

BbsI



1-800-632-7799
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R0539S 053121113111

R0539S



300 units Lot: 0331211 Exp: 11/13

5,000 U/ml Store at **-20°C** (see below)

Recognition Site:

5'... GAAGAC (N)₂... 3'

3'... CTTCTG (N)₆... 5'

Source: *Bacillus laterosporus* (C. Story)

More Units

**-70°C Storage Is Recommended For
Periods Greater Than 30 Days.
Avoid Freeze/Thaw Cycles.**

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Supplied in: 300 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 300 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2.

Reaction Conditions: 1X NEBuffer 2.
Incubate at 37°C.

1X NEBuffer 2:

50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
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 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).

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Quality Control Assays

Ligation: After 20-fold overdigestion with BbsI, > 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 λ 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 50 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 5 units of enzyme with 1 µg pUC19 plasmid DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

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Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 100%
NEBuffer 2 **100%**
NEBuffer 3 25%
NEBuffer 4 75%

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: 50 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: BbsI is an isoschizomer of BbvII.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation

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

CERTIFICATE OF ANALYSIS

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