diagenode
A Hologic Company

TECHNICAL DATASHEET

S. aureus CRISPR/Cas9 antibody

Cat. No. C15200230

Type: Monoclonal

Isotype IgG2bK

Source: Mouse

Lot: 001

Size: 10 µg / 50 µg

Concentration: 2 µg/µl

Specificity: Staphylococcus aureus

Purity: Protein G purified monoclonal antibody in PBS containing 0.05 % Na-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: Monoclonal antibody raised in mouse against the N-terminus of the S. aureus Cas9 nuclease (CRISPR-associated protein 9) using a recombinant protein.

Applications

Applications	Suggested dilution	References
Western blotting	1:4,000	Fig 1
Immunoprecipitation	5 µg/IP	Fig 2
Immunofluorescence	1:400	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 CRISPR/Cas9 has the advantage that it's smaller and therefore easier to transfect cells with, whereas the efficiency and specificity are similar.

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Results

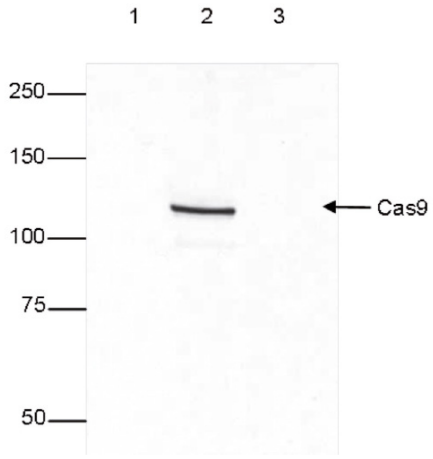

Figure 1. Western blot analysis using the Diagenode monoclonal antibody directed against S. aureus CRISPR/Cas9

Western blot was performed on protein extracts from HEK293 cells (lane 1), HEK293 cells transfected with S. aureus CRISPR/Cas9 (lane 2) and HeLa cells transfected with S. pyogenes Cas9 (lane 3) using the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15200230), diluited 1:4,000 in PBS-T containing 3% NFDM. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

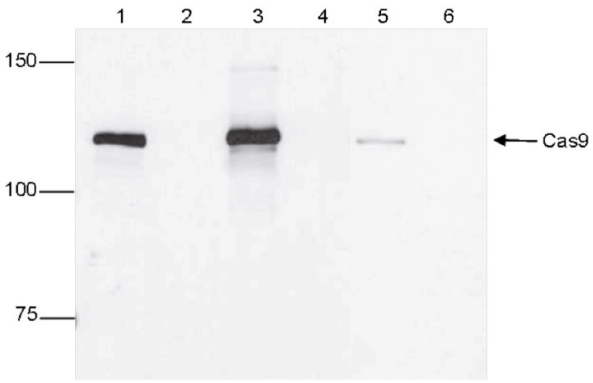

Figure 2. IP using the Diagenode monoclonal antibody directed against S. aureus CRISPR/Cas9

IP was performed on whole cell extracts (200 µg) from HEK293 cells transfected with a Cas9 expression vector (lane 1, 3 and 5), or untransfected cells (lane 2, 4 and 6) using 5 µg of the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15200230). The immunoprecipitated proteins were subsequently analysed by Western blot. The results obtained with the Cas9 antibody are shown in lane 3 and 4. The negative control (IP with beads only) is shown in lane 5 and 6, the input (10 µg) is shown in lane 1 and 2.

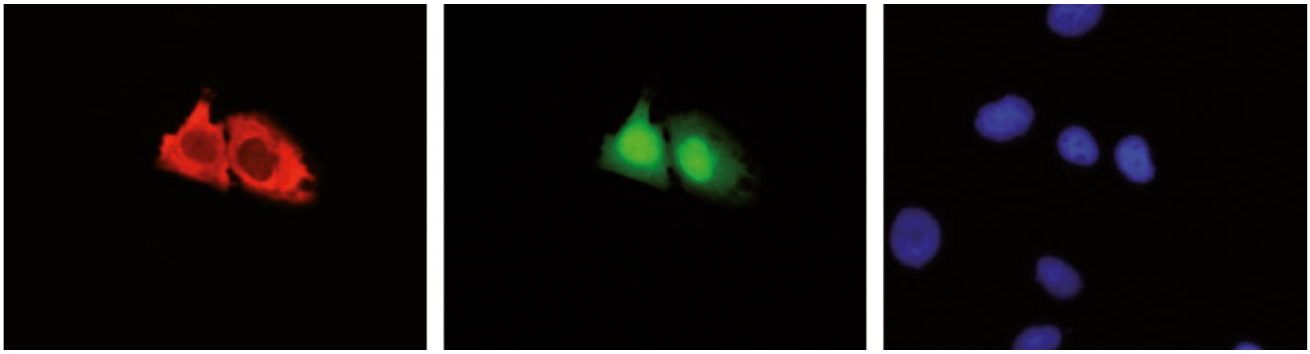

Figure 3. Immunofluorescence using the Diagenode monoclonal antibody directed against S. aureus CRISPR/Cas9

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. C15200230) diluted 1:400 in blocking solution at 4°C o/n, followed by incubation with an anti-mouse 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.