Product Datasheet

CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus - BSA Free
NBP2-36440

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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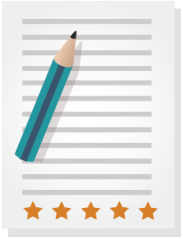
Updated 9/11/2024 v.20.1



NBP2-36440

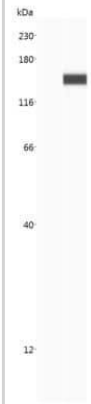
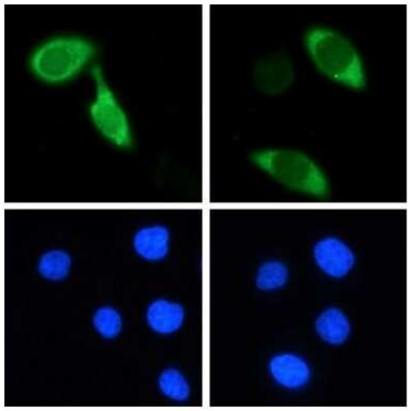
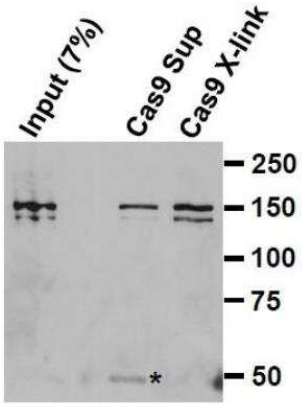
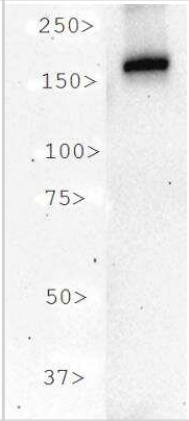
CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus - BSA Free

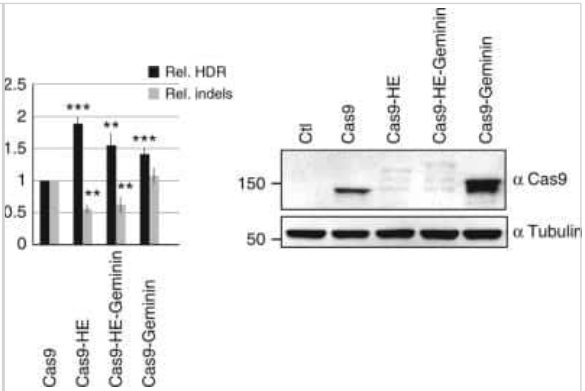
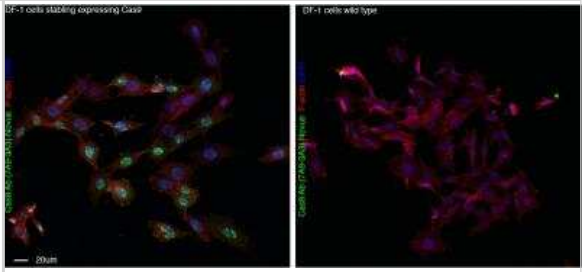
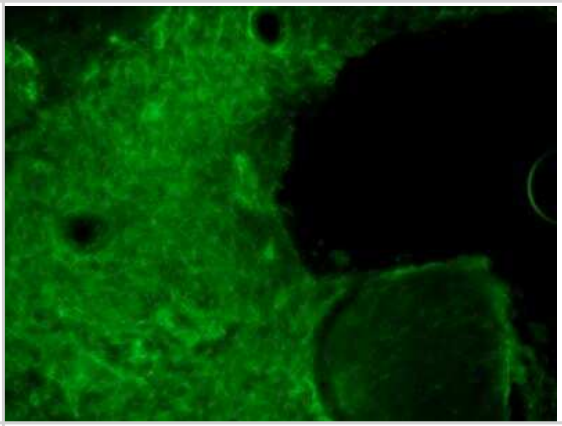
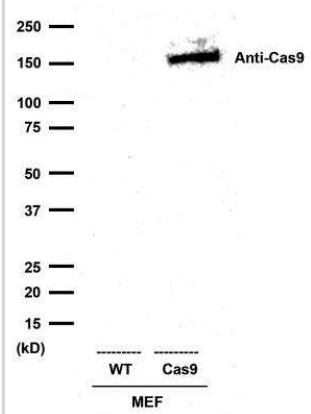
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	7A9-3A3
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	158.4 kDa

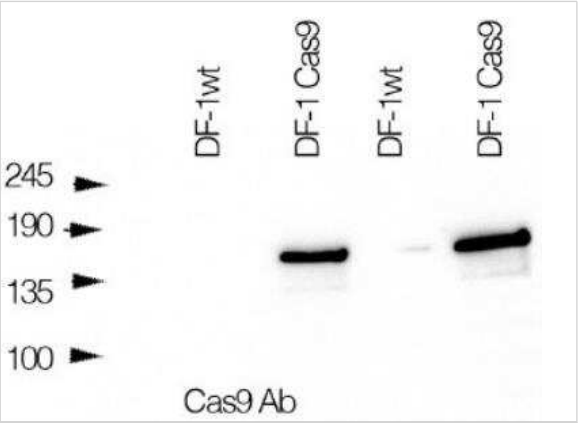
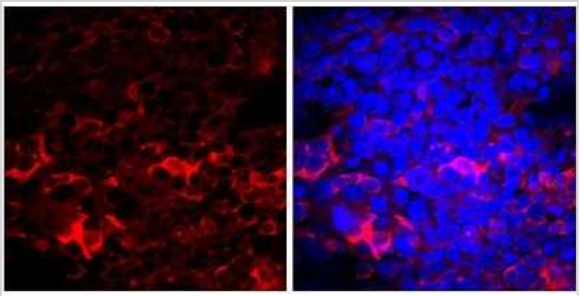
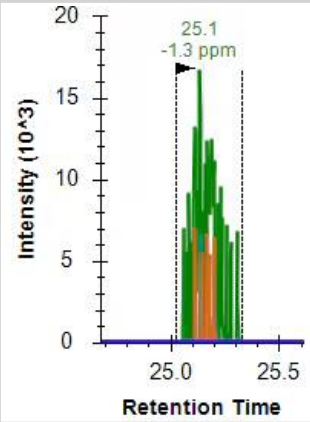
Product Description	
Host	Mouse
Gene ID	901176
Species	Bacteria
Specificity/Sensitivity	This CRISPR-Cas9 antibody (7A9-3A3) - N-Terminus is specific toCas9 protein from Streptococcus pyogene serotype M1.
Immunogen	This CRISPR-Cas9 antibody (7A9-3A3) - N-Terminus was raised against Recombinant Cas9 within the N-terminal region of Streptococcus pyogene. [Uniprot: Q99ZW2].

Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Immunohistochemistry Whole-Mount, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:500, Immunoprecipitation, Immunohistochemistry-Frozen reported in scientific literature (PMID 28153089), Immunoblotting reported in scientific literature (PMID 31959836), Immunohistochemistry Whole-Mount, Knockout Validated reported in scientific literature (PMID 31959836)
Application Notes	IF and IHC use of CRISPR-Cas9 antibody (clone 7A9-3A3) on 4% formaldehyde fixed and 20um thick frozen-/cryo-sections reported in scientific literature

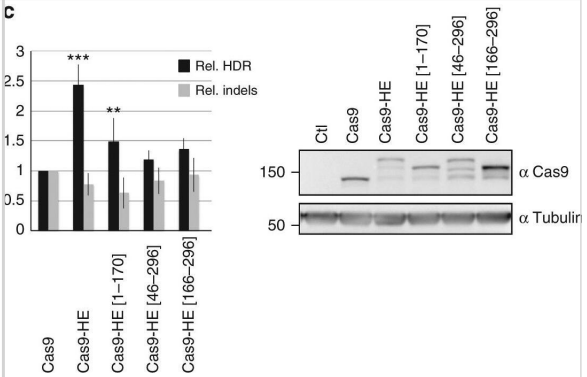
Images

Simple Western: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Image shows a specific band for Cas9 (observed molecular weight ~158 kDa) in HeLa Cas9 lysate but not in Hela WT lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Hela cells were transiently transfected with an N-terminally Flag-tagged S. pyogenes Cas9 expression vector. The cells were stained with the Cas9 antibody followed by anti mouse-AF488 coupled secondary antibody. Nuclei were counter-stained with Hoechst 33342.	
Immunoprecipitation: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - HEK293T expressing N-terminally Flag-tagged S.pyogenes Cas9 were lysed 72h post transfection by resuspending the cells in Hunt buffer and subjecting to 3 freeze-thaw cycles in liquid nitrogen/ice. Proteins were immunoprecipitated from 100ug of whole cell lysate for 1h at 4C with Cas9 supernatant followed by incubation for 1h at 4C with a 1:1 mixture of protein A/G sepharose beads, or for 2h at 4C with Cas9 ab crosslinked to a 1:1 mixture of protein A/G sepharose beads. Beads were washed 2x with Hunt buffer and 1x with TBS. Bound proteins were eluted by boiling in Laemmli, separated by SDS-PAGE and transferred to nitrocellulose. Membrane was blocked, incubated with Cas9 ab, incubated with HRP anti-mouse secondary. *IgG heavy chain	
Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Analysis of lysate from Cas9 transfected HEK-293T cells using Cas9 antibody clone 7A9-3A3 at 2ug/ml concentration. The signal was developed using HRP-labelled anti-mouse secondary antibody and ECL based detection. Observed molecular weight is ~158 kDa.	

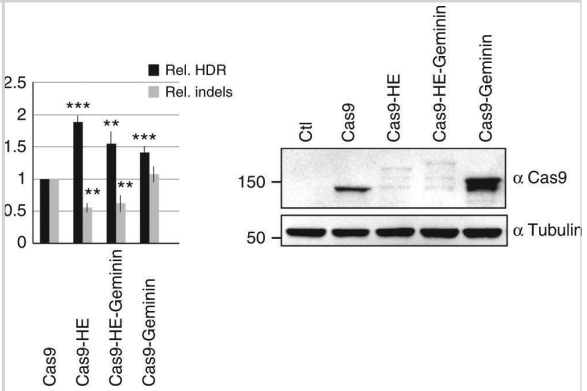
Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Human HEK293 cells were transfected with the indicated CRISPR-Cas9 plasmids, T2 guide RNA, and GFP transgene donor with homology arms to the AAVS1 targeted locus. HDR-mediated transgene integration was measured by FACS analysis of GFP-positive cells, resulting from targeted GFP transgene integration. Indels at the cleavage site were measured by the T7E1 assay. Asterisks indicate that the difference is statistically significant when comparing Cas9-HE, Cas9-HE-Geminin, and Cas9-Geminin to CRISPR-Cas9 in t-test (*P<0.05 or **P<0.005). Relative expression levels of and other fusions were analyzed by western blot with CRISPR-Cas9 and control anti-tubulin antibodies. Protein extracts were obtained with lysis buffer containing 150 mM NaCl. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-018-03475-7) licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - DF-1 stable cell line (chicken fibroblast) expressing Cas9 (left)or wildtype DF-1 cells (right) stained with Cas9 antibody NBP2-36440 and phalloidin and DAPI to visualise F-actin and DNA respectively. Cells fixed with 4% PFA. Antibody at 1:500 overnight at 4C. ICC/IF image submitted by a verified customer review.	
Immunohistochemistry-Frozen: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Analysis of a formalin fixed 20um thick frozen section of mouse brain with GBM xenograft tumor areas (GBM cells over expressing SpyCas9 through lentivirus infection). CRISPR-Cas9 antibody (clone 7A9-3A3) was used at 1:50 dilution. The signal was detected using immunofluoresence labeled secondary antibody via Confocal microscopy. Image submitted via verified customer review.	
Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - 20 ug whole cell lysates from control MEF (MEF-WT) and MEF-Cas9 stable cell line. CRISPR-Cas9 antibody (clone 7A9-3A3) was used at 1:1000 dilution. Observed molecular weight is ~158 kDa. Image submitted via verified customer review.	

Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Chicken fibroblasts DF-1 cells stably expressing Cas9 in lanes 2 and 4, wild type fibroblasts in lanes 1 and 3 blot, probed with Cas9 antibody. WB image submitted by a verified customer review.	
Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - Analysis of Crispr-Cas9 transfected HEK293 cells using CRISPR-Cas9 antibody (clone 7A9-3A3). Red staining represents CRISPR-Cas9 positivity while DAPI stained nuclei are visible in blue color. Image submitted via verified customer review.	
Immunoprecipitation: Mouse Monoclonal CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus [NBP2-36440] - LC-MS/MS signals of a surrogate peptide from Cas9 and a Cas9 variant after immunoprecipitation using CRISPR-Cas9 Antibody (7A9-3A3) [Biotin] (NBP2-36440B). Image from a verified customer review.	

Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus - BSA Free [NBP2-36440] - Identification of “HDR-enhancer” (HE) domain of CtIP. a Schematic diagram of CtIP protein showing different truncated CtIP proteins have been fused to Cas9 & tested for their ability to stimulate HDR. Various sequence features of CtIP, including tetramerization & dimerization domains, & CDK phosphorylation sites S233, T245, & S276, are indicated. b Identification of a domain of CtIP, called HE, spanning aa 1 to 296, which is able to stimulate HDR when fused to Cas9. Human RG37 fibroblasts transfected w/ indicated plasmids expressing Cas9 or Cas9–CtIP derivatives, T2 guide RNA plasmid, & GFP transgene donor w/ homology arms to targeted AAVS1 locus. Expression of fusion proteins examined by WB (Supplementary Fig. 1). Data are from four independent experiments. Error bars indicate standard deviation. c Functional analysis of HE domain. HEK293 cells transfected w/ indicated Cas9 plasmids, T2 guide RNA, & GFP transgene donor w/ homology arms to AAVS1 targeted locus. HDR-mediated transgene integration measured by FACS analysis of GFP-positive cells, resulting from targeted GFP transgene integration. Indels at cleavage site measured by T7E1 assay. Results are expressed as mean of relative HDR or indel frequencies calculated by normalizing every HDR or indel frequency by induced by Cas9, respectively. Asterisks indicate difference is statistically significant when comparing Cas9–CtIP or Cas9–HE derivatives to Cas9 in nonparametric t-test (*P<0.05, **P<0.005, or ***P<0.0005). Data are from four independent experiments. Error bars indicate standard deviation. The relative expression levels of Cas9 & Cas9–HE derivatives analyzed by WB using anti-Cas9 & control anti-tubulin antibodies Image collected & cropped by CiteAb from following publication (https://pubmed.ncbi.nlm.nih.gov/29556040), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus - BSA Free [NBP2-36440] - Stimulation of transgene integration by Cas9–HE & Cas9–Geminin. Relative frequencies of HDR & indels induced by Cas9 or fusion of Cas9 to HE domain, Geminin degron, or both. Human HEK293 cells were transfected with the indicated Cas9 plasmids, T2 guide RNA, & GFP transgene donor with homology arms to the AAVS1 targeted locus. HDR-mediated transgene integration was measured by FACS analysis of GFP-positive cells, resulting from targeted GFP transgene integration. Indels at the cleavage site were measured by the T7E1 assay. The results are expressed as the mean of relative HDR or indel frequency calculated by normalizing every HDR or indel frequency by that induced by Cas9. Asterisks indicate that the difference is statistically significant when comparing Cas9–HE, Cas9–HE–Geminin, & Cas9–Geminin to Cas9 in t-test (*P<0.05 or **P<0.005). Data are from three independent experiments. Error bars indicate standard deviation. Relative expression levels of Cas9 & other fusions were analyzed by western blot with anti-Cas9 & control anti-tubulin antibodies. Protein extracts were obtained with lysis buffer containing 150 mM NaCl, which resulted in inefficient solubilization of Cas9 fusions with the HE domain compared to those of Cas9 & Cas9–Geminin Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29556040), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Yanhong Zhang, Rosalia Rabinovsky, Zhiyun Wei, Rachid El Fatimy, Evgeny Deforzh, Bai Luan et al. Secreted PGK1 and IGFBP2 contribute to the bystander effect of miR-10b gene editing in glioma Molecular Therapy - Nucleic Acids 2023-03-14 [PMID: 36700043]

Nazma F. Ilahibaks, Thomas A. Kluiver, Olivier G. de Jong, Saskia C. A. de Jager, Raymond M. Schiffelers, Pieter Vader, Weng Chuan Peng, Zhiyong Lei, Joost P. G. Sluijter Extracellular vesicle□mediated delivery of CRISPR/Cas9 ribonucleoprotein complex targeting proprotein convertase subtilisin□kexin type 9 (Pcsk9) in primary mouse hepatocytes Journal of Extracellular Vesicles 2024-01-08 [PMID: 38191764]

Li WK, Zhang SQ, Peng WL et al. Whole-brain in vivo base editing reverses behavioral changes in Mef2c-mutant mice Nature neuroscience 2023-11-27 [PMID: 38012399]

Zhang C, Schekman R Syncytin-mediated open-ended membrane tubular connections facilitate the intercellular transfer of cargos including Cas9 protein eLife 2023-03-10 [PMID: 36896791] (Western Blot)

Szczerba M, Johnson B, Acciai F et al. Canonical cellular stress granules are required for arsenite-induced necroptosis mediated by Z-DNA-binding protein 1 Science signaling 2023-03-14 [PMID: 36917643]

Pettitt SJ, Shao N, Zatreanu D et al. A HUWE1 defect causes PARP inhibitor resistance by modulating the BRCA1-?11q splice variant Oncogene 2023-01-01 [PMID: 37491606] (WB)

Abrego J, Sanford-Crane H, Oon C et al. A Cancer Cell-Intrinsic GOT2-PPAR? Axis Suppresses Antitumor Immunity Cancer discovery 2022-10-05 [PMID: 35894778] (WB, Human)

Zelceski A, Francica P, Lingg L et al. MND1 and PSMC3IP control PARP inhibitor sensitivity in mitotic cells Cell reports 2023-05-30 [PMID: 37163373]

Cappellesso F, Orban MP, Shirgaonkar N et al. Targeting the bicarbonate transporter SLC4A4 overcomes immunosuppression and immunotherapy resistance in pancreatic cancer Nature cancer 2022-12-01 [PMID: 36522548] (WB, Mouse)

Zhang Y, Rabinovsky R, Wei Z et al. Secreted PGK1 and IGFBP2 contribute to the bystander effect of miR-10b gene editing in glioma Molecular Therapy - Nucleic Acids 2023-01-01 (ICC/IF, Bacteria)

Wilk A, Hayat F, Cunningham R et al. Extracellular NAD+ enhances PARP-dependent DNA repair capacity independently of CD73 activity Sci Rep 2020-01-20 [PMID: 31959836] (KO, WB)

Wu Y, Yang L, Chang T et al. A Small Molecule-Controlled Cas9 Repressible System Mol Ther Nucleic Acids 2020-01-10 [PMID: 32000033] (WB, KO)

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NBP2-36440B	CRISPR-Cas9 Antibody (7A9-3A3) [Biotin]

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