TINZYME
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LbaCas12a (Cpf1)

Product Number: CAS12A

Shipping and Storage

Store at -30 ~ -15℃ and transport at ≤ 0℃.

Component

Component	CAS12A 100pmol	CAS12A 1000pmol
LbaCas12a (1 μM)	100μl	1000μl
LbaCas12a Reaction Buffer (10 ×)	1ml	2ml

Description

LbaCas12a (Cpf1) nuclease recognizes T rich TTTN PAM sequences, opening up additional genomic regions for gene targeting. The required gRNA is short, with only 40-44 bases. It has two nuclear localization signals, improving the efficiency of transportation to the nucleus. The cutting product is a 5 'protruding end.The activity temperature range of this enzyme is between 16 ℃ and 48 ℃. Compared with the homologous enzyme of Acidaminococcus, it still exhibits good activity at lower temperatures and can be used to edit the genome of cold-blooded animals, such as zebrafish and African Xenopus. High concentration enzymes can be used for micro injection, electroporation, and liposome transfection.

Source

Obtained through E.coli recombination, expression, and purification, the expressed gene is derived from Lachnospiraceae bacterium.

Application

In vitro screening of efficient gRNA sequences, specific double stranded DNA cleavage guided by gRNA, selective linearization of double stranded DNA containing specific sequences, etc.

Unit definition

1 unit refers to the amount of Cas12a enzyme required to cleave 1 pmol ssDNA probe within 1 minute under 37℃ reaction conditions.

Protocol

1. Prepare the system reaction solution according to the following suggestions:

Reagent	Volume
Nuclease-free ddH_2O	20μl
LbaCas12a Reaction Buffer (10×)	3μl
gRNA (300nM)	3μl
LbaCas12a (1μM)	1μl
Total	27μl

Note:1)To ensure the highest cutting efficiency, the molar ratio of LbaCas12a Nuclease and sgRNA to target DNA should be at least 10:10:1 or higher.

2)Generally, use 30μl system , but it can also be scaled up in equal proportions.

3)Please dilute sgRNA to 300nM using Nuclease free ddH_2O before the experiment.

4)Please wear a mask and use Nuclease free consumables and reagents to avoid the degradation of sgRNA during the

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experiment.

2. Incubate at 25℃ for 10 minutes.
3. Add 3μl of 30nM DNA.
   Note: Dilute DNA to 30nM using Nuclease free ddH_2O.
4. Shake well and centrifuge briefly for collection.
5. Incubate at 37°C for 10 minutes.
6. Add 1 μl of protease K to the sample, shake well, and collect briefly by centrifugation.
7. Incubate at room temperature for 10 minutes.
8. The reaction products can be directly analyzed by agarose gel electrophoresis.
   Note: If electrophoresis is not done immediately, EDTA can be added to terminate the reaction.

Note

1. When using this product, it will involve the operation of gRNA and DNA, and attention must be paid to the relevant operations of RNase free and DNase free. All self prepared reagents and consumables should also be Nuclease free. If there may be nuclease contamination, consider treating overnight with 0.01% DEPC and then using it after high-temperature and high-pressure treatment. It is recommended to wear a disposable mask during operation.
2. This product is for scientific research purposes only and shall not be used for clinical medical diagnosis or other unreasonable purposes.