



Miltenyi Biotec

CAR Detection Reagents

Evaluation of CAR expression by flow cytometry



CAR-engineered cell therapies – the principle

Engineered cell therapies are a new and innovative treatment option against cancer. They are based on the principle that immune cells (e.g. T or NK cells) can be genetically engineered to carry a chimeric antigen receptor (CAR) on their surface, which binds a specific antigen expressed on tumor cells, e.g., CD19 antigen (fig. 1). Upon re-infusion into the patient, the engineered immune cells are directed to the tumor cells expressing the target antigen. Recognition of the target antigen leads to activation of the engineered cell resulting in proliferation, cytokine secretion, and tumor cell lysis.

Discover our solutions for CAR detection and choose a highly specific and sensitive **antigen-based** or **antibody-based (idiotype)** CAR Detection Reagent.

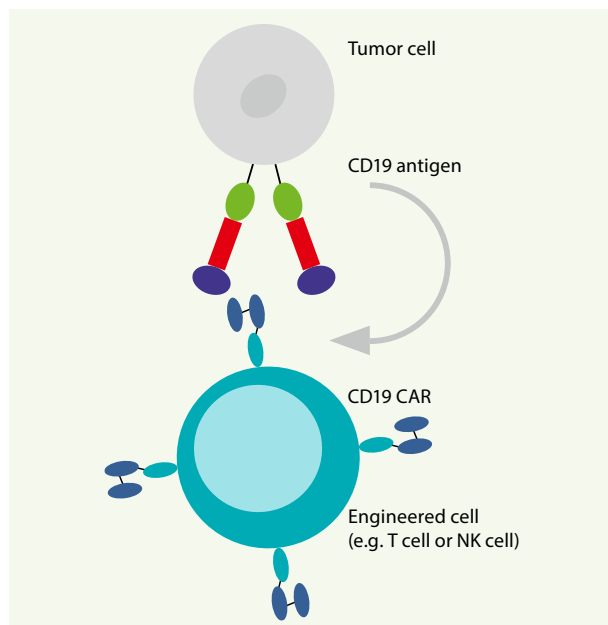


Figure 1: Mechanism of tumor cell recognition by CAR-engineered cells.

Antigen-based CAR Detection Reagents

Antigen-based CAR Detection Reagents consist of the CAR target protein (e.g. CD19) fused to a biotin-labeled mutated Fc backbone. The target protein is recognized by the CAR receptor and can be readily identified using fluorochrome-labeled biotin antibodies, i.e., clone REA746 (fig. 2). This approach multiplies the signal and increases assay sensitivity compared to fluorescence-labeled proteins.

- Robust yet specific CAR detection through multiple binding sites
- Shows functional CAR folding
- Flexibility in panel design for flow cytometry

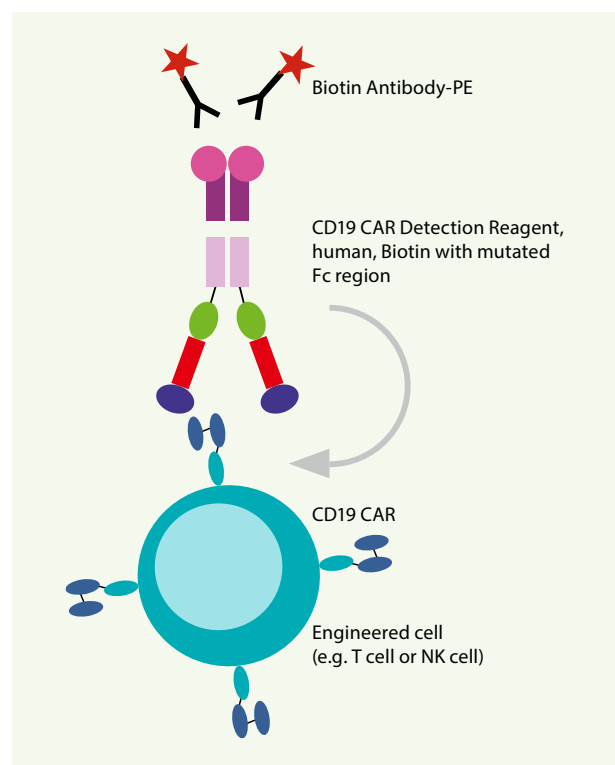


Figure 2: Detection of CAR-engineered cells using antigen-based CAR Detection Reagent.

Antibody-based CAR Detection Reagents

Antibody-based (idiotype) CAR Detection Reagents are antibodies that bind to the unique antigen-binding site of a specific CAR receptor which is typically derived from a monoclonal antibody, e.g., FMC63-derived CD19 CAR (fig. 3). Biotin and fluorochrome-labeled idiotype antibodies can be used for sensitive detection.

- Highly specific CAR detection through unique binding site
- One-step staining possible
- Compatible with flow cytometry, imaging (MICS, IF, IHC), and mass cytometry

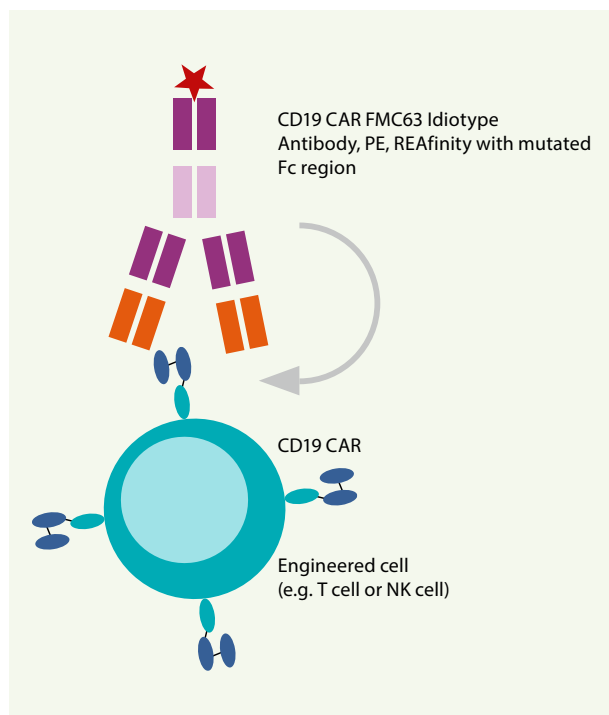


Figure 3: Detection of CAR-engineered cells using antibody-based (idiotype) CAR detection reagents.

Precise and reproducible CAR detection

Miltenyi Biotec CAR Detection Reagents are designed with the proprietary REAfinity™ Recombinant Antibody Technology. The associated benefits are:

- No background signal due to unique mutated Fc region
- Superior lot-to-lot consistency and purity
- Liquid ready-to-use formulation for streamlined workflows



LEARN MORE



Learn more about REAfinity Recombinant Antibody Technology

► miltenyibiotec.com/reafinity





Reproducible



Easy



Fast

In-process and quality control (IPC/QC) during cell manufacturing

Miltenyi Biotec's CAR Detection Reagents enable a clear distinction between CAR⁺ and CAR⁻ cells, which makes them the ideal tool for the assessment of transduction efficiency and viability of CAR-engineered cells during manufacturing. This includes but is not limited to CAR T (fig. 4) and CAR NK cells (fig. 5).

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What I find very useful and interesting about Miltenyi Biotec is the wide range of products and associated protocols dedicated to CAR T cells. We are using CD19 CAR Detection Reagents for our *in vitro* and *in vivo* R&D experiments. They are easy, fast to use, and show far better affinity than other CD19-based detection reagents. I also want to thank the scientific and technical support for their useful help and availability.

”

**Rachel Pacherie, PhD,
R&D Director, IXAKA,
Villejuif, France**

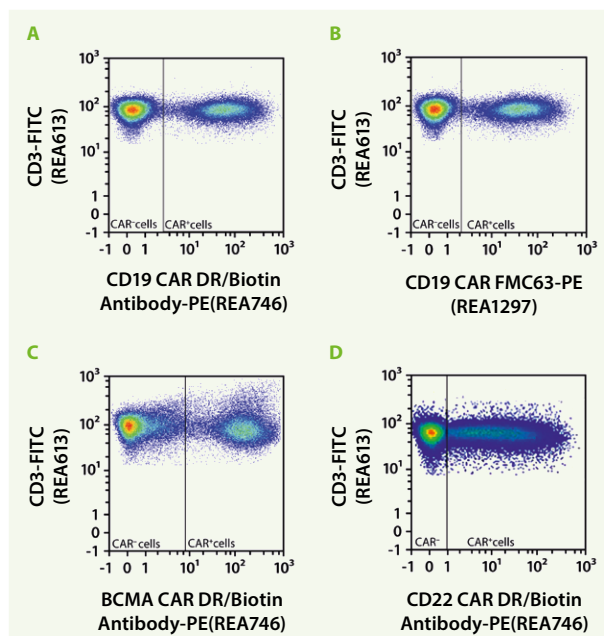


Figure 4: Distinction of CAR⁺ and CAR⁻ cells. CAR T cells were generated within 12 days using the CliniMACS Prodigy® T Cell Transduction process. Cells were stained with CD3-FITC and a suitable CAR Detection Reagent conjugated with Biotin. Biotinylated CAR Detection Reagents were detected using PE-labeled Biotin Antibodies. Cells were detected by flow cytometry using the MACSQuant® Analyzer 10.

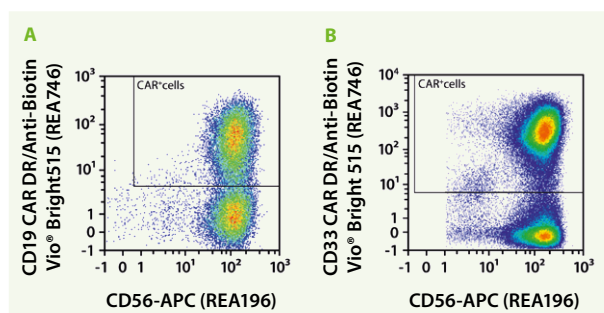


Figure 5: Detection of CAR NK cells. NK cells were isolated using the CliniMACS Prodigy LP-3-56-System. CAR NK cells were generated by lentiviral transduction. On day 13 in culture, cells were stained with CD56-APC and a suitable CAR Detection Reagent. Biotinylated CAR Detection Reagents were identified using Vio Bright 515-labeled Biotin Antibodies. Cells were detected by flow cytometry using the MACSQuant Analyzer 10.

Follow-up of patients undergoing CAR T cell therapy

To model the analysis of CAR T cell persistence after adoptive transfer, CAR T cells can be spiked into whole blood. Miltenyi Biotec's CAR Detection Reagents then enable a clear distinction of CAR⁺ and CAR⁻ cells, even with very low CAR T cell numbers (fig. 6) which may occur during post-infusion monitoring.

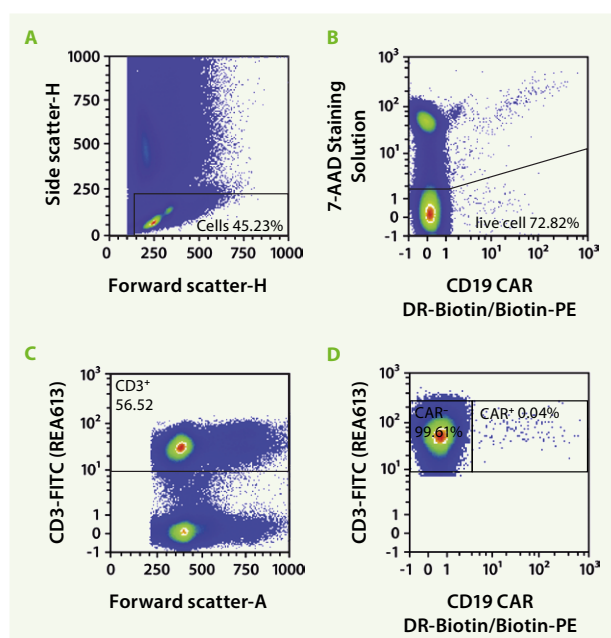


Figure 6: CAR monitoring in whole blood samples spiked with CAR-engineered T cells. Samples were initially gated on lymphocytes based on SSC-H/FSC-H (A). Further gating allowed the discrimination of live, 7-AAD-negative cells (B). T cells were gated as CD3⁺ (C). CAR T cells are displayed based on staining with CAR Detection Reagent (D). Cells were detected by flow cytometry using the MACSQuant Analyzer 10.

LEARN MORE

Discover our Miltenyi Biotec-tested antibody panels to jumpstart your CAR T cell flow analysis

► miltenyibiotec.com/MBTPs

No background signal

The unique REAfinity Recombinant Antibody Technology minimizes non-specific signals in CAR analysis by flow cytometry. The significantly lower background staining in CAR-negative samples, compared to products from other vendors, makes Miltenyi Biotec's CAR Detection Reagents the ideal tool for reliable distinction of CAR-expressing and -negative cells during cell manufacturing and patient monitoring research.

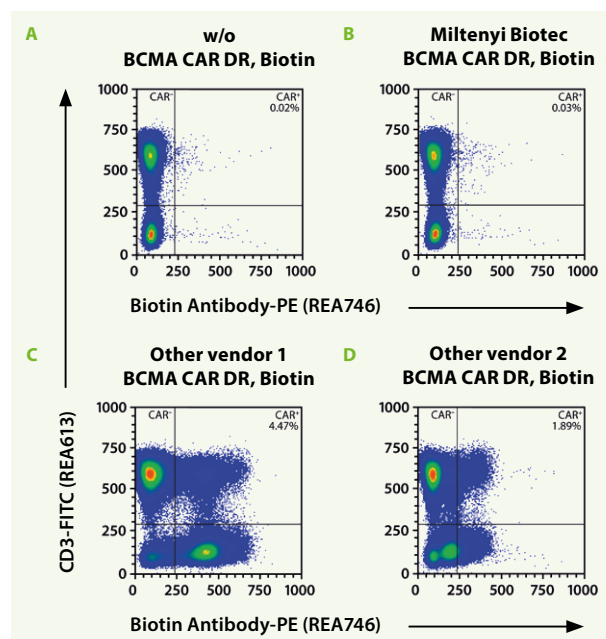


Figure 7: Comparison of the specificity of different commercial CAR detection reagents in CAR-negative samples from healthy donors. (A): w/o BCMA CAR DR, Biotin; (B): Miltenyi Biotec BCMA CAR DR, Biotin; (C): Other vendor 1 BCMA CAR DR, Biotin; (D): Other vendor 2 BCMA CAR DR, Biotin.

Highly specific imaging of CAR T cells

CD19 CAR FMC63 Idiotypic Antibody-PE (REA1297) is a highly specific and sensitive tool to detect FMC63-derived CD19 CAR, not only in flow cytometry but also in a range of other applications, e.g., immunostainings for microscopy (fig. 8).

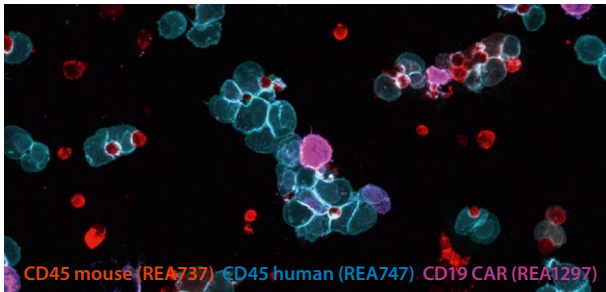


Figure 8: Human primary T cells expressing an FMC63-based CD19 CAR were mixed with murine T cells expressing a Glypican3 CAR in a 1:1 ratio and spun onto a microscope slide. Cells were fixed with 4% PFA and subjected to MACSima™ Imaging Cyclic Staining (MICS) analysis using the MACSima Imaging System. The image shows stainings of CD19 CAR FMC63 Idiotypic Antibody-PE (REA1297, magenta), mouse CD45-FITC (REA737, red); and human CD45-PE (REA747, turquoise). The concurrent staining of CD19 CAR and human CD45, but not mouse CD45, demonstrates specificity of REA1297 for the CD19 CAR construct. Image courtesy of Dr. Christian Seitz, University Children's Hospital Tübingen, Germany.

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Working with both antigen-based and anti-idiotype Miltenyi Biotec CAR Detection Reagents, I value the high quality of the REAfinity Recombinant Antibody Technology. Consistent performance over time, and the elimination of background, allows sensitive detection of CAR⁺ cells in blood samples as well as tissues by immunofluorescence and ultrahigh-content imaging. The convenient, ready-to-use format and reduced pipetting steps support an efficient workflow.

”

Dr. med. Christian Seitz,
University Children's Hospital
Tübingen, Germany



Workflow automation minimizes operator interference

Miltenyi Biotec's CAR Detection Reagents are compatible with the StainExpress™ Dry Antibody Cocktail "CAR T Transduction" (# 130-127-638) for standardized and accelerated CAR T cell identification by flow cytometry. Additionally, they are tested with our CAR T Cell and NK Cell Express Mode packages (# 160-002-376 or # 160-003-341) and panels, a unique add-on for the MACSQuantify™ Software, developed to automate data acquisition and analysis and minimize hands-on time and variation of results caused by different operators.

Product overview

CAR target	Product	Order no.
CD19	CD19 CAR Detection Reagent, human, Biotin	130-129-550
CD19 (FMC63)	CD19 CAR FMC63 Idiotypic Antibody, REAfinity (REA1297)	
	PE	130-127-342
	APC	130-127-343
	Vio Bright B515	130-127-344
	Biotin pure	130-127-345
CD19 (FMC63)	CD19 CAR FMC63 Idiotypic Antibody, REAfinity (REA1298)	
	Biotin pure	130-127-349
BCMA	BCMA CAR Detection Reagent, human, Biotin	130-126-090
CD22	CD22 CAR Detection Reagent, human, Biotin	130-126-727
CD33	CD33 CAR Detection Reagent, human, Biotin	130-127-642

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Our portfolio is constantly growing.
Check the latest product listing under

► miltenyibiotec.com/CARdetection



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