Product Datasheet

CRISPR-Cas9 Antibody
NBP2-52717

Unit Size: 100 ul

Store at -20C. Avoid freeze-thaw cycles.

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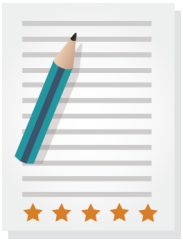
Updated 10/23/2024 v.20.1



NBP2-52717

CRISPR-Cas9 Antibody

Product Information

Unit Size	100 ul
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS (pH 7.3), 1.0% BSA and 50% Glycerol
Target Molecular Weight	158.4 kDa

Product Description

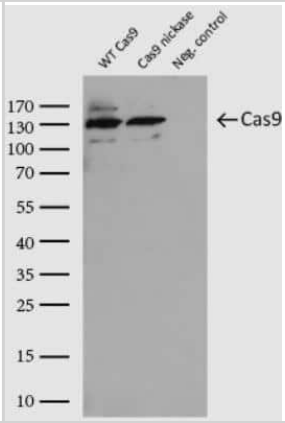
Host	Rabbit
Gene ID	901176
Species	Bacteria
Immunogen	This CRISPR-Cas9 antibody was raised against CAS9 synthetic peptide within S. Pyogenes enzyme cas9 (Used in CRISPR/Cas9 Genome Editing system).

Product Application Details

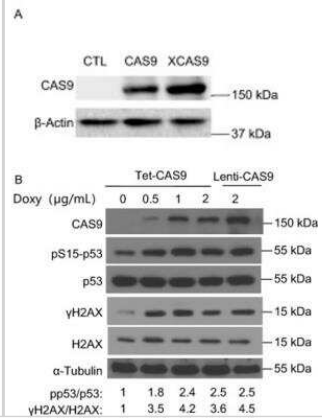
Applications	Western Blot, Immunoprecipitation
Recommended Dilutions	Western Blot 1:500, Immunoprecipitation

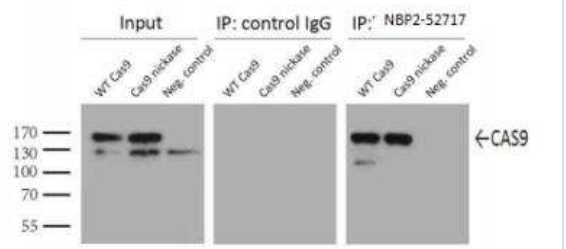
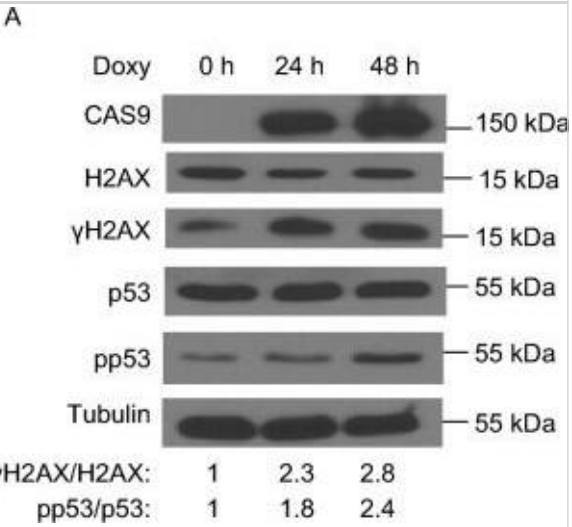
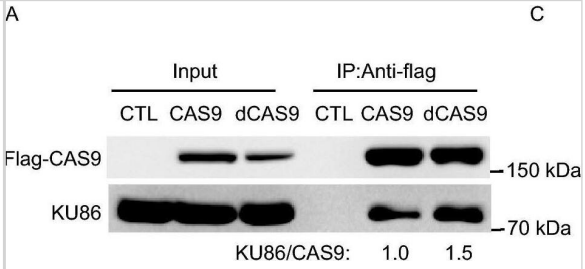
Images

Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - HEK293T were transfected with either WT CAS9 plasmid, CAS9 nickase plasmid or control vector. Equivalent amount of each cell lysates (10ug per lane) were immunoblotted with (1:2000). Theoretical molecular weight ~158 kDa.

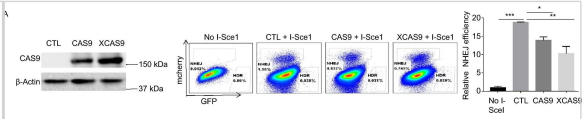


Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - A. After the induction of CAS9 and XCAS9 in 293 cells with 2 ug/mL doxycycline for 3 days, CAS9 expression was accessed by WB. XCAS9 is a CAS9 variant with potentially higher fidelity and broader compatibility. B) The impact of expression levels of CAS9 on DNA DSB damage in hESCs. At the same lentiviral titers, the expression levels of CAS9 in hESCs transduced by standard lentiviral vector are higher than those transduced by the inducible lentiviral vector after 2 ug/mL Doxy treatment. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32170574) licensed under a CC-BY license.

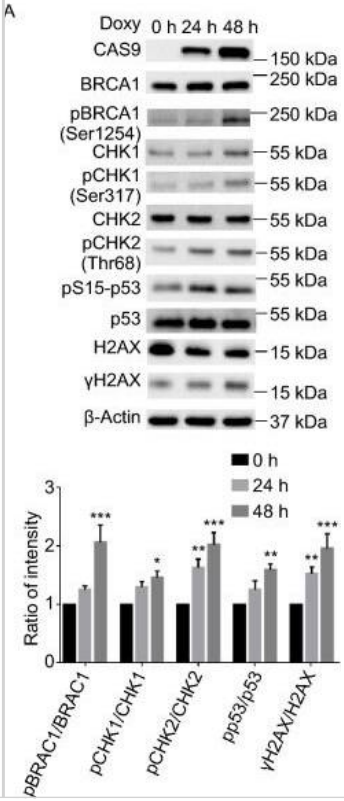


Immunoprecipitation: CRISPR-Cas9 Antibody [NBP2-52717] - HEK293T were transfected with either WT CAS9 plasmid, CAS9 nickase plasmid (GE100019), or negative control vector (PS100001). Input: 10ug total cell lysates; WB was performed (1:2000). IP: 100ug of cell lysates were precipitated with 20ug of NBP2-52717 or control antibody, and 5ug Protein G Beads. Mouse anti-DDK monoclonal antibody was used in WB to detect the immuno-precipitated Cas9 (as Cas9 is Myc-DDK tagged)	
Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - The expression of CAS9 in hiPSCs & hESCs promotes DNA DSB damage. (A) The inducible expression of CAS9 promotes DNA DSB damage responses in hiPSCs after 2 µg/mL Doxy treatment. The relative levels of the phosphorylation of p53 & H2AX are indicated at the bottom. Consistent data were obtained from two independent experiments. (B) The impact of expression levels of CAS9 on DNA DSB damage in hESCs. At the same lentiviral titers, the expression levels of CAS9 in hESCs transduced by standard lentiviral vector are higher than those transduced by the inducible lentiviral vector after 2 µg/mL Doxy treatment. Much lower expression levels of CAS9 can also promote DNA DSB damage in hESCs after the treatment with lower dosages of Doxy. The relative levels of the phosphorylation of p53 & H2AX are indicated at the bottom Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32170574), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - dCAS9 & Cpf1 impair NHEJ & induce genetic mutations. (A) Co-immunoprecipitation assay confirmed the interaction between dCAS9 & KU86. (B) Comet assay analysis of DNA damage in hESCs expressing dCAS9 or treated with doxorubicin. CTL, human fibroblasts with lentiviral empty vector were treated with 2 µg/mL doxycycline for three days; Doxy, Dox, Doxy + Dox, human fibroblasts with lentiviral CAS9 inducible expression vector were treated with 2 µg/mL doxycycline for 3 days or 0.5 µmol/L Dox for 2 h or 2 µg/mL doxycycline for three days + 0.5 µmol/L Dox for 2 h, respectively. Tail length was analyzed using Image J software. Data are represented as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. (C) The expression of dCAS9 induces mutation of endogenous HPRT gene. WT, WT hESCs; CTL, CAS9, dCAS9, hESCs with empty expression vector, CAS9 inducible expression vector. Cells with dCAS9 inducible expression vector were treated with 2 µg/mL doxycycline for 3 days before HAT treatment. n = 3. Data are presented as mean value ± SD. **P < 0.01, ***P < 0.001. (D) The expression of Cpf1 increased the levels of γH2AX. (E) Cpf1 interacts with KU86 as confirmed by Co-immunoprecipitation. Protein extract of Flag-tagged Cpf1 was immunoprecipitated with anti-Flag antibody & the presence of Cpf1 & KU86 in the immunoprecipitate was examined by Western blot Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32170574), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - Both CAS9 & XCAS9 impair NHEJ & induce genetic mutations. (A & B) Expression of CAS9 & XCAS9 impairs NHEJ. Traffic Light Reporter system was established in 293 cells harboring the CAS9 & XCAS9 inducible expression vectors. After the induction of CAS9 & XCAS9 expression with 2 µg/mL doxycycline for 3 days (left panel), the efficiency of NHEJ (mcherry) & HDR (GFP) was analyzed by flow cytometry (middle panel). Statistic analysis of the efficiency of NHEJ (left panel of A) & HDR (B) is presented. n = 3. Data are presented as mean values ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. ns, non-significant. (C) The expression of CAS9 in hESCs induces genomic mutations at the endogenous HPRT locus. After hESCs harboring CAS9 inducible expression vector were selected with HAT medium for 5 days, they were treated with 2 µg/mL doxycycline for CAS9 expression for various time periods, & subsequently, treated with 5 µg/mL 6-TG or mock treated for 4 days. Mutational rate is calculated as the ratio of colony number in 6-TG treated samples versus untreated controls. n = 3. Data are presented as mean values ± SD. ***P < 0.001. (D) XCAS9 interacts with KU86. Protein extracts from 293FT cells expressing Flag-tagged CAS9 or XCAS9 were immunoprecipitated with anti-Flag antibody. The immune precipitates were analyzed for the presence of CAS9, XCAS9 & KU86. The relative ratio of KU86 versus CAS9 or XCAS9 is indicated. (E) The expression of XCAS9 increases the number of γH2AX foci in hESCs. hESCs harboring XCAS9 inducible expression vector were treated with or without 2 µg/mL doxycycline for 3 days. n = 20. Scale bar, 10 µm. Data are presented as mean values ± SD. ***P < 0.01 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32170574), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CRISPR-Cas9 Antibody [NBP2-52717] - The expression of CAS9 in human fibroblasts promotes DNA DSB damage & activates DNA damage response pathways. (A) The expression of CAS9 in human fibroblasts activates DNA damage responses. The expression of CAS9 was induced with 2 µg/mL Doxy treatment. The relative levels of phosphorylation of BRCA1, CHK1, CHK2 & p53 are indicated at the bottom. n = 3. Data are presented as mean value ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. (B) The expression of CAS9 increased the number of γH2AX foci in human fibroblasts. CTL or CAS9, human fibroblasts with CAS9 inducible expression cassette plated on chamber slides were treated with or without 2 µg/mL doxycycline for 3 days. The expression of CAS9 & γH2AX foci was revealed by immunoflourescence analysis. Representative images are shown. Scale bar, 10 µm. Unpaired t test. n = 20. Data are presented as mean values ± SD. ***P < 0.001. (C) CAS9 induces DNA DSB damage in human fibroblasts. CTL, human fibroblasts with lentiviral empty vector were treated with 2 µg/mL doxycycline for three days; Doxy, Dox, Doxy + Dox, human fibroblasts with lentiviral CAS9 inducible expression vector were treated with 2 µg/mL doxycycline for 3 days or 0.5 µmol/L Dox for 2 h or 2 µg/mL doxycycline for three days + 0.5 µmol/L Dox for 2 h, respectively. Representative images are shown. n = 40. Unpaired t test. Data are presented as mean value ± SD. **P < 0.01 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32170574), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Xu S, Kim J, Tang Q et al. CAS9 is a genome mutator by directly disrupting DNA-PK dependent DNA repair pathway Protein Cell 2020-05-01 [PMID: 32170574]

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