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Poly(A) Polymerase Tailing Kit

Cat. No. PAP5104H

1. Introduction

The Poly(A) Polymerase Tailing Kit was developed for the rapid and efficient addition of poly(A)-tails to the 3′-hydroxyl end of any RNA. RNA with 3′ phosphates may be end-repaired using T4 Polynucleotide Kinase (PNK) in the absence of ATP. A simple cleanup will be necessary to remove T4 PNK prior to A-tailing. Polyadenylation increases the stability of RNA in eukaryotic cells and enhances its ability to be translated after transfection or microinjection.[1-3] Poly(A) tails can also provide priming sites for the synthesis of first-strand cDNA, or be used to end-label[4] or quantitate[5] mRNA.

The kit features Poly(A) Polymerase which uses ATP as a substrate for template-independent addition of adenosine monophosphates to the 3′-hydroxyl termini of RNA molecules. The standard protocol was designed to produce a poly(A)-tail length of ~150 b on 60 µg of capped RNA. An alternative protocol is also provided for tailing lesser amounts of RNA as well as suggestions on how to adjust the length of the poly(A)-tails generated.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
Poly(A) Polymerase Tailing Kit	50 Reactions (400 Units)	PAP5104H	Poly(A) Polymerase (4 U/µL)	E0122-4D1	100 µL
			ATP (10 mM)	SS000391-D4	500 µL
			10X Poly(A) Polymerase Reaction Buffer	SS000644-D1	500 µL
			Nuclease-Free Water	SS000772-D3	1 mL

3. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: Poly(A) Polymerase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, and 0.1% Triton® X-100.

Unit Definition: One unit of Poly(A) Polymerase converts 1 nmol of ATP into acid-insoluble material in 10 minutes at 37°C under standard assay conditions.

10X Poly(A) Polymerase Reaction Buffer: 0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, and 0.1 M $MgCl_2$. A 10 mM ATP Solution is also provided.

Quality Control: Poly(A) Polymerase is function-tested in a 50 µL reaction containing 50 mM Tris-HCl (pH 8.0), 250 mM NaCl, 10 mM $MgCl_2$, 5 µg of yeast tRNA, 1 mM ATP, and varying amounts of Poly(A) Polymerase.

Contaminating Activity Assays: All components of the Poly(A) Polymerase Tailing Kit are free of detectable exo- and endonuclease and RNase activities.

4. Notes on Using the Poly(A) Polymerase Tailing Kit

1. **Poly(A)-Tail Length:** The standard protocol will generate a poly(A)-tail length of ~150 b. However, the length of poly(A)-tail which can be synthesized by the Poly(A) Polymerase Tailing Kit is dependent upon several reaction parameters. Accordingly, users can customize poly(A)-tails to a desired length by adjusting one or more of these reaction parameters as outlined below.

   Assuming all other reaction parameters are kept constant, poly(A)-tail length increases with:

   – increasing units of Poly(A) Polymerase (2-16 Units).

   – increasing time of incubation (10-60 minutes).

   – decreasing amount of substrate RNA (60-1 µg).

   – decreasing total reaction volume (100-10 µL).

   Customers wishing to customize the length of the poly(A)-tail generated should set up several test reactions covering a range of the parameters to be changed, in order to find the most appropriate reaction conditions for the tail length desired (see Fig. 1).

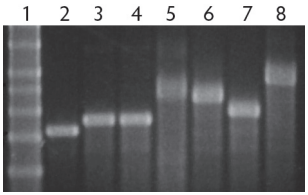

Figure 1. Customized Poly(A)-tail Lengths. A 1.4-kb transcript was poly(A)-tailed using various reaction conditions to demonstrate the affect on poly(A)-tail lengths. Each lane contains 0.1 µg of the completed poly(A)-tailing reaction product.

Lane 1: RNA MW markers (sizes top to bottom: 4 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb).
Lane 2: non-poly(A)-tailed RNA.
Lane 3: 60 µg RNA, 16 U enzyme, 37°C for 30 min., 100 µL total rxn volume.
Lane 4: 60 µg RNA, 6 U enzyme, 37°C for 60 min., 100 µL total rxn volume.
Lane 5: 1 µg RNA, 2 U enzyme, 37°C for 60 min., 50 µL total rxn volume.
Lane 6: 1 µg RNA, 2 U enzyme, 37°C for 30 min., 10 µL total rxn volume.
Lane 7: 10 µg RNA, 4 U enzyme, 37°C for 60 min., 50 µL total rxn volume.
Lane 8: 1 µg RNA, 2 U enzyme, 37°C for 60 min., 10 µL total rxn volume.

2. **Reaction Size:** Poly(A) Polymerase tailing reactions can be scaled up or down as desired by proportionally increasing or decreasing the volumes of reagents.

3. **Stopping the Reaction:** A Poly(A) Polymerase tailing reaction may be stopped in a number of different ways, depending on the subsequent uses of the poly(A)-tailed RNA. These include immediate freezing of the completed reaction at –20°C or –70°C, removal of the enzyme via organic solvent extraction (e.g., phenol/chloroform) or chelation of the Mg^{2+} (e.g. EDTA). We do not recommend heat denaturation of the enzyme to stop the reaction due to the potential for RNA thermal degradation.

4. **Addition to an *In vitro* Translation Reaction:** Poly(A)-tailed RNA should be purified (organic extraction/ethanol precipitation, ammonium acetate precipitation, spin columns, or AMPure® beads) prior to use in *in vitro* translation systems or *in vivo* experiments.

Standard Protocol

The following protocol is designed to produce a poly(A)-tail length of ~150 b.

1. On ice, combine the following reaction components in the order given:

x	µL	RNase-Free Water
10	µL	10X Poly(A) Polymerase Reaction Buffer
10	µL	10 mM ATP
2.5	µL	RiboGuard RNase Inhibitor (optional)
20	µL	*In vitro* transcription capping reaction (60 µg RNA)
2	µL	Poly(A) Polymerase (8 Units)
100	µL	Total reaction volume

2. Incubate at 37°C for 30 minutes.

   (Extending the incubation to 60 minutes results in poly(A)-tails >200 b.)
3. The reaction may be stopped by any one of the following:

   a) immediate storage at –20°C.

   b) addition of EDTA to a final concentration of >11 mM.

   c) phenol/chloroform extraction and salt/alcohol precipitation.

Alternate Protocol

The following protocol is designed to be used as a starting point from which to customize a poly(A)-tailing reaction for use on 1-10 µg of RNA. See the Notes section for the effect of variously altered reaction parameters.

1. On ice, combine the following reaction components in the order given:

x	µL	RNase-Free Water
2	µL	10X Poly(A) Polymerase Reaction Buffer
2	µL	10 mM ATP
0.5	µL	RiboGuard RNase Inhibitor (optional)
1-10	µg	RNA Substrate
1	µL	Poly(A) Polymerase (4 Units)
20	µL	Total reaction volume

2. Incubate at 37°C for 15-20 minutes.
3. The reaction may be stopped by any one of the following:

   a) immediate storage at –20°C.

   b) addition of EDTA to a final concentration of >11 mM.

   c) phenol/chloroform extraction and salt/alcohol precipitation.

5. References

1. Drummond, D.R. *et al.*, (1985) *J. Cell. Biol.* **100**, 1148.
2. Galili, G. *et al.*, (1988) *J. Biol. Chem.* **263**, 5764.
3. Belasco, J. and Brawerman, G. (1993) *Control of Messenger RNA Stability*, Academic Press, San Diego, CA.
4. Lingner, J. and Keller, W. (1993) *Nucleic Acids Res.* **21**, 2917.
5. Krug, M.S. and Berger, S.L. (1987) *Methods Enzymol.* **152**, 262.