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AacCas12b (C2c1)

Product Number: CAS12B

Shipping and Storage

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

Components

Component	CAS12B 100p	CAS12B 1kp
AacCas12b (10μM)	10μl	100μl
AacCas12b Reaction Buffer(10×)	1ml	2ml

Description

AacCas12b nuclease (C2c1) is an RNA mediated endonuclease that can specifically cleave the target double stranded DNA in the presence of PAM (TTN), causing the DNA double stranded to break and generate sticky ends.And AacCas12b specifically cleaves single stranded DNA targets without relying on PAM sequences. In addition, both double stranded and single stranded DNA targets can activate the trans cleavage activity (i.e., bypass cleavage activity/accessory cleavage activity) of AacCas12b. When AacCas12b enzyme binds to sgRNA and target DNA to form a ternary complex, it will activate the trans cleavage activity against non-specific sequence ssDNA, chopping any sequence of ssDNA in the system.

AacCas12b is derived from the acidophilic and heat-resistant bacterium Alicyclobacillus acidoterrestris, and the optimal shear reaction temperature is 48 ℃.AacCas12b relies on the guidance of crRNA and tracrRNA (or sgRNA formed after connection).In addition, AacCas12b can be applied not only to the cleavage of target dsDNA, but also to the rapid detection of target nucleic acids through its trans cleavage activity against ssDNA.

Source

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

Application

Used for the cleavage of target dsDNA, the trans cleavage activity of ssDNA can also be used for rapid detection of target nucleic acids.

Unit definition

1 unit refers to the amount of Cas12b enzyme required to cleave 1 pmol ssDNA probe within 1 minute under reaction conditions of 48℃.

Protocol

1. Prepare the system reaction solution (cis shear experiment) according to the following suggestions:

Reagent	Volume
AacCas12b Reaction Buffer (10×)	2μl
AacCas12b (10μM)	0.5μl
sgRNA (10μM)	0.5μl
Target DNA (1μM)	0.5μl
Nuclease-free ddH_2O	Up to 20μl

Note:1)The target DNA in the cis cleavage system can be ssDNA or dsDNA with PAM sequence.

2)If nucleic acid electrophoresis is used to analyze cis cleavage products, it is recommended to use dsDNA targets, as ssDNA targets will be further fragmented by trans activated Cas12b.For a 20μL cis shear stress system, the recommended amount of target DNA usage is 100ng~500ng, and the length of target DNA fragments is within the range of 300bp~3kb.The amount of Cas enzyme and sgRNA required for different lengths of target DNA needs to be calculated based on the molar amount of target DNA. It is recommended to maintain the molar ratio of Cas enzyme: sgRNA: target DNA at 10:10:10:1 to ensure that the target DNA is completely cleaved as much as possible.

React at 48℃ for 30 minutes to 1 hour, inactivate at 85℃ for 5 minutes, and analyze the cis cleavage products by nucleic acid electrophoresis.

2. Trans shear experiment:

Reagent	Volume
AacCas12b Reaction Buffer (10×)	2μl
AacCas12b (10μM)	0.05~0.5μl
sgRNA (10μM)	0.05~0.5μl
Target DNA (1μM)	0.5~5μl
ssDNA Reporter (10μM)	0.05~0.5μl
Nuclease-free ddH_2O	Up to 20μl

Note:1)The target DNA in the cis cleavage system can be ssDNA or dsDNA with PAM sequence.

2)The crRNA, Target RNA, and ssRNA Reporter can be diluted with Nuclease free ddH2O, but for very low concentrations of Target RNA (such as LOD experiments), it is recommended to dilute with 0.1% Tween 20.

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

Note

1. Cis cleavage of Cas12b: Cas12b specifically cleaves target DNA under the guidance of sgRNA. The dsDNA target needs to carry a PAM site, while the ssDNA target does not rely on the PAM site.
2. Trans cleavage of Cas12b: When target DNA is present, Cas12b/sgRNA forms a ternary complex with target DNA (Cas12b/sgRNA/target DNA), and at the same time, Cas12b is stimulated with trans cleavage activity, chopping up single stranded DNA of any sequence in the reaction system.
3. Conduct molecular diagnostic experiments using the trans shear activity of this product.
4. To prevent RNase pollution, please keep the experimental area clean and tidy. Wear clean gloves and masks during operation, and use RNase free consumables such as gun heads and centrifuge tubes for the experiment.
5. The AacCas12b enzyme is heat sensitive and prone to deactivation. A reaction system should be prepared on ice throughout the entire process, and the enzyme should be immediately stored at -20 ℃ after use.
6. This product is for scientific research purposes only.