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Product Information Sheet *P7460L Rev B*

Product Information	
Poly(A) Polymerase	
Part Number	P7460L 280500
Concentration	5,000 U/mL
Unit Size	1,000 U

Product Specification	
Storage Temperature	-25°C to -15°C
TEST:	**SPECIFICATION:**
Purity (SDS-PAGE)	>95%
Specific Activity	>20,000 U/mg
SS Exonuclease	200 U <5.0% released
DS Exonuclease	200 U <1.0% released
DS Endonuclease	200 U = no conversion
***E.coli* DNA Contamination**	100 U <10 copies
RNAse Contamination	200 U = no detectable non-specific RNAse

Product Description:

Poly(A) Polymerase catalyzes the addition of AMP from ATP to the 3' hydroxyl of RNA. The reaction requires Mg^{2+} and is template independent.

Source of Protein

The gene encoding *E. coli* Poly(A) Polymerase expressed from a plasmid in *E. coli*.

Supplied in

25 mM Tris-HCl
500 mM NaCl
1 mM $MgCl_2$
0.1 mM DTT
0.1 mM EDTA
50% glycerol
pH 8.0 @ 25°C

Supplied with

B7460 (10X Poly(A) Polymerase Reaction Buffer)
N2070-10 (10 mM ATP Solution)

10X Poly(A) Polymerase Reaction Buffer (B7460):
500 mM Tris-HCl
2.5 M NaCl
100 mM $MgCl_2$
pH 7.9 @ 25°C

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 1 nmol of ATP into acid-insoluble material in 10 minutes at 37°C.

Quality Control Analysis:

Unit Characterization Assay

Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer (50 mM Tris-HCl, 250 mM NaCl, 10 mM $MgCl_2$, pH 7.9 @ 25°C, and added to 50 µL reactions containing a 15-mer RNA Oligo, 1X reaction buffer, 1 mM ATP, 2.5 mM $MnCl_2$ and ^{3}H-ATP. 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-2000 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 52,060 and molecular weight of 53,870 Daltons.

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 95% purity of the concentrated sample.

Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 µL reaction containing 10,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution

(additional information on reverse side)

incubated for 4 hours at 37°C resulted in less than 5% 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% 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 µL samples were screened for non-specific RNAse contamination using the RNAse Alert kit, (Integrated DNA Technologies), following the manufacturer's guidelines.

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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.

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