BspEI

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

R0540S 006120914091

R0540S

1,000 units **10,000 U/ml** **Lot: 0061209**

RECOMBINANT **Store at –20°C** **Exp: 9/14**

Recognition Site:

5′. . . T▼CCGGA . . . 3′
3′. . . AGGCC▲T . . . 5′

Source: An *E. coli* strain that carries the cloned BspEI gene from *Bacillus* species (H. Kong)

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.

New Storage Conditions

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°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 of λ DNA (*dam*⁻) in 1 hour at 37°C in 50 µl of reaction buffer.

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 20-fold overdigestion with BspEI, > 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 100 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 ^3H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 30 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

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

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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(See other side)

CERTIFICATE OF ANALYSIS

Survival in a Reaction: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 1 unit.

Notes: BspEI is an isoschizomer of BspMII.

Blocked by overlapping *dam* methylation.

Cleavage of mammalian genomic DNA is impaired by CpG methylation.

Companion Products:

dam⁻/dcm⁻ Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

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

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