



Product Information	
T7 RNA Polymerase	
Part Number:	P718L
Lot Number:	

Rev. B

Product Description:

T7 RNA Polymerase is a DNA-dependent RNA polymerase derived from the T7 bacteriophage which exhibits a high recognition specificity to the T7 promoter and terminator sequences and catalyzes the 5'→3' synthesis of RNA starting at a T7 promoter sequence (1,2).

Source of Protein

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

Supplied in

50 mM Tris-HCl
100 mM NaCl
1 mM dithiothreitol
1 mM EDTA
50% glycerol
0.1% Triton X-100
pH 7.9 @ 25°C

Supplied With

B718 (10X T7 RNA Polymerase Buffer)

10X T7 RNA Polymerase Buffer (B718)

400 mM Tris-HCl
60 mM MgCl₂
100 mM Dithiothreitol
20 mM Spermidine
pH 7.9 @ 25°C

Unit Definition

One 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.

Product Specification*

Unit Size:	50,000 Units
Unit Concentration	50,000 U/mL
Protein Concentration	0.16 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity	312,500 U/mg
SS Exonuclease	500 U <0.1% released
DS Exonuclease	500 U <0.1% released
Endonuclease	500 U <10% converted
RNAse Contamination	500 U = none detected
<i>E. coli</i> 16S rDNA Contamination	500 U <10 copies
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a glycerol (50%) containing T7 RNA Polymerase storage solution ([T7 RNA Polymerase]_f = 64–0.5 μg/μL) and added to 50 μL reactions containing 2 μg T7 promoter-containing plasmid DNA, 1X T7 RNA Polymerase Buffer, 2 μCi/mL α-³²P-ATP and 400 μM NTPs. 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 (OD₂₈₀) Measurement

A 3.0 μL sample of enzyme was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 138,830 and molecular weight of 98,855 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 μL of enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 μL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Endonuclease Activity

A 50 μ L reaction containing 1 μ g of pBR322 DNA and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

Single-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

RNase Contamination

A reaction containing 1 μ g of an RNA ladder substrate and 10 μ L of enzyme solution incubated for 2 hours at 37°C resulted in no detectable substrate degradation as measured by agarose gel electrophoresis.

***E. coli* 16S rDNA Contamination Test**

Replicate 5 μ L samples of enzyme solution were 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. The acceptance criterion for the test is the threshold cycle count (C_t) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control C_t values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

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.



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. MSDS sheets relevant to this product are available upon request.