DNA Polymerase I (*E. coli*)

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

M0209S 0911303150310

M0209S

500 units **10,000 U/ml** **Lot: 0911303**
RECOMBINANT **Store at –20°C** **Exp: 3/15**

Description: DNA Polymerase I (*E coli*) is a DNA-dependent DNA polymerase with inherent 3´→ 5´ and 5´→ 3´ exonuclease activities (1). The 5´→ 3´ exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick-translation.

Source: An *E. coli* strain that carries an overexpressed copy of the *polA* gene.

Applications:

- Nick translation of DNA to obtain probes with a high specific activity (2)
- Second strand synthesis of cDNA (3,4)

Supplied in: 25 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2.

Reaction Conditions: 1X NEBuffer 2. Supplement with dNTPs (not included). Incubate at 37°C.

DNA Polymerase I (*E. coli*) is active in all four NEBuffers when supplemented with dNTPs.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 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 37°C.

Unit Assay Conditions: 1X NEBuffer 2, 33 µM dNTPs including [^{3}H]-dTTP and 70 µg/ml denatured herring sperm DNA.

Molecular Weight: 109,000 daltons.

Heat Inactivation: 75°C for 20 minutes.

Quality Control Assays

Endonuclease Activity: Incubation of a 50 µl reaction in NEBuffer 2 containing a minimum of 25 units of DNA Polymerase I (*E. coli*) 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.

Notes On Use: DNase I is **not** included with this enzyme and must be added for nick translation reactions.

References:

1. Lehman, I.R. (1981). In P.D. Boyer (Ed.), *The Enzymes* Vol. 14A, (pp. 16–38). San Diego: Academic Press.
2. Meinkoth, J. and Wahl, G.M. (1987) *Methods Enzymology* 152, 91–94.
3. Gubler, U. and Hoffmann, B.J. (1983) *Gene* 25, 263–269.
4. D'Alessio, J.M. and Gerard, G.F. (1988) *Nucleic Acids Res.* 16, 1999–2014.

Companion Products Sold Separately:

NEBuffer 2
#B7002S 6.0 ml

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

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

CERTIFICATE OF ANALYSIS

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