

Protein : *E.coli* DNA Polymerase I
Large (Klenow) Fragment, 3'-5' exonuclease (-)

Lot # : 080806HCBM

Concentration : 200,000 units/ml ; 13.48 mg/ml

Package format : 20 μ l = 4000 units enzyme. Also included are 1.0 ml dilution buffer and 2.0ml 10X reaction buffer.

**10X Reaction
Buffer:**

500mM KPO₄ pH 7.0
60mM MgCl₂
50mM 2-Mercaptoethanol

**1X Dilution
Buffer:**

50mM KPO₄ pH 7.0
100mM KCl
1mM DTT
50% v/v Glycerol

Protein Uses : Dideoxy sequencing (2), blunt ending restriction fragments, second strand cDNA synthesis for labeling and for use in mutagenesis protocols (3). The enzyme is extensively utilized for labeling of DNA with nucleotide analogs for use as microarray probes (4).

Description : This protein is an N-terminal truncation of *E.Coli* DNA Polymerase I at amino acid #323 . In this construct, the domain containing the 5'→3' exonuclease activity is deleted, leaving the polymerase function intact. In addition two mutations are introduced (D355A and E357A) which abolish the 3'→5' exonuclease activity (1).

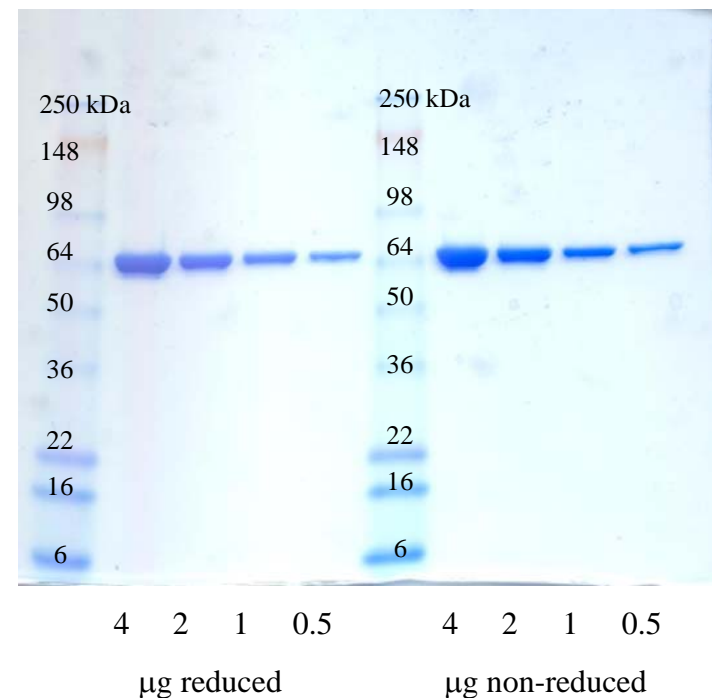
Properties : The enzyme features strand displacement capability , an error rate of @ 100x10⁻⁶ bases and has no intrinsic exonuclease activity.



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Quality Assessment	Assay	Result
ds endonuclease	Degrade ΦX174 RF	None Detected
ss endonuclease	Degrade M13mp18	None Detected
5' ss+ds exonuclease	Removal of labeled nucleotide from 5' end of a ss or ds oligonucleotide	None Detected
3' ss+ds exonuclease	Removal of labeled nucleotide from 3' end of a ss or ds oligonucleotide	None Detected
unit functional assay	1 unit incorporates 10nMol ³² P dTTP into acid insoluble material in 30 minutes at 37°C using poly dA/dT as substrate	Pass

1. Derbyshire, V. et al. (1988) *Science*, 240, 199-201.
2. Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. USA*, 74, 5463-5467.
3. Gubler, U. (1987) S.L. Berger and A.R. Kimmel (Eds.), *Methods in Enzymology*, 152, pp. 330-335. San Diego: Academic Press.
4. Joseph Gilbert, Jeremy Hasseman, Robin Cline, Kathy Munoz, Jon Hnath *Microbial Genomic DNA aminoallyl labeling for microarrays. S.O.P. from The Institute for Genomic Research (TIGR).*

