BsmI

NEW ENGLAND BioLabs Inc.
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R0134S 016121014101

R0134S

500 units **10,000 U/ml** **Lot: 0161210**
RECOMBINANT **Store at –20°C** **Exp: 10/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned BsmI gene from *Bacillus stearothermophilus* NUB 36 (N. Welker)

New Reaction Buffer

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.

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 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 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 Control Assays

Ligation: After 10-fold overdigestion with BsmI, > 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 70 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 65°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	100%
NEBuffer 3	75%
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: Intermediate activity. Suitable for extended digestion, but < 8 hours.

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

Incubation at 37°C results in 20% activity.

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

U.S. Patent No. 6,335,190

CERTIFICATE OF ANALYSIS

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Enzyme Properties
Activity in NEBuffers:
NEBuffer 1 75%
NEBuffer 2 100%
NEBuffer 3 75%
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.
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