

*E. coli*  
Poly(A) Polymerase



M0276S 016160118011

**M0276S**



**100 units**      **5,000 U/ml**      **Lot: 0161601**  
**RECOMBINANT**      **Store at -20°C**      **Exp: 1/18**

**Description:** *E. coli* Poly(A) Polymerase catalyzes the template independent addition of AMP from ATP to the 3' end of RNA.

**Source:** An *E. coli* strain that carries the cloned Poly(A) Polymerase gene from *E. coli* (1).

**Applications:**

- Labeling of RNA with ATP or cordycepin
- Poly(A) tailing of RNA for cloning or affinity purification
- Enhances translation of RNA transferred into eukaryotic cells

Supplied in: 20 mM Tris-HCl (pH 7.5), 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

**Reagents Supplied with Enzyme:**

10X *E. coli* Poly(A) Polymerase Reaction Buffer  
10 mM ATP

**Reaction Conditions:** 1X *E. coli* Poly(A) Polymerase Reaction Buffer and 1 mM ATP. Incubate at 37°C.

**1X *E. coli* Poly(A) Polymerase Reaction Buffer:**

250 mM NaCl  
50 mM Tris-HCl  
10 mM MgCl<sub>2</sub>  
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µl volume in 10 minutes at 37°C.

**Unit Assay Conditions:** 1X *E. coli* Poly(A) Polymerase

Reaction Buffer, 1 mM ATP and 500 ng 5' FAM labeled poly A 20-mer RNA in a 20 µl reaction. After incubation at 37°C for 10 minutes the length of the poly(A) addition is determined either by gel

electrophoresis or with an automated capillary DNA sequencer. In this assay 5 units of enzyme add approximately 60 to 80 adenosines to the RNA primer. In these conditions 20 units of enzyme will deplete the ATP.

**Heat Inactivation:** 65°C for 20 minutes.

**Quality Assurance:** *E. coli* Poly(A) Polymerase contains no detectable DNAses, RNAses and phosphatases. The purified protein contains no detectable DNA or RNA as determined by ethidium staining of an agarose gel.

**Quality Control Assays**

**RNase Activity (Extended Digestion):** A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 5 units of *E. coli* Poly(A) Polymerase is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

**Exonuclease Activity (Radioactivity Release):** A 50 µl reaction in Poly(A) Polymerase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 10 units of *E. coli* Poly(A) Polymerase incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

**Endonuclease Activity (Nicking):** A 50 µl reaction in Poly(A) Polymerase Reaction Buffer containing 1 µg of supercoiled φX174 DNA and a minimum of 10 units of *E. coli* Poly(A) Polymerase incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Protein Purity Assay (SDS-PAGE):** *E. coli* Poly(A) Polymerase is > 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**References:**

1. Cao, G.J. and Sarkar, N. (1992) *Proc. Natl. Acad. Sci. USA.* 89, 10380-10384.

CERTIFICATE OF ANALYSIS

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