

Tinzyme Co., Limited

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BstXI

Product Number: RE0584

Shipping and Storage

-20°C.

Components

Component	Specifications	
BstXI(10U/µL)	50µL	
10×Cut Buffer C	1mL	

Description

BstXI belongs to the Type IIP restriction enzyme and recognizes palindrome sequences. The optimized reaction buffer maximizes the functionality of BstXI, while the reaction buffer contains recombinant albumin, which enhances the stability of various enzymes.

Suggested reaction conditions

- 1. 1×Cut Buffer C;
- 2. Incubate at 37°C;
- 3. Prepare the reaction system according to the "DNA enzyme digestion process".

Inactivation conditions

Incubate at 80°C for 20 minutes.

Definition of enzyme activity

The active unit (U) refers to the amount of enzyme required to completely cleave $1\mu g \lambda$ DNA at 37°C within 1 hour in a 50 μ L reaction system.

Quality control

- 1. Long term incubation detection: At the optimal reaction temperature, 10U BstXI was incubated with $1\mu g \lambda$ DNA for 16 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 BstXI to digest the substrate and recover the enzyme digestion product. Using an appropriate amount of T4 DNA Ligase (Fast) at 22°C can reconnect the enzyme cleavage products. After recycling the connecting product again, the same endonuclease can be used to cleave the connecting product again.

Icon annotation

5'...CCANNNNNTGG...3' 3'...GGTNNNNNACC...5'

- 1. 37 The optimal reaction temperature is 37° C.
- 2. Im For DNA methylated by Dcm, splicing may be hindered.

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3. Inactivation condition: Incubate at 80°C for 20 minutes.

Protocol

1. DNA enzyme digestion process

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

ddH ₂ O	up to 50µL
10× Cut Buffer C	5µL
Substrate DNA	1µg
$BstXI(5U/\mu L)$	1µL
Total	50µL

Note: The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergents, or high concentration salts, otherwise it will affect BstXI 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 37°C for 15 minutes to 1 hour;
- 1.4. Incubate at 80°C 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 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.

The number of enzyme cleavage sites in different DNA

λDNA	ФХ174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
13	3	0	0	0	1	0	10

The influence of methylation modification

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

Activity in different reaction buffers

	Cut One™	Thermo Scientific	NEB	Takara
	Buffer	Fast Digest Buffer	Cut Smart® Buffer	Quick Cut [™] Buffer
Reactivity	25%	100%	25%	100%

Note: The activity data comes from the detection under the restriction enzyme standard reaction system.