Nb.BsmI

1-800-632-7799
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R0706S 001120814081

R0706S

1,000 units **10,000 U/ml** **Lot: 0011208**
RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

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

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

Source: An *E. coli* strain that carries the cloned BsmI gene from *Bacillus stearothermophilus* NUB 36 (N. Welker)

Supplied in: 100 mM NaCl,10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3

Reaction Conditions: 1X NEBuffer 3.
Incubate at 65°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 convert 1 µg of supercoiled plasmid pBR322 DNA to open circular form in 1 hour at 65°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 enzyme incubated for 16 hours showed no degradation of DNA fragments. However, incubation of more than 10 units for 16 hours will result in some double-stranded cleavage at the BsmI site.

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

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: Use of Nb.BsmI in NEBuffer 1 and NEBuffer 4 may result in cleavage of both DNA strands. Incubation times of > 4 hours are not recommended.

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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