Nt.BspQI

1-800-632-7799
info@neb.com
www.neb.com

R0644S 001121113111

R0644S

1,000 units 10,000 U/ml Lot: 0011211

RECOMBINANT Store at –20°C Exp: 11/14

Recognition Site:

5′...GCTCTTCN▼...3′
3′...CGAGAAGN...5′

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

Source: An *E. coli* strain expressing an engineered BspQI variant from BspQI restriction enzyme

Supplied in: 10 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Reagents Supplied with Enzyme:
10X NEBuffer 3

Reaction Conditions: 1X NEBuffer 3.
Incubate at 50°C.

1X NEBuffer 3:
50 mM Tris-HCl
10 mM $MgCl_2$
100 mM NaCl
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 pUC19 DNA to open circular form in 1 hour at 50°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 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 100 units of enzyme incubated for 16 hours at 50°C showed no degradation of DNA fragments.

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

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	10%
NEBuffer 2	75%
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 nicking.

Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Note: Incubation at 37°C results in 80% activity.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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].

CERTIFICATE OF ANALYSIS

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