TliI

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

R0612S 00512061406l

R0612S

NEB3 BSA 75° No1

1,000 units **10,000 U/ml** **Lot: 0051206**

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

Recognition Site:

5′... C▼TCGAG...3′
3′... GAGCT▲C...5′

Source: An *E. coli* strain that carries the cloned TliI gene from *Thermococcus litoralis* (H. W. Jannasch)

Supplied in: 300 mM NaCl, 25 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA. **Incubate at 75°C.**

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 (HindIII digest) in 1 hour at **75°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 2-fold overdigestion with TliI, > 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.

Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at **75°C** in 50 µl reaction buffer released 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	25%
NEBuffer 2	25%
NEBuffer 3	**100%**
NEBuffer 4	10%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Heat Inactivation: No

Notes: TliI is a highly thermostable isoschizomer of XhoI.

Cleavage of mammalian genomic DNA is impaired by CpG methylation.

Incubations of longer than 2 hours are **not** recommended because of the **75°C** optimal incubation temperature.

Incubations at 37°C results in 10% activity.

CERTIFICATE OF ANALYSIS

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