diagenode
A Hologic Company

TECHNICAL DATASHEET

CRISPR/Cas9 C-terminal monoclonal antibody

Cat. No. C15200223-100

Type: Monoclonal	Specificity: Streptococcus pyogenes
Size: 100 µg	Isotype: IgG1
Concentration: 2.2 µg/µl	Source: Mouse
Lot No.: 001	Purity: Protein G purified monoclonal antibody.
Storage buffer: PBS containing 0.05 % Na-azide.	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: August 5, 2015

Description

Alternative name: **Csn1**

Monoclonal antibody raised in mouse against the C-terminus of Cas9 nuclease (CRISPR-associated protein 9)

Applications

Applications	Suggested dilution	References
Western Blotting	1:1,000 - 1:5,000	Fig 1,2
IP	13 µg/IP	Fig 3
IF	1:200	Fig 4

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.

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Validation data

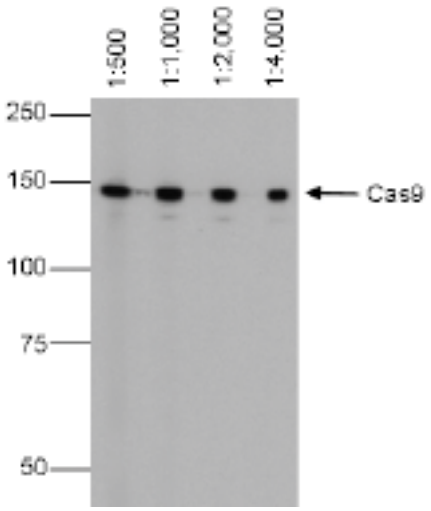

Figure 1. Western blot analysis using the Diagenode monoclonal antibody directed against CRISPR/Cas9
Western blot was performed on protein extracts from HeLa cells transfected with Cas9 using the Diagenode antibody against CRISPR/Cas9 (cat. No. C15200223). The antibody was used at different dilutions. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

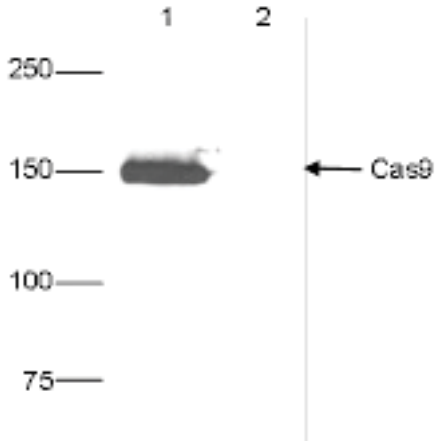

Figure 2. Western blot analysis using the Diagenode monoclonal antibody directed against CRISPR/Cas9
Western blot was performed on 20 µg protein extracts from Cas9 expressing HeLa cells (lane 1) and on negative control HeLa cells (lane 2) with the Diagenode antibody against Cas9 (cat. No. C15200223). The antibody was diluted 1:4,000. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

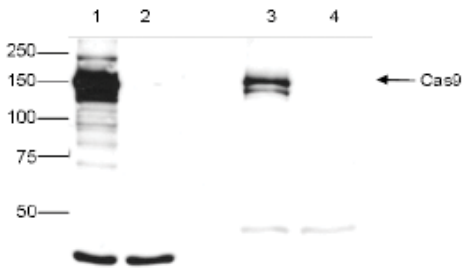

Figure 3. IP using the Diagenode monoclonal antibody directed against Cas9
IP was performed on whole cell extracts (250 & micro;g) from HeLa cells transfected with a Cas9 expression vector (lane 1 and 3), or untransfected cells (lane 2 and 4) using 13 µg of the Diagenode antibody against Cas9 (cat. No. C15200223). The immunoprecipitated proteins were subsequently analysed by Western blot. Lane 3 and 4 show the result of the IP, the input (15 µg) is shown in lane 1 and 2.

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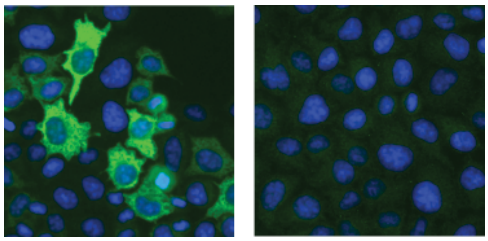

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against CRISPR/Cas9

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 C-terminal antibody (cat. No. C15200223) diluted 1:200, followed by incubation with a donkey anti-mouse secondary antibody coupled to AF488. Nuclei were counter-stained with Hoechst 33342. Figure 4 shows the result in the presence (left) or absence (right) of doxycycline.

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