BspQI

REF: EG23503S

Isoschizomers*: SapI, LguI, PciSI

*Isoschizomers may have different methylation sensitivities.

50 80 ★

Storage Condition

-20℃

Components

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

Description

BspQI is a Type IIS restriction enzyme that recognizes non-palindromic sequences and cuts outside the recognition site. It is commonly used in Golden Gate assembly. The reaction buffer has been optimized to maximize the functionality of BspQI. Additionally, the reaction buffer contains recombinant albumin, which enhances the stability of various enzymes.

Recommended Reaction Conditions

1× Cut Buffer C;
Incubate at 50℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80℃ for 20 minutes.

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 50℃ for 1 hour.

Quality Control Assays

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 10 U BspQI 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 optimal reaction temperature, digest the substrate using 10 U BspQI 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 BspQI as determined by agarose gel electrophoresis.

DNase Activity

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

Icon Descriptions

50 The enzyme's optimum reaction temperature is 50℃ .

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

★ 3 hours incubation did not show star activity, but delayed enzyme digestion might show star activity.

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Protocol

1. Protocol for DNA Digestion

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

Reagents	Volume
ddH_2O	up to 50 μl
10× Cut Buffer C	5 μl
DNA[a]	1 μg
BspQI (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 50℃ for 15 minutes~1 hour.
④ Optional: Inactivate the enzyme by heating at 80℃ for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

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.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
10	1	1	1	1	0	0	7

Methylation Effects on Digestion

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

Activity in Different Buffers*

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

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

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