EZassay

Cas14a (Cas12f1) Protein Instructions

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Catalog Code: CAS-14-001
CAS-14-010
CAS-14-100

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Product Information	
Product name	Cas14a (Cas12f1) Protein
Expression system	Escherichia coli
Form	Liquid
Molecular weight	62 kDa

Product Introduction

Cas14a is an endonuclease that specifically binds to and cleaves target ssDNA under the guidance of sgRNA without the need for a PAM site. Unlike Cas12a, Cas14a can only bind to ssDNA targets, so the amplified and enriched target nucleic acids need to be treated with T7 exonuclease, and one of the amplification primers needs to be phosphorothioated to ensure that only one strand of T7 exonuclease is cleaved, leaving the ssDNA target strand for Cas14a-mediated molecular detection.

Storage

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

Materials supplied

Cat:	Cas-14-001	Cas-14-010	Cas-14-100
Cas14a	10μM*20μl (200pmol)	10μM* 200μl (2,000pmol)	10μM *1000μl (10,000pmol)
1X Diluent Buffer (for Cas14a)	1 ml * 1	1 ml * 2	10 ml *1
10X Reaction Buffer（for Cas14a）	1 ml * 1	1 ml * 4	10 ml *2
Positive Control	50μL*1	50μL*2	50μL*5

Cas14a sgRNA

1. sgRNA is composed up of tranrRNA, crRNA and Spacer. sgRNA and Cas14a form functional complex.

Cas14a sgRNA scaffold sequence: 5’-3’：
CUUCACUGAUAAAGUGGAGAACCGCUUCACCAAAAGCUGUCCCUUAGGGGAUUAGAACUUGAG
UGAAGGUGGGCUGCUUGCAUCAGCCUAAUGUCGAGAAGUGCUUUCUUCGGAAAGUAACCCUC
GAAACAAAUUCAUUUUUCCUCUCCAAUUCUGCACAAGAAAGUUGCAGAACCCGAAUAGACGAA
UGAAGGAAUGCAAC

Recommend EZassay™ High Yield crRNA/sgRNA Synthesis Kit to prepare sgRNA.（Cat.#: SG-RNA-001）

Cas14a(Cas12f1) sgRNA:

Assay procedure

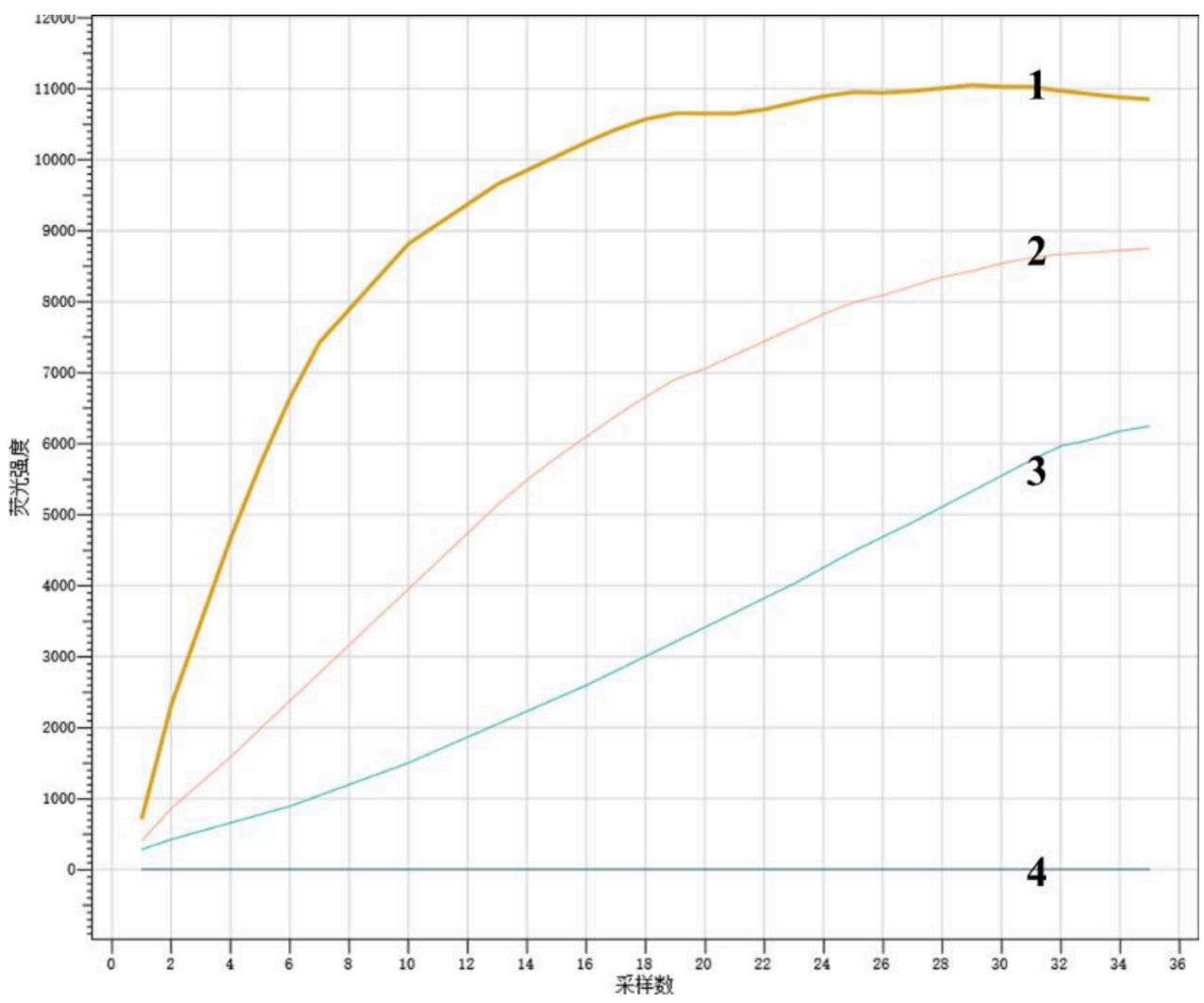
1. Prepare reaction as described in the table below. (Suggest to work on ice.)

Component	Volume	Working concentration
10X Reaction Buffer（for Cas14a）	2 µl	1X
Cas14a (10µM)*	0.5~2 µl	250~1000 nM
10 µM sgRNA	0.5~2 µl	250~1000 nM
10 µM Target DNA**	X µl	-

10 μM ssDNA Reporter (for Cas14a)***	0.5~2 μl	250~1000 nM
Nuclease-free water	Up to 20 μl	-

*Cas14a stock can be diluted with 1X Diluent Buffer. Use immediately after dilution.
**Target DNA should be ssDNA or dsDNA with PAM site.(crRNA、Target DNA and ssDNA Reporter can be diluted with Nuclease-free Water. For very low concentration of Target DNA, it is suggested to use 0.1% Tween 20 to diluent. Consumables of low retention are recommended.)
***Recommend EZassayTM Reporter for Cas14a (Cat.#: DNA-FAM-BHQ-L).It is labelled with FAM and BHQ1.

2. Set the Q-PCR instrument temperature to 37°C and collect fluorescence signals every minute.



1：Cas14a 250nM
2：Cas14a 100nM
3： Cas14a 500nM
4： No template control

Notes

1. Turn off the heat function or set it 40°C if Q-PCR thermal cycler is used.
2. Cis-cleavage of Cas14a: Cas14a specifically cleaves target DNA under the guidance of sgRNA. dsDNA targets require PAM sites, while ssDNA targets do not rely on PAM sites.
3. Trans-cleavage of Cas14a: When target DNA is present, Cas14a/sgRNA forms a complex with target DNA, and the trans-cleavage activity of Cas14a is activated and then it cleaves single-stranded DNA of any sequence in the reaction system. .
4. When Cas14a needs to work with target ssDNA, T7 exonuclease needs to be used to digest the amplified target dsDNA to obtain the target ssDNA for Cas14a-mediated molecular detection. (Note: One of the amplification primers needs to be phosphorothioated to ensure that T7 exonuclease only cuts the other strand, leaving the ssDNA target strand.)

5. Please keep the experimental area clean and tidy, and wear clean gloves and masks when operating. Consumables such as pipette tips and centrifuge tubes used in the experiment are all RNase-free.