CAR Cell Identification and Characterization

Using Novel Cell Surface Antigen-Based Fusion Proteins

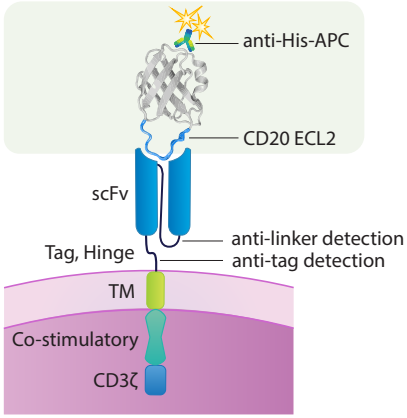
Cell Surface Antigen-Based Fusion Proteins

- Consist of extracellular domains of cell surface proteins, fused with different proprietary scaffold elements
- Mimic binding properties of cell surface proteins, qualified by binding to known ligands or known antibodies
- Bind to CAR molecules expressed on the cell surface
- Have no Fc sequences to reduce the size of the protein molecule, and to eliminate Fc-induced non-specific FcR binding to cells

Target	Target Domain	Design Rationale	Scaffold
CD19	CD19 extracellular domain	Supports native-like, multivalent presentation of a larger extracellular domain from a single-pass membrane protein.	Scaffold 1*
BCMA (TNFRSF17)	BCMA extracellular domain	Supports native-like, multivalent presentation of a short extracellular domain from a single-pass membrane protein.	Scaffold 2*
CD20 (MS4A1)	CD20 extracellular loop 2 (ECL2)	Supports the conformationally constrained presentation of a short extracellular loop from a multi-pass membrane protein.	Scaffold 3*

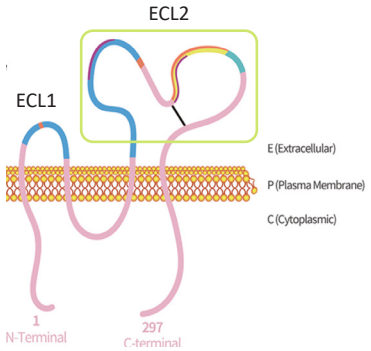
*Scaffolds 1–3 are proprietary, Fc-free designs selected according to the structural architecture of each target protein.

Antigen-Specific CAR Detection

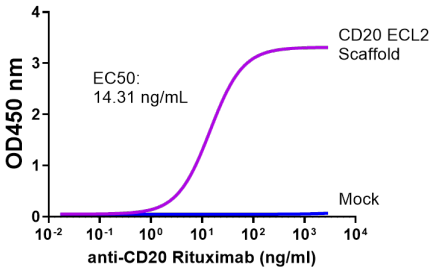


CD20-ECL2 Scaffold Protein for CAR Detection

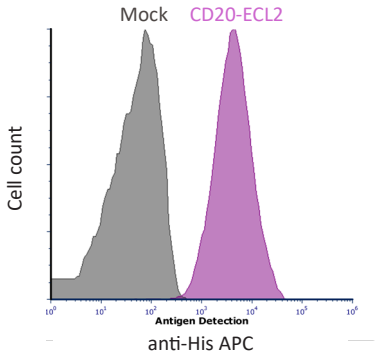
CD20 Protein Structure



CD20-ECL2 Protein Binding to Rituximab



CD20-ECL2 Detecting Rituximab CAR



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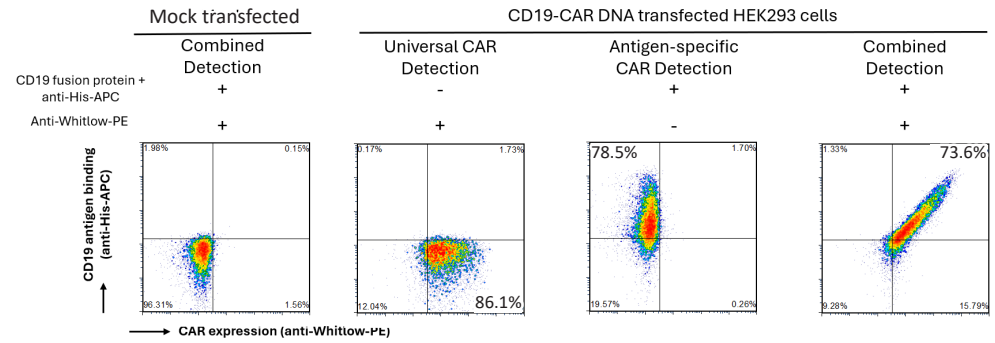
Detecting and Characterizing CAR T Cells Using Flow Cytometry

Universal and antigen-specific detection provides complementary measurements of CAR surface expression and target-binding competency.

Method	Detection Reagent	Flow Cytometry Readout	What it Shows
Universal CAR Detection	Anti-tag antibody, anti-linker antibody, or Protein L	% CAR-positive cells and fluorescence intensity (MFI)	Measures CAR surface expression, but not antigen-binding competency
Antigen-specific CAR Detection	Recombinant target antigen	% antigen-binding cells and antigen-binding intensity (MFI)	Confirms CAR surface expression and recognizes its intended target
Combined Detection	Universal detection reagent plus recombinant target antigen	CAR expression and antigen-specific binding measured together	Distinguishes antigen-binding competency from total CAR surface expression for more complete CAR characterization

Case Study 1: Detection of Anti-CD19 CAR in HEK293 Cells

Comparable results were obtained using CD20- and BCMA-directed CAR constructs with their corresponding Conigen antigen fusion proteins, demonstrating the broader applicability of this antigen-specific CAR detection strategy.



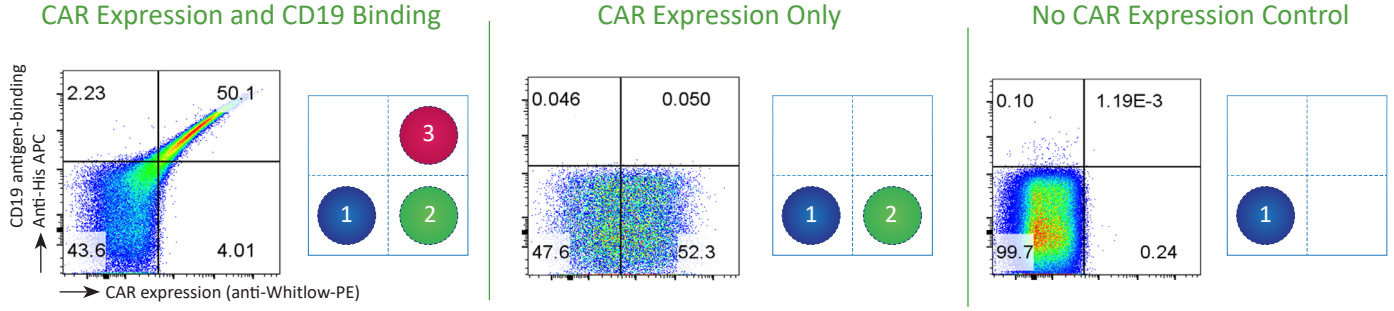
Universal and antigen-specific detection of an anti-CD19 FMC63 CAR. *Transfected HEK293 cells were analyzed 72 hours after transfection using anti-Whitlow–PE, Conigen's CD19 fusion protein followed by anti-His–APC, or both detection reagents. Percentages indicate the proportion of cells within each quadrant.*

Case Study 2: Detection of Anti-CD19 CAR in Primary T Cells

Conigen's CD19 fusion protein identifies cells whose surface CAR can bind CD19. Universal anti-linker staining identifies cells expressing the CAR. When used together, the reagents distinguish three relevant populations:

1 **CAR-negative/CD19-binding-negative** 2 **CAR-positive/CD19-binding-negative** 3 **CAR-positive/CD19-binding-positive**

Combined detection was then used to compare CAR constructs differing in VH/VL orientation, leader sequence, and intracellular domain.

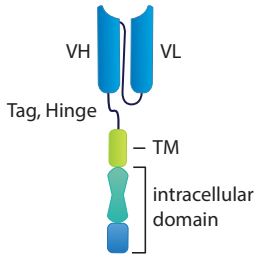


Combined universal and antigen-specific detection of an anti-CD19 FMC63 CAR in primary human T cells. *Primary human T cells were transiently transfected with FMC63 anti-CD19 CAR mRNA or mock transfected. At 24 hours, CD8+ T cells were evaluated by flow cytometry using anti-Whitlow–PE to detect surface CAR expression and Conigen's CD19 fusion protein followed by anti-His–APC to detect CD19 binding. Combined staining identified CAR-negative/CD19-binding-negative, CAR-positive/CD19-binding-negative, and CAR-positive/CD19-binding-positive populations. Representative CD8+ T-cell results are shown; comparable results were observed in CD4+ T cells.*

Not All CAR-Positive Cells Are Functionally Equivalent

Despite similar percentages of CAR-positive cells, the CAR T cells differed in CD19 binding and tumor-cell deletion.

Schematic of a scFv-Based CAR



	Construct Design Pair	CAR + Cells (%)	CD19 Binding Cells (%)	Tumor Deletion[1] (%)
Different VH/VL orientation	A1	100%	91%	67%
	A2	100%	91%	42%
Different leader sequence	B1	97%	45%	43%
	B2	100%	95%	56%
Different intracellular domains	C1	87%	60%	48%
	C2	100%	97%	63%

[1]Measured at an effector-to-target ratio of 0.5:1.

Key takeaway:
CAR expression alone does not define construct performance. Antigen-specific detection adds a direct measure of target recognition.

Supporting CAR Development from Discovery Through Translation

One reagent platform across the CAR development workflow. Conigen's native-like recombinant target antigens support CAR discovery, candidate characterization, analytical assay development, and translational research by directly measuring antigen recognition.

Discovery
Target expression profiling (flow, IHC, single cell)
CAR construct libraries

Development
CAR expression & phenotype analysis

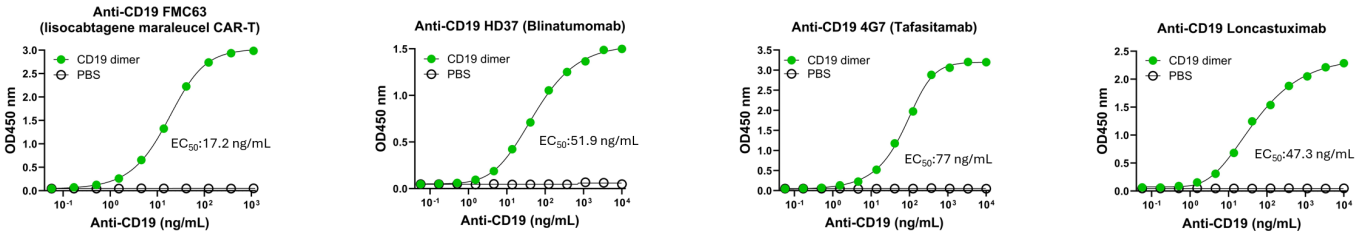
Manufacturing
Product characterization (identity, purity, potency, sterility, viability)

Clinical Research
Long-term follow-up (safety & durability)
CMC & FDA Regulatory Readiness

Protein Quality Shapes CAR Detection

Reliable CAR detection depends on recombinant antigens that preserve biologically relevant structure and binding sites. Conigen proteins are evaluated for recognition by therapeutic antibodies, performance relative to alternative protein designs, and binding to known ligands. Together, these data demonstrate their suitability for CAR detection, characterization, and candidate selection.

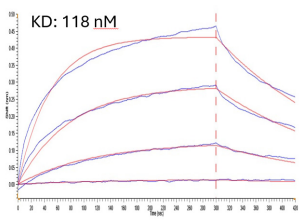
Broad Epitope Recognition for Confident CAR Detection



- CD19 fusion protein preserves known therapeutic antibody binding epitopes and conformation.
- CD19 fusion protein can be used to screen and characterize CD19-specific antibodies.

Binding that Reflects Biological Function

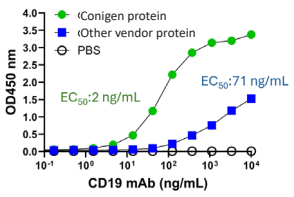
FGF-1 ligand binding (BLI)



FGFR4 fusion protein can be used for target-specific antibody screening/ characterization, receptor/ ligand binding evaluation, and CAR detection.

Protein Design Makes the Difference

Anti-CD19 4G7 (Tafasitamab)



Rational design of the CD19 fusion protein can provide better protein presentation, resulting in improved antibody binding sensitivity for antibody screening and characterization.

Conigen Fusion Proteins for CAR Detection

Target	Species	Tag	Antibody Binding	Ligand Binding	Product Code
B7H3	Human	His-Avi	✓		CSP-240984lg
	Human	His-Avi	✓		CSP-240982lg
	Human	His	✓		CSP-260984-01
BCMA	Human	His	✓	✓	CSP-26402.M4-01
CD117	Human	hFc	✓	✓	CSP-25123-04
	Human	His-Avi	✓	✓	CSP-24123-03
	Mouse	His-Avi	✓	✓	CSP-25193-03
	Rhesus Macaque	His-Avi	✓	✓	CSP-25194-03
CD123	Human	His	✓		CSP-26321-01
CD155	Human	His	✓	✓	CSP-24029
	Mouse	His	✓	✓	CSP-25184-01
CD19	Human	His	✓		CSP-24125-01
	Human	His-Avi	✓		CSP-24125-03
	Human	Biotinylated	✓		BCSP-24125-03
	Mouse	His	✓		CSP-124-01
	Rhesus Macaque	His	✓		CSP-25291-01
CD20	Human	His	✓		CSP-26403.S4-01
CD22	Human	His	✓		CSP-25199-01
CD33	Human	His	✓		CSP-25200-01
CD38	Human	His-Avi	✓		CSP-24096-03
CD4	Human	Biotinylated	✓	✓	BCSP-24004-03
	Human	His	✓	✓	CSP-24004
	Human	His-Avi	✓	✓	CSP-24004-03
	Rhesus Macaque	His	✓		CSP-24038
CD7	Human	His	✓		CSP-26322-01
CD8α	Human	His	✓	✓	CSP-25135-01
EpCAM	Human	His	✓		CSP-26323-01
EphA2	Human	His	✓		CSP-26324-01
FGFR4	Human	His-Avi	✓	✓	CSP-25132-03
IL1RAP	Human	His	✓		CSP-26325-01
MICA	Human	His	✓		CSP-25242-A1B6
MICB	Human	His	✓		CSP-26328-01
NECTIN 4	Human	His	✓	✓	CSP-24016
PD1	Human	His-Avi	✓	✓	CSP-24093-03
PD-L1	Human	hFc	✓	✓	CSP-2594-04
	Human	His-Avi	✓	✓	CSP-24094-03
	Mouse	hFc	✓		CSP-25190-04
	Mouse	His	✓		CSP-25190-01
ROR1	Human	His	✓		CSP-25287
SIGLEC 6	Human	His-Avi	✓		CSP-26203-03
TROP-2	Human	FLAG-His	✓		CSP-24041-02
	Human	His-Avi	✓		CSP-24041-03
	Mouse	FLAG-His	✓		CSP-25191-02
VEGFR2	Human	His-Avi	✓	✓	CSP-25127-03

Don’t see your CAR target? Contact Conigen to discuss a custom fusion protein for your CAR detection needs.

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