# *Tth* Endonuclease IV

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M0294S 0011311115111

# M0294S

**500 units** **10,000 U/ml** **Lot: 0011311**
**RECOMBINANT** **Store at –20°** **Exp: 11/15**

**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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