ApeKI

REF: EG25519S

Isoschizomers*: TseI

*Isoschizomers may have different methylation sensitivities.

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
ApeKI (10 U/μl)	50 μl
10× Cut Buffer C	1 ml

Description

ApeKI is a Type IIP restriction enzyme derived from the *Aeropyrum pernix* K1. It recognizes and cleaves the sequence G/CWGC, generating sticky-end with a 3-base overhang. ApeKI is one of the preferred enzymes for Genotyping-by-Sequencing (GBS) and is widely used in plant and animal genetics, population evolution studies, and functional gene mapping.

Recommended Reaction Conditions

1× Cut Buffer C;
Incubate at 75℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.
This product has <10% activity when performing enzymatic digestion reactions at 37℃ .

Heat Inactivation

This enzyme can not be heat inactivated. Please purify the digested product by phenol/chloroform treatment or column-based purification kit.

Definition of Activity Unit

One unit of activity refers to the amount of enzyme required to completely digest 1 μg of λDNA in a 50 μl reaction system at 75℃ for 1 hour.

Quality Control Assays

Function

10 U of ApeKI can completely digest 1 μg of λDNA within 15 minutes at 75℃ .

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 10 U ApeKI with 1 μg λDNA for 3 hours. No contamination from other nucleases or non-specific substrate degradation caused by star activity was detected. Longer incubation may result in star activity.

Ligation and Recuting

Under the optimal reaction temperature, digest the substrate using 10 U of ApeKI and then recover the digested products. The DNA fragments can be ligated with T4 DNA Ligase at 22℃ . After ligation, these ligated fragments can be recut with ApeKI, as determined by agarose gel electrophoresis.

Icon Descriptions

75 The enzyme's optimum reaction temperature is 75℃ .
CpG Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.
The enzyme can not be thermal inactivated.
3 hours incubation do not show star activity, but longer incubation may result in star activity.

Protocol

1. Protocol for DNA Digestion

① Combine the following components on ice in the following order:

ddH_2O	up to 50 μl
10× Cut Buffer C	5 μl
DNA[a]	1 μg
ApeKI (10 U/μl)	1 μl
Total	50 μl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

② Mix gently and spin down.
③ Incubate at 75℃ for 15 min~3 h.
④ Optional: Phenol/chloroform treatment or column based purification.

2. Notice

① The volume of enzyme added to the reaction mixture should not exceed 10% of the total volume to avoid star activity caused by excessive glycerol in the enzyme storage buffer.
② The additives (e.g., glycerol, salt) in the enzyme storage buffer are the same as the contaminants in the substrate solution (e.g., salt, EDTA, or ethanol, etc.). Therefore, the smaller the reaction volume, the stronger the digestion inhibition effect.

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Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
199	14	21	12	12	22	10	179

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	Some blocked	No effect	No effect

Activity in Different Buffers*

	CutOne® Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	12.5%	50%	12.5%	25%

*The activity data come from the functional test described above.

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