BpiI (BbsI)

REF: EG25525S

Isoschizomers*: BstV2I, BbsI

*Isoschizomers may have different methylation sensitivities.

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
BpiI (10 U/μl)	25 μl
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

Description

BpiI is a Type IIS restriction enzyme. It specifically recognizes the GAAGAC sequence, cleaves downstream, and generates 5' overhangs with 4 bases. It is commonly used in Golden Gate assembly. BpiI belongs to the non-LightNing® restriction enzymes; however, it is compatible with CutOne® buffer, allowing for double digestion with other LightNing® restriction enzymes. It is one of the commonly used enzymes for Golden Gate assembly. It is applicable for scenarios such as routine cloning or restriction digestion and identification other than Golden Gate assembly.

BpiI 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 unit of activity refers to the amount of enzyme required to completely digest 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour.

Quality Control Assays

Function

10 U of BpiI can completely digest 1 μg of λDNA within 15 minutes at 37℃ .

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 10 U BpiI 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 of BpiI and then recover the digested products. The DNA fragments can be ligated with T4 DNA Ligase at 22℃ . After ligation, these ligated fragments can be recut with BpiI, as determined by agarose gel electrophoresis.

Icon Descriptions

 The enzyme's optimum reaction temperature is 37℃ .

 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 or 10× CutOne® Color Buffer	5 μl
DNA[a]	1 μg
BpiI (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 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.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

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
24	3	3	0	0	3	0	27

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%	25%	100%	50%

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

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