

Therminator™ II DNA Polymerase



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



M0266S 003120914091

M0266S



200 units **2,000 U/ml** **Lot: 0031209**
RECOMBINANT **Store at -20°C** **Exp: 9/14**

Description: Therminator II DNA Polymerase is a 9°N™ DNA Polymerase variant with an enhanced ability to incorporate modified substrates such as dideoxynucleotides, ribonucleotides and acyclonucleotides (1,2).

Therminator II DNA Polymerase was derived from Therminator DNA Polymerase and differs in having one additional amino acid change. This change allows more efficient incorporation of ribonucleotides (1) and nucleotides with modified 3' functional groups (3).

Source: An *E. coli* strain that carries the 9°N (D141A / E143A / Y409V / A485L) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus species* 9°N-7.

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Applications:

- DNA sequencing by partial ribosubstitution (4)
- DNA sequencing using dideoxy (5) or acyclo (6) chain terminators
- SNP analysis with dideoxy or acyclo chain terminators (7)

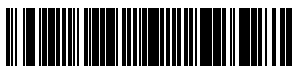
Reagents Supplied with Enzyme:
10X ThermoPol™ Reaction Buffer.

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

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1X ThermoPol Reaction Buffer:

20 mM Tris-HCl
10 mM (NH₄)₂SO₄
10 mM KCl
2 mM MgSO₄
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 dNTP into acid insoluble material in 30 minutes at 75°C.

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

Heat Inactivation: No

Quality Control Assays

Exonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 8 units of Therminator II DNA Polymerase and 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA for 4 hours at 75°C releases < 0.1% of the total radioactivity.

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Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 8 units Therminator II DNA Polymerase with 1 µg of supercoiled φX174 DNA for 4 hours at 37°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.
3. Ruparel, H. et al.(2005) *Proc. Natl. Acad. Sci. USA*, 26, 5932–5937.
4. Barnes, W. F. (1978) *J. Mol. Biol.* 119, 83
5. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA*, 74, 5463–5467.
6. Trainor, G. L. (1996) U.S. Patent # 5, 558, 991.
7. Haff, L. A. and Simirnov, I. P. (1997) *Genome Methods* 7, 378–388.

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CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Companion Products Sold Separately:

Magnesium Sulfate (MgSO₄) Solution
#B1003S 6.0 ml

Diluent E
#B8005S 4.0 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

Acyclonucleotide Set
#N0460S 0.5 µmol each

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Companion Products Sold Separately:

Magnesium Sulfate (MgSO₄) Solution
#B1003S 6.0 ml

Diluent E
#B8005S 4.0 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

Acyclonucleotide Set
#N0460S 0.5 µmol each

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