Product Datasheet

CRISPR-Cas9 Antibody (6G12) - C-Terminus
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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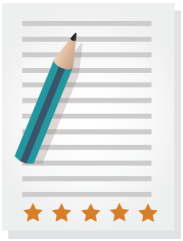
Updated 3/13/2018 v.20.1



NBP2-52398

CRISPR-Cas9 Antibody (6G12) - C-Terminus

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

Product Description

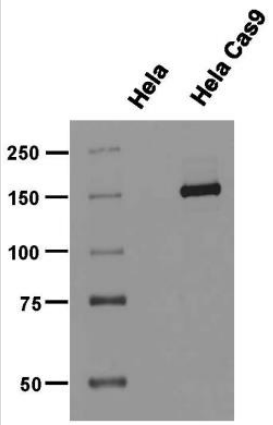
Host	Mouse
Species	Bacteria
Specificity/Sensitivity	Cas9 from Streptococcus pyogene.
Immunogen	Recombinant C-terminal fragment of S.pyogenes CRISPR/Cas9. [UniProt# Q99ZW2]

Product Application Details

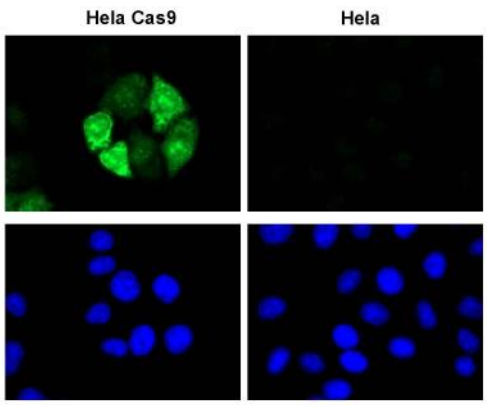
Applications	Western Blot, Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000, Chromatin Immunoprecipitation, Immunocytochemistry/Immunofluorescence 1:500, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)

Images

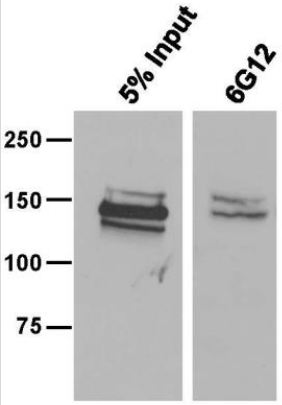
Western Blot: CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - Control Hela cells (un-transfected) and Hela cells expressing Flag-tagged S.pyogenes's CRISPR-Cas9 under the control of PTight (Tet-ON) promoter were treated for 24 hours with 1ug/ul of Doxycyclin and lysed under native conditions. 30ug of the whole cell lysate from each sample type per lane was separated by 7.5% SDS-PAGE. Proteins were transfered to nitrocellulose membrane which were then 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 here is from 1 minute exposure time.



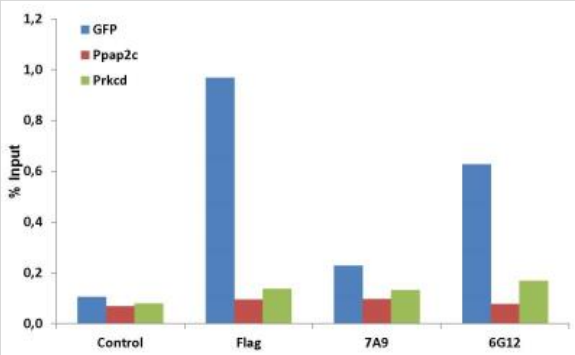
Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) [NBP2-52398] - Hela cells or Hela cells expressing Flag-tagged SpCas9 under the control of the PTight (Tet-ON) promoter were treated for 24h with 1ug/ul Doxycyclin, fixed and permeabilized with Methanol/Acetone and blocked in 2% BSA in PBS for 2 hours at RT. Cells were stained with 6G12 hybridoma supernatant (diluted 1:10) at 4C o/n, followed by incubation with anti mouse-AF488 coupled secondary antibody for 1 h at RT. Nuclei were counter-stained with Hoechst 33342.



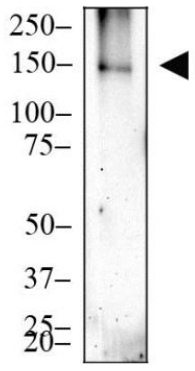
Immunoprecipitation: CRISPR-Cas9 Antibody (6G12) - C-Terminus [NBP2-52398] - CRISPR-Cas9 Antibody (6G12) [NBP2-52398] - HEK293 cells expressing Flag-SpCas9 were lysed under native conditions. SpCas9 was immunoprecipitated at 4C from 300ug of whole cell lysate with the 6G12 antibody and a 1:1 mixture 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.



Chromatin Immunoprecipitation: 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] - 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.



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

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This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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