Product Information	
DNA Polymerase I	
Part Number	P7050L
Concentration	10,000 U/mL
Unit Size	5,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: DNA Polymerase I is a mesophilic DNA polymerase that exhibits 5'-3' DNA synthesis in addition to both 3'→5' and 5'→3' exonuclease activities. The combination of DNA synthesis and 5'→3' nuclease characteristics enable nick-translation during DNA synthesis.

Product Specifications						
P7050						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**200**	**200**	**200**	**200**
Specification	>99%	6,850 U/mg	Functional	Functional	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying the *PolA* gene.

Unit Definition: 1 unit is defined as the amount of polymerase required to convert 10 nmol of dNTPs into acid insoluble material in 30 minutes at 37°C.

Molecular weight: 103,118 Daltons

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X Blue Reaction Buffer and added to 50 µL reactions containing Calf Thymus DNA, 1X Blue Reaction Buffer, ^{3}H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E.coli* 16S rDNA Contamination** is evaluated using 5 µL **r**eplicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in: 25mM Tris-HCl, 1mM DTT, 0.1mM EDTA, 50% glycerol (pH 7.4 at 25ºC).
Supplied with:
10X Blue Buffer (B0110): 500mM NaCl, 100mM Tris-HCl, 100mM $MgCl_2$, 10mM DTT (pH 7.9 at 25ºC).

Usage Instructions:

Filling-in of 5´-overhang (1)

1. Set up the following reaction mixture in a total volume of 50 µl:
   - 1–5 µg digested DNA containing 5´-overhangs
   - 5 µL Buffer 10x Blue
   - 5 µL dNTP mixture (1 mM) (0.1 mM final)
   - 0.25 µL DNA Polymerase I (2.5 units)
   - Nuclease-free water up to 50 µL
2. Incubate 1 hour at 12°C
3. Stop the reaction by heating for 10 minutes at 68°C.

Blunt-Ending of 3´-Overhang (1)

The 3'→ 5' exonuclease activity of DNA Polymerase degrades the 3´-overhang from 3' to 5' direction. The removal of additional nucleotides may occur generating a 5´-protruding end). After addition of dNTP's repair of the 5'-protruding end is performed to generate a blunt end.

1. Set up the following reaction mixture in a total volume of 20 µl:
   - 1–5 µg digested DNA containing 3´-overhangs
   - 2 µL Buffer 10x Blue
   - 0.25 µL DNA Polymerase I (2.5 units)
   - Nuclease-free water up to 20 µL
2. Incubate 10 minutes at 12°C
3. Add 5 µL dNTP (1 mM) mixture
4. Incubate 1 hour at 12°C
5. Stop the reaction by heating for 10 minutes at 68°C.

Heat at 68°C for 10 minutes to inactivate the enzyme.

Nick Translation

Reference:

1. Perbal, B. (1988) A Practical Guide to Molecular Cloning, 2nd ed., John Wiley and Sons.