



Product Information	
T7 DNA Polymerase	
Part Number:	P726L
Lot Number:	

Certificate of Analysis

2010-P726-Rev.B

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* *trx*A gene thioredoxin) (1,2). The enzyme possesses a powerful (3'→5') nuclease 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).

Source of Protein

A recombinant *E. coli* strain carrying the bacteriophage T7 gene 5.

Supplied in

50 mM KPO₄
0.1 mM EDTA
1.0 mM dithiothreitol
50% glycerol
pH 7.0 @ 25°C

Supplied With

B726 (10X T7 DNA Polymerase Buffer)

10X T7 DNA Polymerase Buffer (B726)

400 mM Tris-HCl
200 mM MgCl₂
500 mM NaCl
pH 7.5 @ 25°C

Product Specification*

Unit Size:	3,500 Units
Unit Concentration	10,000 U/mL
Protein Concentration	0.75 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity (est.)	13,333 U/mg
SS Exonuclease	Functional
DS Exonuclease	Functional
Endonuclease	100 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	100 U <10 copies
Storage	-20°C

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

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.

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer ([T7 DNA Pol]_i = 0.009-0.0007 μg/μL) and added to 50 μL reactions containing 40 μg Calf Thymus DNA, 1X T7 DNA Polymerase Reaction Buffer, 4mCi/mL ³H-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 (*Molecular Cloning*, v3, 2001, pp. A8.25-A8.26).

SDS-Page (Physical Purity Assessment)

2.0 μL of concentrated 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.

Protein Concentration (OD₂₈₀) Measurement

A 2.0 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 157,180 and molecular weight of 92,104 Daltons. Acceptance for this assay is +/- 5% of reference sample.

Functional Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 11,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in greater than 50% 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 greater than 50% release of TCA-soluble counts.

Nuclease Contamination Tests:

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 heat denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E. coli* genomic DNA using primers for 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 no template control C_t values, the detection limit of this assay is <10 copies genome/sample.

Notes:

This enzyme is not suitable for DNA sequencing.

Usage Protocol:

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.

In a sterile reaction vessel, combine the following components:

Amount	Description	Final Concentration
20 µL	Pre-annealed primer/template	20 nmol complex
4 µL	10X T7 DNA Pol. Buffer	1X
0.5 µL	25 mM dNTP Solution (N205L)	300 µM
2 µL	T7 DNA Polymerase (P726L)	500 U/mL
2 µL	T4 DNA Ligase (L603-LC-L)	2,400 U/ mL
10 µL	Type I Water	N/A
40 µL	Total Volume	

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

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.



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.