diagenode
A Hologic Company

TECHNICAL DATASHEET

L. bacterium CRISPR/Cpf1 polyclonal antibody

Cat. No. C15310263

Type: Polyclonal

Source: Rabbit

Lot #: A2577-001

Size: 100 µl

Concentration: Not determined

Specificity: *Lachnospiraceae bacterium*

Purity: Whole antiserum from rabbit containing 0.05% 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: Polyclonal antibody raised in rabbit against Lachnospiraceae bacterium (Lb) Cpf1 (CRISPR from Prevotella and Francisella 1) using a recombinant protein.

Applications

	Suggested dilution	Results
Western blotting	1:5,000	Fig 1, 2
IP	2 µl/IP	Fig 3
IF	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. The CRISPR/Cas9 (CRISPR-associated protein 9nuclease) system from S. pyogenes was the first to be 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. Recently, a so-called type V CRISPR system has been identified in several bacteria which contains the Cpf1 (CRISPR from Prevotella and Francisella 1) protein. In contrast to Cas9 systems, CRISPR/Cpf1 systems do not require an additional trans-activating crRNA (tracrRNA), they cleave target DNA proceeded by a short T-rich protospacer-adjacent motif (PAM), in contrast to the G-rich PAM following the target DNA for Cas9, and they introduce a staggered DNA doublestranded break with a 4 or 5-nt 5' overhang. Two of these CRISPR/Cpf1 systems, present in Acidaminococcus sp. and Lachnospiraceae bacterium have been identified as potential candidates for genome editing in mammalian cells.

Results

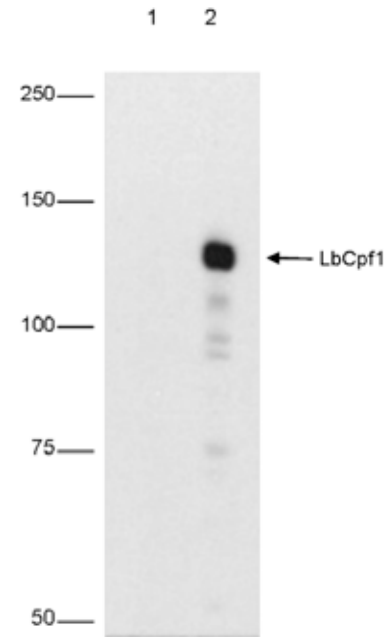

Figure 1. Western blot analysis using the Diagenode antibody directed against LbCRISPR/Cpf1

Western blot was performed on protein extracts from HEK293 cells transfected with LbCRISPR/Cpf1 using the Diagenode antibody against LbCRISPR/Cpf1 (Cat. No. C15310263), diluted 1:5,000 in PBS-T containing 3% NFDM. The marker is shown on the left, the position of the Cpf1 protein is indicated on the right. Lane 1 shows the Western blot analysis with the pre-immune serum, used as a negative control.

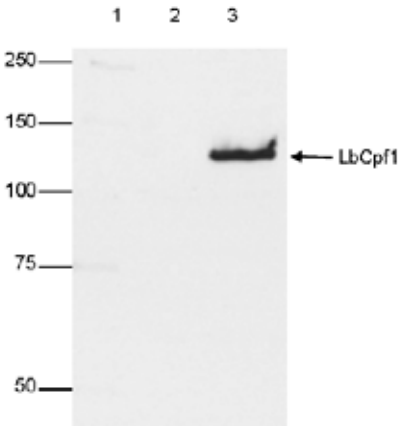

Figure 2. Western blot analysis using the Diagenode antibody directed against LbCRISPR/Cpf1

Western blot was performed on protein extracts from HEK293 cells (lane 1), HEK293 cells transfected with AsCRISPR/Cpf1 (lane 2) and HEK293 cells transfected with LbCRISPR/Cpf1 (lane 3) using the Diagenode antibody against LbCRISPR/Cpf1 (Cat. No. C15310263), diluted 1:5,000 in PBS-T containing 3% NFDM. The marker is shown on the left, the position of the Cpf1 protein is indicated on the right.

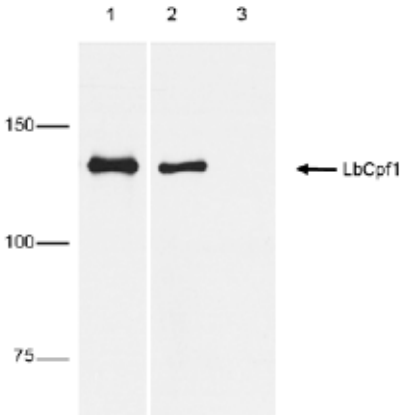

Figure 3. IP using the Diagenode antibody directed against LbCRISPR/Cpf1

IP was performed on whole cell extracts (350 µg) from HEK293 cells transfected with HA-tagged LbCpf1 using 2 µl of the Diagenode antibody against LbCRISPR/Cpf1 (Cat. No. C15310263). The immunoprecipitated proteins were subsequently analysed by Western blot with an anti-HA antibody. Lane 2 and 3 show the result of the IP with the LbCRISPR/Cpf1 antibody and with beads only, respectively. The input (17.5 µg) is shown in lane 1.

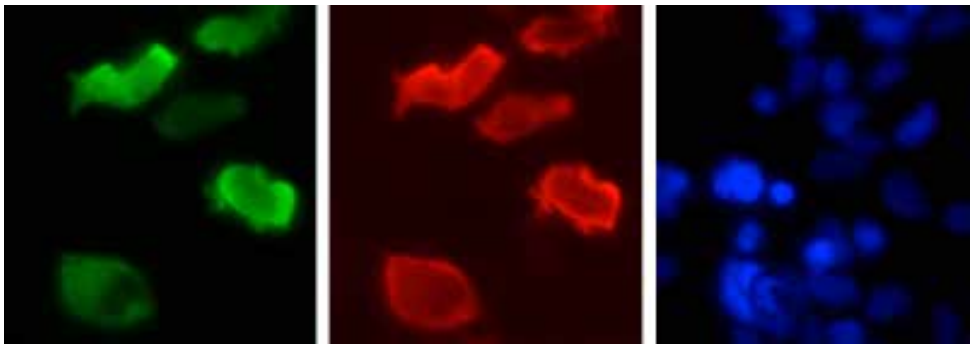

Figure 4. Immunofluorescence using the Diagenode antibody directed against LbCRISPR/Cpf1

Transiently transfected HEK293 cells expressing HA-tagged LbCRISPR/Cpf1 were fixed with 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 LbCRISPR/Cpf1 antibody (Cat. No. C15310263) diluted 1:500 in blocking solution at 4°C o/n followed by incubation with an anti-rabbit secondary antibody coupled to DyLight594 for 1 h at RT (left figure). Nuclei were counter-stained with Hoechst 33342 (right). The middle figure shows IF with an anti-HA antibody.

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