

LbaCas12a Nuclease

Cat. No.: E373-YH01

01/ Product Description

The CRISPR-associated protein Cas9 has been widely applied in genome editing. Compared with Cas9, Cas12a requires no tracrRNA and is guided solely by a single crRNA of 41 – 44 nucleotides. The Cas12a-crRNA complex recognizes the T-rich PAM sequence (5'-TTTN-3') and cleaves target double-stranded DNA. Different from Cas9, Cas12a induces double-strand breaks that generate 4 – 5 nt sticky-end overhangs after cleavage.

This Cas12a protein is derived from *Lachnospiraceae bacterium*. Nuclear localization signals (NLS) are fused at both terminals of the protein, enabling efficient nuclear translocation of Cas12a RNPs after cellular delivery. In addition, Cas12a exhibits unique trans-cleavage collateral activity. Once Cas12a forms a ternary complex with crRNA and target dsDNA and completes specific target cleavage, its trans-cleavage activity is activated to non-specifically degrade arbitrary single-stranded DNA (ssDNA) in the reaction system. This characteristic enables Cas12a to serve as a versatile tool for gene detection and molecular imaging applications.

02/ Product Features

1. Adopts a pure protein reaction system, effectively eliminating interference caused by plasmid or viral vectors during CRISPR gene knockout experiments.
2. Compared with plasmid and RNA delivery modes, Cas12a protein exhibits a shorter intracellular half-life, significantly reducing genome off-target effects.

03/ Applications

1. Applied for gene knockout and knock-in research;
2. Suitable for the development of detection technologies for various pathogenic nucleic acids;
3. Applicable to the development of high-sensitivity detection technologies for protein, small molecule and other biomarkers.

04/ Preservation system

500 mM NaCl, 20 mM sodium acetate, 0.1 mM EDTA, 0.1 mM TCEP, 50% Glycerol, pH 6.0.

05/ Package Contents

Components	E373-YH01-01A (Size: 50 µg)	E373-YH01-01B (Size: 500 µg)
LbaCas12a Nuclease (2.5 µg/µl)	20 µl	200 µl
10×Cas12a-dsDNA Buffer I	500 µl	1 ml×5
10×Cas12a-ssDNA Buffer II	500 µl	1 ml×5

Note: LbaCas12a Nuclease (150 ng = 1 pmol)

06/ Quality Control

After multiple column purifications, only a clear and single target band was visible during SDS-PAGE gel detection, indicating a purity of 90%. qPCR detection showed low levels of E. coli genome residue and no RNase, endonuclease, or exonuclease contamination.

07/ Storage Conditions

-20 °C ± 5 °C.

08/ Operating Instructions

1. In vitro cleavage of target dsDNA (20 µl):

10× Cas12a-dsDNA Buffer I	2 µl
Target DNA (200 nM)	1 µl
crRNA (15 µM)	1 µl
LbaCas12a Nuclease	2-8 pmol
RNase Free Water	To 20 µl

Incubate at 37 °C for 1 h, then react at 70 °C for 10 min.

Note: RNase inhibitor can be added to the reaction system up to 1 U/µl to prevent crRNA degradation.

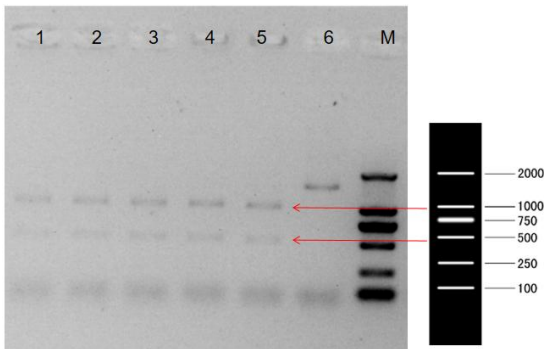
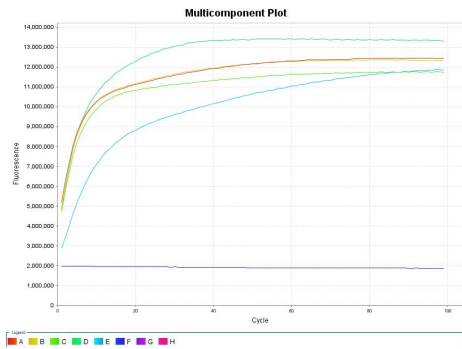
2. In vitro cleavage of probe ssDNA (20 µl):

10× Cas12a-ssDNA Buffer II	2 µl
Target DNA (35 nM)	2 µl
crRNA (350 nM)	2 µl
LbaCas12a Nuclease	1-4 pmol
Probe ssDNA (10 µM)	0.5 µl
RNase Free Water	To 20 µl

Collect fluorescence every 30-60 s at 37 °C for 80-99 cycles.

Note: A molar ratio of Cas12a : crRNA : DNA of 15:10:1 is recommended for optimal cleavage efficiency.

09/ Application examples

In vitro cleavage of target dsDNA	In vitro ssDNA cleavage probe
A 1500 bp DNA fragment containing the target site was used as the target. Experimental results showed that LbaCas12a, guided by crRNA, specifically recognized and cleaved the 1500 bp target sequence, resulting in double-strand breaks in the target DNA and yielding two products with fragment sizes of 1000 bp and 500 bp, respectively.	An ssDNA reporter probe was added to the cleavage system. Experimental results showed that when LbaCas12a formed a ternary complex with the specific target dsDNA under the guidance of crRNA, it was activated to perform highly efficient trans-cleavage activity against ssDNA, cleaving the ssDNA reporter probe in the system and thus emitting a fluorescent signal.
	
(Legend) dsDNA cleavage activity of LbaCas12a Nuclease. M-DL2000; 1-LbaCas12a 1µg; 2-LbaCas12a 900ng; 3-LbaCas12a 600ng; 4-LbaCas12a 300ng; 5-LbaCas12a 100ng; 6-Target dsDNA without LbaCas12a	(Legend) ssDNA cleavage activity of LbaCas12a Nuclease. A - LbaCas12a 1.2 µg/20 µl; B - LbaCas12a 900 ng/20 µl; C - LbaCas12a 600 ng/20 µl; D - LbaCas12a 300 ng/20 µl; E - LbaCas12a 100 ng/20 µl; F - Target DNA without LbaCas12a

10/ Related Products

Cat. No	Product Name	Cat. No	Product Name
EB373-YH	10×Cas12a-ssDNA Buffer II	E381	LwaCas13a Nuclease
E365	NLS-Cas9 Nuclease	E368***	Cas9(D10A,H840A) Nuclease
E376*	NLS-Cas9(D10A)Nickase	E369	sgRNA <i>In Vitro</i> one-step Transcription Kit
E377**	NLS-Cas9(H840A)Nickase	E370	sgRNA Screening and Genotype Confirmation

Note: * indicates Cas9(D10A)Nickase, a mutation in one of the cleavage active sites of Cas9 Nuclease, which creates a cleavage on the DNA strand containing the sgRNA target sequence, resulting in a functional single-strand break.

** indicates Cas9(H840A)Nickase, a mutation in one of the cleavage active sites of Cas9 Nuclease, which creates a cleavage on the DNA strand complementary to the sgRNA target sequence, resulting in a functional single-strand break.

*** indicates Cas9(D10A,H840A) Nuclease, which has target DNA binding activity but no cleavage activity, and can be used for negative controls, etc.