TECHNICAL DATASHEET

LOT: 004

HOLOGIC® Diagenode

CRISPR/Cas9 antibody 4G10

Cat. No. C15200216

Lot:	004
Size:	10 µg / 50 µg / 100 µg
Type:	Monoclonal
Source:	Mouse
Concentration:	1.4 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) using a recombinant protein.

Applications

Applications	Suggested dilution	References
Western blotting	1:1,000–1:5,000	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. 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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Results

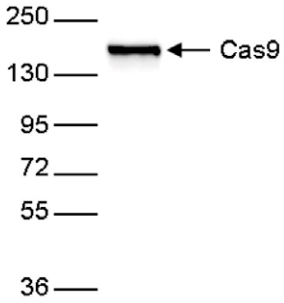

Figure 1: Western blot analysis using the monoclonal antibody directed against CRISPR/Cas9

Western blot was performed on protein extracts from HEK293T cells transfected with Cas9 using the antibody against CRISPR/Cas9 (cat. No. C15200216), diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

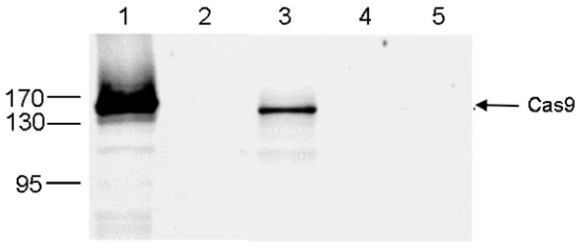

Figure 2: IP using the monoclonal antibody directed against CRISPR/Cas9

IP was performed on whole cell extracts from HEK293T cells transfected with a Cas9 expression vector (lane 1, 3 and 5), or untransfected cells (lane 2 and 4) using 5 µg of the antibody against CRISPR/Cas9 (cat. No. C15200216, lane 3 and 4) or with an equal amount of IgG, used as a negative control (lane 5). The immunoprecipitated proteins were subsequently analysed by Western blot with the polyclonal Cas9 antibody (Cat. No. C15310258, diluted 1:5,000). Lane 1 and 2 show the result of the input.

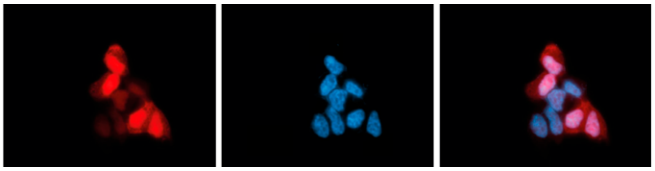

Figure 3: Immunofluorescence using the monoclonal antibody directed against CRISPR/Cas9

HEK293T cells were transiently transfected with a Cas9 expression vector. The cells were fixed with 4% formaldehyde, permeabilized in 0,1% Triton X-100 and blocked in PBS containing 5% BSA. The cells were stained with the Cas9 antibody diluted 1;400 at 4°C o/n, followed by incubation with an anti mouse secondary antibody coupled to AF596 for 1 h at RT (left). Nuclei were counter-stained with DAPI (middle). A merge of the two stainings is shown on the right.