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BsiWI

Product Number: RE0580

Shipping and Storage

-20℃.

Components

Component	Specifications
BsiWI(10U/μL)	30μL
10×Cut Buffer C	1mL

Description

BsiWI belongs to the Type IIP restriction enzyme and recognizes palindrome sequences. The optimized reaction buffer maximizes the functionality of BsiWI, 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 56℃;
3. Prepare the reaction system according to the "DNA enzyme digestion process".

Inactivation conditions

Incubate at 80℃ for 20 minutes.

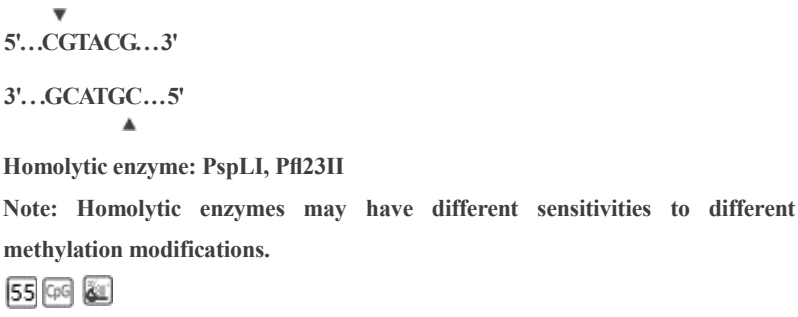
Definition of enzyme activity

1 active unit (U) refers to the amount of enzyme required to completely cleave 1μg p615 at 55℃ within 1 hour in a 50μL reaction system.

Quality control

1. Long term incubation detection: At the optimal reaction temperature, 10U BsiWI was incubated with 1μg p615 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 BsiWI 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.

Icon annotation



Homolytic enzyme: PspLI, Pfl23II

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. Inactivation condition: Incubate at 80℃ 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:

	DNA
ddH_2O	up to 50μL
10× Cut Buffer C	5μL
Substrate DNA	1μg
BsiWI(5U/μL)	1μL
Total	50μL

Note: The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergents, or high concentration salts, otherwise it will affect BsiWI 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 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 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	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	2	0	0	0	0	0	4

The influence of methylation modification

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