TfiI

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

R0546S 0181206140 61

R0546S

500 units **5,000 U/ml** **Lot: 0181206**

RECOMBINANT **Store at –20°C** **Exp: 6/14**

Recognition Site:

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

Single Letter Code: W = A or T

Source: An *E. coli* strain that carries the cloned TfiI gene from *Thermus filiformis* (D. Cowan, University College London)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 0.1 mM EDTA, 0.15% Triton X-100, 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 C
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg ml BSA and 50% glycerol (pH 7.4@ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with TfiI, > 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.

4-Hour Incubation: A 50 µl reaction containing 1 µg of ϕX174 DNA and 5 units of enzyme incubated for 4 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme (see note).

Exonuclease Activity: Incubation of 30 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	100%
NEBuffer 2	100%
NEBuffer 3	100%
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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Note: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

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

U.S. Patent No. 6,133,008

CERTIFICATE OF ANALYSIS

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4-Hour Incubation: A 50 µl reaction containing 1 µg of ϕX174 DNA and 5 units of enzyme incubated for 4 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme (see note).

Exonuclease Activity: Incubation of 30 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.

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