Miltenyi Biotec

CAR T cell research and discovery

Innovative product solutions

CAR T cells – From discovery to therapeutics

Miltenyi Biotec's reagents, instruments, and protocols are used by scientists all over the world. They help to advance CAR T cell research as well as clinical CAR T cell manufacturing.

Field-tested workflow

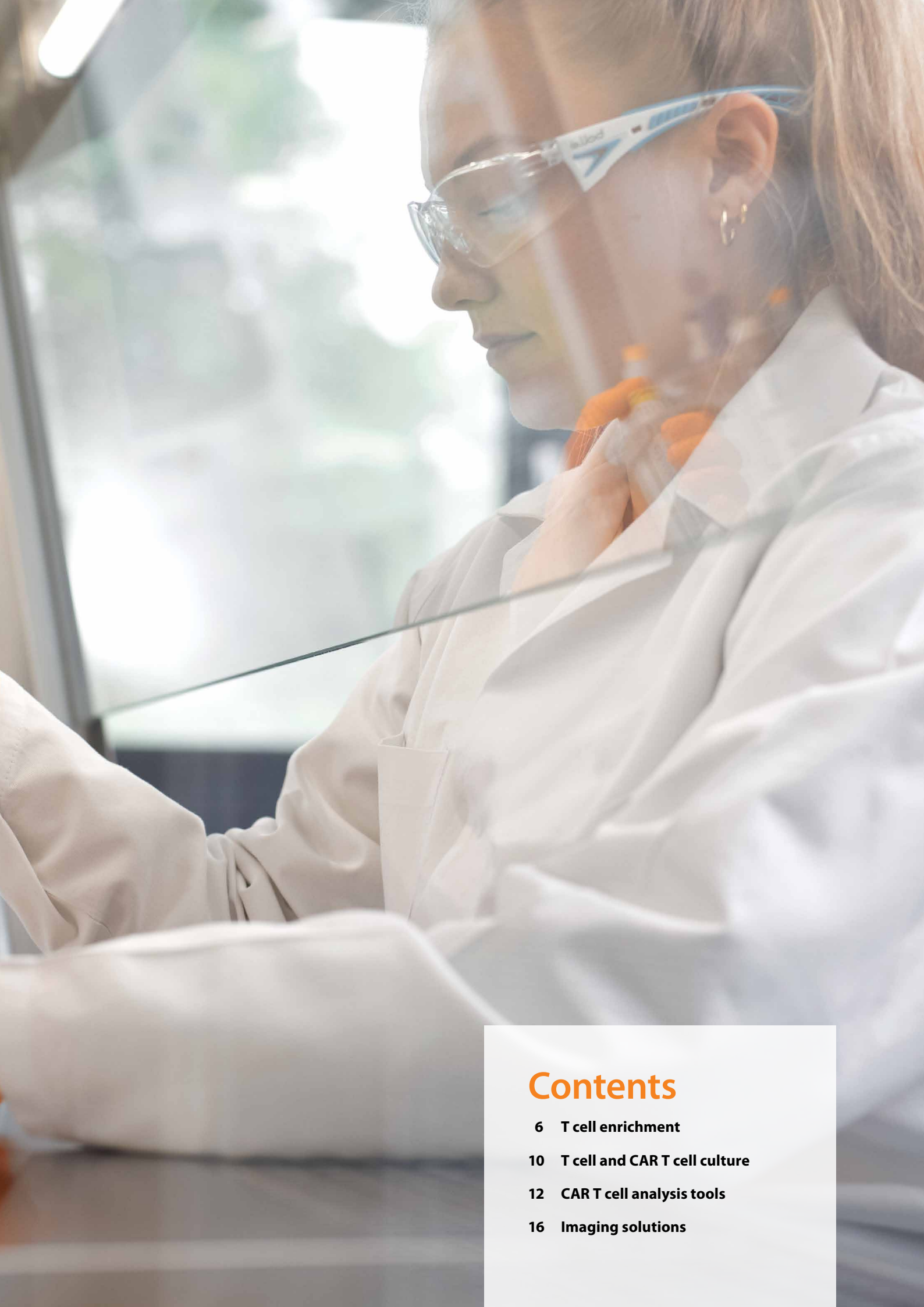
Take advantage of our straightforward translational workflow. The workflow was developed based on our longstanding expertise and experience in CAR T cell research and clinical manufacturing.

Tailored CAR T cell research solutions

Exploit our innovative product solutions for CAR T cell research to prove your theories and to optimize your small-scale workflow to your needs.

Truly translational and easy to out-scale

Transfer your small-scale workflow to the CliniMACS Prodigy® Platform using the corresponding MACS® GMP Portfolio for GMP-compliant production.



Contents

Single-source trusted supplier

No need to look for compatible product solutions or to validate each workflow step. Our experts carefully fine-tuned this workflow specifically for CAR T cell research. We offer the products, the protocols, as well as expert support when needed.

Cell separation

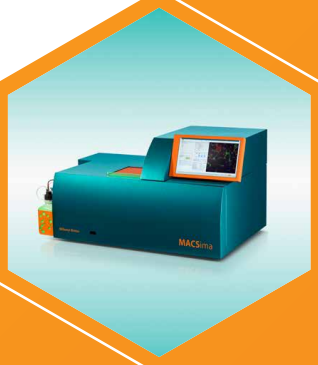
T cell expansion

Gene delivery

Flow analysis

From benchtop research …

Imaging



… to clinical-grade manufacturing.

MACS GMP Products

CliniMACS Prodigy

Cell therapy

IPC & QC

Cryo-preservation

Custom services

Start with a well-defined T cell population

Gentle and efficient T cell isolation with MACS® Technology is the first choice for CAR T cell engineering. In fact, CAR T cells manufactured using MACS Technology are used in clinical trials worldwide. Starting into CAR T cell research with a well-defined T cell population increases transduction efficiency and reduces costs by preventing vector consumption by non-target cells.

T cell isolation

T cell isolation from PBMCs

Nano-sized MACS MicroBeads enable cell isolation with minimal labeling of the target cell population. Gentle column-based isolation prevents cell stress and ensures high cell viability.

- Small bead size and minimal labeling preserve cell functionality.
- Compatibility with the MultiMACS™ Cell24 Separator Plus or the autoMACS® Pro Separator enables automated cell enrichment.
- MACS Technology is a trusted method used in research publications and clinical trials worldwide.

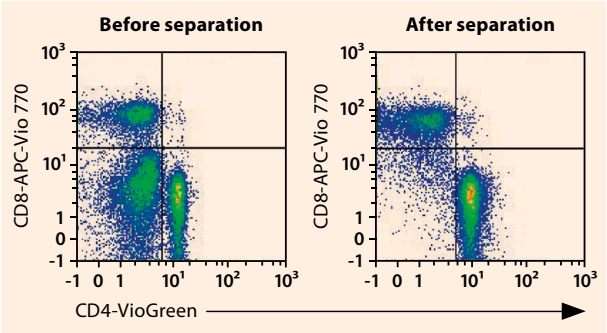


Figure 1: Purity of CD4⁺ and CD8⁺ T cells after isolation with MACS Technology. T cells were enriched by positive selection using a mixture of CD4 and CD8 MicroBeads (1:1) in combination with MACS Technology. Cells were stained with CD45-VioBlue®, CD4-VioGreen™, and CD8-APC-Vio® 770 and analyzed by flow cytometry using the MACSQuant® Analyzer.

T cell isolation from blood products

Based on our trusted MACS Technology, the new StraightFrom® Technology increases throughput and productivity. T cells are directly isolated from whole blood samples or other blood products without the need for lengthy density gradient centrifugation or erythrocyte lysis.

- Direct T cell isolation saves time.
- Optimized protocols for processing, for example, a full buffy coat in only 30 minutes.
- Automation with the MultiMACS Cell24 Separator Plus further reduces hands-on time and user variability.

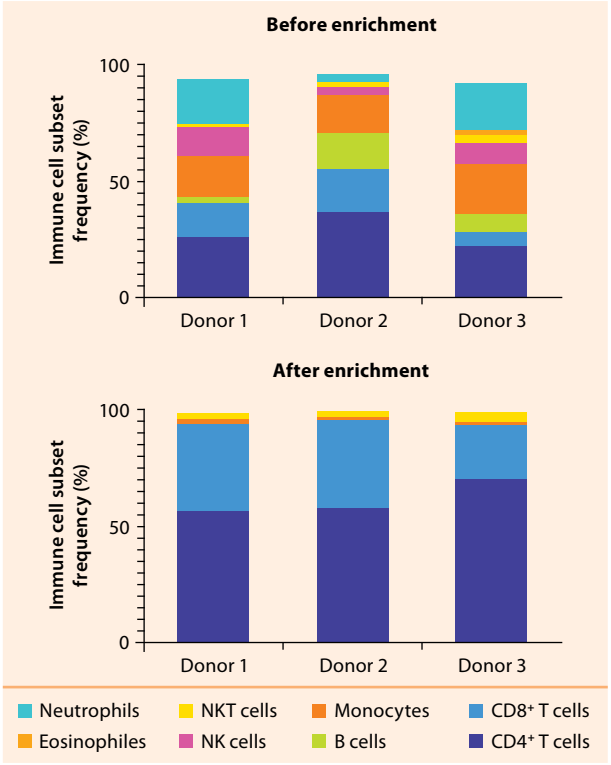


Figure 2: Enrichment from blood products results in high T cell frequencies. Immune cell composition of independent healthy donors (n=3) was determined before and after T cell enrichment with StraightFrom Buffy Coat CD4/CD8 MicroBeads using the MACSQuant Analyzer 10 and the CAR T Cell Express Mode Package (Immune_Cell_Composition_human).

VISIT

Learn more about StraightFrom Technology for T cell isolation on our website:

▸ **miltenyibiotec.com/t-cell-isolation**

CAR T cell enrichment for patient monitoring

The MACSprep™ CD19 CAR MicroBead Kit, human and other CAR T cell enrichment kits are designed to enrich CAR T cell populations prior to flow cytometric analysis or functional and molecular characterization.

- CAR T cell isolation directly from whole blood samples saves time.
- Ready-to-use kits for convenient handling of low frequent CAR T cell populations.
- Purified CAR T cells are suited for any downstream analysis.

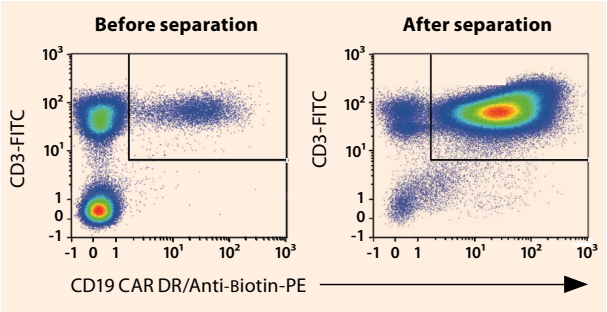


Figure 3: Enrichment of CAR T cells. CD19 CAR T cells were spiked into a whole blood sample from a healthy donor and isolated using the MACSprep CD19 CAR MicroBead Kit, human. Cells were fluorescently stained with CD19 CAR Detection Reagent, human, Biotin, Biotin Antibody-PE, CD45-VioBlue, 7-AAD Staining Solution, and CD3-FITC and analyzed by flow cytometry using the MACSQuant Analyzer 10.

Most cited cell isolation technology

Validated protocols

MACS Cell Separation

MACS Technology

Manual to automated solutions

Patient monitoring

Tailored for CAR T cell research

Workflows from basic to clinical research

Select the best

Maximize your CAR T cell expansion

Maintaining phenotype stability is pivotal for the generation of functional CAR T cells. The strong synergy of T Cell TransAct™, TexMACS™ Medium, and MACS Premium-Grade Cytokines offers optimal cultivation and expansion conditions. Together, these products allow for reliable cultivation of the desired cell phenotype and reproducible results.

T cell and CAR T cell culture

Reliable T cell activation

T cell activation facilitates an efficient CAR transduction and induces cell proliferation. The ready-to-use T Cell TransAct Reagent provides an innovative method for gentle and therewith physiological activation and expansion of human T cells.

- The unique polymeric nanomatrix conjugated to humanized CD3 and CD28 agonists is more gentle than bead-based methods.
- Easy protocol with no need for cell counting, plate coating, or bead removal steps. The reagent is simply washed off.
- Optimized for seamless translation to CAR T manufacturing on an automated closed system like the CliniMACS Prodigy Platform.

Reproducible T cell cultivation and expansion

TexMACS Medium and MACS Premium-Grade Cytokines offer optimal conditions for expansion and differentiation of engineered T cells as well as for functional assays.

- Special T cell media formula is optimized for high expansion rates, even without serum.
- Serum-free media formulation and lot-specific cytokine activities allow for reproducible cell culture conditions.
- Low endotoxin levels and high purity of MACS Premium-Grade Cytokines ensure relevant pre-clinical results and a seamless translation to MACS GMP Products.

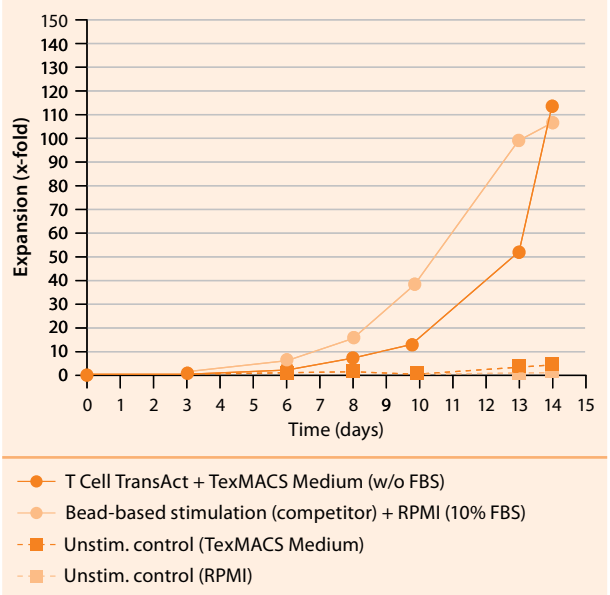


Figure 4: Reliable T cell activation. Comparison of T cell expansion after stimulation with T Cell TransAct and TexMACS Medium or a bead-based product and RPMI was identical. Both media were supplemented with human IL-2 IS, premium grade (50 U/mL).

VIDEO

Watch our video and learn how to optimize your T cell research with T Cell TransAct:

▸ **miltenyibiotec.com/transact-video**

Characterize your engineered cells

The success of CAR T cell therapies has been associated with the phenotype, activation status, and functional profiling of CAR T cells. Miltenyi Biotec offers proven solutions for reliable, reproducible, and standardized phenotypic characterization and *in vitro* potency assays. Our high-quality reagents, instruments, and software streamline CAR T cell analysis from basic research and discovery to IPC/QC in clinical manufacturing.

CAR T cell analysis reagents and tools

Rethink your antibodies

REAfinity™ Recombinant Antibodies and our validated antibody panels are optimized for efficient assessment of immune cell composition, T cell activation and exhaustion, and CAR T cell persistence.

- High purity of REAfinity Antibodies allows for lot-to-lot consistency and reproducible results.
- Antibody panels are designed and validated by our experts.
- Broad spectrum of antibodies available.

VISIT

Find our Miltenyi Biotec Tested Panels (MBTP) to accelerate your CAR T cell flow analysis:

▸ **miltenyibiotec.com/car-t-MBTP**

Dried cocktails for IPC/QC

StainExpress™ Dry Antibody Cocktails offer highest convenience and reproducibility. These dried, pre-formulated REAfinity Antibody cocktails are provided in ready-to-use barcoded tubes for fast and easy application per test and can be combined with a CAR Detection Reagent of choice. The cocktails match the MACSQuant® Express Modes.

- Ready-to-use format reduces technical errors.
- Expert antibody panels for optimal results.
- Easy reagent storage.

REAfinity Recombinant Antibodies

Easy validation of CAR expression

CAR Detection Reagents are specifically developed for the analysis of T cells that are engineered to express a specific CAR construct on the cell surface.

- Flow cytometric detection and quantification of CAR-expressing cells.
- Reagent binding to CAR receptor simulates *in vivo* binding of CAR T cell to tumor antigen.
- Background-free analysis with no need for additional FcR blocking steps saves time.

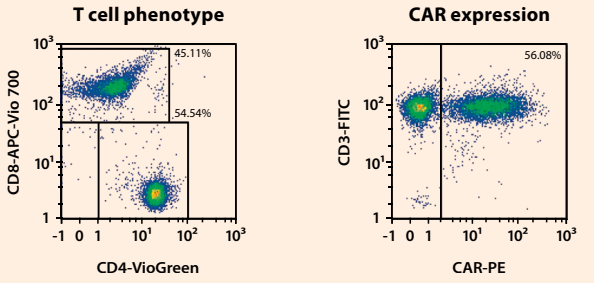


Figure 5: Flow cytometric verification of transduction efficiency. Cells were analyzed for CD4, CD8, and CD19 CAR expression using REAfinity Antibodies, the CAR T Cell Express Mode Package, and the MACSQuant Analyzer 10.

Reliable data at the push of a button

Express Mode tools are unique add-on features for the MACSQuantify™ Software that empower truly standardized flow cytometry assays with MACSQuant Analyzers. Individual needs can be addressed with custom Express Modes.

- Fully automated flow cytometry and data analysis saves time.
- Automated analysis allows for reproducible results even in a multi-user environment.
- Standardized analysis based on mathematic algorithms reduces user variability.

VISIT

Explore our on-demand webinars on standardizing flow cytometry assays in cell manufacturing:

▸ **miltenyibiotec.com/car-t-flow-analysis**

CAR T cell potency assays

An early eye on functionality

Co-culture assays of CAR T cells and target cells allow for an early evaluation of efficacy and side effects. Assess multiple functionally relevant differences between CAR T cells by flow cytometry, including antigen-specific killing ability, effector cytokine secretion, and CAR T cell phenotyping.

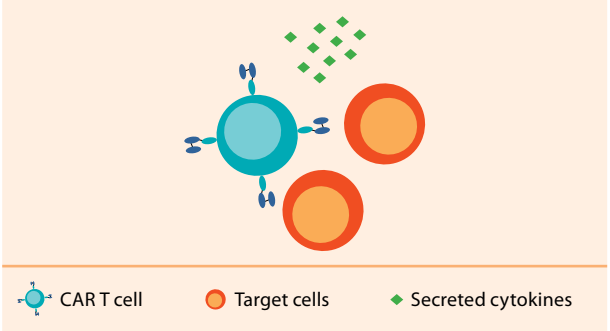


Figure 6: Interaction of CAR T cells and target cells in a co-culture assay.

DOWNLOAD

Get our application note on maximizing the efficiency of CAR T cell functionality assessment with flow cytometry:

▸ **miltenyibiotec.com/car-t-functionality**

CAR T cell viability assessment

The Annexin V-FITC Kit identifies phosphatidylserine molecules exposed on the cell surface of apoptotic cells. The 7-AAD Staining Solution penetrates the cell membrane of dead or dying cells and stains their DNA. Both options can be used to exclude dead cells from flow cytometry analysis and to assess CAR T fitness *in vitro*.

DOWNLOAD

Download our scientific poster on automated analysis for CAR T cell manufacturing and patient immunomonitoring:

▸ **miltenyibiotec.com/car-t-immunomonitoring**

Detection of secreted cytokines

MACSPlex Cytokine Kits enable the analysis of up to 12 cytokines in one sample to verify the functionality and antigen-specific killing of gene-engineered T cells. Secreted proteins are accurately quantified in co-culture supernatant.

- Flow cytometry–based multiplex cytokine screening saves time.
- Kits are compatible with any standard multicolor flow cytometer.
- Preinstalled Express Mode packages on MACSQuant Analyzers enable full automation.

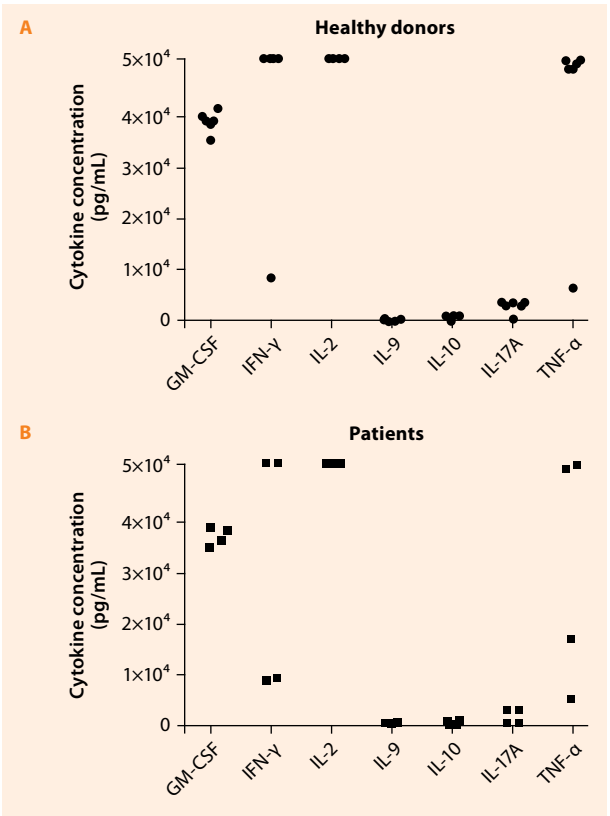


Figure 7: Flow cytometric analysis of CAR T cell functionality. CD20 CAR T cells, derived from healthy donor (A, n = 6) or patient samples (B, n = 4) were co-cultured with JeKo-1 as the target cell line (effector:target = 1:1). Analysis using the MACSPlex Cytokine 12 Kit, human shows full CAR T cell functionality in terms of cytokine secretion.

Intracellular cytokine analysis

Rapid Cytokine Inspector Kits combine surface marker and intracellular cytokine detection. They offer fast and easy analysis of activated cytokine-secreting human $CD4^+$ and $CD8^+$ T cells.

- High-throughput, multiparameter analysis saves time.
- Flexibility to analyze antigen-specific T cell responses upon *in vitro* stimulation with the respective antigen or upon polyclonal restimulation.
- Combined surface marker and intracellular cytokine staining in one experiment allows for detailed immunomonitoring of antigen-specific T cells.
- Optimized for automated and standardized measurement using the Express Mode of MACSQuant Analyzers.

The analyzer fit for CAR T cell research

The MACSQuant Analyzer 16 is engineered for automated flow cytometric cell characterization at a single-cell level. The flexibility of 16 optical parameters and the freedom offered by Express Modes make this instrument the perfect fit for the CAR T cell field.

- Express Modes comprise predefined experiment settings as well as acquisition and analysis templates.
- Multi-instrument alignment during inter-lab collaborations ensures reproducibility.
- Enables 21 CFR Part 11 compliance for data submission to regulatory agencies.

Dive deep into your sample material

Study complex biological systems with innovative MACS Imaging and Microscopy solutions. Ultra-high content and 3D imaging enable CAR T cell characterization for research and development, as well as target discovery and CAR T cell monitoring.

Imaging solutions

Ultra-high content imaging

Ultra-high content imaging with the MACSima™ Imaging Platform allows for in-depth analysis of tumor infiltration, preclinical CAR T cell target safety evaluation, CAR T cell biodistribution assessment, and patient monitoring. Furthermore, it is perfectly applicable for CAR T cell target discovery.

- MICS (MACSima Imaging Cyclic Staining) technology together with a broad spectrum of recombinant antibodies enables the analysis of hundreds of markers on one single sample.
- Failure-free analysis through automated processing and imaging paired with our powerful MACS Qi analysis software.
- Compatibility with any kind of fixed sample allows for highest flexibility.

Section-free 3D tissue analysis

The UltraMicroscope Blaze™ enables 3D visualization and quantification of CAR T cell penetration at a cellular resolution. Localize single disseminated tumor cells and CAR T cells in whole animal models by light sheet microscopy.

- Full automation including an autofocus feature for easy handling.
- Sample chamber allows for imaging of multiple samples in one run.
- High resolutions ensure unprecedented image quality from overview to sub-cellular details.

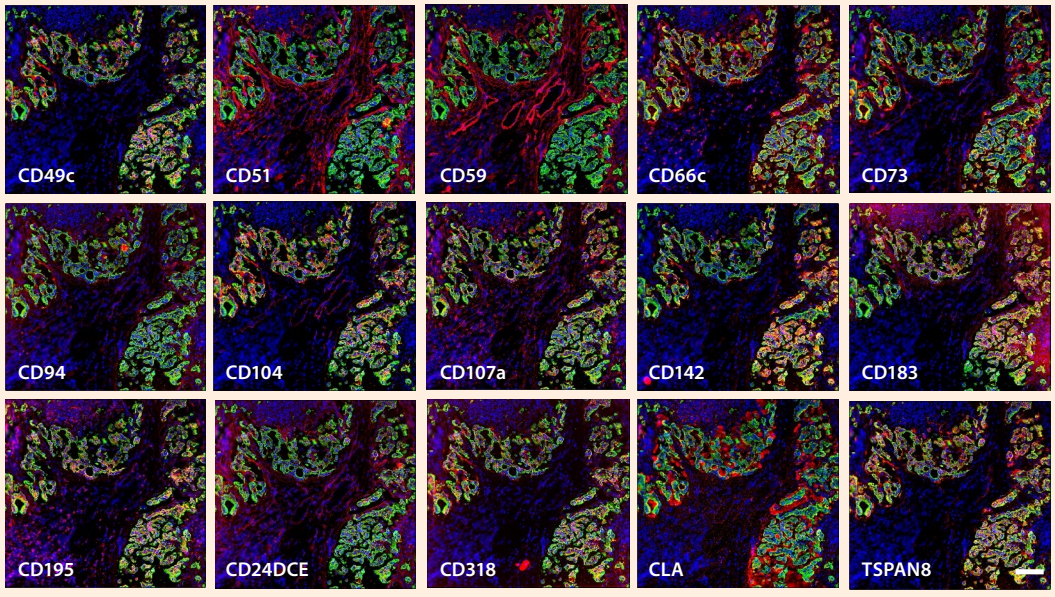


Figure 8: CAR target candidate identification. One tissue section of pancreatic adenocarcinoma was analyzed with the MACSima Imaging Platform. Nuclei are depicted in blue, tumor cells are identified by cytokeratin staining (green), and markers of interest are depicted in red. Scale bar: 100 µm. Image adapted from Schaefer, D. *et al.* (2021) Identification of CD318, TSPAN8 and CD66c as target candidates for CAR T cell based immunotherapy of pancreatic adenocarcinoma. Nat. Commun. 12(1): 1453. Licensed under a Creative Commons Attribution 4.0 International License.

VISIT

Find out more about the MACSima Imaging Platform on our website:

▸ **miltenyibiotec.com/macsima**

VISIT

Learn more about the UltraMicroscope Blaze on our website:

▸ **miltenyibiotec.com/UM-Blaze**

Product highlights

CAR T cells and further engineered immune cells

Product	Order no.
T cells	
CD4 MicroBeads, human	130-045-101
CD8 MicroBeads, human	130-045-201
Pan T Cell Isolation Kit, human	130-096-535
StraightFrom Buffy Coat CD4/CD8 MicroBead Kit, human	130-121-312
T Cell TransAct, human	130-111-160
TexMACS Medium	130-097-196
Human IL-7, premium grade	130-095-361
Human IL-15, premium grade	130-095-762
Human IL-2 IS, premium grade	130-097-744
Vectofusin®-1	130-111-163
MACSPlex Cytokine 12 Kit, human	130-099-169
MACSPlex Cytotoxic T/NK Cell Kit, human	130-125-800
StainExpress Immune Cell Composition Cocktail, human	130-127-637
StainExpress CAR T Transduction Cocktail, human	130-127-638
REAfinity Recombinant Antibodies	www.miltenyibiotec.com
CD19 CAR Detection Reagent, human, Biotin	130-115-965
CD19 CAR FMC63 Idiotype Antibody, REAfinity	130-127-349
BCMA CAR Detection Reagent, human, Biotin	130-126-090
CD22 CAR Detection Reagent, human, Biotin	130-126-727
CAR T Cell Express Mode Package	160-002-376
MACSQuant Analyzer 10	130-096-343
MACSQuant Analyzer 16	130-109-803
Treg cells	
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit, human	130-091-301
$CD4^+CD25^+CD127^{dim/-}$ Regulatory T Cell Isolation Kit II, human	130-094-775
$CD4^+CD25^+$ Regulatory T Cell Isolation Kit, mouse	130-091-041
Treg Expansion Kit, human	130-095-345
Treg Expansion Kit, mouse	130-095-925
Treg Detection Kit (CD4/CD25/CD127), human	130-096-076
Treg Detection Kit (CD4/CD25/FoxP3) (PE), mouse	130-120-674
Treg Suppression Inspector, human	130-092-909

Product	Order no.
NK cells	
CD3 MicroBeads, human	130-050-101
CD56 MicroBeads, human	130-050-401
NK MACS Medium	130-114-429
Human IL-2 IS, premium grade	130-097-746
Human IL-15, premium grade	130-095-765
Human IL-1β, premium grade	130-093-897
MACS BSA Stock Solution	130-091-376
Vectofusin®-1	130-111-163

VISIT

Visit our webpage for our complete CAR T cell product porftolio and scientifc resources:
▸ **miltenyibiotec.com/cartcells**

What's your vision?

Translate your CAR T cell research protocol into an automated GMP-compliant process. The CliniMACS Prodigy performs cell enrichment, activation, genetic modification, and expansion with minimal involvement from the user. All steps take place in a closed system, providing a sterile solution for clinical-grade CAR T cell manufacturing.

Automated CAR T cell manufacturing

Closed system

Minimal hands-on time

CliniMACS Prodigy T Cell Transduction Process

Standardized process

▸ miltenyibiotec.com/cartcells

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