

Rev01
Update: Dec,13,2022

DATASHEET

GenCRISPR™ SpCas9 Antibody (14B6), mAb, Mouse

Cat. No.: A01936

Overview

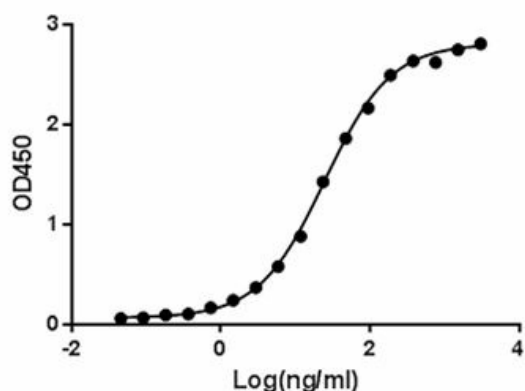
Specificity	The product is specific for Streptococcus pyogenes CRISPR/Cas9. This antibody binds with recombinant Streptococcus pyogenes CRISPR/Cas9 protein in ELISA and Endogenous overexpressed Streptococcus pyogenes CRISPR/Cas9 in immunofluorescence, western blot.
Host Species	Mouse
Immunogen	Recombinant Streptococcus pyogenes CRISPR/Cas9

Properties

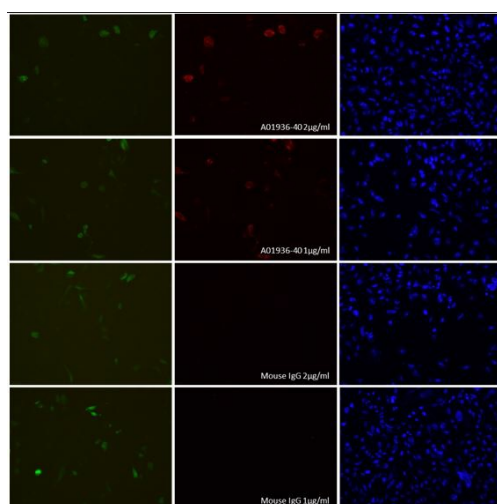
Concentration	0.5 mg/ml, lyophilized with PBS, pH 7.4, contains 0.02% sodium azide. (Might be eligible for customized bulk orders.)
Reconstitution	Reconstitute the lyophilized antibody with deionized water (or equivalent) to a final concentration of 0.5 mg/ml.
Purification	Protein A affinity column
Clone ID	14B6

Examples

Clone 14B6 ELISA binding with SpCas9

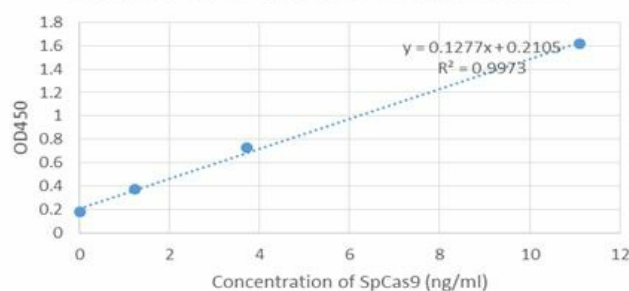


ELISA binding of GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) with recombinant *Streptococcus pyogenes* CRISPR/Cas9 protein. Coating antigen: SpCas9, 1 µg/ml. SpCas9 antibody dilution start from 3000 ng/ml, EC50= 24.51 ng/ml.

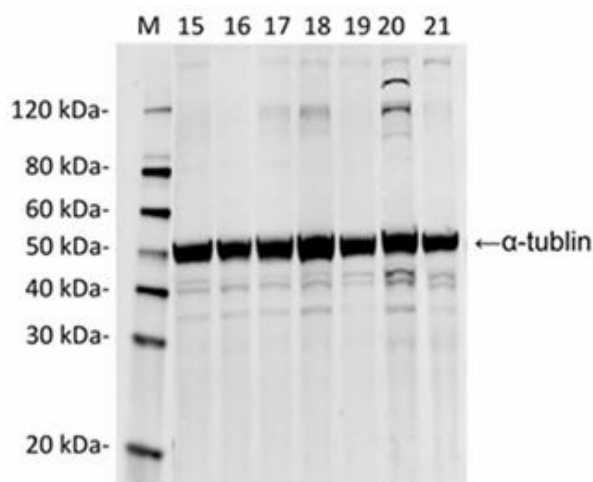


Immunofluorescence staining of GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) with SpCas9 in HeLa cells. HeLa cells transfected with PX458 (pSpCas9(BB)-2A-GFP) (green) were fixed with 4% Poly-Formaldehyde (5min) and then blocked in 3% BSA 30min. The cells were then incubated with GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) at 2 µg/ml, 1 µg/ml and mouse IgG at 2 µg/ml, 1 µg/ml at room temperature for 2h, followed by a further incubation at 37°C for 1h with Goat Anti-Mouse IgG Antibody (H&L) [ifluor 555], pAb (GenScript) (red) at 5 µg/ml. DAPI was used to stain the cell nuclei (blue) at a concentration of 0.2 µg/ml for 2h at room temperature.

Standard curve of SpCas9 Sandwich ELISA



Standard curve of SpCas9 Sandwich ELISA. The SpCas9 Sandwich ELISA assay is developed by using GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) and GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) as capture and detection antibody, respectively. These two antibodies recognize different epitopes. In this ELISA assay, GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) was labeled with Biotin. GenScript can provide customized conjugation service for this product per customer's request. The sensitivity is <1 ng/ml and the detection range is 0-10 ng/ml.



Western Blot analysis of HEK293 transfected with various plasmids with GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40). The different HEK293 with transfected with various plasmids indicate the minimum cross reaction of the antibody.

Loading:

Lane 1: 50µg HEK293 cell lysate transfected with SaCas9(BB)-2A-GFP (J7RUA5, *Staphylococcus aureus*)

Lane 2: 50µg HEK293 cell lysate transfected with StCas9(BB)-2A-GFP (G3ECR1, *Streptococcus thermophilus*)

Lane 3: 50µg HEK293 cell lysate transfected with FnCpf1(BB)-2A-GFP (A0Q7Q2, *Francisella tularensis* subsp. novicida (strain U112))

Lane 4: 50µg HEK293 cell lysate transfected with LbCpf1(BB)-2A-GFP (A0A182DWE3, *Lachnospiraceae* bacterium ND2006)

Lane 5: 50µg HEK293 cell lysate transfected with AsCpf1(BB)-2A-GFP (U2UMQ6, *Acidaminococcus* sp. (strain BV3L6))

Lane 6: 50µg HEK293 cell lysate transfected with pSpCas9(BB)-2A-GFP (PX458, Q99ZW2, *Streptococcus pyogenes* serotype M1)

Lane 7: 50µg HEK293 cell lysate (Non-transfected)

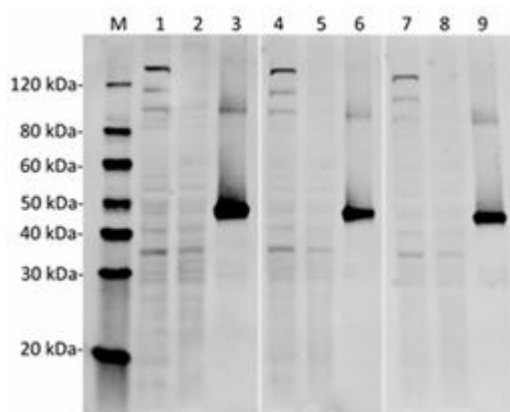
Primary Antibody:

Lane 1~7: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 µg/ml

Lane 1~7: THE™ Anti-α-tubulin mAb (mouse)(1F4) 0.5µg/ml

Secondary antibody:

Goat anti-Mouse IgG (H&L) [IRDye⁸⁰⁰], 0.125 µg/ml



Western Blot of HEK293 transfected with PX458 (pSpCas9(BB)-2A-GFP) or untransfected cell lysates with GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40). The different concentration of antibodies indicates the high affinity and sensitivity of the antibody.

Lane 1: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 2: 50 µg Untransfected HEK293 cell Lysate

Lane 3: 40 ng SpCas9 recombinant protein

Lane 4: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 5: 50 µg Untransfected HEK293 cell Lysate

Lane 6: 40 ng SpCas9 recombinant protein

Lane 7: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 8: 50 µg Untransfected HEK293 cell Lysate

Lane 9: 40 ng SpCas9 recombinant protein

Primary Antibody:

Lane 1~3: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript,

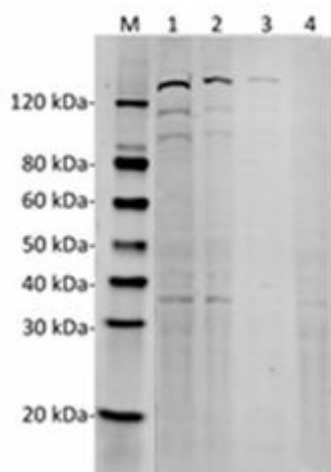
A01936-40) 2 µg/ml

Lane 4~6: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 µg/ml

Lane 7~9: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 0.5 µg/ml

Secondary Antibody:

Goat anti-Mouse IgG (H&L) [IRDye⁸⁰⁰], 0.125 µg/ml



Western Blot of HEK293 transfected with PX458 (pSpCas9(BB)-2A-GFP) or untransfected cell lysates with GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40). The different concentration of cell lysates indicates the high affinity and sensitivity of the antibody.

Lane 1: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 2: 25 µg HEK293 transfected with PX458 cell Lysate

Lane 3: 10 µg HEK293 transfected with PX458 cell Lysate

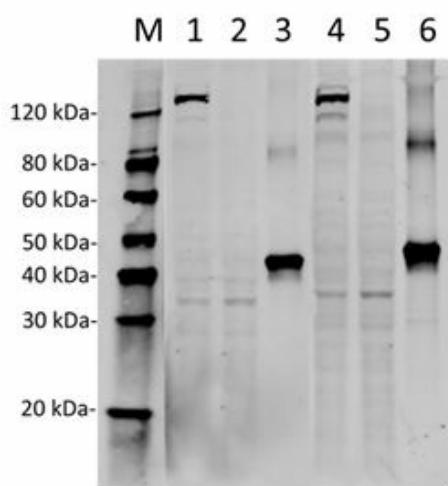
Lane 4: 50 µg Untransfected HEK293 cell Lysate

Primary Antibody:

GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 µg/ml

Secondary Antibody:

Goat anti-Mouse IgG (H&L) [IRDye⁸⁰⁰], 0.125 µg/ml



Western Blot of HEK293 transfected with PX458 (pSpCas9(BB)-2A-GFP) or untransfected cell lysates with two independent antibodies: GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) and GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40). The correlated pattern indicates the high specificity of these two antibodies.

Lane 1: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 2: 50 µg Untransfected HEK293 cell Lysate

Lane 3: 40 ng SpCas9 recombinant protein

Lane 4: 50 µg HEK293 transfected with PX458 cell Lysate

Lane 5: 50 µg Untransfected HEK293 cell Lysate

Lane 6: 40 ng SpCas9 recombinant protein

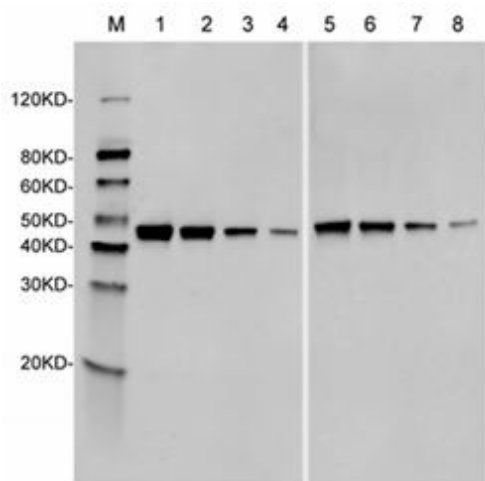
Primary Antibody:

Lane 1~3: GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) 1 µg/ml

Lane 4~6: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 µg/ml

Secondary Antibody:

Goat anti-Mouse IgG (H&L) [IRDye⁸⁰⁰], 0.125 µg/ml



Western Blot of recombinant *Streptococcus pyogenes* CRISPR/Cas9 protein with two independent antibodies: GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) and GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40). The correlated pattern indicates the high specificity of these two antibodies.

Lane 1: 50 ng SpCas9 recombinant protein

Lane 2: 25 ng SpCas9 recombinant protein

Lane 3: 10 ng SpCas9 recombinant protein

Lane 4: 5 ng SpCas9 recombinant protein

Lane 5: 50 ng SpCas9 recombinant protein

Lane 6: 25 ng SpCas9 recombinant protein

Lane 7: 10 ng SpCas9 recombinant protein

Lane 8: 5 ng SpCas9 recombinant protein

Primary Antibody:

Lane 1~4: GenCRISPR™ SpCas9 Antibody (4A1) (GenScript, A01935-40) 1 µg/ml

Lane 5~8: GenCRISPR™ SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 µg/ml

Secondary Antibody:

Goat anti-Mouse IgG (H&L) [IRDye⁸⁰⁰], 0.125 µg/ml

Background

Target Background : Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system provides a robust and multiplexable genome editing tool, enabling researchers to precisely manipulate specific genomic elements, and facilitating the elucidation of target gene function in biology and diseases. CRISPR/Cas9 genome editing allows for double-stranded DNA breaks at specific sequences to efficiently disrupt, excise, mutate, insert, or replace genes. To date, the *Streptococcus pyogenes* Cas9 (SpCas9) has been used broadly to achieve efficient genome editing in a variety of species and cell types. It is important that the precision of transfection and the level of Cas9 expression should be controlled strictly during the editing processes by using specific anti-CRISPR/Cas9 antibodies.

Synonyms : Mouse monoclonal to *Streptococcus pyogenes* Cas9/SpCas9