Product Datasheet

CRISPR-Cas9 Antibody (6G12) - C-terminus - BSA Free
NBP2-52398

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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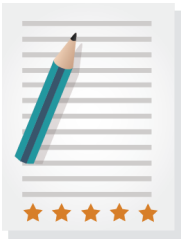
Updated 10/23/2024 v.20.1



NBP2-52398

CRISPR-Cas9 Antibody (6G12) - C-terminus - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	6G12
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	158.4 kDa

Product Description

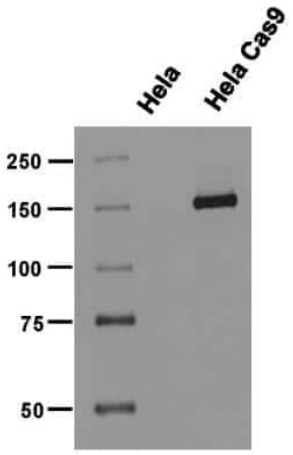
Host	Mouse
Gene ID	901176
Species	Bacteria
Specificity/Sensitivity	This CRISPR-Cas9 antibody (6G12) - C-terminus is specific to Cas9 from Streptococcus pyogene.
Immunogen	This CRISPR-Cas9 antibody (6G12) - C-terminus was raised against recombinant C-terminal fragment of S.pyogenes CRISPR/Cas9. [UniProt# Q99ZW2]

Product Application Details

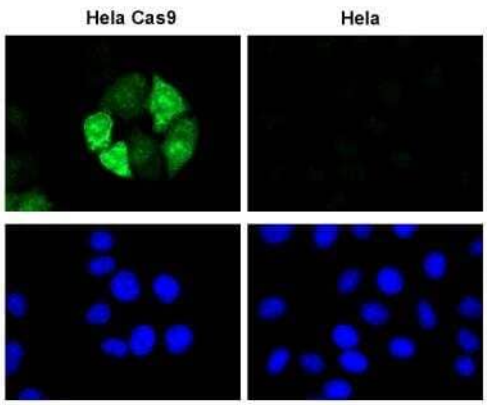
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000, Simple Western 10-20 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)

Images

Western Blot: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - Control HeLa cells (un-transfected) and HeLa cells expressing Flag-tagged S. pyogenes's CRISPR-Cas9 under the control of PTight (Tet-ON) promoter. Samples were treated for 24 hours with 1ug/uL of Doxycyclin and lysed under native conditions. 30 ug of the whole cell lysate from each sample type per lane was separated by 7.5% SDS-PAGE. Nitrocellulose membrane was incubated with CRISPR-Cas9 antibody clone 6G12 (hybridoma supernatant diluted 1:100 at 4C O/N). After washing, the membranes were incubated with secondary HRP-coupled antibody and bands were visualized by ECL and exposure of X-ray films. Prestained marker bands were visualized with Blue Marker Antibody (NBP2-33376). The image shown is from 1 minute exposure time. Observed molecular weight is ~158 kDa.

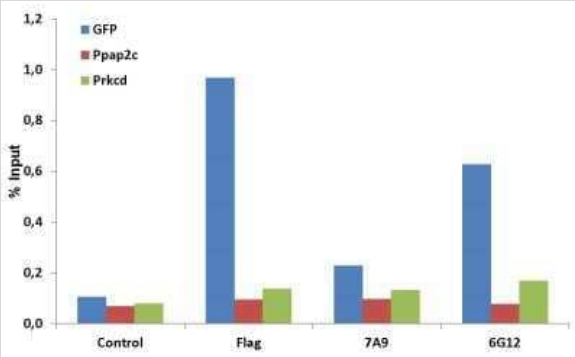


Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - HeLa cells or HeLa cells expressing Flag-tagged SpCas9 under the control of the PTight (Tet-ON) promoter were treated for 24h with 1ug/uL Doxycyclin, fixed and permeabilized with Methanol/Acetone and blocked in 2% BSA in PBS for 2 hours at RT. Cells were stained with 6G12 hybridoma supernatant at 1:10 at 4C O/N, followed by incubation with anti mouse-Alexa Fluor 488 coupled secondary antibody for 1h at RT. Nuclei were counter-stained with Hoechst 33342.

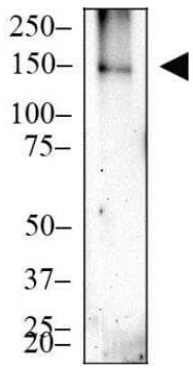


Simple Western: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - Image shows a specific band for Cas9 in 0.2 mg/mL of HeLa Cas9 lysate but not in Hela WT lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Observed molecular weight is ~158 kDa.

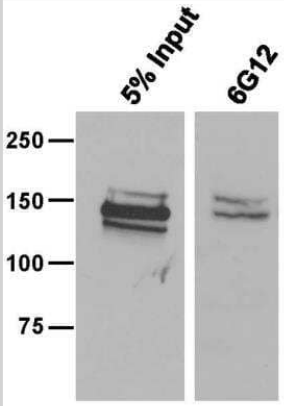
Chromatin Immunoprecipitation: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) [NBP2-52398] - NIH3T3 cells stably expressing GFP-H2B, nuclease dead Cas9, and a GFP-targeting gRNA were fixed with formaldehyde, harvested and sonicated to get 200-500bp DNA fragments. 50ug chromatin was incubated over night at 4C with the indicated antibodies (200ul hybridoma SN, 5ug anti-Flag [M2, Sigma]) followed by incubation with protein G beads for 3h at 4C. After washing chromatin was eluted from the beads and crosslinking was reversed over night at 65C. After a proteinase K digestion step, DNA was separated using phenol/chloroform/isoamyl alcohol, precipitated with ethanol/sodium acetate and dissolved in water. For qPCR, primers either targeting the GFP gene or as negative control non-targeted regions (Ppap2c +7122 and Prkcd +24069 from transcription start) were used.



Western Blot: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - Whole cell protein from 293T cells transfected with Cas9-Flag (~150 kDa) was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2 ug/mL anti-Cas9 (6G12) in 1% milk, and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



Immunoprecipitation: CRISPR-Cas9 Antibody (6G12) - C-terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - HEK293 cells expressing Flag-SpCas9 were lysed under native conditions. SpCas9 was immunoprecipitated at 4C from 300 ug of whole cell lysate with the 6G12 antibody and a 1:1 mixture of protein A/G sepharose. After 4x washing, the bound proteins were boiled off the beads, separated by 7.5% SDS-PAGE and transfered to nitrocellulose membranes, and SpCas9 was detected with a rabbit polyclonal Cas9 antibody. After washing, the membranes were incubated with secondary HRP-coupled antibody and bands were visualized by ECL and exposure of X-ray films.



Publications

Johnston R, Seamon K, Saada E et al. Use of anti-CRISPR protein AcrIIA4 as a capture ligand for CRISPR/Cas9 detection Biosens Bioelectron 2019-06-18 [PMID: 31207570]

Giehrl-Schwab J, Giesert F, Rauser B et al. Parkinson's disease motor symptoms rescue by CRISPRa-reprogramming astrocytes into GABAergic neurons EMBO molecular medicine 2022-04-04 [PMID: 35373464] (WB)

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Products Related to NBP2-52398

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NBP2-52986	CRISPR-Cas9 Antibody Pack

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