BssSI

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

R0587S 010121014101

R0587S

NEB3 BSA 37° Yes

200 units **4,000 U/ml** **Lot: 0101210**
RECOMBINANT Store at –20°C **Exp: 10/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned BssSI gene from *Bacillus stearothermophilus* S719 (Z. Chen)

New Reaction Conditions

Supplied in: 300 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, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
pH 7.9 at 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 BssSI, > 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 50 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 < 0.05% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	50%
NEBuffer 3	**100%**
NEBuffer 4	10%

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.

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

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

NEBuffer 3 is required for optimal performance. When used in lower salt reaction buffers (NEB 1, 2 or 4) with more than 10 units/µg and an incubation time of more than 2 hours, star activity may result. Activity can be increased 50% by incubating at 60°C.

CERTIFICATE OF ANALYSIS

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