E. coli Poly(A) Polymerase

1-800-632-7799
info@neb.com
www.neb.com

M0276S 017170319031

M0276S

RR 37° Yes

100 units	**5,000 U/ml**	**Lot: 0171703**
RECOMBINANT	**Store at –20°C**	**Exp: 3/19**

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 $MgCl_2$
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 approximatley 60 to 80 adenosines to the RNA primer. In these conditons 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 [^{3}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.

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