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DATASHEET

GenCRISPR™ LbCas12a Nuclease

Cat. No.: Z03753

Product Introduction

GenCRISPR™ LbCas12a Nuclease is an RNA-guided DNA endonuclease from *Lachnospiraceae bacterium*. Cas12a (previously known as Cpf1) belongs to the Class 2 Type V CRISPR/Cas system. Different from CRISPR/Cas9 system, the ribonucleoprotein (RNP) complex of CRISPR/Cas12a system is formed by Cas12a and crRNA, it recognizes a T-rich protospacer adjacent motif (PAM) and results in a staggered DNA double-strand break (DSB). After the specific cleavage, Cas12a can also activate collateral cleavage activity towards adjacent non-specific ssDNA sequences. Hence, Cas12a nuclease is a good alternative for Cas9 in certain target DNA editing, and provides a novel strategy for DNA detection.

Source: Recombinant Cas12a with a C-terminal NLS expressed by *E.coli*
Species: *Lachnospiraceae bacterium*
Accession#: 6KL9_A
Tag: C-terminal 6× His Tag
Apparent Molecular Weight: 146 kDa, on SDS-PAGE under non-reducing conditions
Concentration: 4 mg/ml

Storage Buffer: 10 mM Tris-HCl, 300 mM NaCl, 0.5 mM DTT, 50% glycerol, pH 7.4.
Storage& Stability: Store at -20 °C for up to 12 months from the date of manufacture. Avoid repeated freeze-thaw cycles. **Do not store below -20 °C!**
Application: crRNA-dependent DNA cleavage

Quality Control Specifications

Assay	Specifications
Appearance	Clear, colorless liquid
Purity by SDS-PAGE	≥ 90%
Concentration by A280	4(± 10%) mg/ml
Bioactivity (*in vitro*)	≥ 90%
Residual DNase	Undetectable
Residual RNase	Undetectable
Endotoxin Level	< 0.1 EU/μg

Reagents Supplied

Components	Size			Storage
GenCRISPR™ LbCas12a Nuclease	100 µg	500 µg	1 mg	-20 °C
10 × Cas12a Reaction Buffer*	1.5 ml	1.5 ml	1.5 ml	-20 °C

* The reaction buffer is intended for *In vitro* digestion assays described in this manual only, and not intended for other applications.

Protocols for *In vitro* digestion of DNA

1. Reagents preparations

1) Synthesize specific crRNA; we recommend GenScript's CRISPR Synthetic Guide RNA Services (**see www.genscript.com**) for crRNA synthesis.
2) Synthesize specific substrate DNA for cleavage; we recommend custom DNA synthesis using GenScript's DNA Synthesis Service (**see www.genscript.com**), or customers can also apply PCR method to obtain substrate DNA (**Note:** the PCR product needs to be purified prior to further assays).
3) Refer to the following procedure to anneal crRNA before preparing the crRNA working solution.

95 °C	5 min	-
94 °C	10s	Decrease 1 °C per cycle, and 69 cycles in total
25 °C		
4 °C	Hold	-

4) Prepare the crRNA working solution at an appropriate concentration (e.g., 5 ng/µl) by diluting the stock solution with DEPC-Treated Water (nuclease-free) on ice.
5) Prepare the substrate DNA working solution at an appropriate concentration (e.g., 30 ng/µl) by diluting the stock solution with DEPC-Treated Water (nuclease-free) on ice.
6) The reaction volume is recommended to be set at 20 µl, and the reaction conditions should be optimized according to your specific applications.

2. Assay procedures

1) Assemble the reaction in a nuclease-free PCR strip tube at room temperature on a clean bench, mix the following reagents thoroughly, and incubate for 10 min at 25 °C.

Components	Volume
10 × Cas12a Reaction Buffer	2 µl
10 ng crRNA	2 µl (5 ng/µl)
100 ng GenCRISPR™ LbCas12a Nuclease (Cat. No. Z03753)	2 µl (50 ng/µl)
DEPC-Treated Water (nuclease-free)	12 µl

2) Add substrate DNA into the mixture and mix again thoroughly, then incubate for 30 min at 37 °C;

Components	Volume
60 ng Substrate DNA	2 µl (30 ng/µl)
Total reaction volume	20 µl

3) Add 1 µl Proteinase K (20 µg/µl), incubate for 30 min at 55 °C. After incubation, determine the digestion efficiency by agarose gel electrophoresis.

Product Validation

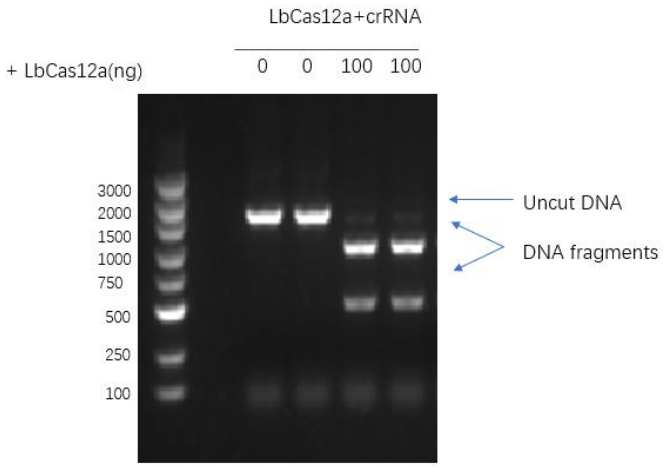

Figure 1: *In vitro* digestion efficiency analysis by agarose gel electrophoresis. A 20 µl reaction in 1 × Cas12a Nuclease Reaction Buffer containing 60 ng linearized plasmid, 10 ng crRNA, and 100 ng GenCRISPR™ LbCas12a Nuclease (Cat. No. Z03753) for 30 min at 37°C results in a digestion efficiency of linearized plasmid higher than 90%, as determined by agarose gel electrophoresis.

References

1. Zetsche, Bernd, *et al.* "Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system." *Cell* 163.3 (2015): 759-771.
2. Ledford, Heidi. "Alternative CRISPR system could improve genome editing." *Nature News* 526.7571 (2015): 17.
3. Chen, Janice S., *et al.* "CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity." *Science* 360.6387 (2018): 436-439.

For laboratory research use only. Direct human use, including taking orally and injection and clinical use are forbidden.