TECHNICAL DATASHEET

LOT: 006

HOLOGIC® Diagenode

CRISPR/Cas9

Cat. No. C15200203

Lot:	006
Size:	50 µg
Type:	Monoclonal
Isotype:	IgG1 kappa
Source:	Mouse
Concentration:	2 µg/µl

Specificity:	Streptococcus pyogenes
Purity:	Protein A purified monoclonal antibody
Storage buffer:	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).

Applications

Applications	Suggested dilution	References
Western blotting	1:1,000 – 1:6,000	Fig 1, 2
Immunoprecipitation	1:200	Fig 3
Immunofluorescence	1:100 – 1:500	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 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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Results

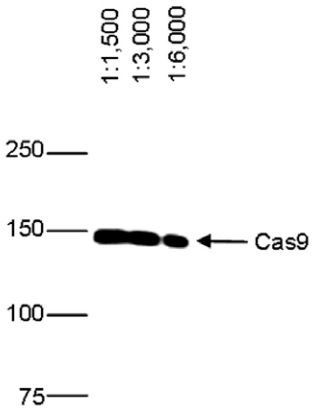

Figure 1: Western blot analysis using the Hologic Diagenode monoclonal antibody directed against Cas9

Western blot was performed on protein extracts from HeLa cells transfected with a flag-tagged Cas9 using the Hologic Diagenode antibody against Cas9 (cat. No. C15200203). The antibody was used at different dilutions. The marker is shown on the left, position of the flag-tagged Cas9 protein is indicated on the right.

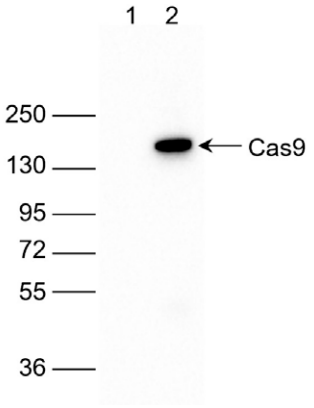

Figure 2: Western blot analysis using the Hologic Diagenode monoclonal antibody directed against Cas9

Western blot was performed on protein extracts from HeLa cells (lane 1) and on 293T cells transfected with dCas9 protein (lane 2) using the Hologic Diagenode antibody against Cas9 (cat. No. C15200203). The antibody was diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right, the marker (in kDa) is shown on the left.

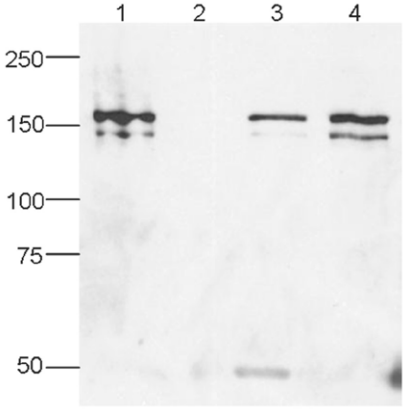

Figure 3: IP using the Hologic Diagenode monoclonal antibody directed against Cas9

IP was performed on whole cell extracts (100 µg) from HEK293 cells transfected with a Flag-tagged Cas9 using the Hologic Diagenode antibody against Cas9 (cat. No. C15200203). The immunoprecipitated proteins were subsequently analysed by Western blot with the antibody. Lane 3 and 4 show the result of the IP; a negative IP control (IP on untransfected cells) and the input (15 µg) are shown in lane 2 and 1, respectively

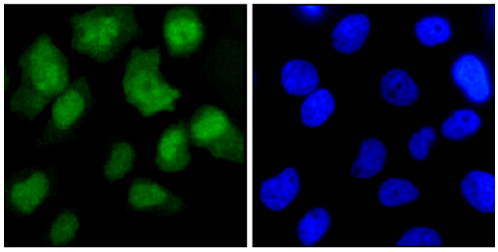

Figure 4: Immunofluorescence using the Hologic Diagenode monoclonal antibody directed against Cas9

Hela cells were transiently transfected with a Flag-tagged Cas9 expression vector. 48 hours post transfection the cells were fixed in 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 Cas9 antibody at 4°C o/n, followed by incubation with an anti mouse secondary antibody coupled to AF488 for 1 h at RT (left). Nuclei were counter-stained with DAPI (right).