Poly(A) Polymerase (Cloned)

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Contents	Quantity	Storage conditions
Poly(A) Polymerase, 2 U/µL	80 Units [1]	Store at –20°C. *Do not store in a frost-free freezer.*
5X *E*-PAP Buffer	800 µL	
25 mM $MnCl_2$	400 µL	
DEPC treated Water	1 mL	

[1] One unit is the amount of enzyme that incorporates 1 nmol of AMP into an acid insoluble tail on tRNA in 10 min at 37°C at pH 7.9 with ATP as the substrate.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Poly(A) Polymerase (PAP) catalyzes the addition of adenosine to the 3′ end of RNA in a sequence-independent fashion. The polymerase is purified from an *E. coli* strain overexpressing *E. coli* Poly(A) Polymerase I, and supplied in 25 mM Tris (pH 8.0), 500 mM NaCl, 0.1 mM DTT, 1 mM $MgCl_2$, 0.1 mM EDTA and 50% glycerol (v/v).

Using Poly(A) Polymerase

Poly(A) Polymerase can be used to add a poly(A) tail to RNA transcripts. A poly(A) tail may increase mRNA stability and therefore translation efficiency when tailed RNA is used in transfection or micro-injection experiments. It can also be used to label the 3′ end of RNA molecules by addition of radiolabeled adenosine.

Poly(A) tailing reactions

Use 8 U Poly(A) Polymerase to generate ≥150 nt-long poly(A) tails. Use less Poly(A) Polymerase to produce shorter poly(A) tails.

In a total reaction volume of 100 µL:

- ≤40 µg RNA
- 1X PAP Buffer
- 2.5 mM $MnCl_2$
- 1 mM ATP
- 2–8 U Poly(A) Polymerase

For additional information on poly(A) tailing reactions, refer to the Poly(A) Tailing Kit (Cat. no. AM1350) user guide, available at **thermofisher.com**.

Other applications

PAP can be used to append radiolabeled adenosine to the 3′ end of RNA molecules to generate labeled RNAs.

In conjunction with terminal transferase, reverse transcriptase, and thermostable polymerase, PAP can be used to clone RNAs of unknown sequence (see the following figure). For RNA cloning, first add a poly(A) tail to the 3′ end of the RNA using PAP. Reverse transcribe the RNA using reverse transcriptase (for example, Cat. No. 28025013) and an oligo(dT) primer. Add a poly(dA) tail to the 3′ end of the cDNA using terminal transferase. Then amplify the modified cDNA using Platinum™ *Taq* High Fidelity DNA Polymerase (Cat. No. 11304011) and PCR primers specific to the homopolymer tails appended by PAP and terminal transferase. The dsDNA can then be cloned and sequenced. Using PCR primers with restriction sites can facilitate cloning.

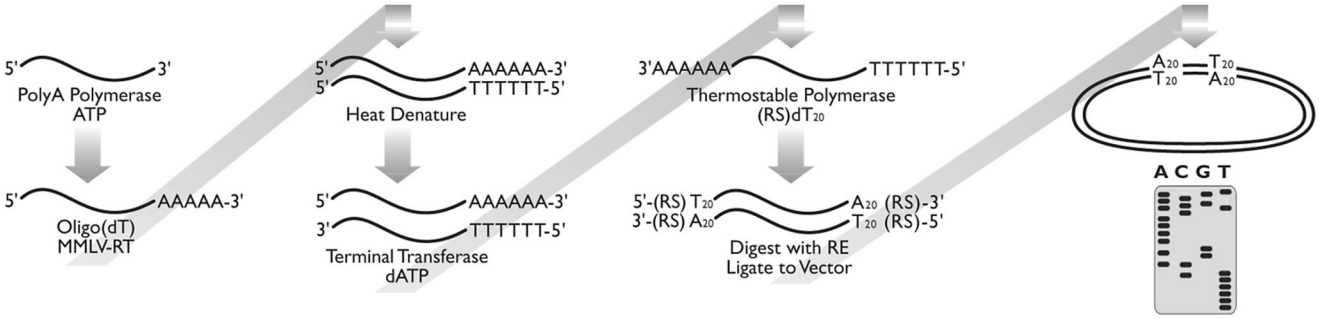

Figure 1 Cloning RNAs with PAP

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References

Biebricher , C.K. and Luce, R. 1993. Sequence analysis of RNA species synthesized by Q beta replicase without template. *Biochemistry* 32: 4848–4854.

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