diagenode
A Hologic Company

TECHNICAL DATASHEET

S. aureus CRISPR/Cas9 polyclonal antibody (C-terminal)

Other name: Csn1

Cat. No. C15310259

Type: Polyclonal

Source: Rabbit

Lot #: A2555-001

Size: 100 µl

Concentration: not determined

Specificity: Staphylococcus aureus

Purity: Whole antiserum from rabbit containing 0.05% azide.

Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against the C-terminus of the S. aureus Cas9 nuclease (CRISPR-associated protein 9) using a recombinant protein.

Applications

	Suggested dilution	Results
Western blotting	1:10,000	Fig 1
IP	1 µg/IP	Fig 2
IF	1:1,000	Fig 3

Target description

CRISPR systems are adaptable immune mechanisms which are present in many bacteria to protect themselves from foreign nucleic acids, such as viruses, transposable elements or plasmids. The CRISPR/Cas9 (CRISPR-associated protein 9 nuclease) system from S. pyogenes was the first to be adapted for inducing sequence-specific double stranded breaks and targeted genome editing. This system is unique and flexible due to its dependence on RNA as the moiety that targets the nuclease to a desired DNA sequence and can be used to induce indel mutations, specific sequence replacements or insertions and large deletions or genomic rearrangements at any desired location in the genome. In addition, Cas9 can also be used to mediate upregulation of specific endogenous genes or to alter histone modifications or DNA methylation, Recently, the CRISPR/Cas9 from S. aureus (UniProtKB/Swiss-Prot entry J7RUA5) was also shown to be suitable for humane genome editing. The S. aureus Cas9 has the advantage that it's smaller and therefore easier to transfect cells with, whereas the efficiency and specificity are similar.

Results

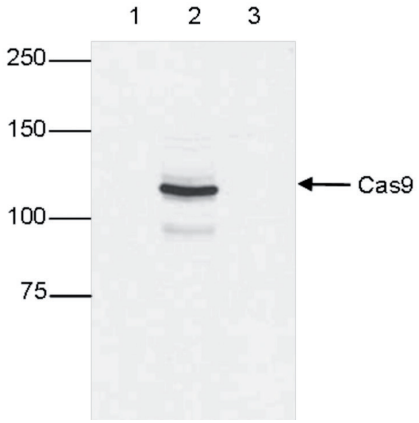

Figure 1. Western blot analysis using the Diagenode antibody directed against S. aureus CRISPR/Cas9 (C-terminal)

Western blot was performed on protein extracts from HEK293 cells (lane 1), HEK293 cells transfected with S. aureus Cas9 (lane 2) and HeLa cells transfected with S. pyogenes Cas9 (lane 3) using the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15310259), diluted 1:10,000 in PBS-T containing 3% NFDM. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

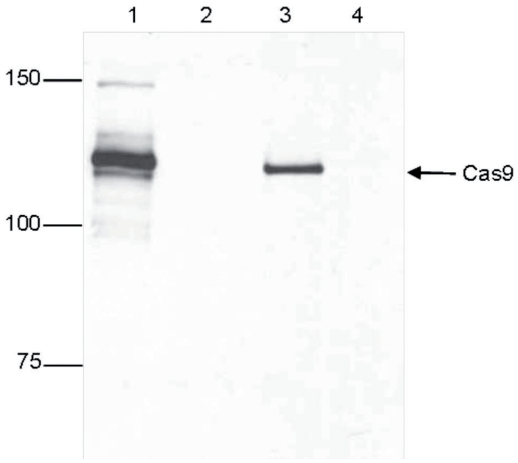

Figure 2. IP using the Diagenode antibody directed against S. aureus CRISPR/Cas9 (C-terminal)

IP was performed on whole cell extracts (200 µg) from HEK293 cells transfected with a Cas9 expression vector (lane 1 and 3), or untransfected cells (lane 2 and 4) using 1 µl of the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15310259). The immunoprecipitated proteins were subsequently analysed by Western blot with the monoclonal CRISPR/Cas9 antibody (C15200230). Lane 3 and 4 show the result of the IP, the input (10 µg) is shown in lane 1 and 2.

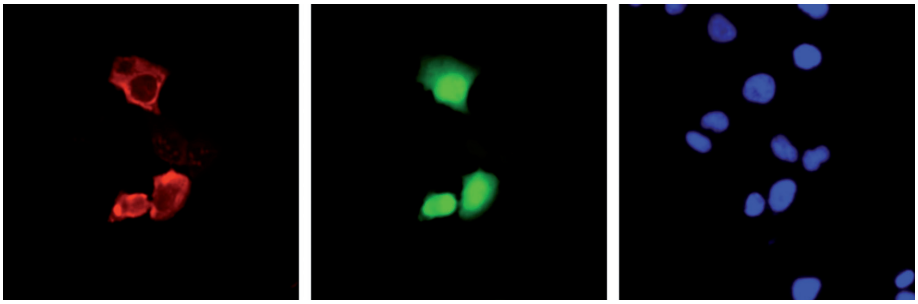

Figure 3. Immunofluorescence using the Diagenode antibody directed against S. aureus CRISPR/Cas9 (C-terminal)

Transiently transfected U2OS cells expressing SaCas9-T2A-GFP were fixed with 3.7% formaldehyde, permeabilized in 0.5% Triton-X-100 and blocked in PBS containing 2% BSA for 2 hours at RT. The cells were stained with the S. aureus CRISPR/Cas9 antibody (Cat. No. C15310259) diluted 1:1,000 in blocking solution at 4°C o/n, followed by incubation with an anti-rabbit secondary antibody coupled to DyLight594 for 1 h at RT (left figure). Nuclei were counter-stained with Hoechst 33342 (right). The middle figure shows IF with an anti-GFP antibody.

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Last update: January 22, 2016