E. coli Poly(A) Polymerase

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

M0276S 0101307150715

M0276S

RR 37° Yes

100 units	**5,000 U/ml**	**Lot: 0101307**
RECOMBINANT	**Store at –20°C**	**Exp: 7/15**

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 $MgCl_2$
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 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 ^3H *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 RFII as determined by agarose gel electrophoresis.

References:

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

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CERTIFICATE OF ANALYSIS