BssHII

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

R0199S 028121014101

R0199S

500 units **4,000 U/ml** **Lot: 0281210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

5′. . . G▼C G C G C . . . 3′
3′. . . C G C G C▲G . . . 5′

Source: An *E. coli* strain that carries the cloned BssHII gene from *Bacillus stearothermophilus* H3 (N. Welker)

New Storage Conditions

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

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

Ligation: After 100-fold overdigestion with BssHII, > 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 200 units of enzyme incubated for 16 hours at 50°C resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 300 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at 50°C in 50 µl reaction buffer released < 0.2% radioactivity.

Endonuclease Activity: Incubation of 300 units of enzyme with 1 µg pBR322 for 4 hours at 50°C in 50 µl reaction buffer resulted in < 10% conversion to linear.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^\alpha$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
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.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Notes: BssHII produces ends that are compatible with AscI.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Incubation at 37°C results in 75% activity.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity.

Optimum pH is 7.0 at 25°C. Incubation is at 50°C under paraffin oil in a capped vial.

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent No. 5,786,195

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