Nt.BstNBI

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R0607S 012120814081

R0607S

RR NEB3 55° Yes

1,000 units **10,000 U/ml** **Lot: 0121208**

RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′... GAGTCNNNN▼N...3′
3′... CTCAGNNNNN...5′

Description: Nt.BstNBI is a site specific endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate. The nicking endonuclease catalyzes a single strand break 4 bases beyond the 3′ side of the recognition sequence.

Source: An *E. coli* strain that carries the cloned Nt.BstNBI gene from *Bacillus stearothermophilus* 33M (Z. Chen)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3.
Incubate at 55°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg T7 DNA in 1 hour at 55°C in a total reaction volume of 50 µl.

The phage T7 DNA is a particularly convenient substrate for assaying the activity because of the proximity of GAGTC sequences. Nt.BstNBI cleavage of phage T7 DNA yields 5 bands; 13 kb, 9 kb, 7 kb, 6 kb and 4 kb.

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

Quality Control Assays

Ligation: After 10-fold overdigestion with Nt.BstNBI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 10 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 60 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	10%
NEBuffer 3	**100%**
NEBuffer 4	10%

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 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Plasmid Nicking: 10 units of enzyme are required to convert 1 µg of supercoiled pBR322 or pUC19 DNA to open circular form in 1 hour at 55°C.

Note: Incubation at 37°C results in 10% activity.

The nomenclature of this enzyme has been changed.

References:

1. Song, Q. et al. (2010). *Anal. Chem.* [Epub ahead of print].
2. Zhang, P. et al. (2010) *Protein Expr. Purif.* 69, 226–234. [Epub 2009 Sep 9].

U.S. Patent No. 6,191,267 B1

CERTIFICATE OF ANALYSIS

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