Product Information Sheet *P7180L Rev C*

Product Information	
T7 RNA Polymerase	
Part Number	P7180L 280362
Concentration	50,000 U/mL
Unit Size	50,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>99%
Specific Activity	312,500 U/mg
SS Exonuclease	500 U <1.0% released
DS Exonuclease	500 U <1.0% released
DS Endonuclease	500 U = No conversion
RNAse Contamination	500 U = No Detectable non-specific RNase
***E.coli* DNA Contamination**	500 U <10 copies

Product Description:

T7 RNA Polymerase is a DNA-dependent RNA polymerase having high specificity for the T7 promoter. After promoter initiation, it catalyzes the Mg2+ dependent synthesis of RNA from rNTPs.

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 DTT
0.1 mM EDTA
50% glycerol
0.1% Triton X-100
pH 7.9 @ 25°C

Supplied With

B7180 (10X T7 RNA Polymerase Buffer)

10X T7 RNA Polymerase Buffer (B7180):
400 mM Tris-HCl
60 mM $MgCl_2$
100 mM DTT
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.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was 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 (*Molecular Cloning, v3, 2001, pp. A8.25-A8.26*)

Protein Concentration (OD_{280}) Measurement

A 2.0 µL sample of enzyme was analyzed at OD_{280} 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.

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.

Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 10,000 cpm of a radiolabeled

(additional information on reverse side)

single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity
A 50 µL reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in less than 1.0% release of TCA-soluble counts.

Double-Stranded Endonuclease Activity
A 50 µL reaction containing 0.5 µg of pBR322 DNA and 10 µL 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.

***E.coli* 16S rDNA Contamination Test**
Replicate 5 µL 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.

Non-Specific RNAse Assay
Replicate 10 uL samples were screened for non-specific RNAse contamination using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines

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.

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