

Claudin18.2-VLP: designed for CAR detection

1. Objective

Specific detection of Anti-GPRC5D-CAR-Expi293 cells by Flow Cytometry (FC) analysis

2. Materials & Reagents

- Fluorescent Human CLDN18.2 Full Length Protein-VLP (EGFP) (Catalog No. FLP100017)
- An Irrelevant Biotin Labeled Protein L (Negative Control Protein)
- Anti-GPRC5D CAR-Expi293 cells (Negative Control Cells)
- Anti-CLDN18.2 CAR-Expi293 cells
- Streptavidin-APC
- FACS buffer: 1XPBS buffer with 0.2% BSA
- Blocking Buffer: NBS Blocking Buffer

3. Experimental Procedure

3.1 Cell Collection and Treatment: Collect Anti-Claudin-18.2 CAR cells and negative control cells directly into centrifuge tubes, centrifuge at 1500rpm for 5 minutes, discard the culture medium, wash cells once with 1X PBS, centrifuge at 1500rpm for 5 minutes to remove supernatant.

3.2 Blocking: Resuspend cells in 1X PBS, add 5% volume of NBS to block cells, mix well, incubate at room temperature for 15 minutes, centrifuge at 1500rpm for 5 minutes, remove supernatant.

3.3 Washing: Resuspend cells in freshly prepared 1XPBS buffer with 0.2% BSA, centrifuge at 1500rpm for 5 minutes, remove supernatant.

3.4 Cell Seeding: Count the cells number and the viability. Resuspend and aliquot up 2×10^5 live cells into each well of the 96-well plate (50 μ l/well). Note: the cell viability must $\geq 95\%$.

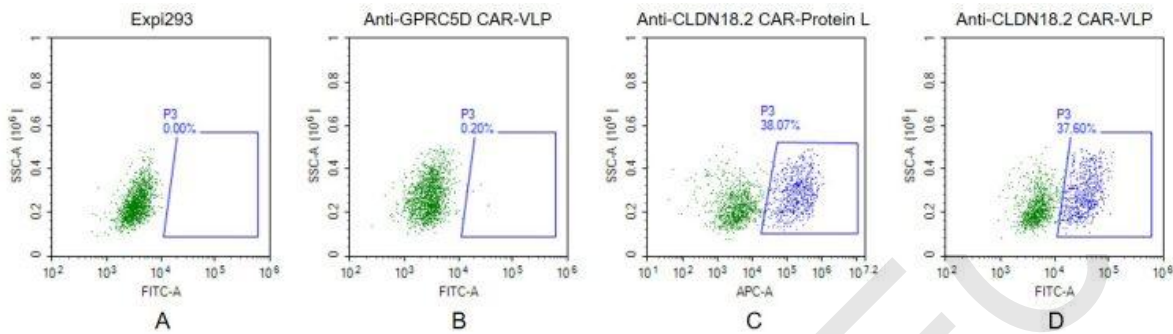
3.5 Reaction: Dilute Fluorescent Human CLDN18.2 Full Length Protein-VLP (Cat. No. FLP100017) or negative control protein L in FACS buffer to a concentration of 25-50 μ g/ml working solution. Add 50 μ l per well and mix well with resuspend cells. Incubate on ice for 30 minutes.

3.6 Washing: Centrifuge the plate at 2390rpm for 3 minutes, gently flick off the supernatant, add FACs buffer per well, centrifuge at 2390rpm for 3 minutes, gently flick off the supernatant, finally add 100-150µl FACs buffer per well.

3.7 Detection: Detect the cells by Flow cytometry. Analyze the results.

DIMA BIOTECH

FACS analysis of CLDN18.2 VLP



- A. Negative Control 1: Expi293 cells were stained with Fluorescent Human CLDN18.2 Full Length Protein-VLP (EGFP).
- B. Negative Control 2: Anti-GPRC5D-CAR-Expi293 cells (an irrelevant CAR) were stained with Fluorescent Human CLDN18.2 Full Length Protein-VLP (EGFP).
- C. Positive Control: Anti-CLDN18.2-CAR-Expi293 cells were stained with biotin labeled Protein L, followed by streptavidin-APC antibody.
- D. Anti-CLDN18.2-CAR-Expi293 cells were stained with Fluorescent Human CLDN18.2 Full Length Protein-VLP (EGFP).