



AapCas12b(C2c1) Protein Instructions

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EZassay Biotechnology Ltd.

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

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Product Information	
Product name	AapCas12b
Expression system	Escherichia coli
Quality	Recombinant protein
Form	Liquid
Molecular weight	136 kDa

Product Introduction

Cas12b and sgRNA form functional complex and it searches for target sequence. The trans-cleavage activity of Cas12b is activated when sgRNA binds target sequence. Cas12b efficiently cuts ssDNA around. Cas12b features resistance of high temperature of 60~65°C, which makes it an ideal partner of LAMP.

Storage

-20°C. Suggest aliquot after receiving. Avoid repeated freeze-thaw.

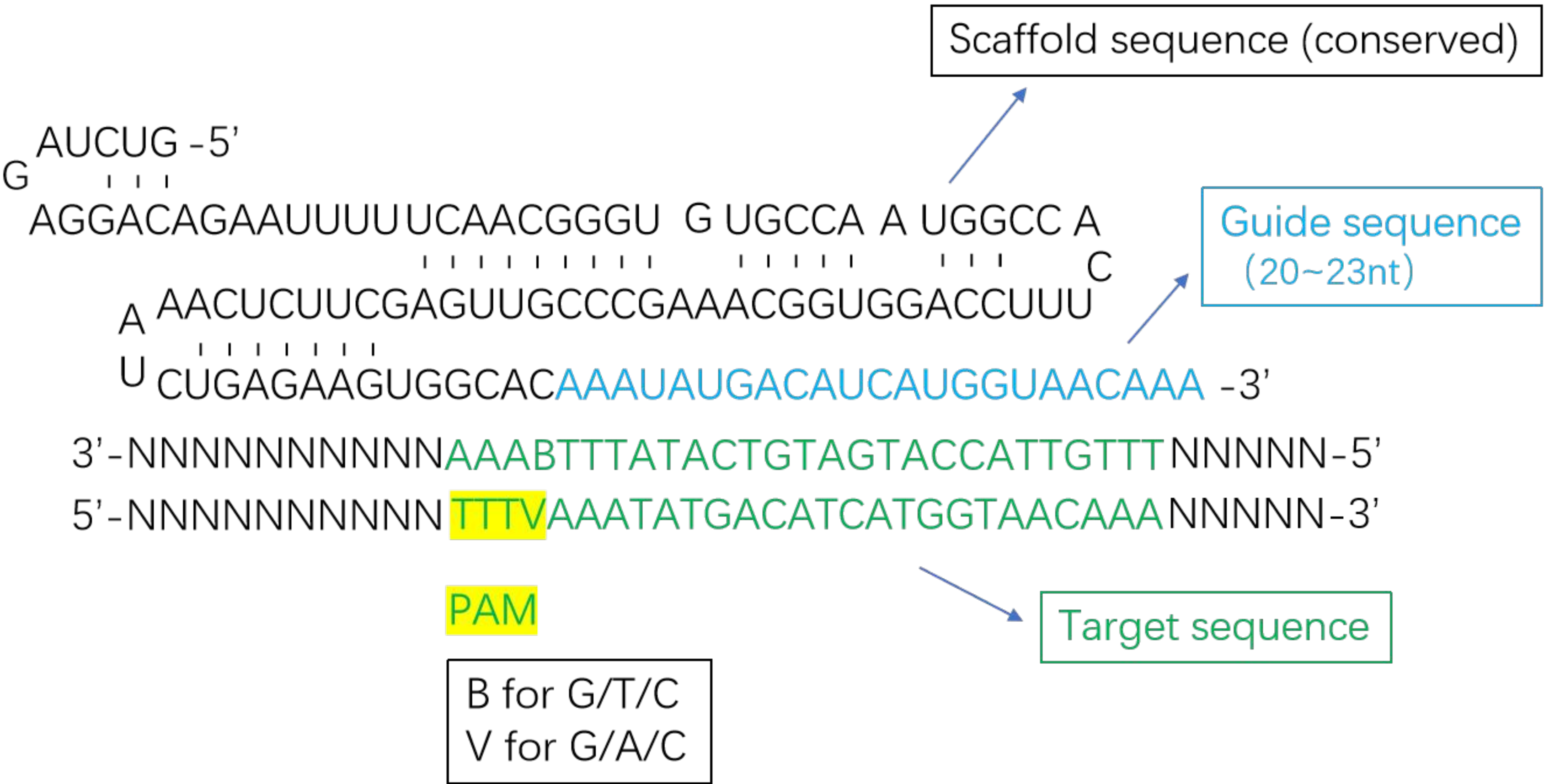
Materials supplied

Cat:	CAS-12P-000	CAS-12P-001	CAS-12P-010	CAS-12P-100
AapCas12b protein	10μM*10μL (100pmol)	10μM*20μL (200pmol)	10μM* 200μL (2,000pmol)	10μM *1000μl (10,000pmol)
Cas12b Reaction Buffer(10X)	1 ml * 1	1 ml * 1	1 ml * 4	10ml*2
Diluent Buffer (1X) (Cas12b)	1 ml * 1	1 ml * 1	1 ml * 2	10 ml * 1

Other materials required

- 1.Reporter: substrate gives signals when digested by Cas12b. Self-design, for example FAM-BHQ labelled ssDNA. Optional select EZassay™ products (Reporter DNA FAM label (for Cas12b) Cat.#. DNA-FAM-BHQ-B.)
2. sgRNA: forms functional complex with AapCas12b.
(AapCas12b sgRNA scaffold sequence: 5’ -
GUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCCAGGUGGCAAAGCCCCG
UUGAGCUUCUCAAUCUGAGAAGUGGCAC -3’)
Recommend **EZassay™ High Yield crRNA/sgRNA Synthesis Kit** to prepare sgRNA.
(Cat.#: SG-RNA-001)
Any question about design, contact info@ezassay.com.

AapCa12b sgRNA



3. DNA samples such as amplification products. Recommend EZassay™ LAMP isothermal amplification kit. (Cat.#.: LAM-LQ-1)

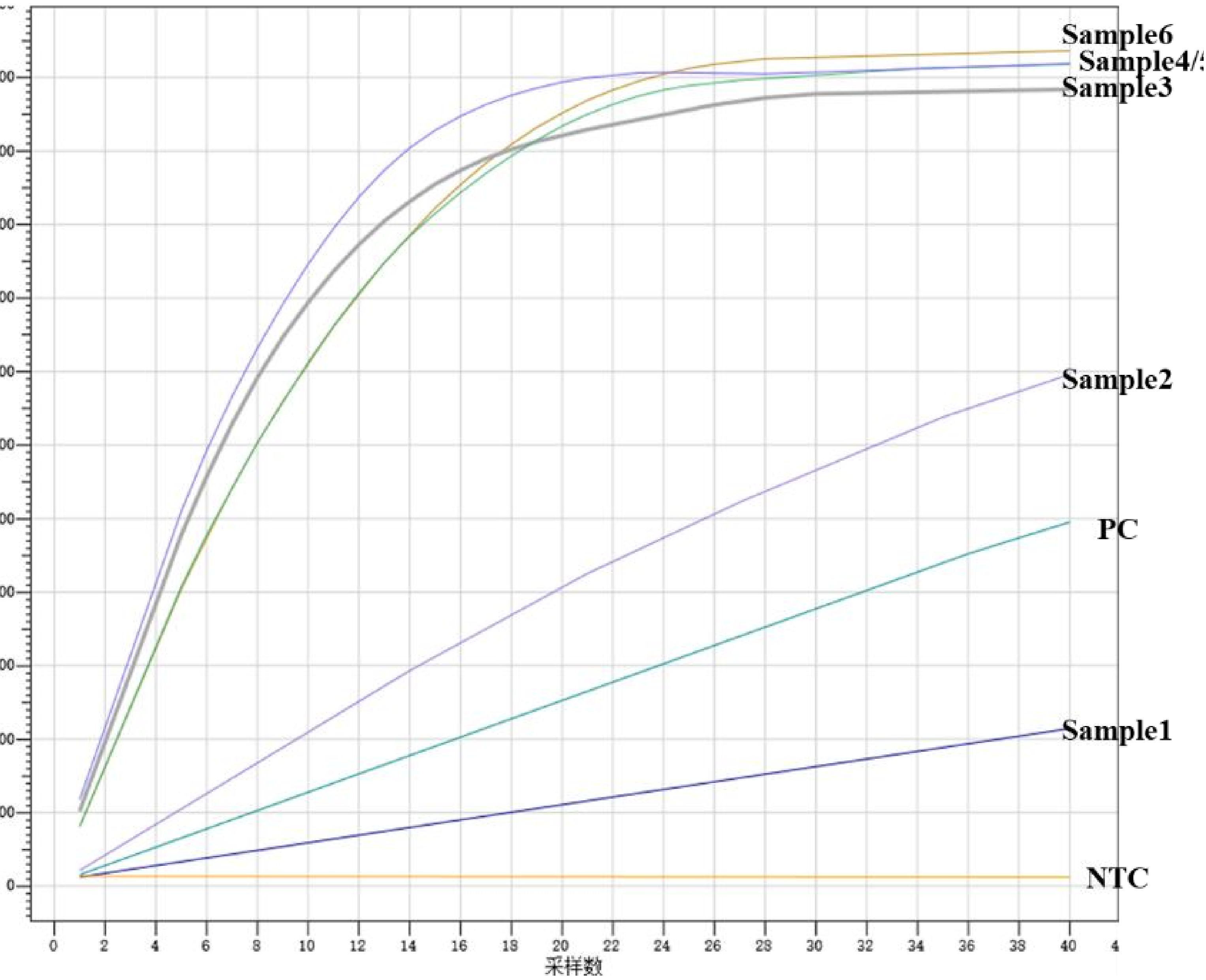
Assay procedure

- 1、Amplification of target DNA (reverse transcription firstly for RNA target) by LAMP. (Optimal)
- 2、Prepare reaction as described in the table below.

Component	Recommend concentration	Optimization range
Cas12b Reaction Buffer (10X)	1×	-
Reporter ssDNA (fluorescence)	200 nM	100 ~ 500 nM
sgRNA	500 nM	400~ 600 nM
AapCas12b	300 nM (=0.3uM)	200 ~ 600 nM (=0.2~0.6uM)
Target sequence	optimal	0-5ul
H2O	Up to 20 μL	-

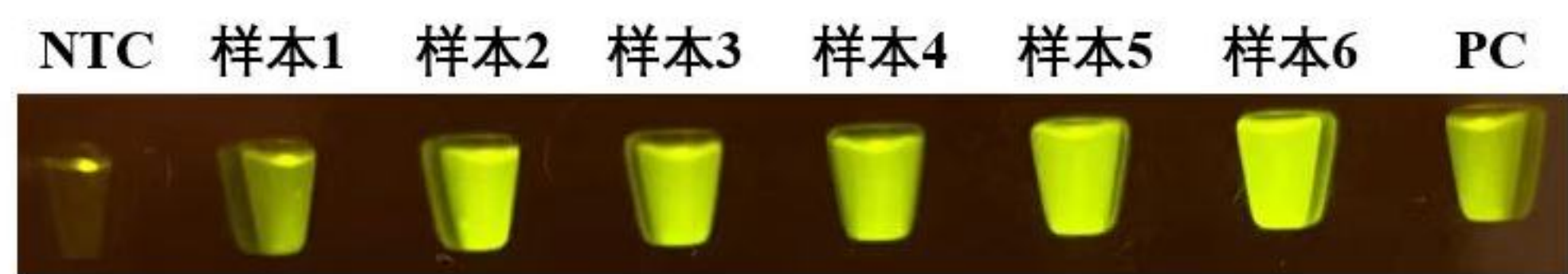
Set temperature at 60~ 65°C, read fluorescence every 1 min for 20~40 minutes.

3、Typical result:



Positive control (PC), Test samples (sample1-6): Cas12b efficiently cuts reporter and gives strong fluorescence signal after activated by LAMP amplicons.
No template control (NTC): No fluorescence signal for no template LAMP amplification group.

Example of fluorescent result at end point.



Reporter: FAM labeled ssDNA (absorption wavelength 492nm, emission wavelength 518nm).

Picture was taken with mobile phone under orange filter.

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.