AapCas12b

REF: EG23202-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG23202S	EG23202M
AapCas12b (10 μM)	10 μl	100 μl
10× Cas12b Buffer	1 ml	1 ml

Description

AapCas12b is a Type V Cas nuclease, exhibiting good activity across 37~60℃ . Guided by crRNA and tracrRNA (or sgRNA), it exhibits cis-cleavage activity against both double-stranded and single-stranded DNA targets. For double-stranded DNA targets, AapCas12b recognizes the site complementary to the crRNA (or sgRNA) spacer sequence downstream of the PAM sequence (5'-TTN) in target DNA, specifically cleaves the target double-stranded DNA to generate sticky ends. For single-stranded DNA targets, its specific cleavage is PAM-independent.

After AapCas12b, target DNA and sgRNA form a ternary complex, the enzyme exhibits *trans*-cleavage activity, namely non-specific cleavage of single-stranded DNA with any sequence. AapCas12b exhibits good activity in common isothermal amplification buffers, making it suitable for rapid nucleic acid detection.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 10 pmol of AapCas12b incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 10 pmol of AapCas12b incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 pmol of AapCas12b incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the E.coli 16S rDNA , and the results show that the *E.coli* genome residues less than 1 copy.

Heat Inactivation

Incubation at 85℃ for 5 minutes.

Protocol

1. Cis-Cleavage Assay

① Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10× Cas12b Buffer	2 μl	1×
AapCas12b (10 μM)[a]	0.5 μl	250 nM
sgRNA (10 μM)[a]	0.5 μl	250 nM
Target DNA (1 μM)[a]	0.5 μl	25 nM
Nuclease-free water	To 20 μl	

a. For detection of cis-cleavage products by gel electrophoresis, double-stranded DNA (dsDNA) is recommended as the target, with a length ranging from 300 bp to 3 kb. Maintain a molar ratio of AapCas12b : sgRNA : Target DNA at 10:10:1 to ensure complete cleavage of the target DNA.

② Incubate the reaction at 60 ℃ for 30 min to 1 h, then inactivate the enzyme by heating at 85℃ for 5 min.

③ Use agarose-gel electrophoresis to check the product. To prevent abnormal electrophoresis caused by AapCas12b binding with DNA, ensure heat inactivation is performed. If abnormal electrophoresis persists, treat the product with Proteinase K before conducting electrophoresis analysis.

2. Trans-Cleavage Assay

① Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10× Cas12b Buffer	2 μl	1×
AapCas12b (10 μM)[a]	0.05~0.5 μl	25~250 nM
sgRNA (10 μM)[a]	0.05~0.5 μl	25~250 nM
Target DNA (1 μM)[b]	0.5~5 μl	25~250 nM
ssDNA Probe (10 μM)[a]	0.05~0.5 μl	25~250 nM
Nuclease-free water	To 20 μl	

a. The amounts of each component in the trans-cleavage assay can be adjusted according to experimental objectives. For small volumes, a pre-mixed solution can be prepared first.

b. In the trans-cleavage experiment, the target DNA can be either single-stranded DNA or double-stranded DNA containing a PAM sequence.

② Place the tube in a qPCR instrument. Select the channel matching the fluorophore on the ssDNA probe. Perform isothermal reaction at 60℃ , with each cycle lasting 30 seconds. The number of cycles can be set between 30 and 60.

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