



Product Information	
Φ29 DNA Polymerase	
Part Number:	P702L
Lot Number:	

Certificate of Analysis

2010-P702-Rev.E

Product Description:

Φ29 DNA Polymerase responsible for the replication of the *Bacillus Subtilis* phage Φ29 (1). The enzyme is a highly processive DNA polymerase (up to 70,000 base insertions per binding event) with a powerful strand displacement activity (2) and a 3' → 5' proofreading exonuclease function (3).

Source of Protein

A recombinant *E. coli* strain carrying the Φ29 DNA Polymerase gene from bacteriophage Φ29.

Supplied in

10 mM Tris-HCl
100 mM KCl
0.1 mM EDTA
1 mM dithiothreitol
0.5% Tween-20
0.5% NP-40
50% glycerol
pH 7.4 @ 25°C

Supplied With

B702 1X Φ29 DNA Polymerase Reaction Buffer

10X Φ29 DNA Polymerase Buffer (B702)

500 mM Tris-HCl
100 mM (NH₄)₂SO₄
40 mM Dithiothreitol
100 mM MgCl₂
pH 7.5 @ 25°C

Unit Definition

1 unit is defined as the amount of polymerase required to convert 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Product Specification*

Unit Size:	2,000 Units
Unit Concentration	10,000 U/mL
Protein Concentration	0.12 mg/ml
Purity (SDS-PAGE)	>99%
Specific Activity	83,333 U/mg
SS 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

Quality Control Analysis:

Unit Characterization Assay

Unit activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X Φ29 DNA Polymerase reaction buffer ([Φ29 Pol]_f = 0.01-0.0008 μg/μL) and added to 50 μL reactions containing 0.02 mg/ml λ Hind III DNA, 1X Φ29 DNA Polymerase Reaction Buffer, 4mCi/mL ³H-dTTP, 0.2 μM dTTP and 200 μM dATP, dCTP, dGTP. Reactions were incubated 10 minutes at 30°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 μL sample of enzyme solution was analyzed using a NanoDrop ND-1000 spectrophotometer in A₂₈₀ protein analysis mode. An extinction coefficient of 109,510 and molecular weight of 66,389 Daltons were used to calculate the protein concentration of 3 replicate samples.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 μ L reaction containing 11,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in greater than 80% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 0.5 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.

***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. Blanco, L. and Salas, M. (1984) *Proc. Natl. Acad. Sci. USA*, 81, 5325-5329.
2. Blanco, L. et al. (1989) *J. Biol. Chem.*, 264, 8935-8940.
3. Garmendia, C. et al. (1992) *J. Biol. Chem.*, 267, 2594-2599.



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.