DNA Polymerase I, Large (Klenow) Fragment

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

M0210S 088120914091

M0210S

RR NEB2 Yes

200 units	**5,000 U/ml**	**Lot: 0881209**
RECOMBINANT	**Store at –20°C**	**Exp: 9/14**

Description: DNA Polymerase I, Large (Klenow) Fragment is a proteolytic product of *E. coli* DNA Polymerase I which retains polymerization and 3´→ 5´ exonuclease activity, but has lost 5´→ 3´ exonuclease activity (1). Klenow retains the polymerization fidelity of the holoenzyme without degrading 5´ termini.

Source: Purified from a strain of *E. coli* that carries the DNA Polymerase I, Large (Klenow) Fragment gene.

Applications:

- DNA sequencing by the Sanger dideoxy method (2)
- Fill-in of 5´ overhangs to form blunt ends (3)
- Removal of 3´ overhangs to form blunt ends (3)
- Second strand cDNA synthesis
- Second strand synthesis in mutagenesis protocols (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).

Klenow Fragment is also active in all four NEBuffers and T4 DNA Ligase Reaction Buffer 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.

DNA Sequencing: When this preparation is used to sequence DNA using the dideoxy method of Sanger et al., 1 unit/5 µl reaction volume is recommended.

Molecular Weight: 68,000 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 50 units of DNA Polymerase I, Large (Klenow)Fragment 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: Protocol for blunting ends by 3´ overhang removal and fill-in of 3´ recessed (5´ overhang) end: DNA should be dissolved in 1X NEBuffer 1–4 or T4 DNA Ligase Reaction Buffer supplemented with 33 µM each dNTP. Add 1 unit Klenow per microgram DNA and incubate 15 minutes at 25°C. Stop reaction by adding EDTA to a final concentration of 10 mM and heating at 75°C for 20 minutes.

CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

(see other side)

CERTIFICATE OF ANALYSIS

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References:

1. Jacobsen, H., Klenow, H. and Overgaard-Hansen, K. (1974) *Eur. J. Biochem.* 45, 623–627.
2. Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
3. Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 5.40–5.43). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
4. Gubler, U. (1987). In S.L. Berger and A.R. Kimmel (Eds.), *Methods in Enzymology,* Vol.152, (pp. 330–335). San Diego: Academic Press.

Companion Products Sold Separately:

NEBuffer 2
#B7002S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol of each

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

References:

1. Jacobsen, H., Klenow, H. and Overgaard-Hansen, K. (1974) *Eur. J. Biochem.* 45, 623–627.
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