EZassay

LbaCas12a(Cpf1) Protein (Enhanced v2) Instructions

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Catalog Code: CAS-12E-000
CAS-12E-001
CAS-12E-010
CAS-12E-100

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Product Information	
Product name	LbaCas12a(Cpf1) Protein (Enhanced v2)
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid
Molecular weight	144 kDa

Product Introduction

Cas12a forms a functional complex with crRNA, "gliding" over the amplification product of the DNA, and upon successful pairing, Cas12a is specifically activated, trans-cleaving the surrounding reporter molecule. This feature can be used in the field of molecular diagnostics to detect pathogens. LbaCas12a enhanced is modified from the wild type LbaCas12a (Cas12a protein is derived from Lachnospiraceae). Compared with wild type, the enhanced LbaCas12a showed an elevated activity.

Storage

-20°C. Aliquot after receiving. Avoid repeated freeze-thaw.

Materials supplied

Cat:	CAS-12E-000	CAS-12E-001	CAS-12E-010	CAS-12E-100
LbaCas12a(Cpf1) Protein (Enhanced v2)*	10μM*10μL (100pmol)	10μM*20μL (200pmol)	10μM* 200μL (2,000pmol)	10μM *1000μl (10,000pmol)
Cas12a Reaction Buffer(10X)	1 ml * 1	1 ml * 1	1 ml * 4	10ml*2
Diluent Buffer (Cas12a) (1X) **	1 ml * 1	1 ml * 1	1 ml * 2	10 ml * 1

* LbaCas12a(Cpf1) Protein (Enhanced v2) is supplied in a buffer containing 50% glycerol.
** Diluent Buffer (Cas12a)(1X) is a protein dilution buffer containing 50% glycerol.
A glycerol-free version can be customized for lyophilization applications upon request.

Other materials required

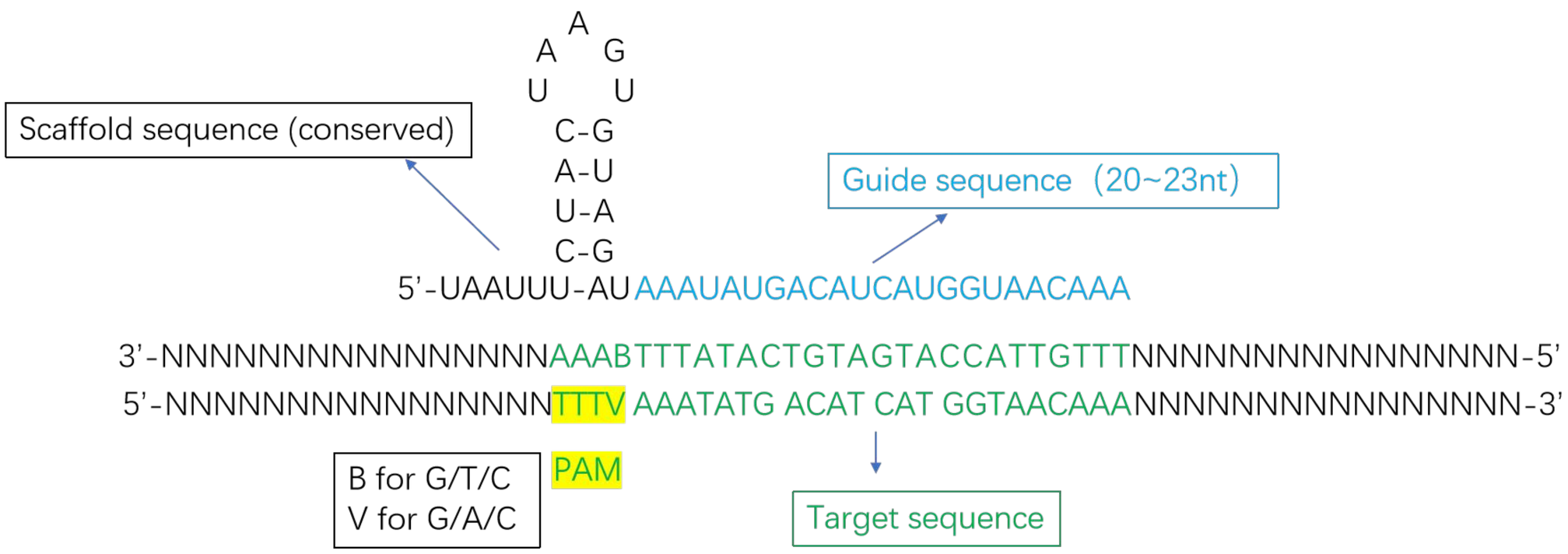
1. Reporter： substrate gives signals when digested by Cas12a. Self-design, for example FAM-BHQ labelled ssDNA. Optional select EZassayTM biotech. products (Reporter DNA FAM label (for Cas12a) Cat.#. DNA-FAM-BHQ.)

Cat:	Item
DNA-FAM-BHQ	Reporter DNA-FAM label
DNA-FAM-BIO	Reporter DNA-FAM-Biotin label

2. crRNA/gRNA：
LbaCas12a crRNA scaffold sequence:
5’ -UAAUUUCUACUAAGUGUAGAU-3’

Any question about design, contact info@ezassay.com

LbaCas12a crRNA



Assay procedure

1、Amplification of target DNA. (reverse transcription firstly for RNA target) (Optimal step)

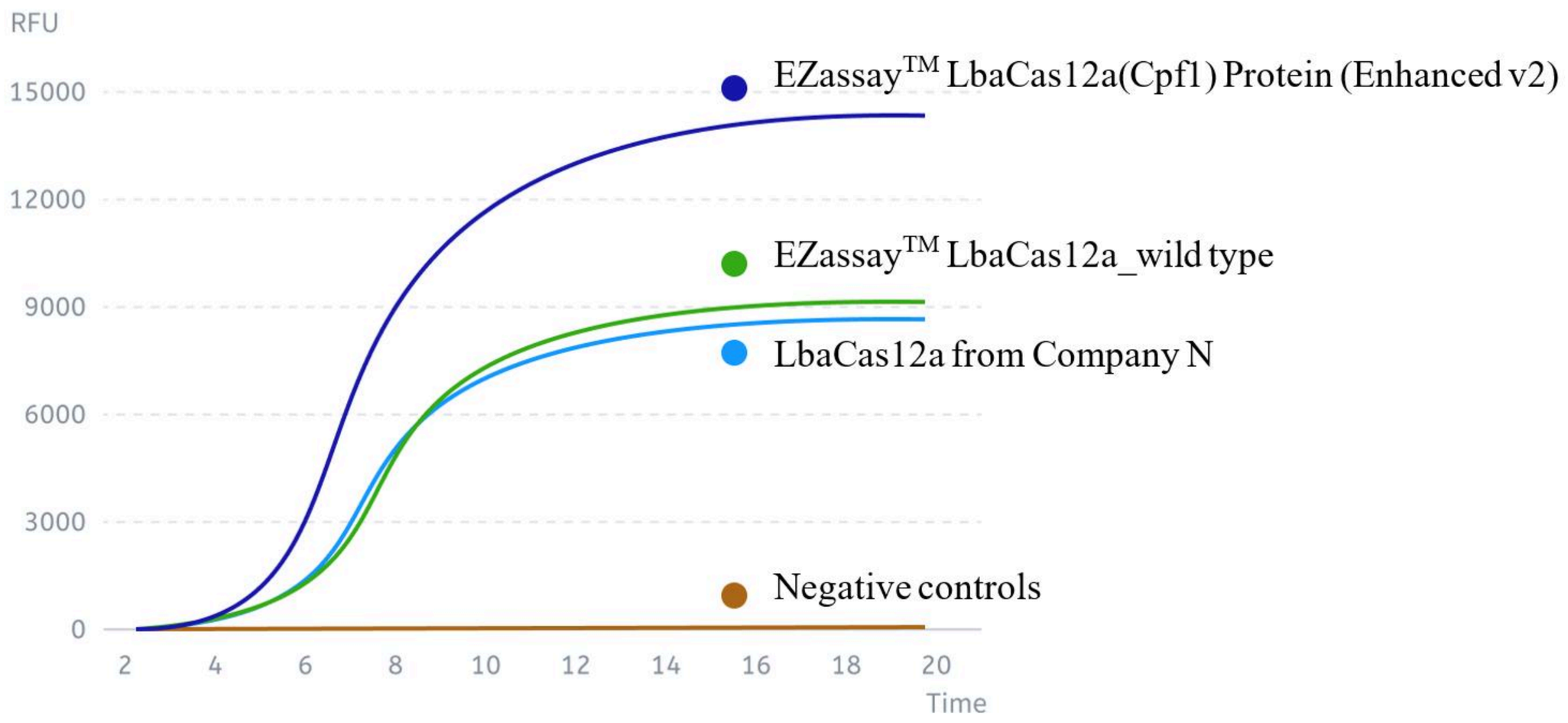
2、Prepare reaction as described in the table below.

Component	Recommend	Optimization
Cas12a Reaction Buffer（10X）	1×	-
Reporter (fluorescence)	120 nM	40 ~ 200 nM
crRNA	50 nM	20 ~ 500 nM
LbaCas12a(Cpf1) Protein (Enhanced v2)	20 nM*(=0.02uM)	10 ~ 100 nM (=0.01uM~0.1uM)
DNA amplicons (from 1st step)	optimal	0-5ul
H2O	up to 20 µL	-

*The optimal enzyme usage varies among manufacturers due to differences in enzymatic activity and reaction buffer systems. Consequently, the ideal concentration of Cas12a must be determined on a per-project basis. For initial use, we recommend performing a comparative test across a concentration gradient ranging from 5 nM to 100 nM.

3、Set temperature at 37°C， read fluorescence every 1 min for 20~60 minutes.

4、Compare LbaCas12a enhanced with wild type：



- DNA amplification using EZassay Isothermal DNA amplification basic kit (Cat.# BA-LYO-96) according to instruction book
- Compare LbaCas12a(Cpf1) Protein (Enhanced v2), LbaCas12a_wild type from EZassay and LbaCas12a from Company N using products from last step. Read fluorescence in Q-PCR thermal cycler FAM channel at 37°C for 20 mins.
- Group of LbaCas12a(Cpf1) Protein (Enhanced v2) showed significantly increased fluorescence signal.

Notes

1. This product is for scientific research only, please read this manual carefully before use.
2. Please strictly follow the management norms of gene amplification laboratory to conduct experimental operations and pay attention to prevent contamination of amplification products.
3. After the end of the experiment, the waste generated during the testing process shall be disposed of in accordance with relevant regulations.