Tth Endonuclease IV

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

M0294S 001140816081

M0294S

500 units **10,000 U/ml** **Lot: 0011408**
RECOMBINANT **Store at –20°** **Exp: 8/16**

Description: *Tth* Endonuclease IV is a thermostable apurinic/apyrimidinic (AP) endonuclease from *Thermus thermophilus*. *Tth* Endo IV will hydrolyze an AP site at the first phosphodiester bond 5' to the lesion leaving a 3' hydroxyl and a deoxyribose 5'-phosphate at the 5' terminus. The enzyme also has a 3'-diesterase activity.

Source: An *E. coli* strain that carries the cloned *Thermus thermophilus* endonuclease IV gene.

More Units

Applications:

- Alkaline elution (1)
- Alkaline unwinding (2)

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mm DTT, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X ThermoPol Reaction Buffer.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 65°C.

1X ThermoPol Reaction Buffer:
10 mM KCl
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
2 mM $MgSO_4$
0.1% Triton X-100
pH 8.8 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 65°C.

* An AP site is created by treating 10 pmol of a 60-mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Diluent Compatibility: Diluent D
100 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50 % glycerol.

Unit Assay Conditions: 1X ThermoPol Reaction Buffer containing 5 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 100 units of *Tth* Endonuclease IV incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 100 units of *Tth* Endonuclease IV in NEBuffer 1 with 1 µg of a mixture of single and double-stranded [^{3}H] *E.coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction containing 100 units of *Tth* Endonuclease IV with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Heat Inactivation: no.

Usage Note: Enzyme stability above 80°C is assured by adding $ZnCl_2$ to a final concentration of 25 µM in the reaction.

References:

1. Pflaum, M. et al. (1998) *Free Rad. Res.* 29, 585–594.
2. Hartwig, A. et al. (1996) *Toxicology Letters* 88, 85–90.

CERTIFICATE OF ANALYSIS

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