Tinzyme Co., Limited

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Nt.BbvCI

Product Number: RE05881

Shipping and Storage

Store at -20℃ for 2 years.

Components

Component	Specifications
Nt.BbvCI (5 U/μL)	30μL
10×FlashCut™ Buffer	1mL
10×FlashCut™ Color Buffer	1mL

Description

Nt.BbvCI is a type of endonuclease that cleaves only one strand of dsDNA substrate; Create incisions on the dsDNA substrate without incising the dsDNA. Nt.BbvCI is commonly used for nucleic acid isothermal amplification (such as SDA, RCA), where Nt.BbvCI generates DNA gaps that trigger polymerase chain displacement reactions, repeating cutting, displacement, and extension processes to achieve nucleic acid index amplification.

Nt.BbvCI in Universal FlashCut™ or FlashCut™ Color buffers have excellent activity. FlashCut™ Color Buffer includes red and yellow tracer dyes, which can be directly used for gel electrophoresis. FlashCut™ The migration rate of red dye of Color Buffer and 2500bp double stranded DNA fragment in 1% agarose gel is close to each other; The migration rate of yellow dye and 10bp double stranded DNA fragment in 1% agarose gel is similar.

Suggested reaction conditions

1. 1×FlashCut™ Buffer.
2. Incubate at 37℃.
3. Prepare the reaction system according to the "DNA rapid enzyme digestion process".

Inactivation conditions

Incubate at 80℃ for 20 minutes.

Definition of Activity

The active unit (U) refers to the amount of enzyme required to completely convert 1μg of supercoiled p615 DNA into an open-loop form within 1 hour at 37 ℃ in a 50μL reaction system.

Quality control

1. Functional activity testing: at 37℃, in 20μL of universal FlashCut™ In the reaction system, 5U Nt.BbvCI can convert 1μg p615 into an open-loop form within 15 minutes.
2. Ultra long incubation test: incubate 5U Nt. BbvCI and super spiral p615 DNA substrate at 37℃ for 16 h, and detect no change of open loop DNA by agarose gel electrophoresis.

Icon annotation

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1. The optimal reaction temperature is 37℃.
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.

Protocol

1. DNA rapid enzymatic 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×FlashCut™ Buffer or 10×FlashCut™ Color Buffer	5μL
Substrate DNA	1μg
Nb.BbvCI(10 U/μL)	1μL
Total	20μL

The DNA substrate should not contain phenol, chloroform, ethanol EDTA、 Detergent or high concentration salt, otherwise it will affect the activity of Nt.BbvCI enzyme;

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℃ for 30 minutes to 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.

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 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
7	3	0	0	0	0	2	9

The influence of methylation modification

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

Activity in different reaction buffers

	FlashCut™ Buffer	Thermo Scientific Fast Digest Buffer	NEB Cut Smart® Buffer	Takara Quick Cut™ Buffer
Reactivity	100%	100%	100%	100%

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