BsiWI

REF: EG24506S

Isoschizomers*: PspLI, Pfl23II

*Isoschizomers may have different methylation sensitivities.

55 CpG 80

Storage Condition

-20℃

Components

Component	Amount
BsiWI (10 U/μl)	30 μl
10× Cut Buffer C	1 ml

Description

BsiWI is a Type IIP restriction enzyme that recognizes palindromic sequences. The reaction buffer has been optimized to maximize the functionality of BsiWI. 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 25~50% activity when performing enzymatic digestion reactions at 37℃ .
This product has 25~50% activity in CutOne® reaction buffer.

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 p615 in a 50 μl reaction system at 55℃ for 1 hour.

Quality Control Assays

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 10 U BsiWI with 1 μg p615 for 16 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 BsiWI 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 BsiWI 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.

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

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
BsiWI (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 55℃ 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
1	2	0	0	0	0	0	4

Methylation Effects on Digestion

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

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