Product Information	
T4 DNA Polymerase	
Part Number	P7080L
Concentration	3,000 U/mL
Unit Size	2,000 U
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: T4 DNA Polymerase catalyzes the extension of a primed DNA template in the 5'→3' direction. This enzyme exhibits a powerful 3'→5' exonuclease activity, while lacking any inherent 5'→3' exonuclease or strand displacement functions.

Product Specifications						
P7080						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**30**	**30**	**30**	**30**
Specification	>99%	5,555 U/mg	Functional	Functional	No Conversion	<10 copies

Source of Protein: Purified from a strain of *E. coli* that expresses the recombinant T4 DNA Polymerase gene.

Unit Definition: 1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-precipitable material in 30 minutes at 37°C (1).

Molecular weight: 103,609 Daltons

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer and added to 50 µL reactions containing Calf Thymus DNA, 1X Blue Buffer, ^{3}H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (Molecular Cloning, v3, 2001, pp. A8.25-A8.26).

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: 100 mM KPO_4,1mM DTT, 0.1mM EDTA, 50% glycerol (pH 6.5 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 and converting 3'-Overhang to a blunt-end (2, 3)

1. Set up the following reaction mixture in a total volume of 50 µl:
   - 5µl Buffer 10x Blue
   - 5µl dNTP mixture (1 mM) to a final concentration of 100 µM
   - 1–5µg digested DNA containing 5´ or 3'-overhangs
   - 1 Unit T4 Polymerase per microgram DNA
   - Nuclease-free water up to 50 µl
2. Incubate at 12°C for 15 minutes.
3. Stop the reaction by adding EDTA to 10 mM final concentration and inactivate the enzyme by heating for 20 minutes at 75°C.

References:

1. Panet, A., van de Sande, J.H., Loewen, P.C. and Khorana, H.G. (1973) Biochemistry, 12, 5045-5050.
2. Ausubel, F.M., et al., Current Protocols in Molecular Biology, vol. 1, John Wiley & Sons, Inc., Brooklyn, New York, 3.5.11-3.5.12, 1994-2004.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, (2nd Ed.), 5.44-5.47.

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.