Nt.BbvCI

REF: EG25520S



37 CpG EB 65

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
Nt.BbvCI (5 U/μl)	30 μl
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

Description

Nt.BbvCI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate. It creates nicks on dsDNA substrates without cutting through the dsDNA. Nt.BbvCI is commonly used in isothermal nucleic acid amplification (e.g., Strand Displacement Amplification [SDA], Rolling Circle Amplification [RCA]). DNA nicks generated by Nt.BbvCI trigger the strand displacement reaction of DNA polymerase, and the repeated processes of cleavage, displacement, and extension enable exponential nucleic acid amplification.

Nt.BbvCI shows superior activity in the universal CutOne® and CutOne® Color Buffer. CutOne® Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the CutOne® Color Buffer migrates with 2.5 kb double-strand DNA fragments in a 1% agarose gel, and the yellow dye migrates with 10 bp double-strand DNA fragments in a 1% agarose gel.

Recommended Reaction Conditions

1× CutOne® Buffer;
Incubate at 37℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 65℃ for 5 minutes.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to completely convert 1 μg of supercoiled p615 DNA into the open-circular form in a 50 μl reaction system at 37℃ for 1 hour.

Quality Control

Function

A 20 μl CutOne® reaction system containing 5 U of Nt.BbvCI can convert 1 μg of supercoiled plasmid DNA p615 into an open circular form at 37℃ for 15 minutes.

Prolonged Incubation

A 10 μl reaction containing 5 U of Nt.BbvCI and the supercoiled p615 DNA substrate were incubated at 37℃ for 16 hours, and no change in the open-circular DNA was detected by agarose gel electrophoresis.

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

CpG Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.

EB Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the EcoBI methylase.

65 The enzyme can be heat inactivated at by incubation 65℃ for 5 minutes.

Method of application

1. Protocol for DNA Digestion

① Combine the following reaction components on ice in the order indicated:

ddH_2O	up to 50 μl
10× CutOne® Buffer or 10× CutOne® Color Buffer	5 μl
DNA[a]	1 μg
Nt.BbvCI (5 U/μl)	1 μl
Total	50 μl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

② Mix gently and spin down.
③ Incubate at 37℃ for 15 min~3 h.
④ Optional: Inactivate the enzyme by heating at 65℃ for 5 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

2. Notice

① The volume of enzyme added to the reaction mixture should not exceed 10% of the total volume to avoid star activity caused by excessive glycerol in the enzyme storage buffer.
② The additives (e.g., glycerol, salt) in the enzyme storage buffer are the same as the contaminants in the substrate solution (e.g., salt, EDTA, or ethanol, etc.). Therefore, the smaller the reaction volume, the stronger the digestion inhibition effect.

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Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
7	3	0	0	0	0	2	9

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	No effect	No effect	Blocked

Activity in Different Buffers*

	CutOne® Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	100%	100%	100%	100%

*The activity data come from the functional test described above.

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