BssKI

1-800-632-7799
info@neb.com
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R0592S 0141202140 21

R0592S

BSA 60° dcm Yes

250 units **Lot: 0141202** **Exp: 2/14**

10,000 U/ml **Store at –20°C**

Recognition Site:

5′. . . ▼CCNGG. . . 3′
3′. . . GGNCC▲. . . 5′

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

Now Recombinant

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 7.6), 0.1 mM EDTA, 1 mM DTT, 200 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 60°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
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 60°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 DTT, 200 µg/ml BSA, and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with BssKI, > 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 36 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in one hour with one unit of enzyme.

Exonuclease Activity: Incubation of 20 units for 4 hours at 60°C in 50 µl assay buffer with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) released < 0.5% of the radioactivity.

Enzyme Properties

Activity in NEBuffers:

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

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: BssKI is an isoschizomer of ScrFI but leaves a 5-base 5′extension.

Blocked *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.

Incubation at 37°C results in 10% activity.

Companion Products:
dam⁻/dcm⁻ Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

CERTIFICATE OF ANALYSIS

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