



Product Information

Mako DNA Polymerase (3'→5' exo-)

Part Number:	P709L
Lot Number:	

Certificate of Analysis

2010-P709-Rev.F

Product Description:

Mako DNA Polymerase (3'→5' exo-) catalyzes the extension of a primed DNA template in the 5'→3' direction. This enzyme lacks any inherent 3'→5' and 5'→3' exonuclease activities and exhibits no measurable strand displacement function.

Source of Protein:

Purified from a strain of *E. coli* that expresses the recombinant Mako DNA Polymerase (3'→5' exo-) gene.

Supplied in:

100 mM KPO₄
1.0 mM dithiothreitol
0.1 mM EDTA
50% glycerol
pH 6.5 @ 25°C

Supplied With:

B011 (10X Blue Buffer)

10X Blue Buffer (B011)

500 mM NaCl
100 mM Tris-HCl
100 mM MgCl₂
10 mM DTT
pH 7.9 @ 25°C

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-precipitable material in 30 minutes at 37°C.

Product Specification*

Unit Size:	3,000 Units
Unit Concentration	30,000 U/mL
Protein Concentration	4.92 mg/mL
Purity (SDS-PAGE)	>99%
Specific Activity	6,100 U/mg
SS Exonuclease	300 U <5.0% released
DS Exonuclease	300 U <0.5% released
Endonuclease	300 U <10% converted
<i>E. coli</i> 16S rDNA Contamination	300 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 reaction buffer ([Mako]_f = 0.25-0.002 μg/μL) and added to 50 μL reactions containing 10 μg denatured Calf Thymus DNA, 1X Blue Reaction Buffer, 4mCi/mL ³H-dTTP and 100 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).

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 128,440 and molecular weight of 103,565 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:

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 less than 5.0% 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 0.5% release of TCA-soluble counts.

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.



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.