Therminator™ DNA Polymerase

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

M0261S 018120914091

M0261S

200 units **2,000 U/ml** **Lot: 0181209**

RECOMBINANT Store at –20°C Exp: 9/14

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

Source: An *E. coli* strain that carries the 9°N (D141A / E143A / 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 (3)
- DNA sequencing using dideoxy (4) or acyclo (5) chain terminators
- SNP analysis with dideoxy or acyclo chain terminators (6)

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 DNA polymerase in a total reaction volume of 100 µl.

1X ThermoPol Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
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 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 [^{3}H]-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 20 units of Therminator DNA Polymerase and 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 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 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. Barnes, W. F. (1978) *J. Mol. Biol.* 119, 83–99.
4. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA,* 74, 5463–5467.
5. Trainor, G. L. (1996) U.S. Patent # 5, 558, 991.
6. Haff, L. A. and Simirnov, I. P. (1997) *Genome Methods* 7, 378–388.

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) 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

(see other side)

CERTIFICATE OF ANALYSIS

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Diluent E
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ThermoPol Reaction Buffer Pack
#B9004S 6.0 ml

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

(see other side)

CERTIFICATE OF ANALYSIS

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

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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