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BspQI

Product Number: RE0583

Shipping and Storage

-20℃.

Components

Component	Specifications
BspQI (5U/μL)	50μL
10×HN Buffer	1mL

Description

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

Suggested reaction conditions

1. 1×HN buffer solution;
2. Incubate at 50℃;
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 reaction in a 50μL reaction system, The amount of enzyme required to completely cleave 1 μg λ DNA within 1 hour at 50℃.

Quality control

1. Ultra long incubation detection: Incubate 10U BspQI with 1μg λDNA for 3 hours at the optimal reaction temperature, No non-specific degradation of substrates caused by other nuclease contamination or star activity was detected, and delayed enzyme digestion may result in star activity.
2. Enzyme digestion ligation re digestion detection: At the optimal reaction temperature, use 10U BspQI 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 BspQI 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

5'…GCTCTTC (N)$_1$…3'

3'…CGAGAAG (N)$_4$…5'

Homolytic enzyme: SapI, LguI, PciSI

Note: Homolytic enzymes may have different sensitivities to different

methylation modifications.

1. The optimal reaction temperature is 50℃.
2. Inactivation condition: Incubate at 80℃ for 20 minutes.
3. 3 hours of incubation did not show star activity, and longer 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:

	DNA
ddH_2O	up to 50μL
10× HN Buffer	5μL
Substrate DNA	1μg
Sgel	1μL
Total	50μL

Note: The DNA substrate should not contain phenol, chloroform, ethanol EDTA、 Detergent or high concentration salt, otherwise it will affect BspQI 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 50℃ for 1 hour;

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.

Note

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. The additives (such as glycerol, salt) in the storage buffer for 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 on the enzyme cleavage reaction.

The number of enzyme cleavage sites in different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
10	1	1	1	1	0	0	7

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	no effect	no effect	no effect