BspMI

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

R0502S 034120814081

R0502S

100 units **2,000 U/ml** **Lot: 0341208**

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

Recognition Site:

5′. . . A C C T G C $(N)_4$▼. . . 3′
3′. . . T G G A C G $(N)_8$▲. . . 5′

Source: An *E. coli* strain that carries the cloned BspMI gene from *Bacillus* species M (R. Morgan)

Supplied in: 500 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

BfuAI Is An Isoschizomer Of BspMI. It Cleaves Plasmid DNAs More Efficiently.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C.

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

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°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

Ligation: After 10-fold overdigestion with BspMI, > 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 2 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 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	NR
NEBuffer 2	NR
NEBuffer 3	**100%**
NEBuffer 4	NR

NEBuffers 1, 2 and 4 are **not** recommended (NR) due to star activity.

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

Notes: BfuAI is an isoschizomer of BspMI. Sites in some plasmid DNAs that are resistant to cleavage by BspMI may be cleaved by BfuAI .

When more than 8 units are incubated overnight star activity appears to cleave the sequence ACCTGT at a rate of 100–1,000-fold slower than ACCTGC.

BspMI requires two copies of its recognition sequence for cleavage to occur. Thus, the single BspMI sites in pBR322 and pUC18 and 19 are resistant to cleavage. A 100-fold overdigestion cuts less than half of the DNA. Some other plasmid DNAs are also resistant to BspMI cleavage.

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

Overnight digestion with this enzyme may result in star activity and is not recommended.

CERTIFICATE OF ANALYSIS

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