

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

Lot # : 080806BM

Concentration : 40,000 units/ml ; 2.93 mg/ml

Package format : 50µl = 2000 units enzyme. Also included are 1.0 ml dilution buffer and 1.0ml 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).

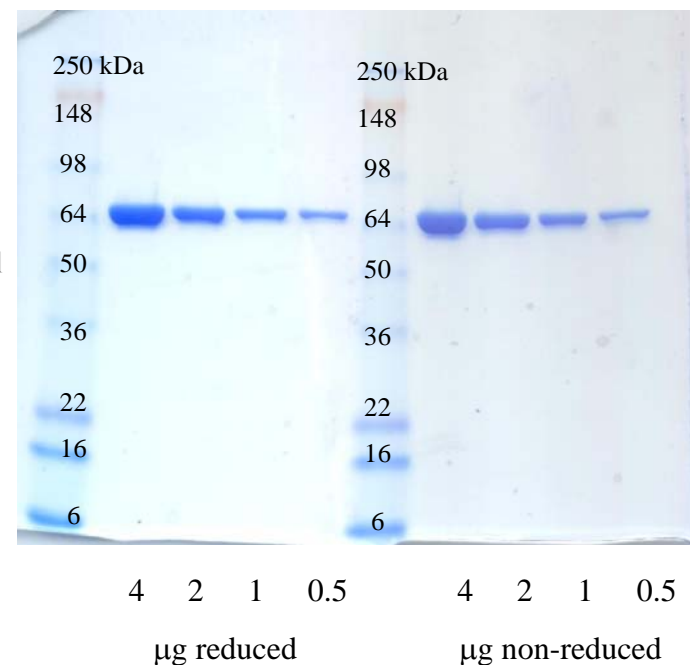
Properties : The enzyme features strand displacement capability , an error rate of @ 100x10⁻⁶ bases and has no intrinsic exonuclease activity.



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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 ³²P 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).*

