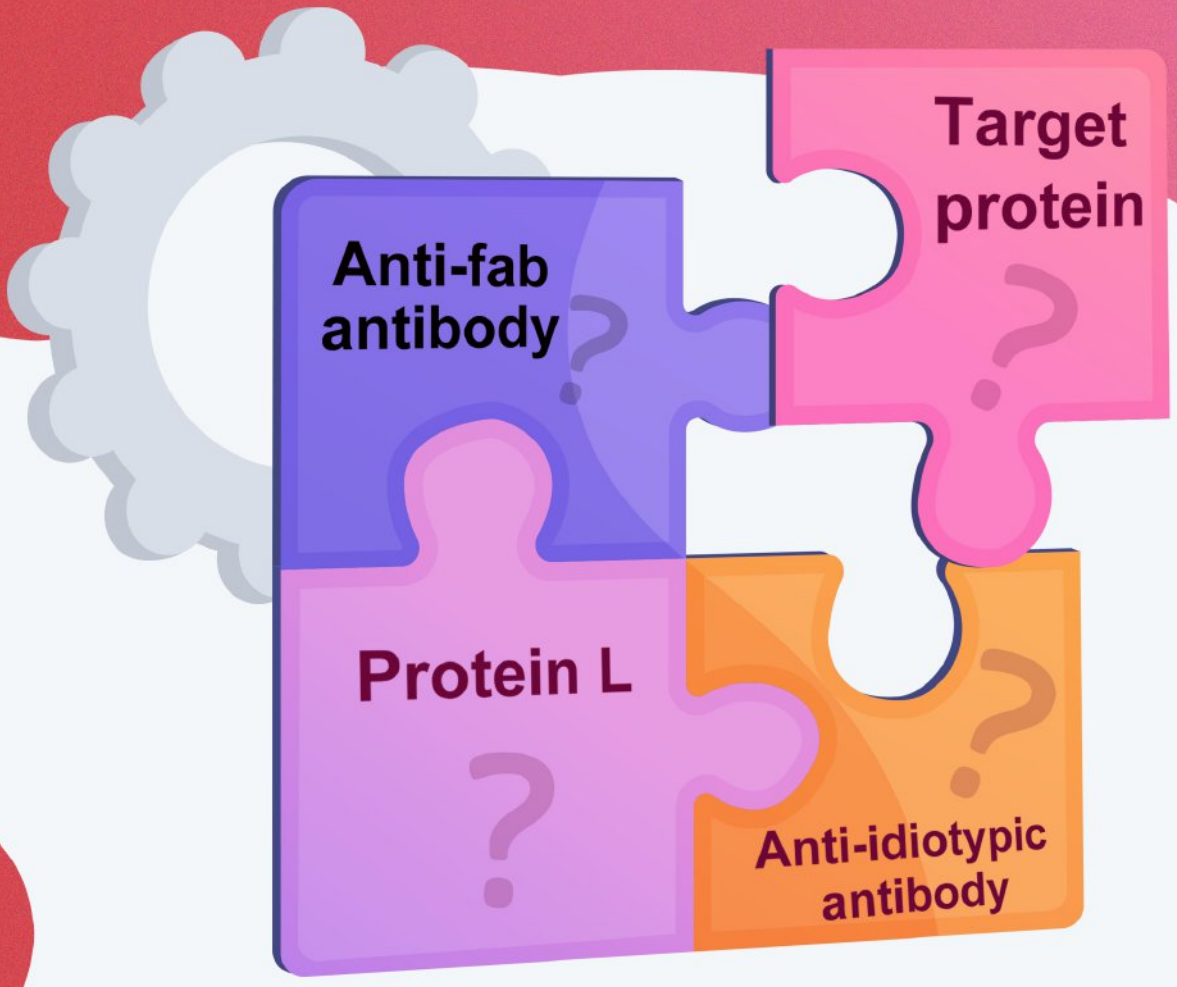
Deciding on the right option for your CAR detection?



Let 's check the pros and cons among these methods and find out the advices and protocols from ACROBiosystems!

Evaluating CAR expression is an essential step in the production of CAR-T cells. This is often done by flow cytometry, using protein L, anti-Fab antibodies, anti-idiotypic antibody or target antigens as detection antibodies. Among these common choices, you may ask which one is a suitable option for my CAR detection?

In this article, we will summarize the key points of each staining agent's general properties for CAR detection. Answers to the following questions will be clear after reading the text:

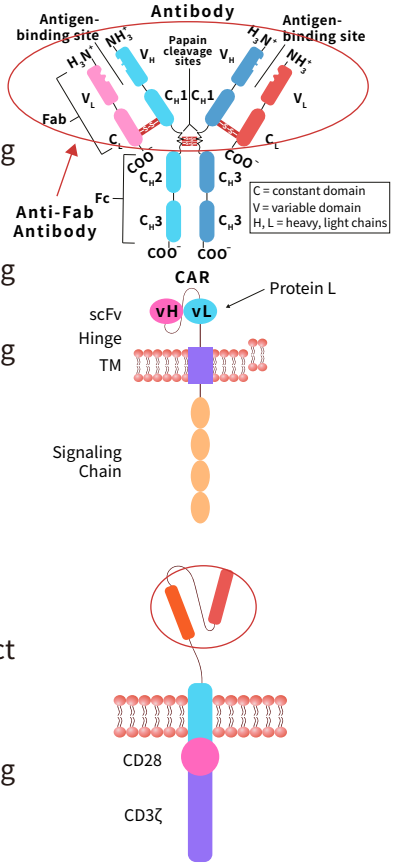
- What are the advantages and disadvantages of various CAR detection reagents?
- What's the binding site of each staining reagent?
- How to choose CAR staining reagents at different stages in the development of CAR -T cell therapy?
- What are the detailed steps for CAR detection by flow cytometry?

Reagents for CAR detection

★ General-purpose reagents: Protein L and anti-Fab antibody

Although both anti-Fab antibodies and Protein L are relatively cheap CAR-staining reagents, they share significant shortcomings:

- Cross-reactivity with non-CAR IgG like proteins on the cell surface, requiring stringent washing before and after staining.
- Incompatibility with antibodies and many FcX blocking reagents during multicolor flow cytometry, requiring multiple staining and washing steps.
- Cannot independently stain different CARs on a dual-CAR expressing T cell.
- Cannot stain CARs with synthetic scFv.

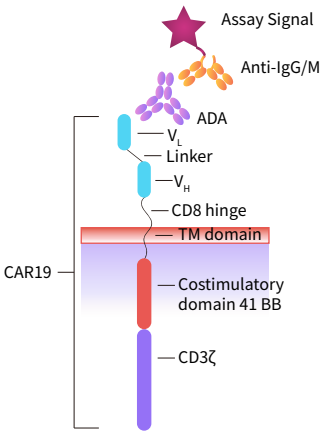


★ Target protein

- Specifically binds the antigen-binding domains of CARs and has functional effect verification.
- Can detect the expression of single CAR in dual-target CAR-T cells.
- Fluorescent-labeled proteins can detect CAR expression by one-step staining with minimal background and saves time.

★ Anti-idiotypic antibody

- Specifically binds the antigen-binding domains of CARs.
- High specificity, sensitivity and minimal background; Suitable for detecting clinical samples.
- Anti-idiotypic antibodies with neutralizing activity can detect free CAR-positive T cells, which have not yet bound to the tumor antigen.
- Each unique CAR has to be stained with corresponding anti-idiotypic antibody. Most need to be customized and customization is very time-consuming.



Combining with the properties and application situation of each CAR staining reagent, and we provided some suggestions for reference:

① Try not use Protein L or anti-Fab antibodies in quality control step and PK studies to avoid high non-specificity background, making the results difficult to analyze.

② Strongly recommend using antigen protein at the stages of antibody screening, methods development and quality control to ensure the functional effect of screened scFv and the stability of the quality control method.

③ For the clinical stage, antigen protein is the first choice to ensure the reliability of PK assay results, and anti-idiotypic antibody is also a preferred option for the clinical samples with few CAR positive cells and complex components.

To support the development of CAR-T cell therapy. Here we show some detailed steps, key points and result analysis of selected case studies about using target protein and anti-idiotypic antibody for CAR detection by flow cytometry.

Case studies

▶ Case No.1 Evaluation of anti-CD19 CAR expression using FITC-labeled CD19 protein

Reagents

FITC-labeled Human CD19 (20-291) Protein (ACROBiosystems, Cat. No. CD9-HF2H2).

Samples

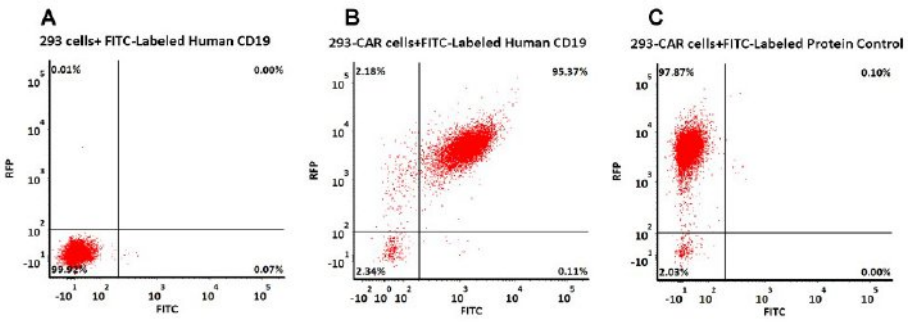
Anti-CD19 CAR-293 cells.

Brief Protocol

- Culture anti-CD19 CAR-293 cells in DMEM medium with 10% FBS in the CO_2 incubator (at 37°C, 5% CO_2).
- Harvest the cells and wash the cells once by wash buffer.
- Count the cells number and the viability, aliquot up 2e5 live cells (Anti-CD19-scFv positive cell is 98%) into each tube. (Note: the cell viability must be ⩾ 95%.)
- Add 100 µl, 10 µg/ml of FITC-labeled Human CD19 (20-291) Protein (ACROBiosystems, Cat. No. CD9-HF2H2) or FITC-labeled Protein control into each tube, incubating at 4°C for 1 hour.
- Wash the cells 3 times by wash buffer and resuspend the cells in 200 µl PBS per sample.
- Transfer the cells into flow tube and detect by Flow cytometry.
- Analyze result using FACS Celesta software and FCS Express 6 Flow software.

Results

The data showed that the expression level of anti-CD19 scFv on the surface of anti-CD19 CAR-293 cells is 95.37%.



293 cells were transfected with anti-CD19-scFv and RFP tag. 2e5 of the cells were stained with B. FITC-Labeled Human CD19 (20-291) (Cat. No. CD9-HF2H2, 10 μg/ml) and C. FITC-labeled protein control. A. Non-transfected 293 cells and C. FITC-labeled protein control were used as negative control. RFP was used to evaluate CAR (anti-CD19-scFv) expression and FITC was used to evaluate the binding activity of FITC-labeled Human CD19 (20-291) (Cat. No. CD9-HF2H2).

Protocol

▶ Case No.2 Evaluation of anti-CD19 CAR expression using FITC-labeled anti-FMC63 scFv antibody

Reagents

FITC-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (ACROBiosystems, Cat. No. FM3-FY45).

Samples

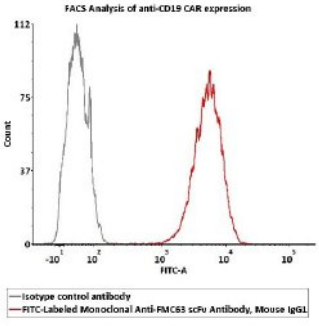
Anti-CD19 CAR-293 cells

Brief Protocol

- Culture Anti-CD19 CAR-293 cells in DMEM medium with 10% FBS in the CO_2 incubator (at 37 °C, 5% CO_2).
- Harvest the cells and wash the cells once by FACS buffer.
- Count the cells number and the viability, aliquot up 2e5 live cells into each tube. (Note: the cell viability must ⩾ 95%.)
- Dilute FITC-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (ACROBiosystems, Cat. No. FM3-FY45) in FACS buffer to get the working solution just before the assay, and then add 100 μL of the working solution into the tube with cell pellet. Mix well and incubate at 4°C for 60 minutes.
- Wash the cells 3 times by FACS buffer and resuspend the cell pellet in 200 μL PBS per sample.
- Transfer the cell suspension into flow tube and detect the cells by Flow cytometry.
- Analyze the result data using FCS Express 7 Plus and GraphPad Prism 5 software.

Results

The data showed that the expression level of anti-CD19 scFv on the surface of anti-CD19 CAR-293 cells was 100%.



2e5 of Anti-CD19 CAR-293 cells were stained with 100 μL of 1:50 dilution (2 μL stock solution in 100 μL FACS buffer) FITC-Labeled Monoclonal Anti-FMC63 scFv Antibody, Mouse IgG1 (Cat. No. FM3-FY45) and isotype control respectively. FITC signal was used to evaluate the binding activity .

Protocol

Case No.3 Evaluation of anti-MSLN CAR expression using PE-labeled MSLN protein

Reagents

PE-labeled Human Mesothelin / MSLN (296-580) Protein (ACROBiosystems, Cat. No. MSN-HP2H5).

Samples

Anti-MSLN CAR-293 cells

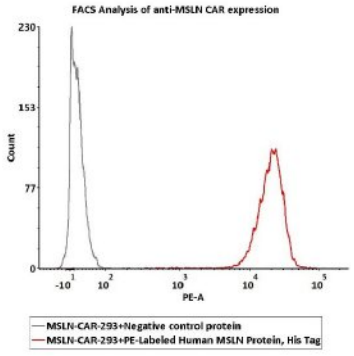
Brief Protocol

- Culture anti-MSLN CAR-293 cells in DMEM medium with 10% FBS in the CO_2 incubator (at 37 °C, 5% CO_2).
- Harvest the cells and wash the cells once by wash buffer.
- Count the cells number and the viability, aliquot up 1e6 live cells into each tube.
- Add 100 µl of diluted PE-labeled Human Mesothelin (296-580) Protein (Cat. No. MSN-HP2H5) (prepared in dilution buffer at 1:50 dilution) into each tube, incubating at 4°C for 1 hour.
  Wash the cells 3 times by wash buffer and resuspend the cells in 200 µl PBS per sample.
- Transfer the cells into flow tube and detect by Flow cytometry.
- Analyze result using FACS Celesta software and FCS Express 6 Flow software.

Results

The data showed that the expression level of anti-MSLN scFv on the surface of anti-MSLN CAR-293 cells was 100 %.

1e6 of the anti-MSLN CAR-293 cells were stained with 100 µL of 1:50 dilution (2 µL stock solution in 100 µL FACS buffer) of PE-Labeled Human Mesothelin (296-580) Protein, His Tag (Cat. No. MSN-HP2H5) and negative control protein respectively, PE signal was used to evaluate the binding activity.



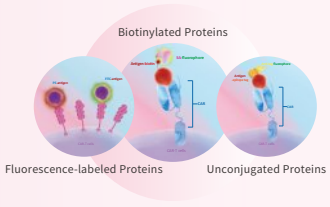
Protocol

>>> About ACROBiosystems

ACROBiosystems is a leading manufacturer of recombinant proteins and other critical reagents to support the development of target therapeutics, vaccines, and diagnostics. Our high quality and batch-to-batch consistency is targeted to satisfy the rigorous standards of antibody drug and CAR-T therapy research and development.

Solutions for Evaluation of CAR Expression

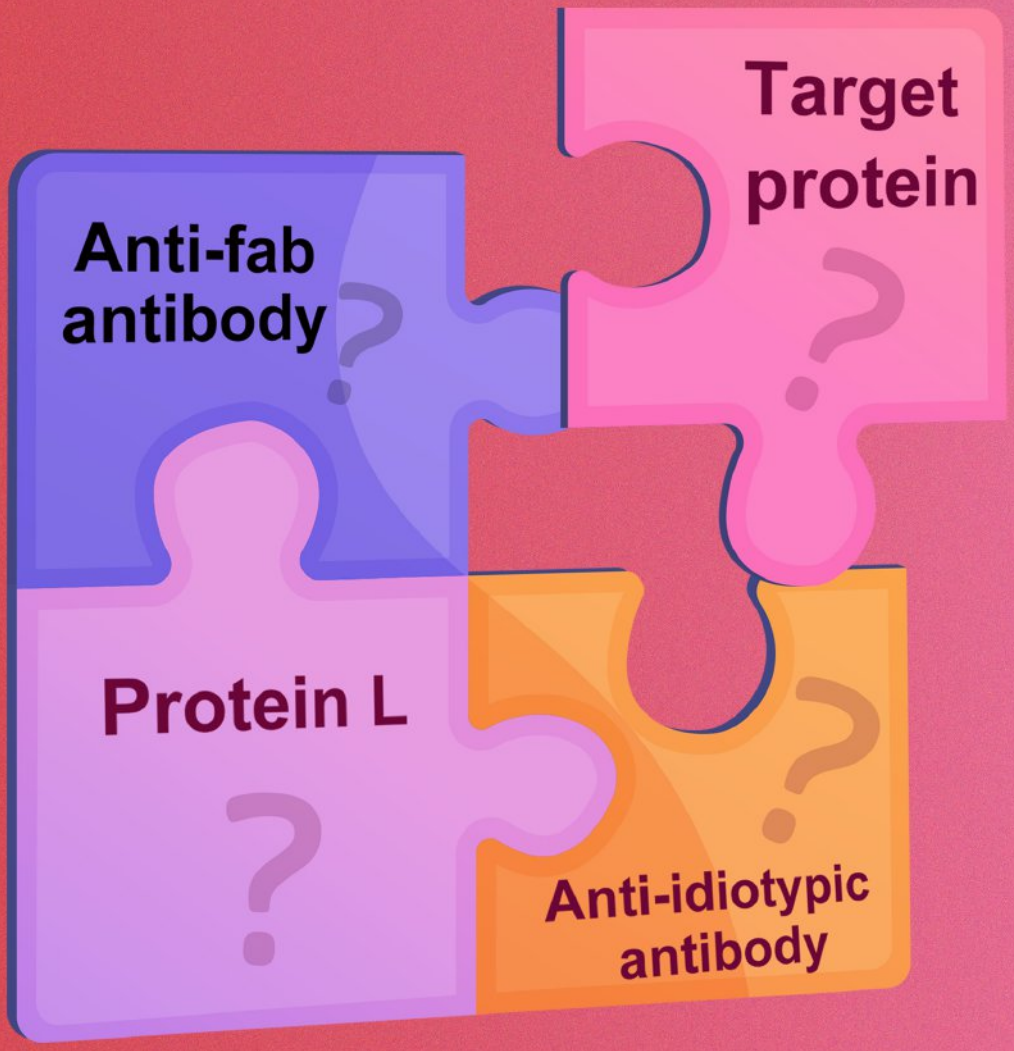
- 50+ CAR-T targets
- Designed for CAR detection
- Free Protocols



Find out more

>>> References

[1] Hu Y, Huang J. The Chimeric Antigen Receptor Detection Toolkit. Front Immunol. 2020;11:1770. Published 2020 Aug 11.



Try target antigens for CAR expression evaluation with high specificity and minimal background staining.

BCMA	CD19	Her2	FMC63	Mesothelin
CD22	CLEC12A	CD33	FAP	ROR1

BIOSYSTEMS Acro

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