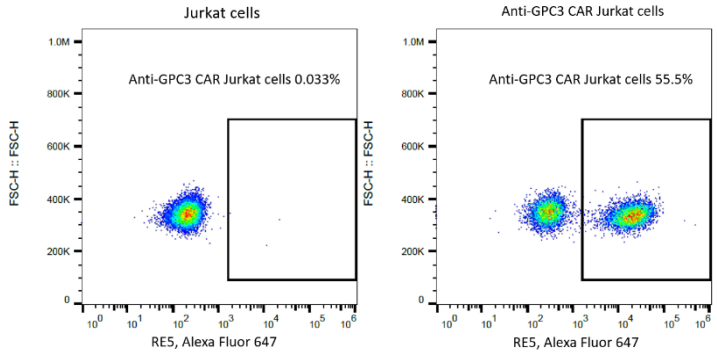
Technical Data Sheet

Anti-GPC3 CAR Idiotype Antibody, Alexa Fluor 647

Product Information	
Product No.	213556
Size	25 tests
Recommended Vol. per Test	5 μL
Antibody Types	Monoclonal
Antibody Format	Whole IgG
Clone	RE5
Immunogen	scFv region of a GPC3-specific mouse mAb clone GC33
Conjugate	Alexa Fluor 647
Excitation/Emission Max	651/667nm
Host Species	Rabbit
Storage Buffer	Aqueous buffered solution containing protein stabilizer and ≤0.05% ProClin 300
Storage conditions	2-8°C, store in dark

Description

RE5 is a specialized rabbit monoclonal antibody with high specificity for binding to the extracellular domain of the GC33 scFv within Chimeric Antigen Receptors (CARs). The GC33 scFv fragment is derived from a mouse monoclonal antibody that targets Glypican-3 (GPC3), a cell surface antigen implicated in hepatocellular carcinoma (HCC). GPC3's elevated presence in liver cancer is linked to tumor progression and metastasis, underscoring its significance as a biomarker and therapeutic target. Ongoing clinical trials are exploring the potential of GPC3-targeted CAR-T cells in treating HCC.



Flow cytometric analysis of anti-GPC3 CAR expression on human cell line Jurkat cells. *Jurkat cells were transduced with lentivirus encoding anti-GPC3 CAR and cultured. 2×10^5 cells were stained for the expression of Anti-GPC3 CAR Idiotype Antibody, Alexa Fluor 647 (Product No. 213556, right panel). Non-transduced Jurkat cells were used as a control for gating of CAR expression (left panel).*

Preparation & Storage

- Store undiluted at 2-8°C.
- Avoid prolonged exposure to light.
- Avoid freeze/thaw cycle of the reagent.
- The monoclonal antibody was purified by Protein A.
- The antibody was conjugated with Alexa Fluor 647 under optimum conditions, and unincorporated dye was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Antibodies to Include in the Detection Process

Product name	Product No.
Anti-human CD45 Antibody	602139/602140
Anti-human CD14 Antibody	602241
Anti-human CD8 Antibody	602006
Anti-human CD3 Antibody	603938/604045
Anti-human CD4 Antibody	601940/604240

FACS Protocol

(Optional) For Whole Blood Sample

1. Pipette 5 µL Anti- GPC3 CAR Idiotype Antibody, Alexa Fluor 647 into the bottom of the tube.
2. Add dead cell staining solution and additional fluorochrome conjugated antibodies into the bottom of the tube.
3. Pipette 100 µL of well-mixed, anticoagulated whole blood into the bottom of the tube. Mix gently and thoroughly.

   Note Avoid smearing sample down the side of the tube. If the sample remains on the side of the tube, it will not be stained with the reagents.
4. Incubate for 25 minutes in the dark at room temperature (18-25°C).
5. Pipette Red Blood Cell Lysis Solution to the tube. Mix gently and thoroughly. Incubate for 15 minutes in the dark at room temperature (18-25°C).
6. Add 500 µL FACS buffer to the tube. Mix well and centrifuge at 300g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
7. Repeat step 6 twice.
8. Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

(Optional) For Cell Sample

1. Harvest the cells and wash the cells twice by FACS buffer.
2. Count the cells number and the viability.
3. Resuspend the cell suspension to a concentration up to 1×10^6 nucleated cells per 100 µL of buffer.
4. Add 5 µL Anti- GPC3 CAR Idiotype Antibody, Alexa Fluor 647, dead cell staining solution and additional fluorochrome. Mix gently and thoroughly.
5. Incubate for 25 minutes in the dark at room temperature (18-25°C).
6. Add 500 µL FACS buffer to the tube. Mix well and centrifuge at 300 g for 5 minutes at room temperature (18-25°C). Aspirate supernatant completely.
7. Repeat step 6 twice.
8. Add a suitable amount of FACS buffer to resuspend cell and analysis by flow cytometry.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Antibody solutions containing ProClin 300 should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.

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