diagenode
A Hologic Company

TECHNICAL DATASHEET

CRISPR/Cas9 Antibody - ChIP-seq Grade

Cat. No. C15310258-100

Type: Polyclonal, **ChIP grade, ChIP-seq grade**	Specificity: Streptococcus pyogenes
Size: 100 µl	Isotype: NA
Concentration: Not determined	Host: Rabbit
Lot No.: A2508-004	Purity: Whole antiserum from rabbit containing 0.05% azide.
Storage buffer: NA	Storage conditions: 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.	

Last Data Sheet Update: January 29, 2021

Description

Polyclonal antibody raised in rabbit against the **Cas9** nuclease (**CRISPR**-associated protein 9) using a recombinant protein.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq *	2-5 µl/ChIP	Fig 1, 2
Western Blotting	1:5,000	Fig 3
Immunoprecipitation	1 µl/IP	Fig 4
Immunofluorescence	1:1,000	Fig 5

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

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Validation data

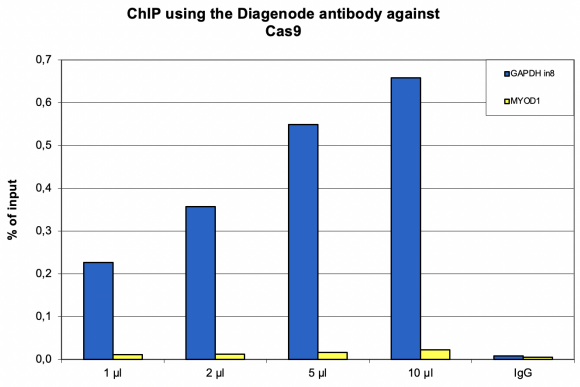

Figure 1. ChIP using the Diagenode antibody directed against Cas9

ChIP was performed on sheared chromatin from 4 million HEK293T cells stably expressing nuclease dead Cas9 and sgRNA targeting a sequence in intron 8 of the GAPDH gene, using the iDeal ChIP-seq kit for transcription factors. A titration consisting of 1, 2, 5 and 10 µl of the Diagenode antibody against Cas9 (cat. No. C15310258) was tested. IgG (2 µg/IP) was used as negative IP control. qPCR was performed with primers specific for the targeted sequence in the GAPDH gene, and for the MYOD1 gene, 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).

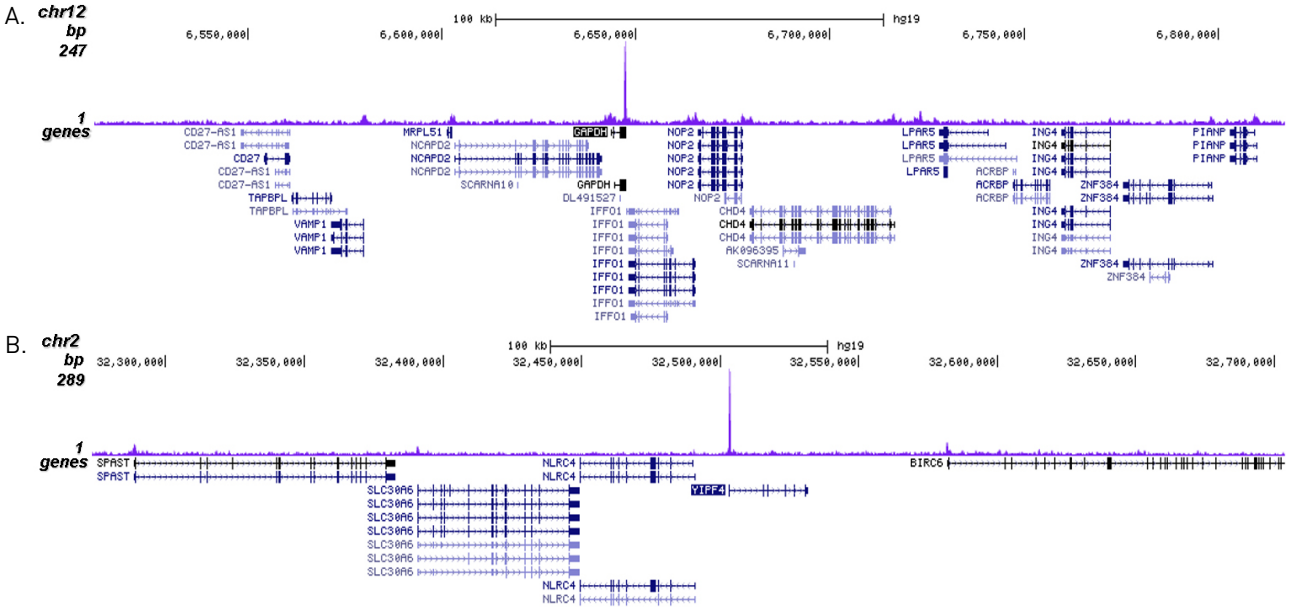

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against Cas9

ChIP was performed on sheared chromatin from 4 million HEK293T cells stably expressing dCas9 and a GAPDH sgRNA cells using 2 µl of the Diagenode antibody against Cas9 (cat. No. C15310258) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the ChIP-seq profile in a region of chromosome 12 surrounding the GAPDH gene (fig 2B) and in a region of chromosome 2 surrounding an off-target peak in the YIPF4 gene.

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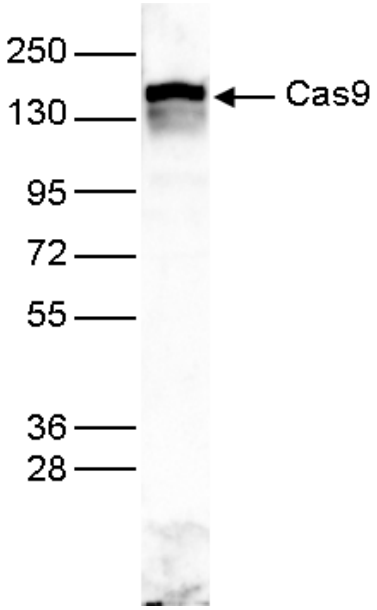

Figure 3. Western blot analysis using the Diagenode antibody directed against Cas9

Western blot was performed on protein extracts from HEK293 cells transfected with dCas9 using the Diagenode antibody against CRISPR/Cas9 (cat. No. C15310258). The antibody was diluted 1:5,000. The marker is shown on the left, the position of the Cas9 protein is indicated on the right.

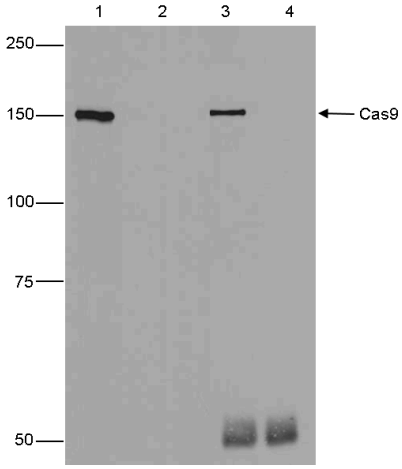

Figure 4. IP using the Diagenode antibody directed against Cas9

IP was performed on whole cell extracts (500 µg) from HeLa 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 Cas9 (cat. No. C15310258). The immunoprecipitated proteins were subsequently analysed by Western blot. Lane 3 and 4 show the result of the IP, the input (25 µg) is shown in lane 1 and 2.

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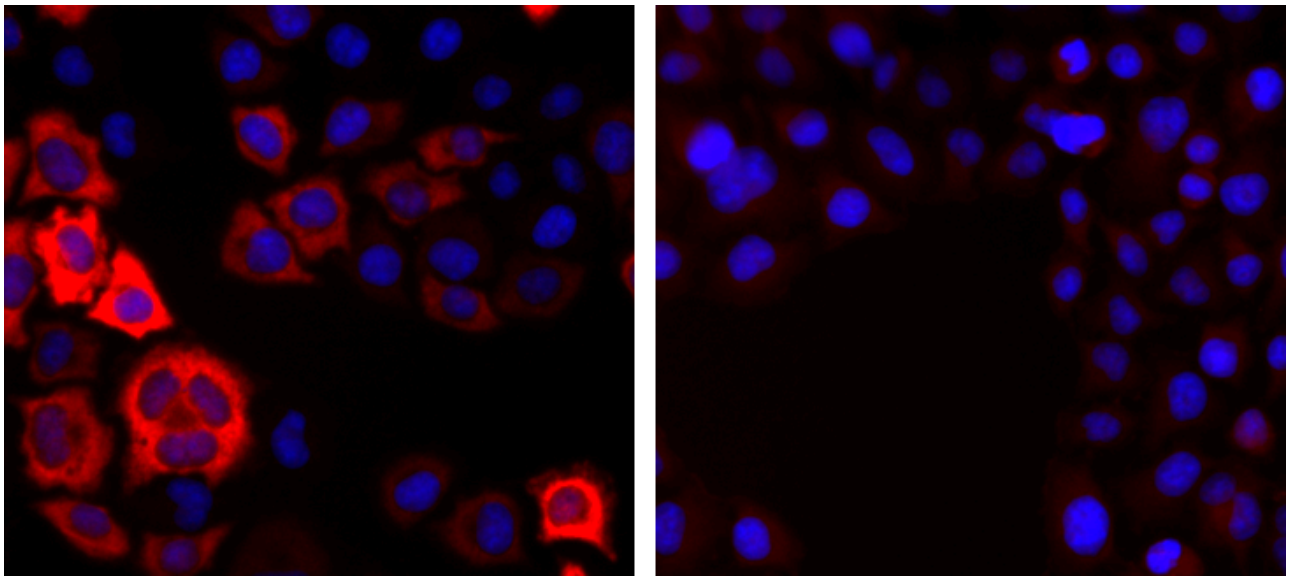

Figure 5. Immunofluorescence using the Diagenode antibody directed against Cas9
HeLa cells expressing Cas9 under the control of the tight TRE promoter were fixed in methanol at -20°C, permeabilized with acetone at -20°C and blocked with PBS containing 2% BSA. The cells were stained with the Cas9 antibody (cat. No. C15310258) diluted 1:1000, followed by incubation with a goat anti-rabbit secondary antibody coupled to AF594. Nuclei were counter-stained with Hoechst 33342. Figure 5 shows the result in the presence (left) or absence (right) of doxycycline.

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