diagenode
Innovating Epigenetic Solutions

TECHNICAL DATASHEET

CRISPR/Cas9 C-terminal monoclonal antibody

Cat. No. C15200231
Type: Monoclonal
Isotype: IgG1, κ
Source: Mouse
Lot #: 001
Size: 50 µg/23 µl
Concentration: 2.2 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 C-terminus of Cas9 nuclease (CRISPR-associated protein 9).

Applications

	Suggested dilution	Results
ChIP*	5 µg/ChIP	Fig 1
Western blotting	1:6,000	Fig 2
IF	1:100	Fig 3

* 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

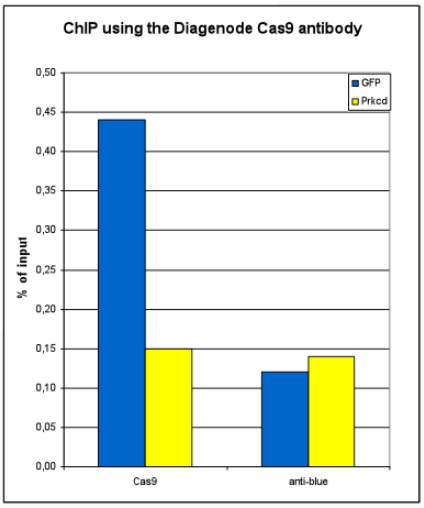

Figure 1. ChIP using the Diagenode monoclonal antibody directed against Cas9

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 µg of either the Diagenode antibody against Cas9 (cat. No. C15200231) or the anti-blue antibody (C15200213), 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).

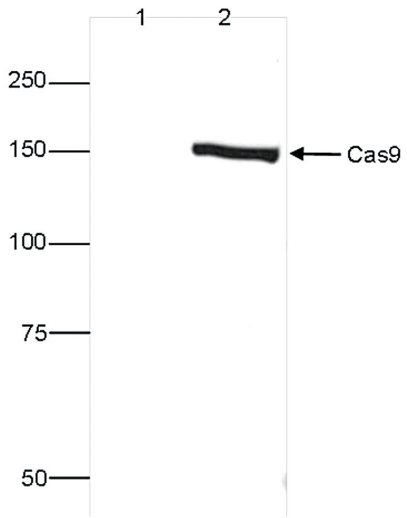

Figure 2. Western blot analysis using the Diagenode monoclonal antibody directed against CRISPR/Cas9

Western blot was performed on protein extracts from HeLa cells transfected with Cas9 (lane 2) or on untransfected control cells (lane 1) using the Diagenode antibody against CRISPR/Cas9 (cat. No. C15200231). The antibody was diluted 1:6,000 in TBS-T containing 3% NFDM. The marker is shown on the left, position of the Cas9 protein is indicated on the right.

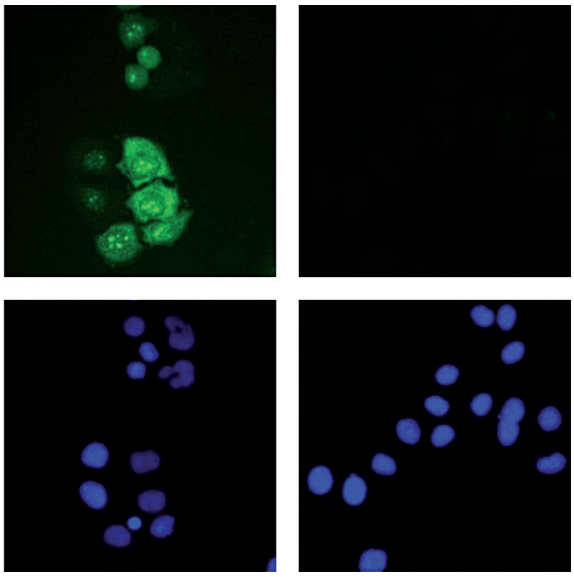

Figure 3. Immunofluorescence using the Diagenode monoclonal antibody directed against CRISPR/Cas9

HeLa cells expressing Cas9 under the control of the tight TRE promoter and untransfected control cells 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. C15200231) diluted 1:100, followed by incubation with a donkey anti-mouse secondary antibody coupled to AF488 (upper panel). Nuclei were counter-stained with Hoechst 33342 (lower panel)

Diagenode sa. BELGIUM | **EUROPE**

LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | **NORTH AMERICA**

400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

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