

# *E. coli* Poly(A) Polymerase



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M0276S 010130915091

## M0276S



**100 units**      **5,000 U/ml**      **Lot: 0101309**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 9/15**

**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 Assay:** Incubation of a 10 µl reaction containing 5 units of *E. coli* Poly(A) Polymerase with 40 ng of RNA transcript for 2 hours at 37°C resulted in no detectable degradation of the RNA as determined by gel electrophoresis.

**DNA Exonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of *E. coli* Poly(A) Polymerase with 1 µg of a mixture of single and double-stranded <sup>3</sup>H *E. coli* DNA (200,000 cpm/µg) for 3 hours at 37°C released < 0.1% of the total radioactivity.

**DNA Endonuclease Activity:** Incubation of a 50 µl reaction containing 10 units of *E. coli* Poly(A) Polymerase with 1 µg of φX174 RF I DNA for 3 hours at 37°C resulted in < 10% conversion to RFI as determined by agarose gel electrophoresis.

### References:

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

CERTIFICATE OF ANALYSIS

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