Cas9 antibody (mAb)

Catalog Nos: 61757, 61957, 61758
RRID: AB_2793760
Clone: 8C1-F10
Isotype: IgG2b
Application(s): ChIP, IF, IP, WB
Reactivity: Human, Mouse, Not Species Specific

Quantities: 100 µg, 50 µg, 10 µg
Purification: Protein A Chromatography
Host: Mouse
Concentration: 1 µg/µl
Molecular Weight: 160 kDa

Background: Cas9 is a nuclease from *Streptococcus pyogenes* that can be targeted to particular DNA sequences through a guide RNA that results in double-stranded breaks in DNA. Cas9 is part of the CRISPR/Cas9 gene-editing system that can create a DNA break at a specific location with the genome.

CRISPR (clustered regularly interspaced short palindromic repeat) is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA) Probable. In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and this protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed by 3'-5' exonucleolytically. DNA-binding requires protein and both RNA species. Cas9 probably recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus nonself.

Immunogen: This antibody was raised against a recombinant protein within the N-terminal region of *Streptococcus pyogene* Cas9. This antibody should recognize Cas9 and dCas9 based on the antigen design.

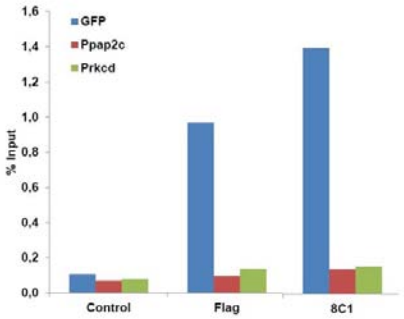
Buffer: Purified IgG in PBS with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

Application Notes:
Applications Validated by Active Motif:
ChIP
ICC/IF
IP
WB

Validation was done using crude hybridoma supernatant. Individual optimization required. For Cas9, we also offer AbFlex® Cas9 Recombinant Antibody (rAb). For details, see Catalog No. 91123.

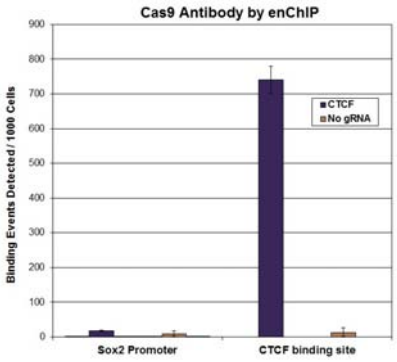
Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product is guaranteed for 12 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.



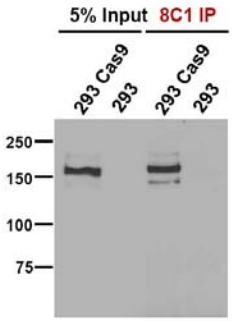
Cas9 antibody (mAb) tested by ChIP.

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. 50µg chromatin was incubated O/N at 4°C with Cas9 antibody (200µl hybridoma SN, 5µg α-Flag) followed by incubation with protein G beads for 3h at 4°C. After washing chromatin was eluted from the beads and crosslinking was reversed)/N at 65°C. After a proteinase K digesting step DNA was separated using phenol/chloroform/isoamyl alcohol, precipitated with ethanol/sodium acetate and dissolved in water. For the qPCR primers either targeting the GFP gene or as negative control non-targeted regions (Ppap2c +7122 and Prkcd +24069 from transcription start) were used.



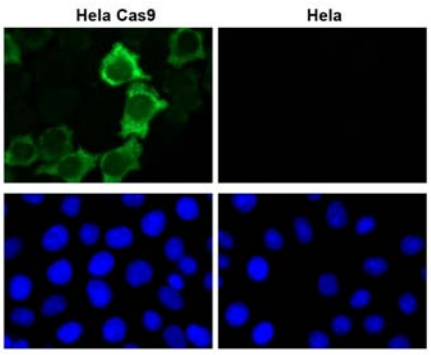
Cas9 antibody (mAb) tested by enChIP

HEK293T cells transfected with vectors encoding both AM-tagged dCas9 and a gRNA targeting a CTCF binding site, or just AM-tagged dCas9 (No gRNA control) were analyzed by enChIP (Active Motif Cat. 53125) using Cas9 mAb clone 8C1-F10 in place of the AM-tag antibody. DNA eluted from enChIP was subjected to qPCR analysis using primers against the CTCF binding targeted by the enChIP gRNA and the Sox2 promoter (negative control site).



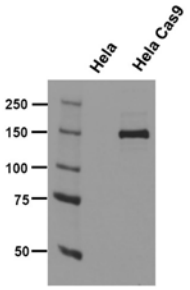
Cas9 antibody (mAb) tested by Immunoprecipitation.

HEK293 cells or HEK293 cells expressing Flag-Cas9 were lysed under native conditions. Cas9 was immunoprecipitated at 4°C from ~300µg of whole cell lysate with Cas9 clone 8C1-F10 and a 1:1 mixture of protein A and protein G sepharose. After 4x washing, the bound proteins were boiled off the beads, separated by 7.5% SDS-PAGE and transfered to nitrocellulose membranes.



Cas9 antibody (mAb) tested by Immunofluorescence.

Hela cells or Hela cells expressing Flag-Tagged Cas9 under the control of the PTight (Tet-ON) promoter were treated for 24h with 1 µg/µl Doxycyclin, fixed and permeabilized with Methanol/Acetone and blocked in 2% BSA in PBS for 2 hours at RT. Cells were stained with 8C1-F10 hybridoma supernatant diluted 1:10 at 4°C o/n, followed by incubation with anti mouse-AF488 coupled secondary antibody for 1 h at RT. Nuclei were counter-stained with Hoechst 33342.



Cas9 antibody (mAb) tested by Western blot.

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 1 µg/µl Doxycyclin and lysed under native conditions. ~30 µg of whole cell lysate per lane was separated by 7.5% SDS-PAGE, transfered to nitrocellulose membrane and incubated with crude hybridoma supernatant (diluted 1:100) of Cas9 specific monoclonal antibody, 8C1-F10. All incubations were done at 4°C o/n.