diagenode
A Hologic Company

TECHNICAL DATASHEET

CRISPR/Cas9 monoclonal antibody

Other name: Csn1

Cat. No. C15200229
Type: Monoclonal **ChIP-grade**
Isotype: IgG2b, κ
Source: Mouse
Lot #: 002
Size: 100 µg
Concentration: 2 µg/µl

Specificity: Streptococcus pyogenes
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 Cas9 nuclease (CRISPR-associated protein 9) using a recombinant protein.

Applications

	Suggested dilution	Results
ChIP*	5 µg/ChIP	Fig 1
Western blotting	1:1,000 - 1:10,000	Fig 2
IP	6 µg/IP	Fig 3
IF	1:400	Fig 4

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µg per IP.

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. Recently, the CRISPR/Cas9 (CRISPR-associated protein 9 nuclease, UniProtKB/Swiss-Prot entry Q99ZW2) system from S. pyogenes has been 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.

Results

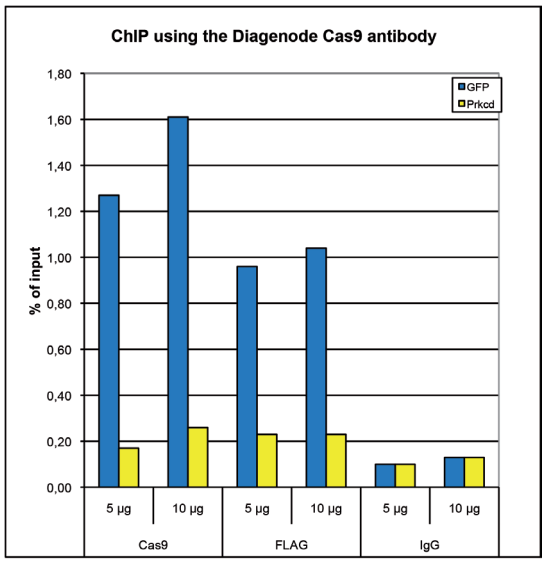

Figure 1. ChIP using the Diagenode monoclonal antibody directed against Cas9

ChIP was performed on NIH3T3 cells stably expressing GFP-H2B, nuclease dead Cas9, and a GFP-targeting gRNA. 50 µg chromatin was incubated overnight at 4°C with 5 or 10 µg of either an anti-FLAG antibody or the Diagenode antibody against Cas9 (Cat. No. C15200229). Mouse IgG was used as a negative IP control. qPCR was performed with primers specific for the GFP gene, and for a non-targeted region (protein kinase C delta, Prkcd), used as negative control. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

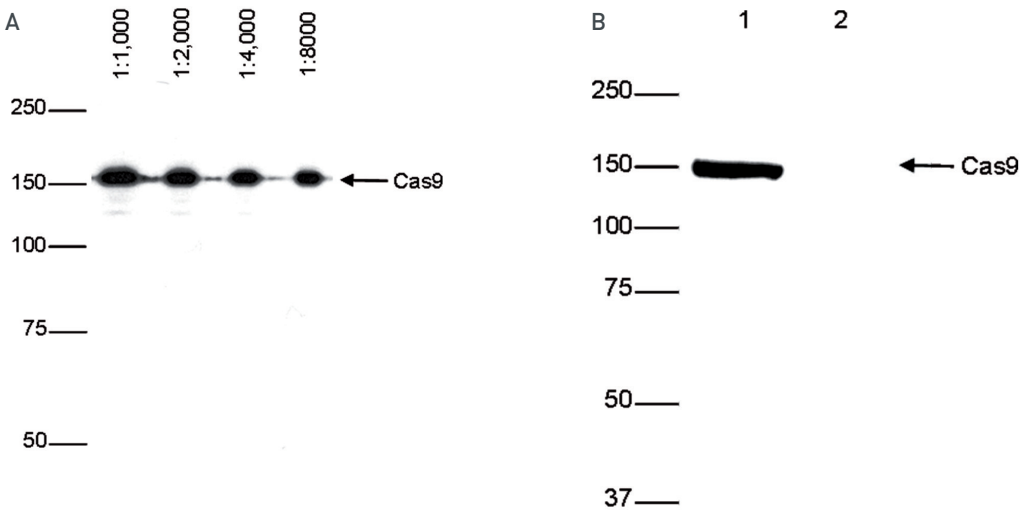

Figure 2. Western blot analysis using the Diagenode monoclonal antibody directed against CRISPR/Cas9

Figure 1A: Western blot was performed on protein extracts from HeLa cells transfected with Cas9 using the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15200229). The antibody was used at different dilutions. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

Figure 1B: Western blot was performed on protein extracts from HeLa cells transfected with Cas9 (lane 1) or from untransfected cells (lane 2) using the Diagenode antibody against CRISPR/Cas9 (Cat. No. C15200229), diluted 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.

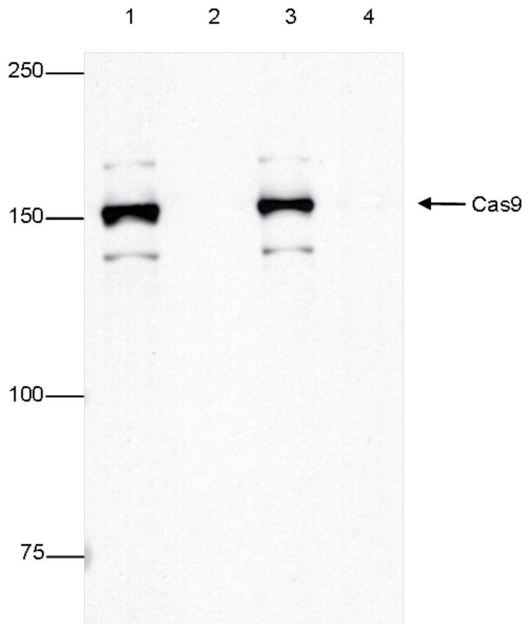

Figure 3. IP using the Diagenode monoclonal antibody directed against Cas9

IP was performed on whole cell extracts (300 µg) from HEK293 cells transfected with a Cas9 expression vector (lane 1 and 3), or untransfected cells (lane 2 and 4) using 6 µg of the Diagenode antibody against Cas9 (Cat. No. C15200229). The immunoprecipitated proteins were subsequently analysed by Western blot with the polyclonal Cas9 antibody (Cat. No. C15310258, diluted 1:8,000). Lane 3 and 4 show the result of the IP, the input (15 µg) is shown in lane 1 and 2.

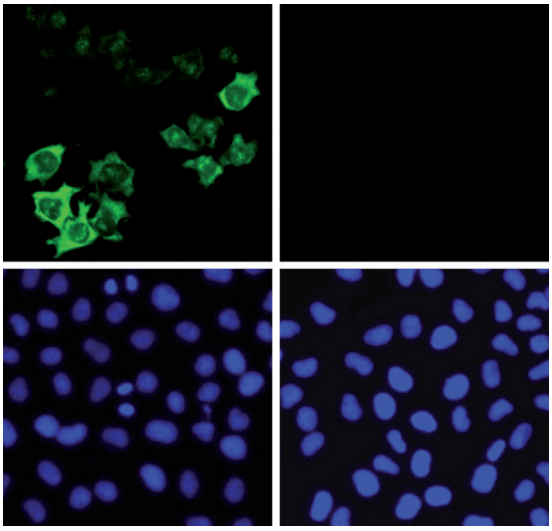

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against CRISPR/Cas9

HeLa cells transfected with a Cas9 expression vector (left) or untransfected cells (right) were fixed in methanol at -20°C, permeabilized with acetone at -20°C and blocked with PBS containing 2% BSA. The cells were stained with the Cas9 N-terminal antibody (Cat. No. C15200229) diluted 1:400, followed by incubation with an anti-mouse secondary antibody coupled to AF488. The bottom images show counter-staining of the nuclei with Hoechst 33342.

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