



## Nb.BbvCI

**Product Number: RE05879**

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### Shipping and Storage

Store at -20°C for 2 years.

### Components

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

### Description

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

Nb.BbvCI exhibits excellent activity in both general FlashCut™ and FlashCut™ Color Buffer. FlashCut™ Color Buffer includes red and yellow tracer dyes, which can be directly used for gel electrophoresis. The migration rate of red dye of FlashCut™ Color Buffer and 2500bp double stranded DNA fragment in 1% agarose gel is similar; 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°C.
3. Prepare the reaction system according to the "DNA rapid enzyme digestion process".

### Inactivation conditions

Incubate at 80°C 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°C in a 50μL reaction system.

### Quality control

1. Functional activity testing: at 37°C, in 20μL of universal FlashCut™ In the reaction system, 10U Nb.BbvCI can convert 1μg p615 into an open-loop form within 15 minutes.
2. Ultra long incubation test: incubate 10U Nb.BbvCI and super spiral p615 DNA substrate at 37°C for 16 h, and detect that the ring opening DNA has no change by agarose gel electrophoresis.




### Icon annotation

5'...CCTCAGC...3'

3'...GGAGTCG...5'





1.  The optimal reaction temperature is 37°C.
2.  For DNA methylated by EcoBI, splicing may be hindered.
3.  Inactivation condition: Incubate at 80°C 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 <sub>2</sub> O	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 Nb.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°C for 30 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.

### 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	no effect	no effect	Shear obstruction