Product Information	
T7 DNA Polymerase	
Part Number	P7260L
Concentration	10,000 U/mL
Unit Size	3,500 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T7 DNA Polymerase is the mesophilic, highly processive, replicative DNA polymerase from bacteriophage T7 that is responsible for the rapid and accurate replication of the virus' genome during its infection cycle. T7 DNA Polymerase is a two-subunit protein, consisting of a polymerase domain (gene 5 from the T7 bacteriophage) and a processivity factor (*E. coli* trxA gene thioredoxin) (1,2). The enzyme possesses a powerful (3'→5') exonuclease activity that acts on both single and double stranded DNA and appears to be responsible for the high fidelity of this enzyme and prevents strand displacement synthesis (3,4,5).

Product Specifications						
P7260						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**100**	**100**	**100**	**100**
Specification	>99%	13,333 U/mg	Functional	Functional	No Conversion	<10 copies

Source of Protein: Recombinant *E. coli* strains carrying the bacteriophage T7 gene 5 and *E. coli* trxA gene.

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

Molecular weight: 92.1 kDa

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 T7 DNA Polymerase Unit Characterization Buffer (20 mM Tris-HCl, 100 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol), ^{3}H-dTTP and 150 µM dNTPs. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (6).

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:
50 mM KPO_4, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.0 at 25°C)

Supplied with:
10X T7 DNA Polymerase Buffer (B7260): 400 mM Tris-HCl, 200 mM $MgCl_2$, 500 mM NaCl (pH 7.5 at 25°C)

Usage Instructions:
The following workflow can be applied to either the production of a second DNA strand following first-strand synthesis off an RNA template, or to a site-directed mutagenesis experiment where the mutagenic primer is extended such that the entire template strand is copied.

1. In a sterile reaction vessel, set up the following reaction mixture in a total volume of 40 µL:

Components	Final Concentration	Volume
Nuclease Free Water	N/A	13.5 µL
Pre-annealed/template	20 nmol complex	20 µL
10X DNA Pol Buffer (B7260)	1X	4 µL
25 mM dNTP solution	300 µM	0.5 µL
T7 DNA polymerase (P7260L)	500 U/mL	2 µL
	Total Volume =	40 µL

2. Incubate 60 minutes at 37°C. Incubate 10 minutes at 70°C to stop the reaction.

Notes:
This enzyme is not suitable for DNA sequencing.

References:
1. Grippo, P. et al. (1971) J. Biol. Chem., 246, 6867-6873.
2. Modrich, P. et al. (1975) J. Biol. Chem., 250, 5515-5522.
3. Adler, S. et al. (1979) J. Biol. Chem., 254, 11605-11614.
4. Hori, K. et al. (1979) J. Biol. Chem., 254, 11598-11604.
5. Lechner, R. L. et al. (1983) J. Biol. Chem., 258, 11185-11196.
6. Sambrook, J. et al. (1989) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., (2nd ed.), 5.40-5.43.

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