For Research Use Only

Anti-GGGGS Linker Rabbit Recombinant Antibody

Catalog Number: 98262-1-RR



Basic Information

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Concentration: 100ug, 1000 µ g/ml

Source: Rabbit Isotype: IgG GenBank Accession Number:

GeneID (NCBI):

Full Name:

Purification Method:

Protein A purfication

CloneNo.: 242306C8

Recommended Dilutions: WB: 1:1000-1:6000 IHC: 1:40000-1:160000 IF/ICC: 1:2000-1:8000

FC: 0.25 ug per 10⁶ cells in 100 μ l

suspension

Applications

Tested Applications: WB, IHC, IF/ICC, FC Species Specificity: recombinant protein

Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0 **Positive Controls:**

WB: Transfected HEK-293T cells, IHC: Transfected HEK-293T cells,

IF/ICC: Transfected HEK-293T cells, Transfected CHO-

K1

FC: Transfected HEK-293T cells,

Background Information

As a crucial element in the design of recombinant fusion proteins, linkers play an increasingly vital role in the construction of stable, bioactive fusion proteins (PMID: 23026637). GGGGS linker (G4S linker) is a flexible linker made of 4 glycine repeats followed by a serine amino acid (PMID:3045807). Due to its flexibility and resistance to proteases, GGGGS and its repeats are commonly used when engineering a protein, particularly in the construction of single-chain Fv (ScFv) domains expressed on the surfaces of CAR-T cells (PMID:23581628; 36874404). This antibody, raised against a synthetic peptide (GGGGSGGGGGGGGGGG), can recognize G4S, G4S*2, G4S*3, G4S*4, and G4S*5.

Storage

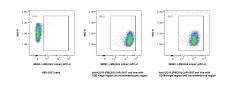
Storage:

Store at 2 - 8° C. Stable for one year after shipment.

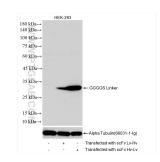
Storage Buffer:

PBS with 0.09% sodium azide, pH7.3

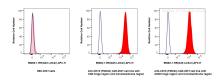
Selected Validation Data



1x10^6 HEK-293T cells or anti-CD19 (FMC63) CAR with CD28 or CD8 hinge region and transmembrane region transfected HEK-293T cells were surface stained with 0.25 ug Anti G4S Linker Rabbit RecAb (98262-1-RR, Clone:242306C8) and Multi-rAb CoraLite Plus 647-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (RGAR005). Cells were not fixed.



Various lysates were subjected to SDS PAGE followed by western blot with 98262-1-RR (GGGGS Linker antibody) at dilution of 1:3000 incubated at room temperature for 1.5 hours.



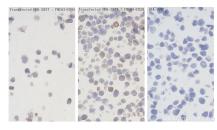
1x10^6 HEK-293T cells or anti-CD19 (FMC63) CAR with CD28 or CD8 hinge region and transmembrane region transfected HEK-293T cells were surface stained with 0.25 ug Anti G45 Linker Rabbit RecAb (98262-1-RR, Clone:242306C8) and Multi-rAb CoraLite Plus 647-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (RGAR005) (red), or Rabbit 1gG Isotype Control RecAb (98136-1-RR, Clone: 240953C9) (blue). Cells were not fixed



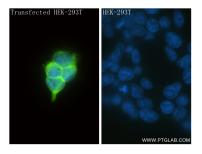




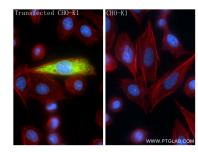
1x10^6 Jurkat cells or NK92 cells or Raji cells were surface stained with 0.25 ug Anti G4S Linker Rabbit RecAb (98262-1-RR, Clone: 242306C8) (red) or Rabbit IgG Isotype Control RecAb (98136-1-RR, Clone: 240953C9) (blue), and Multi-rAb CoraLite Plus 647-Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (RGAR005). Cells were not fixed



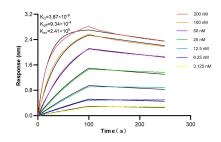
Immunohistochemical analysis of paraffinembedded Transfected HEK-293T cells slide using 98262-1-RR (GGGS Linker antibody) at dilution of 1:80000 (under 40x lens).



Immunofluorescent analysis of (-20°C Ethanol) fixed Transfected HEK-293T cells using GGGGS Linker antibody (98262-1-RR, Clone: 242306C8) at dilution of 1:4000 and Coralite® 488-Conjugated Goat Anti-Rabbit IgG(H+L) (SA00013-2).



Immunofluorescent analysis of (-20°C Ethanol) fixed Transfected CHO-K1 using GGGGS Linker antibody (98262-1-RR, Clone: 242306C8) at dilution of 1:2000 and Coralite®488-Conjugated Goat Anti-Rabbit IgG(H+L) (SA00013-2), CL594-Phalloidin (red).



Biolayer interferometry (BLL) kinetic assays of 98262-1-RR against scFv (single chain fragment variable) format with variable regions of heavy (VH) and light (VL) chains joined together by G4S linker were performed. The affinity constant is 3.87 pm