# Nt.BbvCI, Nicking Endonuclease

**Catalogue number:** MB09401, 1000 U
MB09402, 5000 U

## Description

Nt.BbvCI is a Nicking Endonuclease purified from an *Escherichia coli* strain that carries the BbvCI fragment from *Brevibacillus brevis* (*Bacillus brevis*). The enzyme cleaves only one strand of DNA on a double-stranded DNA substrate. Nt.BbvCI activity is blocked by some combinations of overlapping CpG methylation. The recognition sequence and site of cutting are indicated below:

5'...CC↓TCAGC...3'
3'...GG AGTCG...5'

## Storage temperature

Nt.BbvCI Nicking Endonuclease should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

## Unit definition

One unit is defined as the amount of this enzyme required to completely digest 1 µg of plasmid DNA in 50 µL of the reaction mixture at 37 °C for one hour.

## Enzyme concentration: 10 U/µL

## Inactivation

Nt.BbvCI is heat inactivated at 80 °C for 20 min.

## System components and Reaction conditions

Nt.BbvCI Nicking Endonuclease is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 37 °C.

## Standard protocol

The following standard protocol serves as a general guideline for DNA cleavage using Nt.BbvCI Nicking Endonuclease. Preferably the enzyme should be added last.

**1.** Prepare the following 20 µL reaction:

| Component | Volume |
|---|---|
| Substrate DNA | ≤ 1 µg |
| Nt.BbvCI reaction buffer (10x) | 2 µL |
| Nt.BbvCI Nicking Endonuclease | 1 µL |
| Nuclease-free $H_2O$ (Cat. No. MB11101) | up to 20 µL |

***Note:*** *It may be required to titrate the enzyme or test different incubation periods for more specific results or partial digestions.*

**2.** Gently mix and pulse.

**3.** Incubate at 37 °C for 1 hour.

**4.** If required, heat inactivate at 80 °C for 20 minutes.

## Quality control assays

### Purity

Nt.BbvCI Nicking Endonuclease is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

### Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 10 U of Nt.BvCI for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible degradation of the DNA.

### Functional assay

Nt.BbvCI is tested for performance in a digestion of 0.2-0.3 µg of a proprietary plasmid using 10 U of enzyme. The resulting digestion is visualized in an agarose gel as two sites are in close proximity allowing for strand separation after heating at 80 °C.

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*For research use only*

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