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BsmBI

Product Number: RE0581

Shipping and Storage

-20℃.

Components

Component	Specifications V	Specifications S
BsmBI(10U/μL)	20μL	100μL
10×Cut Buffer C	1mL	1mL

Description

BsmBI belongs to the Type IIs restriction enzyme, which can recognize non palindromic sequences and perform cleavage outside of the recognized sequence. It is commonly used for Golden Gate assembly. The optimized reaction buffer maximizes the functionality of BsmBI, while the reaction buffer contains recombinant albumin, which enhances the stability of various enzymes.

Suggested reaction conditions

1. 1 x HN buffer solution;
2. Incubate at 55℃;
3. Prepare the reaction system according to the "DNA enzyme digestion process".

Inactivation conditions

Incubate at 80℃ for 20 minutes.

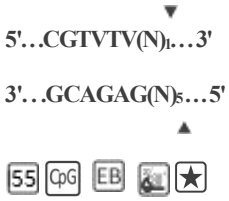
Definition of enzyme activity

1 active unit (U) refers to the amount of enzyme required to completely cleave 1μg λ DNA at 55℃ within 1 hour in a 50μL reaction system.

Quality control

1. Long term incubation detection: At the optimal reaction temperature, 10U BsmBI was incubated with 1μg λ DNA for 3 hours, and no non-specific degradation of the substrate caused by other nuclease contamination or star activity was detected.
2. Enzyme digestion ligation re digestion detection: At the optimal reaction temperature, use 10 U BsmBI to digest the substrate and recover the enzyme digestion product. Using an appropriate amount of T4 DNA Ligase (Fast) at 22℃ can reconnect the enzyme cleavage products. After recycling the connecting product again, the same endonuclease can be used to cleave the connecting product again.
3. DNase residue detection: Incubate 10U BsmBI with double stranded DNA substrate at 37℃ for 16 hours, and detect no change in the double stranded DNA substrate by DNA electrophoresis.

Icon annotation



Homolytic enzyme: Esp3I

Note: Homolytic enzymes may have different sensitivities to different methylation modifications.

1. The optimal reaction temperature is 55℃.
2. For DNA methylated by CpG, splicing may be hindered.
3. For DNA methylated by EcoBI, splicing may be hindered.
4. Inactivation condition: Incubate at 80℃ for 20 minutes.
5. 3 hours of incubation did not show star activity, and delayed enzyme digestion may result in star activity.

Protocol

1. DNA enzyme digestion process

1.1. Prepare the reaction system on ice according to the recommended sample addition sequence as follows:

ddH_2O	up to 50μL
10× HN Buffer	5μL
Substrate DNA	1μg
BsmBI(10U/μL)	1μL
Total	50μL

Note: The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergents, or high concentration salts, otherwise it will affect BsmBI enzyme activity.

1.2. Gently suck or tap the tube wall to mix well (without vortexing), then centrifuge instantly to collect hanging wall droplets;

1.3. Incubate at 55℃ for 15 minutes to 1 hour. It is generally recommended to use 5U~10 U enzyme/μg plasmid DNA and 10 U~20U enzyme/μg genomic DNA. Incubate in a warm bath for 1 hour. If overnight enzyme digestion reaction is required, adjust the enzyme amount to 1 U;

1.4. Incubate at 80℃ for 20 minutes to inactivate the enzyme, stop the reaction, or terminate the reaction through adsorption column or phenol/chloroform purification.

2. Note

2.1. The volume of enzyme added to the reaction system should not exceed 10% of the total volume to avoid excessive glycerol in the enzyme causing star activity,

2.2. The additives (such as glycerol, salt) in the storage buffer of restriction endonucleases are the same as the pollutants (such as salt, EDTA, or ethanol) in the substrate solution. The smaller the reaction volume, the stronger the inhibitory effect of enzyme cleavage reaction.

3. Recommended sample addition system for small volume

DNA	0.1μg	0.5μg
BsmBI(10U/μL)	1U	5U
10× HN Buffer	1μL	2.5μL
ddH_2O	up to 10μL	up to 25μL
Total	10μL	25μL

The number of enzyme cleavage sites in different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
14	0	1	2	2	0	1	21

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	Shear obstruction	no effect	Shear effect