Nb.BtsI

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R0707S 0021207130713071

R0707S

1,000 units **10,000 U/ml** **Lot: 0021207**
RECOMBINANT **Store at –20°C** **Exp: 7/13**

Recognition Site:

5′... G C A G T G N N ... 3′
3′... C G T C A C N N ... 5′

Description: Nb.BtsI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate.

Source: An *E. coli* strain that expresses only the large subunit of the BtsI restriction gene from *Bacillus thermoglucosidasius* (X.Pan).

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4
100X BSA

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA.
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 convert 1 µg of supercoiled plasmid ϕX174 RF I DNA to open circular form 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 10 units of Nb.BtsI incubated for 16 hours at 37°C resulted in a pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of 100 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	100%
NEBuffer 3	75%
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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 10 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Note: Nb.BtsI is very efficient. Under most conditions a one to two hour incubation with 1 µl of enzyme and 1 µg of DNA is recommended.

Nb.BtsI is 100% active at 55°C.

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

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].

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