BstAPI

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

R0654S 003120814081

R0654S

200 units **Lot: 0031208** **Exp: 8/14**
RECOMBINANT **5,000 U/ml** **Store at –20°C**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned BstAPI gene from *Bacillus stearothermophilus* AP (S.K. Degtyarev)

Supplied in: 50 mM NaCl, 20 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 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 60°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
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 60°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.5 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with BstAPI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ 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 Assay: Incubation of a 50 µl reaction containing 50 units of BstAPI with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (20^5 cpm/µg) for 4 hours at 60°C released < 0.1% of the total radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	100%
NEBuffer 3	100%
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: 80°C for 20 minutes.

Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

Incubation at 37°C results in 10% activity.

CERTIFICATE OF ANALYSIS

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