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

Cat. No.: A01936-40

Host: Mouse

Size: 40 µg

Ig Subclass: IgG1,K

Clone: 14B6

Immunogen: Recombinant *Streptococcus pyogenes* CRISPR/Cas9

UniProt Accession: Q99ZW2

Gene ID: 901176

Purification: Protein A affinity column

Conjugation: Unconjugated

Version: 10/16/2017

PRODUCT INFORMATION

Description

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.

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.

Concentration

0.5 mg/ml, lyophilized with PBS, pH 7.4, containing 0.02% sodium azide. (It may be customized for bulk orders.)

Note

GenScript can customize this product per the customer's request including product size, buffer components, etc.

Reconstitution

Reconstitute the lyophilized antibody with deionized water (or equivalent) to a final concentration of 0.5 mg/ml.

Storage

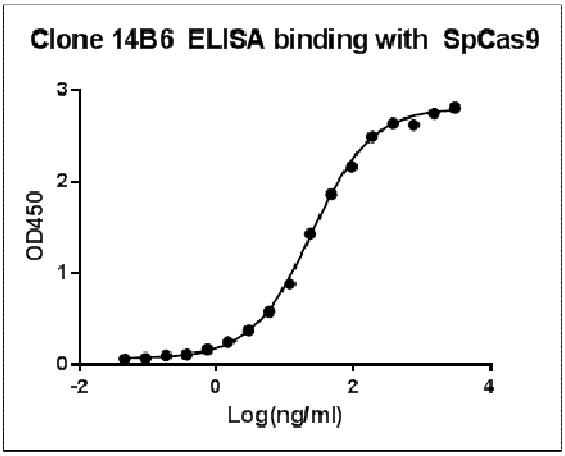
The lyophilized product remains stable up to 1 year at -20 °C from date of receipt. Upon reconstitution, it can be stored for 2-3 weeks at 2-8 °C or for up to 12 months at -20 °C or below. Avoid repeated freezing and thawing cycles.

Applications

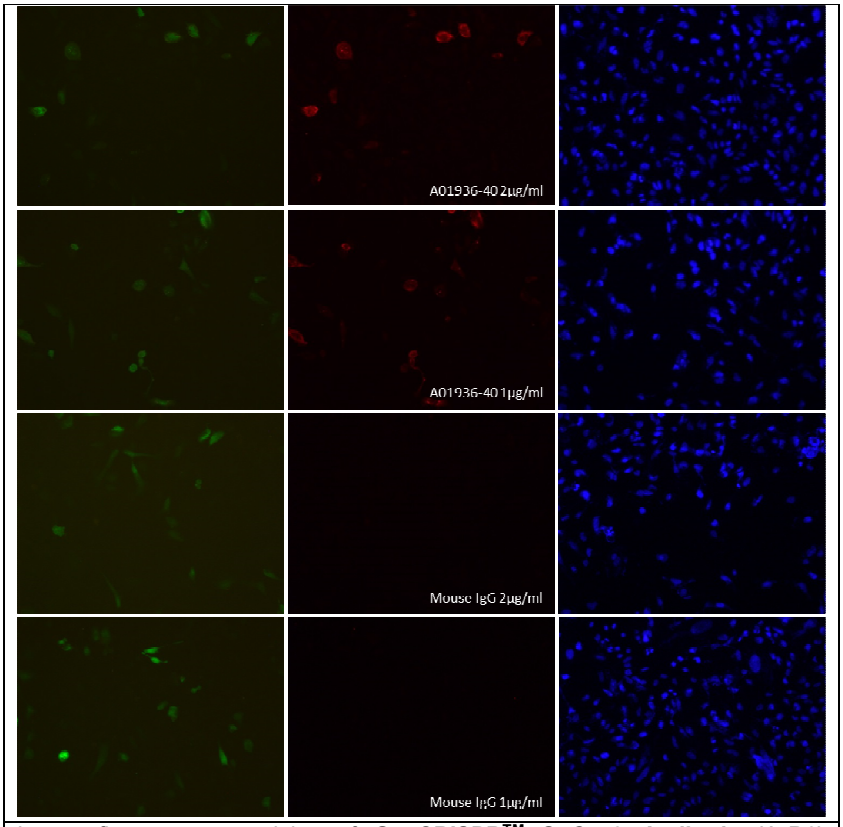
Working concentrations for specific applications should be determined by the investigators. The appropriate concentration may be affected by secondary antibody affinity, antigen concentration, the sensitivity of the method of detection, temperature, the length of the incubations, and other factors. The suitability of this antibody for applications other than those listed below has not been determined. The following concentration ranges are recommended starting points for this product.

ELISA detection: 0.05-1 μg/ml
Immunofluorescence: 1-2 μg/ml
Western blot: 0.5-2 μg/ml
Other applications: user-optimized

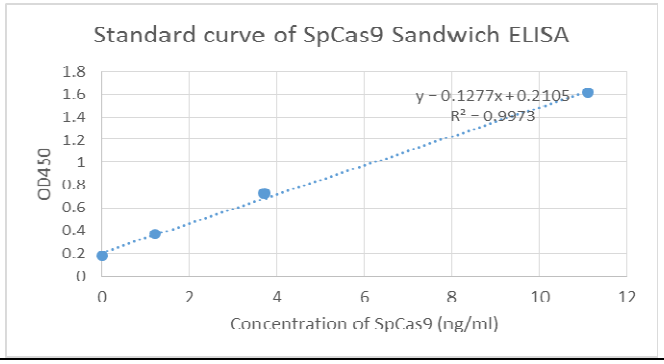
Example



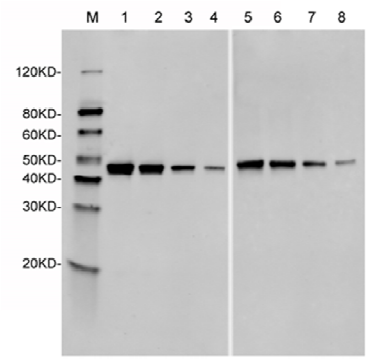
ELISA binding of **GenCRISPR™ SpCas9 Antibody (14B6)** (GenScript, A01936-40) with recombinant *Streptococcus pyogenes* CRISPR/Cas9 protein.
Coating antigen: SpCas9, 1 μg/ml.
SpCas9 antibody dilution start from 3000 ng/ml,
EC50= 24.51 ng/ml.



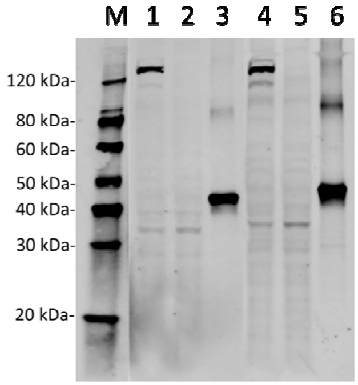
Immunofluorescence staining of **GenCRISPR™ SpCas9 Antibody (14B6)** (GenScript, A01936-40) with SpCas9 in Hela cells.
HeLa cells transfected with PX458 (pSpCas9(BB)-2A-GFP) (green) were fixed with 4% Poly-Formaldehyde (5min) and then blocked in 3% BSA 30min.
The cells were then incubated with GenCRISPR™ 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℃ 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. 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.

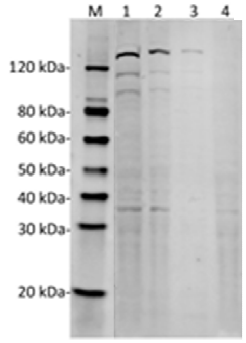


Western Blot of recombinant *Streptococcus pyogenes* CRISPR/Cas9 protein with two independent antibodies: **GenCRISPR™ SpCas9 Antibody (4A1)** (GenScript, A01935-40) and **GenCRISPR™ SpCas9 Antibody (14B6)** (GenScript, A01936-40). The correlated pattern indicates the high specificity of these two antibodies.
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] (Licor,926-32211) 0.125 μg/ml

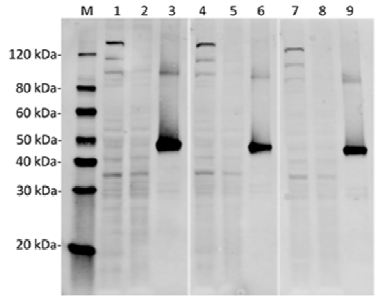


Western Blot of HEK293 transfected with PX458 (pSpCas9(BB)-2A-GFP) or untransfected cell lysates with two independent antibodies: **GenCRISPR™ SpCas9 Antibody (4A1)** (GenScript, A01935-40) and **GenCRISPR™ SpCas9 Antibody (14B6)** (GenScript, A01936-40). The correlated pattern indicates the high specificity 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] (Licor,926-32211) 0.125 μg/ml

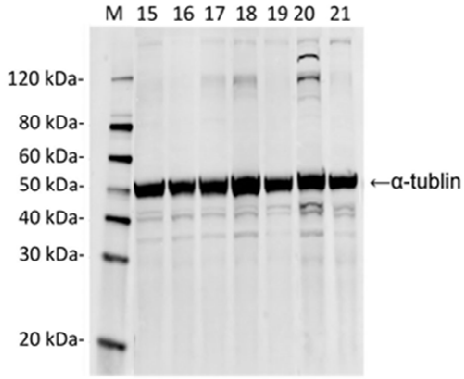


Western Blot of HEK293 transfected with PX458 (pSpCas9(BB)-2A-GFP) or untransfected cell lysates with **GenCRISPR™ SpCas9 Antibody (14B6)** (GenScript, A01936-40). The different concentration of cell lysates indicates the high affinity and sensitivity of the antibody.
Lane 1: 50 μg HEK293 transfected 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] (Licor,926-32211) 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) [IRDye^{800}] (Licor,926-32211) 0.125 μg/ml



Western Blot analysis of HEK293 transfected with various plasmids with **GenCRISPR^{TM} 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 thermophiles*)
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^{TM} SpCas9 Antibody (14B6) (GenScript, A01936-40) 1 μg/ml
Lane 1~7: THE^{TM} Anti-α-tubulin mAb (mouse)(1F4) 0.5μg/ml
Secondary antibody：
Goat anti-Mouse IgG (H&L) [IRDye^{800}] (Licor,926-32211) 0.125 μg/ml