BstBI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

R0519S 0141206140 61

R0519S

2,500 units **20,000 U/ml** **Lot: 0141206**

RECOMBINANT **Store at –20°C** **Exp: 6/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned BstBI gene from *Bacillus stearothermophilus* B225 (Z. Chen)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Now Recombinant

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4. **Incubate at 65°C**.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
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 65°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Controls Assays

Ligation: After 20-fold overdigestion with BstBI, > 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 80 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 100 units for 4 hours at 65°C in 50 µl assay buffer with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 20 units of enzyme with 1 µg pUC19 plasmid DNA for 4 hours at 65°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	50%
NEBuffer 3	25%
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 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Notes: BstBI is an isoschizomer of FspII.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Incubation at 37°C results in 10% activity.

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

CERTIFICATE OF ANALYSIS

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