Catalog # G4S-BLM25G0



#### Source

Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade is a rabbit monoclonal antibody recombinantly expressed from human 293 cells (HEK293), it shows superior performance in various applications such as ELISA assays and flow cytometry assays with higher affinity and better specificity.

It is produced under our rigorous quality control system that incorporates a comprehensive set of tests including sterility and endotoxin tests. Product performance is carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage. When ready to transition into later clinical phases, we also offer a custom GMP protein service that tailors to your needs. We will work with you to customize and develop a GMP-grade product in accordance with your requests that also meets the requirements for raw and ancillary materials use in cell manufacturing of cell-based therapies.

## Isotype

Rabbit IgG | Rabbit Kappa

## Conjugate

Biotin

### Application

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

2. Immunoassay of bispecific antibody.

## 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.

## Endotoxin

Less than 0.01 EU per  $\mu g$  by the LAL method / rFC method.

# Sterility

Negative

# Mycoplasma

Negative

## Formulation

Lyophilized from 0.22  $\mu m$  filtered solution in PBS, 0.2% rHSA, 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

For long term storage, the product should be stored at lyophilized state at -20°C or lower.

This product is stable after storage at:

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

**Bioactivity-ELISA** 



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#### **Bioactivity-FACS**



Flow cytometric analysis of Anti-MSLN CAR-293 cells staining with Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade (Cat. No. G4S-BLM25G0) at 3  $\mu$ g/mL (0.3  $\mu$ g corresponds to labeling of 1e6 cells in a final volume of 100  $\mu$ L) and PE-SA. PE signal was used to evaluate the binding activity (QC tested).







Immobilized Recombinant Protein containing (G4S)2, Recombinant Protein containing (G4S)3 at 1  $\mu$ g/mL (100  $\mu$ L/well) can bind Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade (Cat. No. G4S-BLM25G0) with a linear range of 0.1-8 ng/mL (Routinely tested).



Flow cytometric analysis of Anti-CD22 CAR-293 cells staining with Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade (Cat. No. G4S-BLM25G0) at 3  $\mu$ g/mL (0.3  $\mu$ g corresponds to labeling of 1e6 cells in a final volume of 100  $\mu$ L) and PE-SA. PE signal was used to evaluate the binding activity (Routinely tested).





Flow cytometric analysis of Anti-CD19 CAR-293 cells staining with Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016),





Non-specificity of Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade (Cat. No. G4S-BLM25G0) binding to CD3+ cells

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premium grade (Cat. No. G4S-BLM25G0) at 3  $\mu$ g/mL (0.3  $\mu$ g corresponds to labeling of 1e6 cells in a final volume of 100  $\mu$ L) and PE-SA. PE signal was used to evaluate the binding activity (Routinely tested).

present in human PBMC. 5e5 of human PBMCs were simultaneously stained with FITC-labeled anti-CD3 antibody and Biotinylated Monoclonal Anti-G4S linker Antibody, Rabbit IgG (016), premium grade (0.3  $\mu$ g corresponds to labeling of 5e5 cells in a final volume of 100  $\mu$ L) and washed and then analyzed with FACS. Both FITC and PE positive signals was used to evaluate the non-specific binding activity to human CD3+ cells (QC tested).

## Background

The poly-Glycine-Serine (G4S) linker is a type of flexible, unstructured synthetic peptide linker sequence often leveraged to connect the variable heavy (VH) domain and variable light (VL) domain of single-chain variable fragments (scFvs) and chimeric antigen receptors (CARs) that utilize an extracellular domain scFv for target antigen recognition. The linker itself consists of a core pentapeptide sequence, Gly-Gly-Gly-Ser, that is repeated and commonly found as either a 15-mer (G4S)3 or20-mer(G4S) 4 within scFv-based CARs and scFv fragments. The linker sequence length plays a role in controlling scFv stability and the noncovalent association between the VH and VL domains.



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