Tth111I

NEW ENGLAND BioLabs Inc.
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www.neb.com

R0185S 034120714071

R0185S

400 units **4,000 U/ml** **Lot: 0341207**
RECOMBINANT **Store at –20°C** **Exp: 7/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned Tth111I gene from *Thermus thermophilus* 111 (T. Oshima)

Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 7.4), 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 dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBC4 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 dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 4-fold overdigestion with Tth111I, approximately 25% 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 4 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.

Endonuclease Activity: Incubation of 2 units of enzyme with 1 µg pUC19 RF I DNA for 4 hours at 65°C in 50 µl reaction buffer resulted in < 20% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	**50%**
NEBuffer 2	25%
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 0.25 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: pBR322 = 2 units.

Notes: Tth111I produces DNA fragments that have a single-base 5′ extension which are more difficult to ligate than blunt-ended fragments.

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

Incubation at 37°C results in 10% activity.

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5% or pH > 8.0 may result in star activity. PflFI, an isoschizomer of Tth111I, does not exhibit star activity.

The activity in NEBuffer 1 is sensitive to pH. Slightly acidic pH conditions can cause a dramatic decrease in activity.

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

CERTIFICATE OF ANALYSIS

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