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CRISPR/Cas9 (SaCas9) Monoclonal Antibody [6H4]

(Catalog No. A-9001)

Background

The discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR associated system or CRISPR associated protein 9 nuclease) found in bacteria to work as a defense mechanism against foreign DNA has proven to be an invaluable tool to target and modify a genetic sequence in gene editing and genome engineering applications. The system, known as CRISPR/Cas9, allows for sequence-specific cleavage of a targeted genomic locus by delivering the RNA-guided Cas9 nuclease and appropriate guide RNAs (gRNA) into a cell. In addition, Protospacer Adjacent Motif (PAM) sequence immediately following the specificity sequence is necessary for successful binding of the Cas9 nuclease. It is important and critical to monitor the level of Cas9 editing protein or track the Cas9 editing protein in transfected cells, as it will tell transfection efficiency and optimize the editing process in the total cell population. Staphylococcus aureus Cas9 (SaCas9)-mediated genome editing has been reported in human cells and Arabidopsis. Because SaCas9 (1053 a.a.) is smaller than Streptococcus pyogenes Cas9 (SpCas9) (1368 a.a.), SaCas9 could have substantial advantages for delivering and expressing Cas9 protein, especially using virus vectors.

Description

CRISPR/Cas9 (SaCas9) Monoclonal Antibody, Clone 6H4. Unconjugated. Raised in: Mouse.

Specificity

Clone 6H4 detects Cas-9 in Staphylococcus aureus

Reactivity

Species Independent

Uniprot ID

J7RUA5

Isotype

IgG2bκ

Immunogen

His-tagged recombinant Cas-9 from S. aureus

Formulation

Buffer containing 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide

Storage

Store at 4°C (short-term) or −20°C (long-term). Avoid multiple freeze/thaw cycles

Purity

Protein G Purified

Application

WB (1:500- 2000), IP (1-2 µg/10^6 cells), IF (1:500- 2000), ELISA (1:1000- 2000)

Ordering Information

Products	Size	Cat. No.
CRISPR/Cas9 (SaCas9) Monoclonal Antibody [6H4]	10 µg	A-9001-010
	50 µg	A-9001-050
	100 µg	A-9001-100

This product is for research purposes only. Not intended for use in diagnostic procedures.

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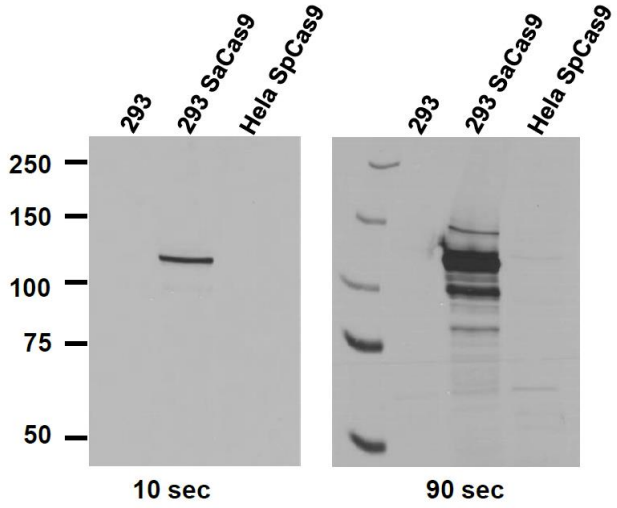
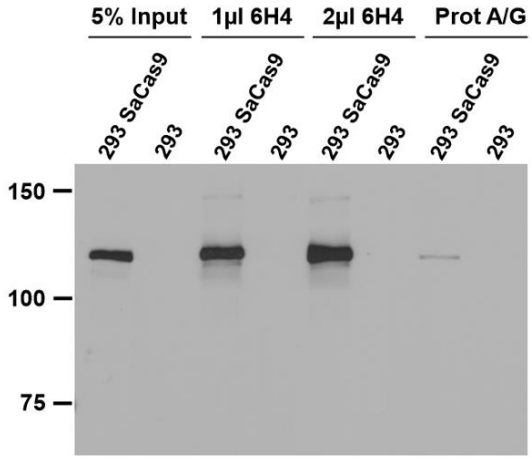
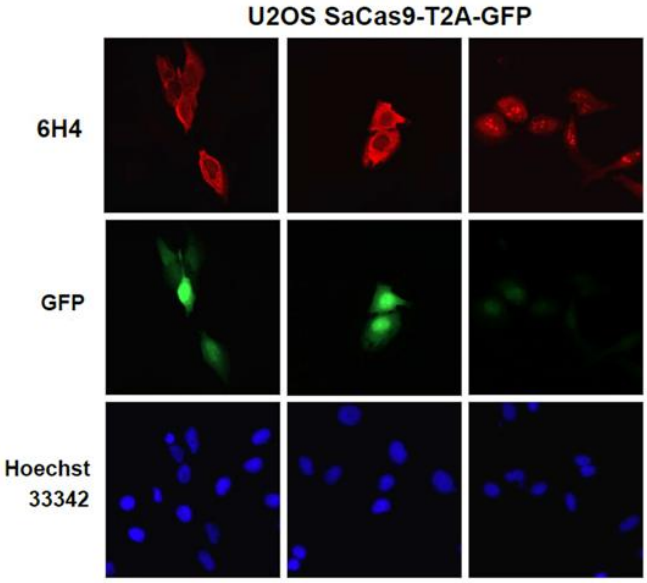


▲Whole cell lysates of the shown cell lines were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membrane. After blocking in 3% NFDM in PBS-T, the membrane was incubated with purified CRISPR/Cas9 (SaCas9) Monoclonal Antibody [6H4] (1:10000 diluted) at 4°C o/n and Cat. #A12003 for 1 hour at room temp. Protein expressions were visualized using ECL exposed to X-ray film.



▲SaCas9 was immunoprecipitated from 200 µg of whole cell lysate of the shown cell lines using 1 µl or 2 µl of purified CRISPR/Cas9 (SaCas9) Monoclonal Antibody [6H4] and a 1:1 mixture of protein A/G beads or with a 1:1 mixture of protein A/G beads alone for 90 min at 4°C. After washing 1x each with lysis buffer, RIPA buffer, LiCl wash buffer, and Tris/EDTA, bound proteins were boiled off the beads with Laemmli buffer, separated by 7.5% SDS-PAGE together with 5% of input cell lysate and transferred to nitrocellulose membrane. The membrane was blocked in 3% NFDM in PBS-T and incubated with SaCas9 rabbit polyclonal antibody (1:10000 diluted) at 4°C o/n. After incubation with anti-rabbit HRP coupled secondary antibody for 1 hour at room temperature, protein expressions were visualized using ECL exposed to X-ray film for 10 seconds.



▲Transiently transfected U2OS cells expressing SaCas9-T2A-GFP were fixed with 3.7% formaldehyde and permeabilized with 0.5% Triton-X at room temperature. After blocking in 2% BSA in PBS, cells were stained with purified CRISPR/Cas9 (SaCas9) Monoclonal Antibody [6H4] (1:1000 diluted) in 1% BSA/PBS at 4°C o/n. After washing, cells were incubated with anti-mouse DyLight594 secondary antibody for 1 hour at room temperature, nuclei were counterstained with Hoechst 33342 and slides were mounted with Vectashield.

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