AarI

REF: EG25526S

Isoschizomers*: PaqCI

*Isoschizomers may have different methylation sensitivities.

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
AarI (5 U/μl)	20 μl
AarI Activator	20 μl
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

Description

AarI is an isoschizomer of PaqCI. Type IIS restriction enzyme which recognizes the sequence CACCTGC and cleaves outside of their recognition sequence, generating 4-base 5' overhangs. Especially, AarI requires two or more sites for digestion. AarI Activator supplied to improve cleavage. It recognizes a 7-basesequence that occurs infrequently in genes, is particularly suitable for Golden Gate assembly.

AarI shows superior activity in the universal CutOne® and CutOne® Color Buffer. CutOne® Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the CutOne® Color Buffer migrates with 2.5 kb double-strand DNA fragments in a 1% agarose gel, and the yellow dye migrates with 10 bp double-strand DNA fragments in a 1% agarose gel.

Recommended Reaction Conditions

1× CutOne® Buffer;
Incubate at 37℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Definition of Activity Unit

One activity unit (U) is defined as the amount of enzyme required to completely digest 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour in the presence of activator.

Quality Control Assays

Function

5 U of AarI can completely digest 1 μg of p615 DNA within 1 hour at 37℃ .

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 5 U AarI with 1 μg p615 DNA 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 optimal reaction temperature, digest the substrate using 5 U AarI and recover the digested products. >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃ .Of these ligated fragments, >95% can be recut with AarI as determined by agarose gel electrophoresis.

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

CpG Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.

The enzyme can be heat inactivated at by incubation 80℃ for 20 minutes.

★ 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× CutOne® Buffer 或 10× CutOne® Color Buffer	5 μl
DNA[a]	1 μg
AarI (5 U/μl)	1 μl
AarI Activator[b]	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;
b. Complete digestion requires adding activator in a 1:1 ratio equal to the enzyme volume. The activator appears as a band of around tens of bp during electrophoresis, please avoid misidentifying it as non-specific cleavage.

② Mix gently and spin down.
③ Incubate at 37℃ for 1~3 h.
④ Optional: Inactivate the enzyme by heating at 80℃ for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

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2. Notice

① AarI is a complete isoschizomer of PaqCI.
② AarI requires two or more recognition sites for complete digestion.
③ 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.
④ 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.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
12	0	0	0	0	0	0	9

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	Impaired	No effect	No effect

Activity in Different Buffers*

	CutOne® Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	100%	＜ 12.5%	100%	＜ 12.5%

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

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