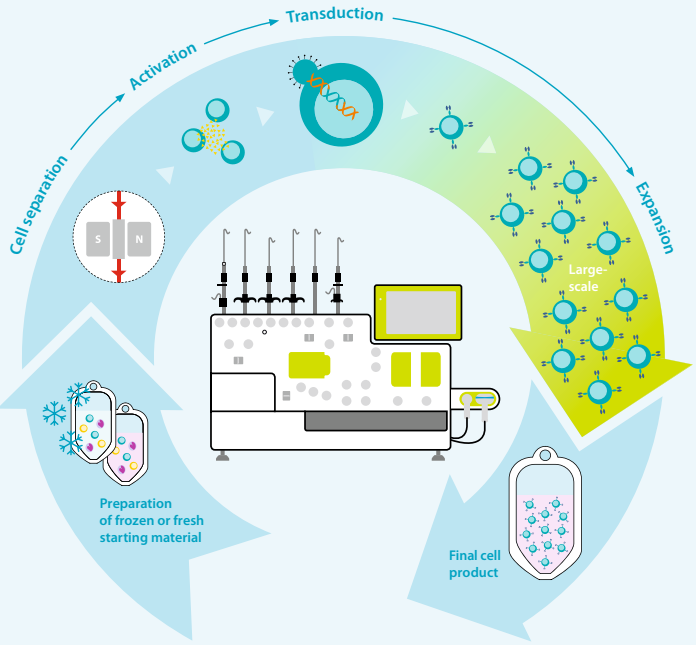
**One platform. Endless possibilities.**
Get an overview of our manufacturing options for gene-engineered T cells.

▸ **miltenyibiotec.com/engineeredTcells**

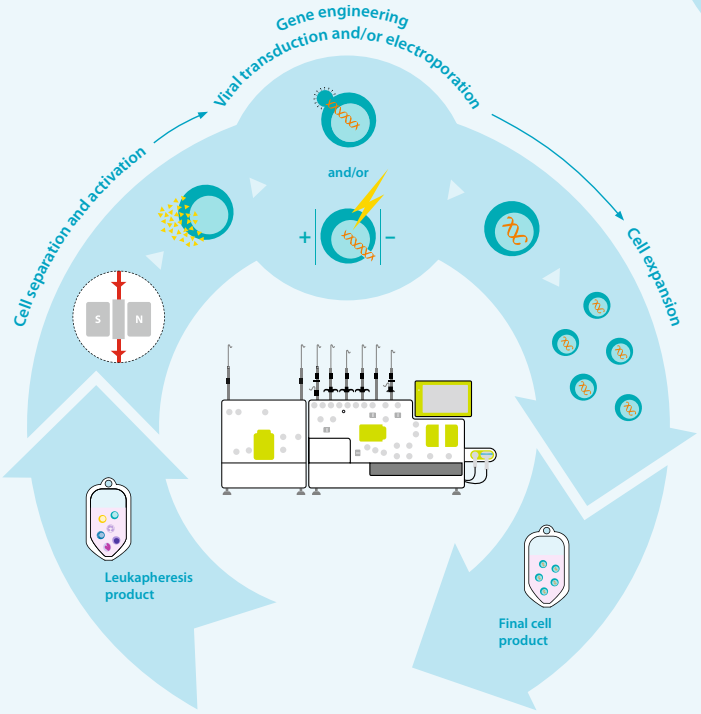
## T Cell Transduction (TCT)




## T Cell Transduction – Large Scale (TCT-LS)




## T Cell Engineering (TCTe)



# No hurdles or limits.
# Just answers and options.

## Integrated cell processing from collection to harvest

CliniMACS Prodigy TCT, TCT-LS, and TCTe integrate optimized cell processing conditions within the protective, sterile environment of the closed CliniMACS Prodigy Platform. Each step overcomes known hurdles in T cell engineering to generate reproducible end products. Plus, automation eliminates variable material handling for standardized outcomes.

## High-purity start. Reproducible results.

Effective cell manufacturing starts with a well-defined, enriched T cell population. Our three processes use clinical-scale magnetic separation to yield highly pure and viable T cells, even from patient material (internal data; fig. 1). The low monocyte content of the enriched T cells enhances transduction efficiency (fig. 2), cutting the amount and cost of vector input.

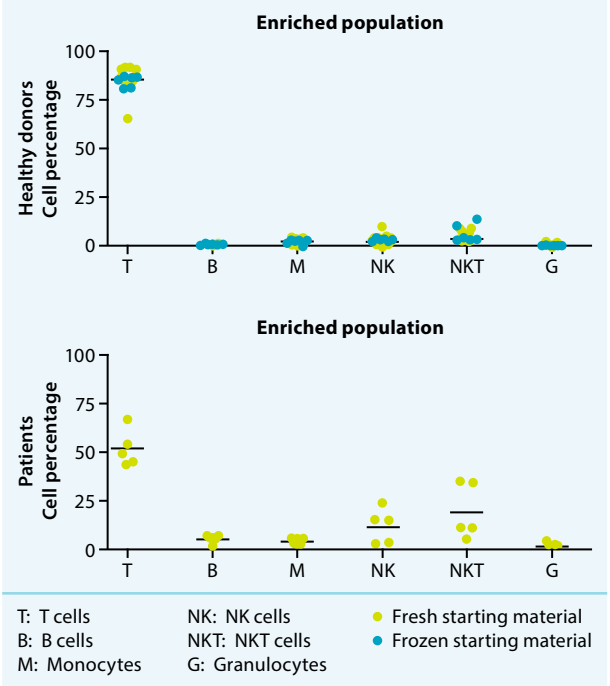



**Figure 1:** Automated enrichment with CliniMACS Prodigy TCT, TCT-LS, or TCTe generates highly reproducible and pure T cell populations. The composition of enriched material from fresh (green) and frozen (blue) samples of healthy donors (top) and patients (bottom) is optimal for downstream steps.

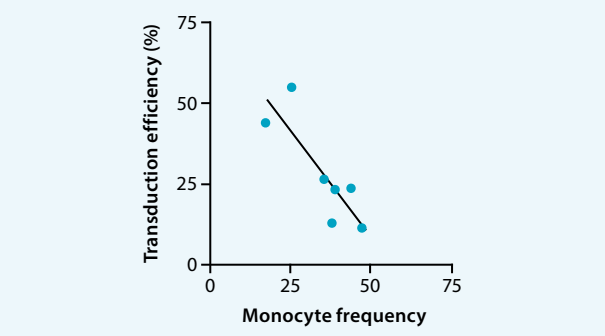



**Figure 2:** Unwanted cells reduce transduction efficiency significantly. A doubling in the relative proportion of monocytes in a sample lowers transduction efficiency from 50 to under 20%.

## Robust and straightforward stimulation

The ready-to-use reagent MACS® GMP T Cell TransAct™ and its large-scale counterpart achieve potent polyclonal activation of cells in preparation for gene modification. Designed for use in a closed manufacturing system, MACS GMP T Cell TransAct is easy to use. Excess of the unique colloidal polymeric nanomatrix is simply washed away after stimulation.

## Transduction or transfection? Your choice.

Our three T cell manufacturing processes cover lentivirus-, retrovirus-, and transfection-based gene modification (figs. 3 and 4). The process occurs in the closed CliniMACS Prodigy Tubing Set, ensuring safety when using viral vectors. Choose one or combine methods to efficiently perform even complex editing. The engineering step is customizable, granting full control to adapt timing, volumes, multiplicity of infection (MOI), electroporation settings, and more. Whether gene transduction, knockout, augmentation, silencing, or editing, the possibilities are endless.

## Simple and convenient T cell activation

**Practical application**

- Volumetric dosage
- Ready to use
- Removal by simple washing

**Robust stimulation**

- Highest cell viability
- Physiological and stable stimulation

**Convenient compatibility**

- Available for research and GMP T cell workflows
- Optimized for CAR T cell production on the CliniMACS Prodigy
- Can be sterile filtered

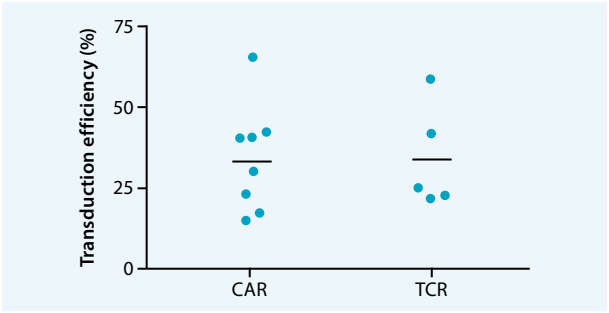



**Figure 3:** Enriched CD4+ and CD8+ T cells were stimulated for 24 hours before transduction using lentiviral vectors encoding CAR or TCR. The CliniMACS Prodigy TCT-LS achieved adequate and stable transduction rates.

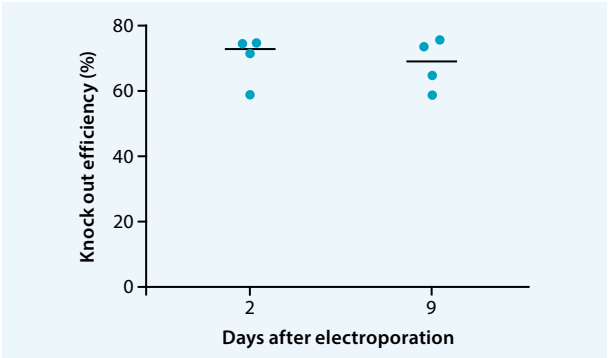



**Figure 4:** The gene for CCR5 was edited using transcription activator-like effector nucleases (TALENs) combined with the CliniMACS Electroporator. Editing rates at two and nine days after electroporation were comparable. Rates were calculated from gene-editing frequency digital PCR (GEF-dPCR) data (n = 4).

LEARN MORE

**Simplified T cell activation**
Discover the unique features of MACS GMP T Cell TransAct.
▸ **miltenyibiotec.com/GMP-TransAct**

# No slowdowns or complications. Just seamless progress.

## Optimal expansion to meet dosing goals

Two in-line cultivation chamber sizes house the fitting conditions to achieve standard and large-scale cell expansion (fig. 5). Each process features a ready-to-use default protocol that quickly and robustly grows the preferable T cell phenotype (fig. 6) from fresh or frozen material (fig. 7). However, cultivation parameters are programmable. Customize the culture duration, medium supply, and supernatant exchange to optimize growth or shorten manufacturing.

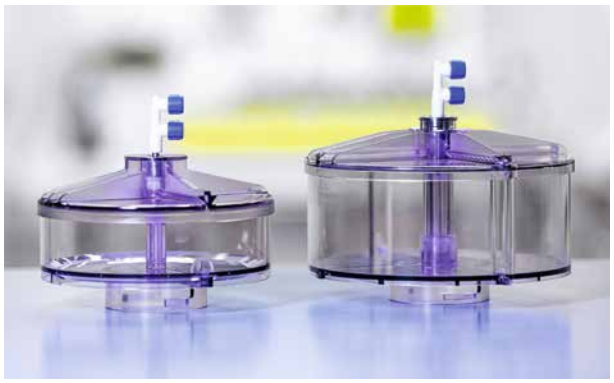


**Figure 5:** CliniMACS Prodigy TCT-LS features an expanded culture chamber (right) for large-scale production compared to CliniMACS Prodigy TCT and TCTe (left).

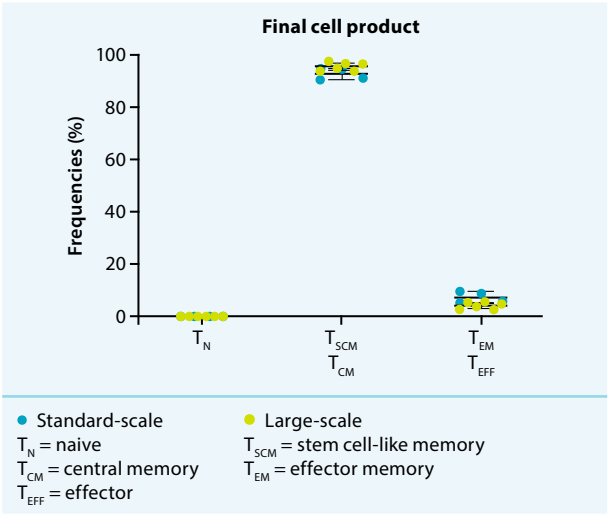



**Figure 6:** Frequencies of T cell phenotypes among viable CD45⁺ cells were determined for the final cell product from enriched CD4⁺/CD8⁺ T cells that were expanded in the standard and large-scale chamber of the CliniMACS Prodigy after polyclonal stimulation with MACS GMP T Cell Transact. After 12 days of culture, phenotype frequencies were comparable between the two culture conditions.

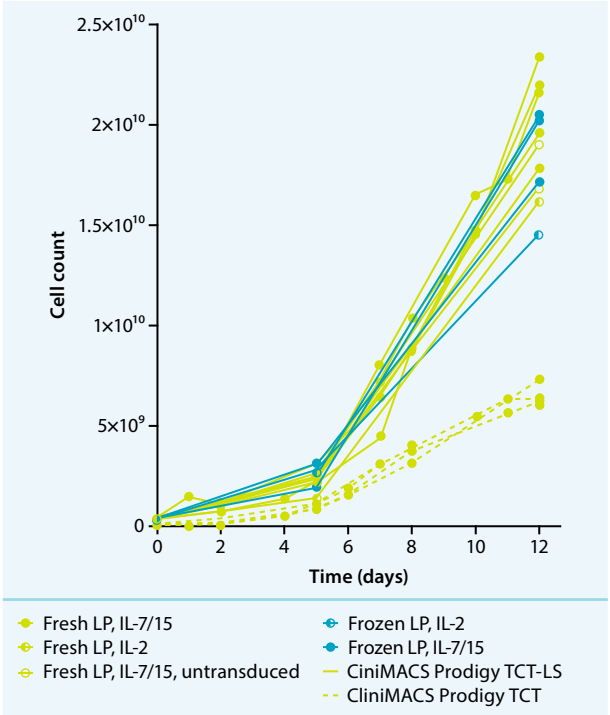



**Figure 7:** After polyclonal stimulation, enriched CD4⁺/CD8⁺ T cells from frozen and fresh samples were expanded in the presence of different cytokines using the standard chamber with CliniMACS Prodigy TCT (dotted lines) and the large-scale chamber with CliniMACS Prodigy TCT-LS (solid lines). Cell expansion was comparable between fresh material, frozen material, and different cytokine combinations. That consistency underscores the robustness of the process.

VISIT

Read other clinical research papers that reference the CliniMACS Prodigy.

▸ **miltenyibiotec.com/Tcell-engineering-refs**

VISIT

Learn about your options for formulation and filling.

▸ **miltenyibiotec.com/TCT-process**

# Reliable and harmonized end-to-end quality control

Whether for in-process control (IPC) or release testing (RT), flow cytometric analysis of cell phenotype, count, function, purity, identity, and other critical attributes goes hand in hand with manufacturing processes on the CliniMACS Prodigy Platform.

Our comprehensive analytics solutions make precise and consistent analytics simple and fast. The MACSQuant® Analyzer 16 and Express Mode software tools seamlessly interface with your production workflow to automate analyses using high-quality reagents like:

**StainExpress™ Dry Antibody Cocktails** for easily standardized and accurate T cell purity analysis, transduction detection, CAR T cell monitoring, and more (fig. 8).

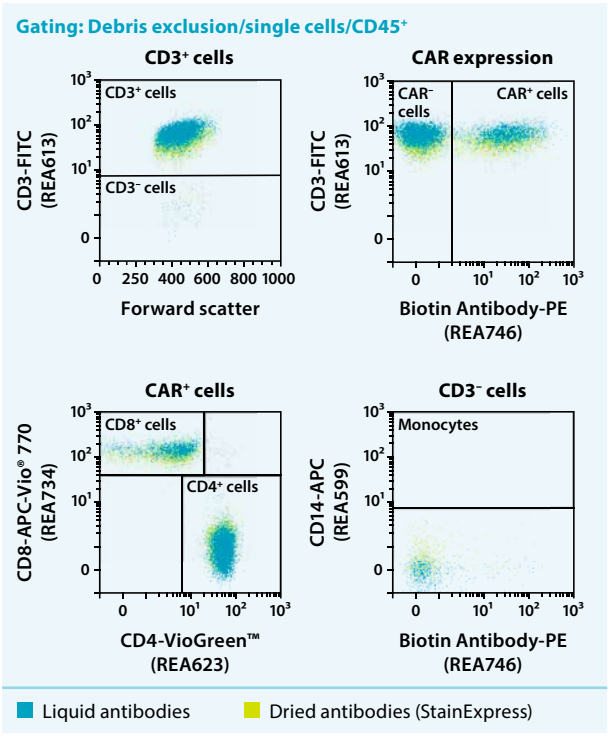



**Figure 8:** StainExpress Dry Antibody Cocktails match the reliable performance of their liquid counterparts. In a direct comparison staining CAR T cells generated with CliniMACS Prodigy TCT, both antibody formats delivered precise analyses on the MACSQuant Analyzer.

**CAR Detection Reagents** to quantify CAR T cells (e.g., CD19, CD22, BCMA) with trial-ready specificity and analyze CAR T cell expansion and persistence during patient monitoring research (fig. 9).

**Peptide-loaded MHC MACSimers** for sensitive, antigen-specific detection of TCR T cells (e.g., NY-ESO-1, MAGE-A4).

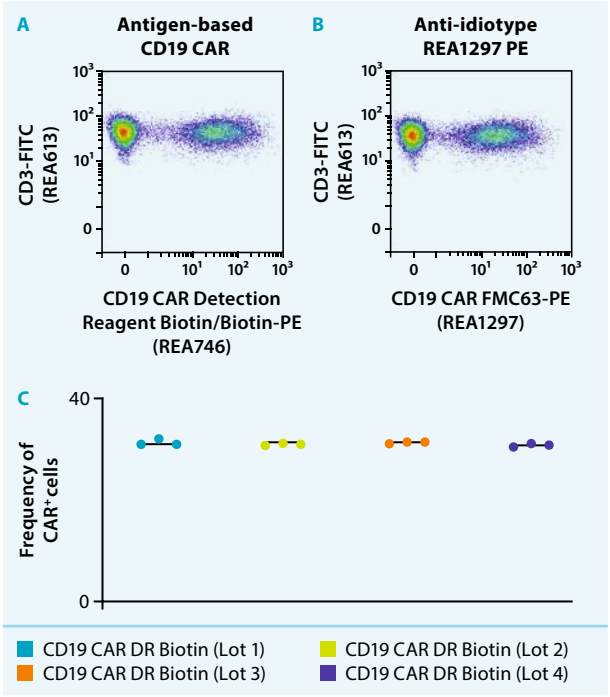



**Figure 9:** Both antigen-based (A) and anti-idiotype (B) detection methods distinguish primary CD19 CAR (FMC63) T cells reliably. Miltenyi Biotec's antigen-based CAR Detection Reagents (CAR DR) are high-quality reagents designed for minimal background noise and exceptional lot-to-lot consistency (C).

LEARN MORE

Discover our complete toolkit for standardized and reliable CGT analytics.

▸ **miltenyibiotec.com/reliable-IPC-RT**

## References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Miltenyi Biotec


**Germany/Austria**
Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macsde@miltenyi.com

**USA/Canada**
Miltenyi Biotec, Inc.
1201 Clopper Road
Gaithersburg, MD 20878, USA
Phone 800 FOR MACS
Phone +1 866 811 4466
Fax +1 877 591 1060
macsus@miltenyi.com

**Australia**
Miltenyi Biotec
Australia Pty. Ltd.
Unit 11, 2 Eden Park Drive
Macquarie Park, NSW 2113
Australia
Phone +61 2 8877 7400
Fax +61 2 9889 5044
macsau@miltenyi.com

**Benelux**
Miltenyi Biotec B.V.
Dellaertweg 9C
2316 WZ Leiden
The Netherlands
macsnl@miltenyi.com
**Customer service for:**
**The Netherlands**
Phone 0800 4020120
Fax 0800 4020100
**Belgium**
Phone 0800 94016
Fax 0800 99626
**Luxembourg**
Phone 800 24971
Fax 800 24984

**China**
Miltenyi Biotec Technology & Trading (Shanghai) Co., Ltd.
Room A401, 4/F
No. 1077, Zhangheng Road
Pudong New Area
201203 Shanghai, P.R. China
Phone +86 21 6084 0210
Fax +86 21 6235 0953
macscn@miltenyi.com.cn

**France**
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris, France
Phone +33 1 56 98 16 16
macsfr@miltenyi.com

**Hong Kong**
Miltenyi Biotec Hong Kong Ltd.
Unit 301, Lakeside 1
No. 8 Science Park West Avenue
Hong Kong Science Park
Pak Shek Kok, New Territories
Hong Kong
Phone +852 3751 6698
Fax +852 3619 5772
macshk@miltenyi.com.hk

**India**
Miltenyi Biotec India Pvt. Ltd.
Vatika Business Centre,
Floor No. 6
Divya Sree Omega
Kondapur, Serilingampally
K.V. Rangareddy
Telangana 500084, India
Phone +91 040 45175910
macsin@miltenyi.com

**Italy**
Miltenyi Biotec S.r.l.
Via Paolo Nanni Costa, 30
40133 Bologna, Italy
Phone +39 051 6 460 411
Fax +39 051 6 460 499
macsit@miltenyi.com

**Japan**
Miltenyi Biotec K.K.
NEX-Eitai Building 5F
16-10 Fuyuki, Koto-ku
Tokyo 135-0041, Japan
Phone +81 3 5646 8910
Fax +81 3 5646 8911
macsjp@miltenyi.com

**Nordics and Baltics**
Miltenyi Biotec Norden AB
Medicon Village
Scheeletorget 1
223 81 Lund, Sweden
macsse@miltenyi.com
**Customer service for:**
**Sweden**
Phone 0200 111 800
Fax +46 280 72 99
**Denmark**
Phone 80 20 30 10
Fax +46 46 280 72 99
**Norway, Finland, Iceland, and Baltic countries**
Phone +46 46 280 72 80
Fax +46 46 280 72 99

**Singapore**
Miltenyi Biotec Asia Pacific Pte Ltd.
438B Alexandra Road, Block B
Alexandra Technopark
#06-01
Singapore 119968
Phone +65 6238 8183
Fax +65 6238 0302
macssg@miltenyi.com

**South Korea**
Miltenyi Biotec Korea Co., Ltd.
Donggeuk 7F,
562 Nonhyeon-ro
Gangnam-gu
Seoul 06136, South Korea
Phone +82 2 555 1988
Fax +82 2 555 8890
macskr@miltenyi.com

**Spain**
Miltenyi Biotec S.L.
C/Virgilio 2, Edificio II, Planta -1
28223 Pozuelo de Alarcón
Madrid, Spain
Phone +34 91 512 12 90
Fax +34 91 512 12 91
macses@miltenyi.com

**Switzerland**
Miltenyi Biotec Swiss AG
Soodstrasse 52
8134 Adliswil
Switzerland
Phone +41 32 623 08 47
Fax +49 2204 85197
macsch@miltenyi.com

**United Kingdom**
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR, UK
Phone +44 1483 799 800
Fax +44 1483 799 811
macsuk@miltenyi.com

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