M0209S

500 units 10,000 U/ml Lot: 0911309
RECOMBINANT Store at −20°C Exp: 9/15

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)

Molecular Weight: 103,000 daltons.

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:


DNA Polymerase I (E. coli)

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 MgCl2
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 [3H]-dTTP and 70 µg/ml denatured herring sperm DNA.

Molecular Weight: 103,000 daltons.

Heat Inactivation: 75°C for 20 minutes.

Quality Control Assays

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

References:

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

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