LbuCas13a Nuclease Instruction Manual

Product Number	LbuCas13a Nuclease	Product Number	EDE0002
Molecular Weight	141.6 KDa	Form	Liquid

I. Product Description

LbuCas13a nuclease (also known as C2c2) is derived from *Leptotrichia buccalis* bacterium strain. LbuCas13a belongs to class II type VI CRISPR effector proteins, which are crRNA-mediated nuclease that activate accessory cleavage activity upon recognition and cleavage of target RNA, and can non-specifically cleave single-stranded RNA (ssRNAs) in the system. By designing single-stranded RNA labeled with fluorescent moieties or other small molecules at both ends, the detection and signal amplification of the RNA template by CRISPR/Cas13a can be realized. The results can be observed by fluorometer and test strips.

II. Product Information

· Product Components

Component	EDE0002-100	EDE0002-500	EDE0002-1000
LbuCas13a Nuclease	5 μM*20 μL (100pmol)	5 μM*100 μL (500 pmol)	5 μM*200 μL (1000 pmol)
LbuCas13a Cleavage Buffer (5×)	500 μL*1 tube	500 μL*4 tube	1mL*4 tube

· Storage Conditions and Shelf Life

The product is stable for 1 year when stored at -20°C. For long-term storage, it is recommended to store at -80°C. It is advised to aliquot the product based on the frequency of use to avoid repeated freeze-thaw cycles.

· Product Features

The product is prepared using a one-step purification process, retaining maximum enzymatic activity. It has been tested to show significantly higher activity compared to similar products.

· Activity Definition

In a 25 μL reaction system at 37°C, the amount of Cas13a enzyme required to cleave 1 pmol of ssRNA probe within 1 minute defines 1 transU. For example, if a batch of LbuCas13a enzyme exhibits trans-cleavage activity of 9 transU/pmol, it indicates that 1 pmol of this batch of LbuCas13a enzyme can cleave 9 pmol of ssRNA probe under the specified reaction conditions within 1 minute.

· Quality Assurance

Sample Purity: ~95% (verified by SDS-PAGE).

III. Detection Steps

Required Reagents:

1. ssRNA reporter probe (labeled with FAM at the 5' end and BHQ1 at the 3' end).

Note: You can use the ssRNA reporter from our company or design your own.

Product Name	Product Number
ssRNA reporter（RNA probe）	EDR0001

2. crRNA/gRNA: Forms a functional complex with Cas13a, which is specifically activated by the target sequence.

Note: Our company offers crRNA synthesis or you can custom-design crRNA.

Name	Specification	Product Number
crRNA Biosynthesis	2OD	EDR0002
crRNA Chemical Synthesis	2OD	EDR0003

For free design consultation, please contact: info@editxor.com

3. RNase Inhibitor (optional): Inhibit RNase and prevent RNA degradation.
4. Isothermal Amplification Reaction Kit.

Reaction System

Component	Final Concentration	Volume (μL)
5 μM LbuCas13a	50 nM	0.25
5 × Cleavage Buffer	1×	5
25 U RNase Inhibitor	1 U	1
500 nM crRNA	50 nM	2.5
4 μM ssRNA Reporter	400 nM	2.5
1 μM RNA target	50 nM	1.25
DEPC H_2O		
Total	25 μL	25 μL

Reaction Conditions:

Use a real-time fluorescence quantitative PCR machine or an isothermal amplification instrument to detect the fluorescence signal. The reaction should occur at 37°C, and the fluorescence signal should be collected every 30 seconds. Alternatively, the fluorescence signal can be directly observed under a UV light.

Comparison of Enzyme Activity

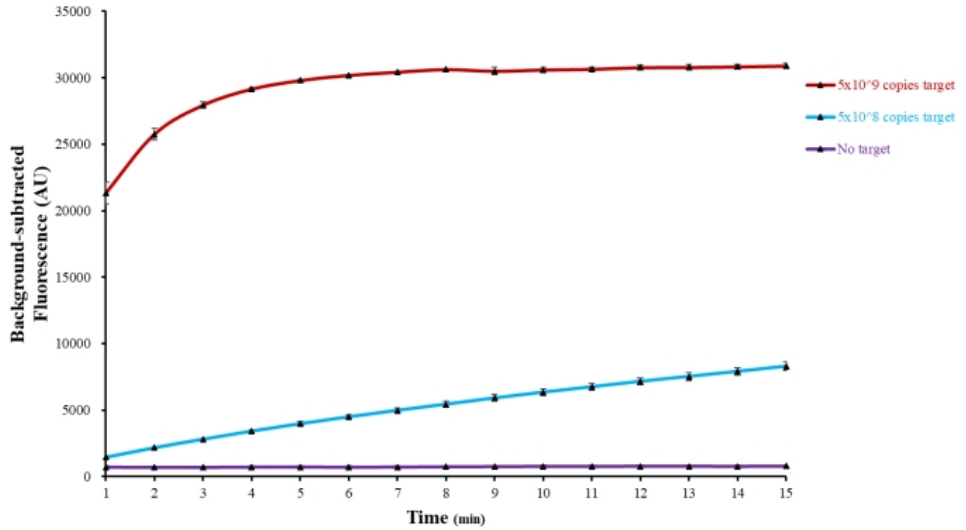

Fig 1. Results of Cas13 collateral cleavage at different concentration.

The horizontal coordinate refers to reaction time, while the vertical coordinate refers to detection result of PCR. As can be seen from the figure, 5×10^{9} copies of target RNA can efficiently activate LbuCas13a enzyme, which can completely cleave the RNA reporter probe and reach the fluorescence peak in 10 minutes.

Precautions

1. To prevent contamination by RNase, please keep the experimental area clean and tidy. Wear clean gloves and masks during operations. All consumables, such as pipette tips and centrifuge tubes, should be RNase-free.
2. Cas13a enzymes are prone to inactivation; store the enzyme at -20°C immediately after use.

Publishing Requirements

When using this product in publications, please acknowledge our company: Guangzhou Editgene Co. Ltd, China. Or EDITGENE CO.LTD if used within U.S. or Europe territory.