Datasheet for ABIN5563965

anti-CRISPR-Cas9 antibody

3 Images

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Overview

Quantity:	100 µg
Target:	CRISPR-Cas9
Reactivity:	Streptococcus pyogenes
Host:	Mouse
Clonality:	Monoclonal
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunoprecipitation (IP), Chromatin Immunoprecipitation (ChIP)

Product Details

Clone:	8C1-F10
Isotype:	IgG2b
Specificity:	Mouse
Purification:	Protein A Chromatography

Target Details

Target:	CRISPR-Cas9
Alternative Name:	Cas9
Molecular Weight:	160 kDa

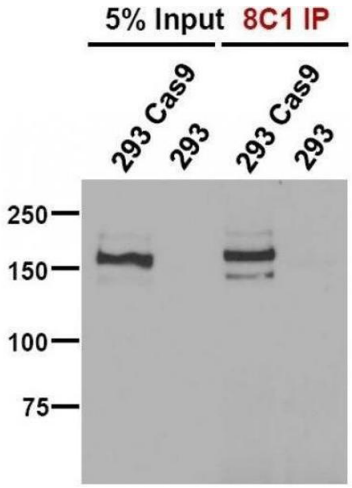
Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

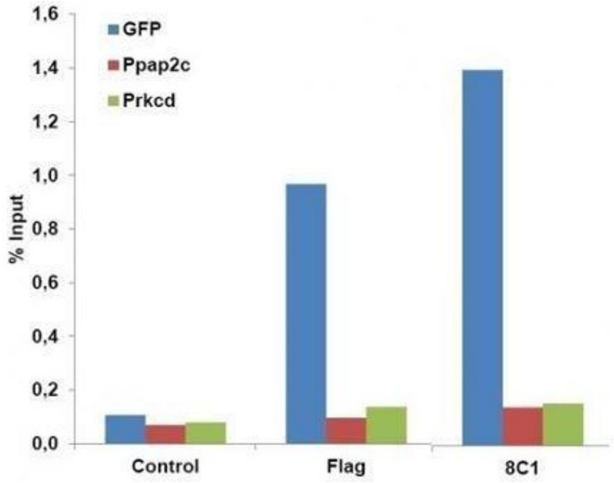
Format:	Liquid
Buffer:	Purified IgG in PBS with 30 % glycerol and 0.035 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C

Images



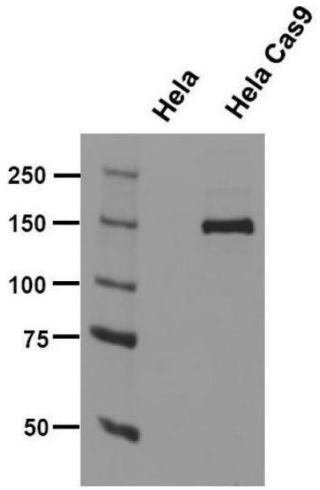
Immunofluorescence

Image 1. 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.



Chromatin Immunoprecipitation

Image 2. 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 over night at 4°C with the 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 over night 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.



Western Blotting

Image 3. 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.