Product Information	
Poly(A) Polymerase	
Part Number	P7460L
Concentration	5,000 U/mL
Unit Size	1,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

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.

Product Specifications							
P7460							
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**	**Non-specific RNAse**
Units Tested	**n/a**	**n/a**	**200**	**200**	**200**	**100**	**200**
Specification	>95%	>20,000 U/mg	<5.0% Released	<1.0% Released	No Conversion	<10 copies	No detectable non-specific RNAse

Source of Protein: A recombinant *E. coli* strain carrying the cloned Poly(A) polymerase gene from *E. coli* (1).

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.

Molecular weight: 53.9 KDa

Quality Control Analysis:

Unit Activity is 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 MgCl2, pH 7.9 at 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 in ice, and analyzed using the method of Sambrook and Russell (2).

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E. coli* 16S rDNA Contamination** is evaluated using 5 µL replicate samples of enzyme solution 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.

Non-Specific RNAse contamination is assessed using the RNAse Alert kit (Integrated DNA Technologies), following the manufacturer's guidelines.

Supplied in:
25 mM Tris-HCl, 500 mM NaCl, 0.1 mM DTT, 0.1 mM EDTA, 1 mM $MgCl_2$, 50% glycerol (pH 8.0 at 25°C)

Supplied with:
10X Poly(A) Polymerase Reaction Buffer (B7460): 500 mM Tris-HCl, 2.5 M NaCl, 100 mM $MgCl_2$ (pH 7.9 at 25°C)
N2070-10 (10 mM ATP Solution)

Usage Instructions:
1. Set up the following reaction mixture in a total volume of 20 µL in the order listed:

- 1-10 µg purified RNA in 15 µL of nuclease free water
- 2 µL 10X Poly(A) Polymerase Reaction Buffer (B7460)
- 2 µL 10mM ATP (N2070-10)
- 1 µL Poly(A) Polymerase (P7460)

2. Incubate reaction mixture at 37°C for 30 minutes.
3. Stop the reaction by adding EDTA (final concentration of 10mM) or proceed to cleanup step.

References:
1. Cao, G.J. and Sarkar, N. (1992) PNAS, 89, 10380-10384.
2. Sambrook, J. and Russell, D.W. (2001) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., v3, A8.25-A8.26.

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