BsmBI

REF: EG22507-V/S

Isoschizomers*: Esp3I

*Isoschizomers may have different methylation sensitivities.

55 CpG EB 80' ★

Storage Condition

-20℃

Components

Component	EG22507V	EG22507S
BsmBI (10 U/μl)	20 μl	100 μl
10× Cut Buffer C	1 ml	1 ml

Description

BsmBI 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 BsmBI. Additionally, the reaction buffer contains recombinant albumin, which enhances the stability of various enzymes.

Recommended Reaction Conditions

1× Cut Buffer C;
Incubate at 55℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.
This product has 50% activity when performing enzymatic digestion reactions at 37℃ .

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

Under optimal reaction temperature, incubate 10 U BsmBI 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 BsmBI 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 BsmBI as determined by agarose gel electrophoresis.

DNase Activity

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

Icon Descriptions

55 The enzyme's optimum reaction temperature is 55℃ .

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

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

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
BsmBI (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 Incubation at 55℃ for 15 minutes~1 hour, generally recommended 5 U~10 U enzyme/μg plasmid DNA, 10 U~20 U enzyme/μg genomic DNA, warm bath for 1 hour, if you need to overnight digestion reaction, please adjust the enzyme amount to 1 U;
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
14	0	1	2	2	0	1	21

Methylation Effects on Digestion

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

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