

## Therminator™ III DNA Polymerase



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



M0333S 005120914091

# M0333S



200 units 2,000 U/ml Lot: 0051209

RECOMBINANT Store at -20°C Exp: 9/14

**Description:** Therminator III DNA Polymerase is a 9°N™ DNA Polymerase variant with an enhanced ability to incorporate modified nucleotides such as 3'-amino-dNTPs, 3'-azido-dNTPs and other nucleotide analogs modified at the 3' ribose position.

**Source:** An *E. coli* strain that carries the 9°N (D141A / E143A / L408S / Y409A / P410V) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus species* 9°N-7.

Supplied in: 20 mM KHPO<sub>4</sub> (pH 6.5 @ 25°C), 350 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 50% glycerol.

### Applications:

- Incorporation of nucleotide analogs with 3'-OH substitutions.
- DNA sequencing using 3'-azido-ddNTP chain terminators.
- SNP analysis with 3'-azido-ddNTP chain terminators.
- DNA labeling with 3'-amino-ddNTP.

**Reagents Supplied with Enzyme:**  
10X ThermoPol™ Reaction Buffer.

**Reaction Conditions:** 1X ThermoPol Reaction Buffer, DNA template, primer, 200 μM dNTPs and 0.5–2 units of Therminator III DNA Polymerase in a total reaction volume of 100 μl.

### 1X ThermoPol Reaction Buffer:

20 mM Tris-HCl  
10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
10 mM KCl  
2 mM MgSO<sub>4</sub>  
0.1% Triton® X-100  
pH 8.8 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTPs into acid insoluble material in 30 minutes at 75°C.

**Unit Assay Conditions:** 1X ThermoPol Reaction Buffer, 200 μM dNTPs including [<sup>3</sup>H]-dTTP and 15 nM primed single-stranded M13mp18.

**Heat Inactivation:** No

### Enzyme Properties:

3'→5' Exonuclease: No  
5'→3' Exonuclease: No  
Strand Displacement: Yes

**Molecular Weight:** 90,000 Daltons

### Quality Control Assays

**Exonuclease Activity:** Incubation of a 50 μl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Therminator III DNA Polymerase with 1 μg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA for 4 hours at either 37°C or 75°C releases < 0.1% of the total radioactivity.'

**Endonuclease Activity:** Incubation of a 50 μl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of Therminator III DNA Polymerase with 1 μg of supercoiled φX174 DNA for 4 hours at either 37°C or 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

### References:

1. Gardner, A.F. and Jack, W.E. (1999) *Nucleic Acids Research* 27, 2545–2555.
2. Gardner, A.F. and Jack, W.E. (2002) *Nucleic Acids Research* 30, 605–613.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

**Companion Products Sold Separately:**

Magnesium Sulfate (MgSO<sub>4</sub>) Solution  
#B1003S 6.0 ml

ThermoPol Reaction Buffer Pack  
#B9004S 6.0 ml

ThermoPol II (Mg-free) Reaction Buffer Pack  
#B9005S 6.0 ml

ThermoPol DF (Detergent-free) Reaction Buffer Pack  
#B9013S 6.0 ml

Deoxynucleotide Solution Set  
#N0446S 25 µmol each

Deoxynucleotide Solution Mix  
#N0447S 8 µmol each  
#N0447L 40 µmol each

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ThermoPol DF (Detergent-free) Reaction Buffer Pack  
#B9013S 6.0 ml

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#N0446S 25 µmol each

Deoxynucleotide Solution Mix  
#N0447S 8 µmol each  
#N0447L 40 µmol each

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