BsmBI

Cat #: C-BSM2507S

Size: 1000 U

Storage: -20°C

5'...C G T C T C (N)$_1$▼...3'
3'...G C A G A G (N)$_5$▲...5'

Isoschizomers*: Esp3I

*Isoschizomers may have different methylation sensitivities.

Components

Components	Amount
BsmBI (10 U/μl)	100 μl
10× HN Buffer	1 ml

Description

BsmBI belongs to Type IIs restriction enzyme that recognizes and cuts non-palindromic sequences and is commonly used in Golden Gate assembly. The optimized reaction buffer maximizes the function of BsmBI, while the reaction buffer contains recombinant albumin, which enhances the stability of multiple enzymes.

Recommended Reaction Conditions

1× HN Buffer;

Incubate at 55°C;

Refer to "Protocol for Fast DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80°C for 20 minutes.

Unit Definition

One unit is defined as the amount of BsmBI required to digest 1 µg of λDNA in 1 hour at 55°C in 50 µL of recommended reaction buffer.

Quality Control

Prolonged Incubation / Star Activity Assay

Incubate 10 U of BsmBI with 1 µg of λDNA at the optimal reaction temperature for 3 hours. No other nucleases contamination or non-specific degradation caused by star activity was detected. Longer incubation may result in star activity.

Ligation and Recuting

After digestion with 10 U of BsmBI at optimal reaction temperature, the DNA fragments can be ligated with T4 DNA Ligase (Fast) at 22°C. Of these ligated fragments, most of them can be recut with BsmBI as determined by agarose gel electrophoresis.

DNase residues

Incubate 10 U of BsmBI with double-stranded DNA at 37°C for 16 hours, no changes for the DNA were detected in DNA electrophoresis.

Icon Descriptions

55 The enzyme's optimum reaction temperature is 55°C.

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.

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

3 hours incubation do not show star activity, but longer incubation may result in star activity.

Method of application

1. Protocol for Fast DNA Digestion

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

	DNA
ddH_2O	Up To 50 µl
10× HN Buffer	5 µl
DNA[a]	1 µg
BsmBI (10 U/µl)	1 µl
Total	50 µl

a. The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergent or high concentration salt, otherwise it will affect the enzyme activities of BsmBI.

② Mix gently(Do not vortex) and spin down;

③ Incubate at 55°C for 15 min-60 min, generally recommended 5~10 U enzyme /µg DNA, 10~20 U enzyme /µg genomic DNA, warm bath for 1 h, if overnight enzyme digestion is required, please adjust the enzyme amount to 1U;

④ Inactivate the enzyme by heating for 20 minutes at 80°C, or terminate the reaction by column-based or phenol/chloroform purification.

⑤ The volume of enzyme added to the reaction should not exceed 10% of the total volume to avoid excessive glycerol, which may lead to star activity.

⑥ The additives in the storage buffer of restriction endonucleases (such as glycerol and salts) and contaminants in the substrate (such as salts, EDTA, or ethanol, etc.) are similar. The smaller the reaction volume, the stronger the inhibitory on the enzyme digestion reaction.

2. Scaling up Plasmid DNA Digestion Reaction

DNA	0.1 µg	0.5 µg
EcoRI, ADCF	1 U	5 U
10× EcoRI Buffer	1 µl	2.5 µl
ddH_2O	Up To 10 µl	Up To 25 µl
Total	10 µl[b]	25 µl

b. To avoid evaporation, the incubation time of the 10 µl reaction buffer should not exceed 1 h.

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	Blocked	No effect	Impaired