Product Datasheet

CRISPR-Cas9 Antibody Pack
NBP2-52986

Unit Size: 3 Vials

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

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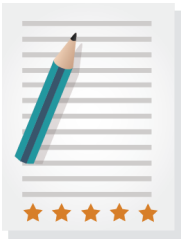


NBP2-52986

CRISPR-Cas9 Antibody Pack

Product Information

Unit Size	3 Vials
Concentration	Concentration of individual antibodies may be found on the vial label. If unlisted please contact technical services.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

Product Description

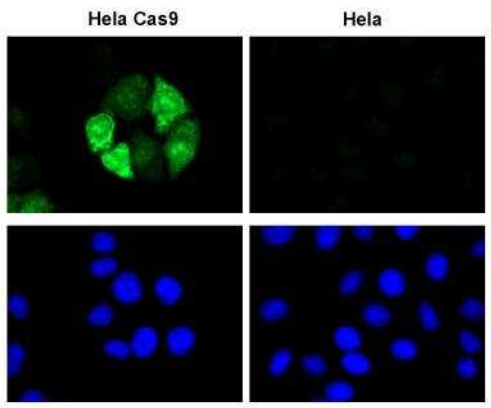
Description	This pack contains 1 vial each of: NBP2-36440SS (0.025 mL), NBP2-52398SS (0.025 mL).
Gene ID	901176
Species	Bacteria
Reactivity Notes	See individual datasheets of components for their validated species
Immunogen	See individual datasheets.
Kit Components	NBP2-52398: CRISPR-Cas9 Antibody (6G12) - C-terminus - BSA Free, NBP2-36440: CRISPR-Cas9 Antibody (7A9-3A3) - N-Terminus - BSA Free, HAF007: Goat anti-Mouse IgG Secondary Antibody [HRP]

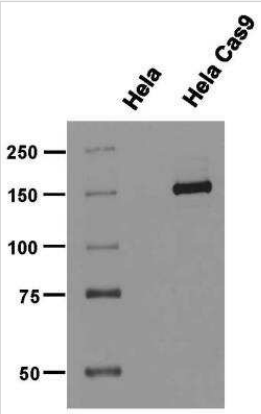
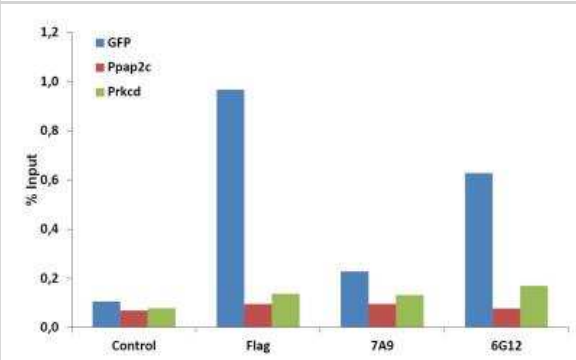
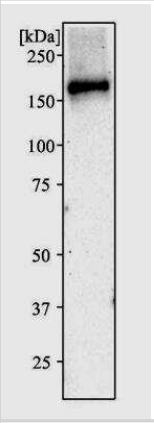
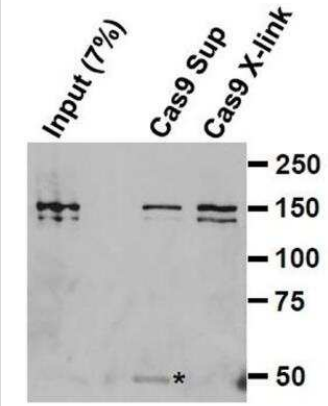
Product Application Details

Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot, Simple Western, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Application Notes	See individual datasheets of components for their validated applications

Images

Immunocytochemistry/Immunofluorescence: CRISPR-Cas9 Antibody Pack [NBP2-52986] - 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.



Western Blot: CRISPR-Cas9 Antibody Pack [NBP2-52986] - Hela cells and Hela cells expressing Flag-tagged S.pyogenes Cas9 under the control of the PTight (Tet-ON) promoter were treated for 24h with 1ug/ul Doxycyclin and lysed under native conditions. 30ug of whole cell lysate per lane was separated by 7.5% SDS-PAGE, transfered to nitrocellulose membrane and incubated with 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). A 1 min exposure is shown. Note the specific band of CRISPR-Cas9 with a molecular weight of 158.4 kDa.	
Chromatin Immunoprecipitation (ChIP): CRISPR-Cas9 Antibody Pack [NBP2-52986] - ChIP analysis of CRISPR-Cas9 antibody clones 7A9-3A3 and 6G12 on NIH3T3 cells which were stably expressing GFP-H2B, nuclease dead Cas9 (Flag tagged), and a GFP-targeting gRNA. The cells 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 supernatants of clones 7A9 and 6G12; 5ug Flag antibody) 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 Pack [NBP2-52986] - WB analysis of Cas9 protein in lysate of CRISPR-Cas9 transfected 293T cells using anti-Cas9 antibody clone 7A9-3A3 at 2ug/ml concentration.	
Immunoprecipitation: CRISPR-Cas9 Antibody Pack [NBP2-52986] - 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.	

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Products Related to NBP2-52986

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

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

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