Nt.CviPII

NEW ENGLAND BioLabs Inc.
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R0626S 002121113111

R0626S

RR NEB4 37° 55°

100 units **5,000 U/ml** **Lot: 0021211**
RECOMBINANT **Store at –20°C** **Exp: 11/13**

Recognition Site:

5′...▼CCD...3′
3′...GGH...5′

Description: Nt.CviPII is a nicking endonuclease that cleaves only one strand of DNA on a double stranded DNA substrate. The final product on pUC19 is an array of bands from 25–200 bp. CCT is cut less efficiently than CCG and CCA. Some of the CCT sites are not cleaved.

Source: An *E. coli* strain that expresses a fusion of MxeGyrA intein, chitin-binding domain and a truncated form of the Nt.CviPII nicking endonuclease gene from Chlorella virus NYs-1.

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 8.0) and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4

Reaction Conditions: 1X NEBuffer 4
Incubate at 37°C.

1X NEBuffer 4:
20 mM Tris-acetate
10 mM magnesium acetate
50 mM potassium acetate
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pUC19 DNA resulting in a stable pattern of fragments between 25 and 200 bp in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 1 unit of enzyme incubated for 16 hours showed no degradation of DNA fragments.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	100%
NEBuffer 3	50%
NEBuffer 4	**100%**

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Survival in a Reaction: A minimum of 1.0 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 30 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Note: Tests have suggested that an exonuclease activity is an inherent part of the enzyme. Thus a one hour incubation time is thought to be the optimal time for most procedures.

To run on an electrophoresis gel, add loading dye to a final concentration of 0.4% SDS.

Not sensitive to *dam* or *dcm* methylation.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

References:

1. Song, Q. et al. (2010). *Anal. Chem.* [Epub ahead of print].
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