

BsiWI



1-800-632-7799
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R0553S 031120314031

R0553S



300 units **10,000 U/ml** **Lot: 0311203**

RECOMBINANT **Store at -20°C** **Exp: 3/14**

Recognition Site:

5'... C[▼]G T A C G... 3'
3'... G C A T G[▲]C... 5'

Source: An *E. coli* strain that carries the cloned BsiWI gene from *Bacillus* species (D. Clark)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3.

Reaction Conditions: 1X NEBuffer 3.
Incubate at 55°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 MgCl₂
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 φX174 DNA in 1 hour at 55°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 BsiWI,
> 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 25 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 20 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 55°C in 50 µl reaction buffer released 0.1% radioactivity.

Endonuclease Activity: Incubation of 20 units of enzyme with 1 µg pUC19 plasmid DNA for 4 hours at 55°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	100%
NEBuffer 3	100%
NEBuffer 4	25%

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: Suitable for an extended or overnight digestion. Enzyme is active > 8 hours.

Heat Inactivation: 150 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: 3 units.

Notes: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Incubation at 37°C results in 50% activity.

Conditions of low ionic strength, high enzyme concentration, glycerol concentrations > 5% or pH > 8.0 may result in star activity.

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

CERTIFICATE OF ANALYSIS

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