

TspRI



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



R0582S 009120814081

R0582S



1,000 units **10,000 U/ml** **Lot: 0091208**
RECOMBINANT **Store at -20°C** **Exp: 8/14**

Recognition Site:

5'... NNC**A**STGNN... 3'
3'... NNGT**S**ACNN... 5'

Single Letter Code: S = C or G

Source: An *E. coli* strain that carries the cloned TspRI gene from *Thermus* species R (R.A.D. Williams)

New Storage Conditions

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Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA.
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 B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

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Quality Control Assays

Ligation: After 10-fold overdigestion with TspRI, > 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 (10⁵ cpm/µg) for 4 hours at 65°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1 25%
NEBuffer 2 50%
NEBuffer 3 25%
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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NEBuffer 3 25%
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: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 5 units, pBR322 = 2 units.

Notes: TspRI produces DNA fragments that have a 9-base 3' extension.

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

Incubation at 37°C results in 10% activity.

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

U.S. Patent No. 6,589,769

CERTIFICATE OF ANALYSIS

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