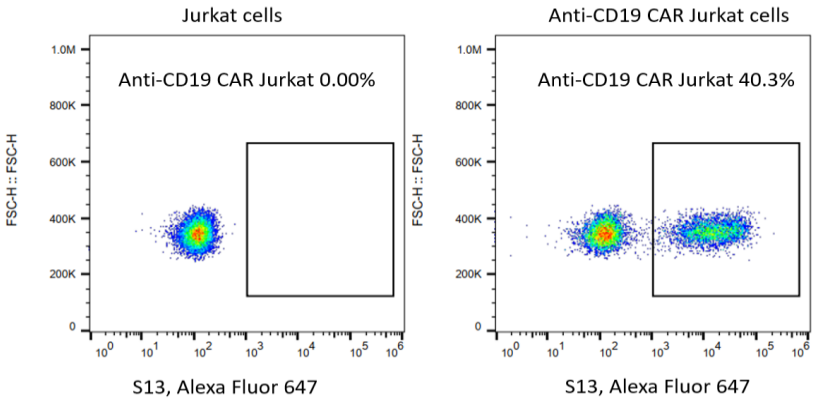
Technical Data Sheet

Anti-G4S Linker Monoclonal Antibody, hIgGC, Alexa Fluor 647

Product Information	
Product No.	211302
Size	100 Tests
Recommended Vol. per Test	1 μL
Antibody Types	Recombinant Monoclonal Antibodies
Antibody Format	Recombinant Human Fc Chimera
Clone	S13
Immunogen	Synthetic 15-mer (G4S) polypeptide
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

This antibody reacts with cell membrane-expressed CARs of varying specificity containing a G4S linker within the scFv of the extracellular domain. The poly-Glycine-Serine (G4S) linker is a kind of synthetic peptide linker sequence that is flexible and unstructured. It is frequently used to link the variable heavy (VH) domain and the variable light (VL) domain of single-chain variable fragments (scFvs) and chimeric antigen receptors (CARs) that have an extracellular domain scFv for recognizing target antigens. The linker is made up of a core pentapeptide sequence, Gly-Gly-Gly-Gly-Ser, that is repeated and usually seen as either a 15-mer (G4S) or 20-mer (G4S) in scFv-based CARs and scFv fragments.



Flow cytometric analysis of a mixed population containing live wild-type Jurkat cells and Jurkat cells engineered to express an scFv-based Anti-CD19 CAR containing a G4S linker. Jurkat cells were transduced with lentivirus encoding anti-CD19 CAR containing a G4S linker and cultured for 7 days. 2×10^5 cells were stained for the expression of anti-CD19 CAR with Anti-G4S Linker Monoclonal Antibody, hIgGC, Alexa Fluor 647 (Product No. 211302, 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 1 µL Anti-G4S Linker Monoclonal Antibody, hIgGC, 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 1 µL Anti-G4S Linker Monoclonal Antibody, hIgGC, 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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