Product Information	
T7 RNA Polymerase	
Part Number	P7180L
Concentration	50,000 U/mL
Unit Size	50,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: T7 RNA Polymerase is a DNA-dependent RNA polymerase with high specificity for the T7 promoter. After promoter initiation, it catalyzes the Mg^{2+} dependent synthesis of RNA from rNTPs (1,2).

Product Specifications							
P7180							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNAse**
Units Tested	**n/a**	**n/a**	**500**	**500**	**500**	**500**	**500**
Specification	>99%	312,500 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies	No detectable non-specific RNAse

Source of Protein: Purified from a strain of *E. coli* that expresses the recombinant T7 RNA Polymerase gene.

Unit Definition: 1 unit is defined as the amount of enzyme that will incorporate 1 nmol of ATP into acid-precipitable material in 1 hour at 37°C.

Molecular weight: 98.9 kDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 50% glycerol containing T7 RNA Polymerase storage solution and added to 50 µL reactions containing a T7 promoter-containing plasmid DNA, 1X T7 RNA Polymerase Buffer, ^{3}H-ATP and 400 µM each ATP, GTP, CTP and UTP. Reactions were incubated 10 minutes at 37°C, plunged on ice, and analyzed using the method of Sambrook and Russell (3).

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.

Non-Specific RNAse contamination is assessed using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

Supplied in:
50 mM Tris-HCl, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 50% glycerol (pH 7.9 at 25°C)

Supplied with:
10X T7 RNA Polymerase Buffer (B7180): 400 mM Tris-HCl, 60 mM $MgCl_2$, 100 mM DTT, 20 mM Spermidine (pH 7.9 at 25°C)

Usage Instructions: Synthesis of non-labeled RNA

1. Set up the following reaction mixture in a total volume of 20 µL:

Components	Final Concentration
Nuclease Free Water	N/A
10X Reaction Buffer (B7180)	1X
rNTP	500 µM each
Template DNA	0.2-1 µg
T7 RNA polymerase (P7180L)	100 U

2. Incubate reaction mixture at 37°C for 1 hour.

References:
1. Chamberlin, M. et al. (1973) J. Biol. Chem., 248, 2235-2244, 2245-2250.
2. Chamberlin, M. et al. (1982) in The Enzymes, 3rd edition, ed. P. D. Boyer (Academic Press, New York), 15, 87-108.
3. Sambrook, J. et al. (1989) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., (2nd ed.), 5.40-5.43.

Disclaimer:
Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.