Source

Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016) is a rabbit monoclonal antibody recombinantly expressed from human 293 cells (HEK293), which provides higher batch consistency and long term security of supply.

Application

Flow Cytometry (Evaluation of cell surface expressed CARs of varying specificity containing a G4S linker within the scFv of the extracellular domain).

Clone

016

Species

Rabbit

Isotype

Rabbit IgG | Rabbit Kappa

Specificity

It can specifically recognize proteins or ScFv containing (G3S)n or (G4S)n linkers, where n = 2, 3, 4... That is, it can recognize linkers with a minimum length of 2 repeats for both (G3S)n and (G4S)n.

Conjugate

AF555

Excitation Wavelength: 561 nm

Emission Wavelength: 572 nm

Recommended Dilution

1:50

Formulation

Lyophilized from 0.22 μm filtered solution in PBS, 0.03% Proclin 300, pH7.4 with trehalose as protectant.

Contact us for customized product form or formulation.

Reconstitution

Please see Certificate of Analysis for specific instructions.

*For best performance, we strongly recommend you to follow the reconstitution protocol provided in the CoA.*

Storage

Please protect from light and avoid repeated freeze-thaw cycles.

This product is stable after storage at:

- -20°C to -70°C for 24 months in lyophilized state;
- -70°C for 12 months after reconstitution.
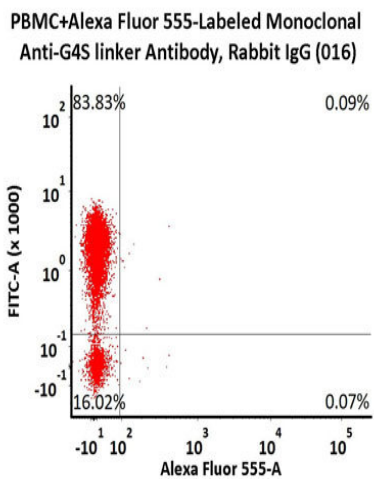- 2-8 °C for 12 months after reconstitution.

Bioactivity-FACS
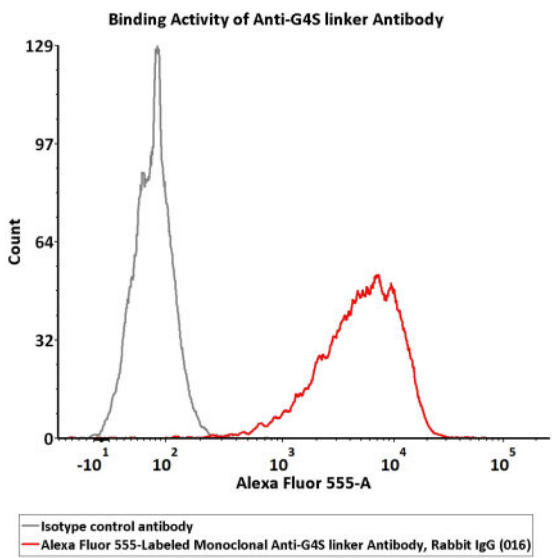
Flow cytometric analysis of Anti-MSLN CAR-293 cells staining with Alexa Fluor 555-Labeled Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016) (Cat. No. G4S-AEFYP1) at 1:50 dilution (2 μL of the antibody stock solution corresponds to labeling of 1e6 cells in a final volume of 100 μL), compared
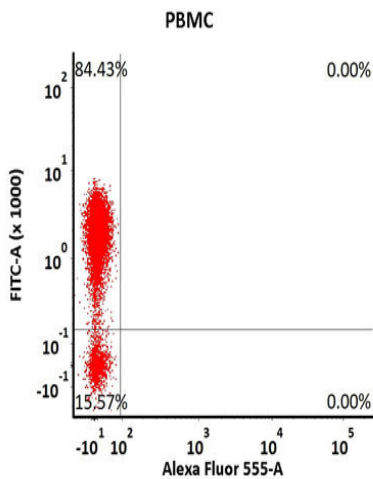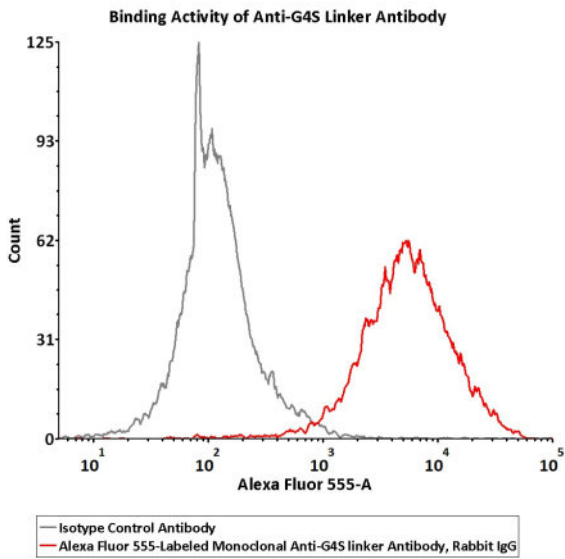
Non-specificity of Alexa Fluor 555-Labeled Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016) (Cat. No. G4S-AEFYP1) binding to CD3+ cells present in human PBMC. 5e5 of human PBMCs were simultaneously stained with FITC anti-CD3 antibody and Alexa Fluor 555-Labeled Monoclonal Anti-

with isotype control antibody. Alexa Fluor 555 signal was used to evaluate the binding activity (QC tested).

G4S linker Antibody, Rabbit IgG (016) (2 μL of the antibody stock solution corresponds to labeling of 5e5 cells in a final volume of 100 μL) and washed and then analyzed with FACS. Both FITC and Alexa Fluor 555 positive signals was used to evaluate the non-specific binding activity to human CD3+ cells (QC tested).

Flow cytometric analysis of Anti-CD19 CAR-293 cells staining with Alexa Fluor 555-Labeled Monoclonal Anti-G4S linker Antibody, Rabbit IgG1 (016) (Cat. No. G4S-AEFYP1) at 1:50 dilution (2 μL of the antibody stock solution corresponds to labeling of 1e6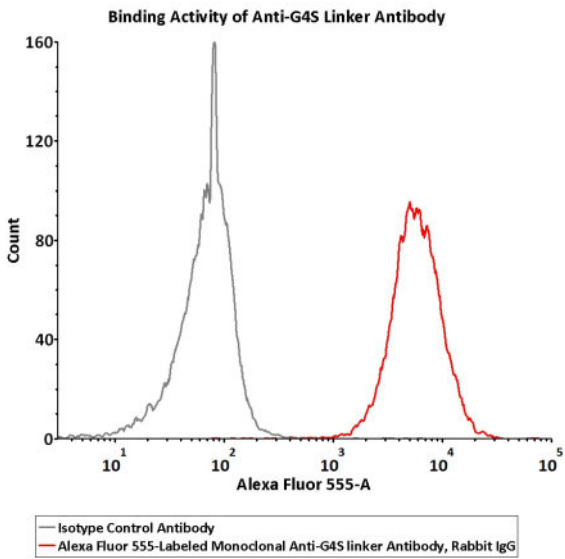 cells in a final volume of 100 μL), compared with isotype control antibody. Alexa Fluor 555 signal was used to evaluate the binding activity (Routinely tested).

Flow cytometric analysis of Anti-CD22 CAR-293 cells staining with Alexa Fluor 555-Labeled Monoclonal Anti-G4S linker Antibody, Rabbit IgG1 (016) (Cat. No. G4S-AEFYP1) at 1:50 dilution (2 μL of the antibody stock solution corresponds to labeling of 1e6 cells in a final volume of 100 μL), compared with isotype control antibody. Alexa Fluor 555 signal was used to evaluate the binding activity (Routinely tested).

Background

The G4S linker is emerged as the most common linking peptides for scFvs. It is composed of the repeated sequence (Gly-Gly-Gly-Gly-Ser) and commonly found as either a 15-mer (G4S)3 or 20-mer(G4S)4 within scFv fragments. The G4S linker has become an integral part of the vast majority of CARs as it links the VH and VL recognition domains of scFvs. The G4S linker is commonly used in CARs targeting solid tumor antigens, such as HER2 and CEA, while the whitlow/218 linker peptide is predominantly used in CAR constructs targeting hematological malignancies such as CD19 CAR products.