RNA production kit

Poly(A) Tailing Kit

Ready-to-use kit for poly(A) tailing of capped or non-capped mRNA.

Protocol

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Poly(A) Tailing Kit | Specifications

Package content	**PolyA-K25** **Number of reactions : 25**
Shipping conditions	The kit is shipped in dry-ice.
Storage conditions	Upon receipt and for long-term use, store the kit at -20°C.
Stability	1 year at -20°C.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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Poly(A) Tailing Kit	Specifications

Notes :

1. Technology

1.1 Description

This kit is **designed for the enzymatic addition of a poly(A) tail to the 3'-hydroxyl termini of capped or non-capped RNAs independently of the template.** The Poly(A) Tailing Kit allows the addition of approximately 200 nucleotides per reaction (based on 100µg of RNA) in 45 minutes at 37°C.
Presence of a poly(A) tail is known to enhance mRNA translation, stability and nucleo-cytoplasmic transport.

1.2 Kit composition

Components	Quantity	Storage
Poly(A) polymerase 5 u/µl	75 µl	-20°C
ATP (10 mM)	500 µl	-20°C
RNase Inhibitor 40 u/µl	50 µl	-20°C
Polyadenylation Buffer 10X	500 µl	-20°C
Nuclease-free water	2 ml	-20°C

Material not provided with the kit:

- mRNA to be polyadenylated.
- 70% ethanol for RNA washing.
- Nuclease-free buffers (1mM citrate buffer pH 6.4, TE buffer or RNAse-free water) for RNA resuspension.
- Materials or kits for purification of the RNA product.

2. Applications and Protocols

2.1 General conditions

Unit Definition:

- One unit (u) is defined as the amount of enzyme that incorporates 1 nmol of AMP (A) into RNA in a 20 µL volume in 10 minutes at 37°C under standard assay conditions.

Standard protocol:

The standard protocol **is designed for the addition of approximately 200 A to 100 µg of RNA in 45 minutes.** This protocol can be scaled up or down by modifying the amount of enzyme units and/or increasing the incubation time (for example, use 8 u of enzyme during two hours incubation), or decreasing the quantity of substrate (from 100 µg to 20 µg) and/or the reaction volume (from 200 µl to 50 µl).
To find the best reaction conditions and the desired poly(A) tail length, we recommend to set up some test reactions covering these different parameters.

RNA Handling:

RNAs are very sensitive to degradation and these simples precautions can limit their degradation:

- Wear clean gloves and lab coat.
- Clean work area and pipette with a surface decontamination agent or with 0,5% SDS and rinse thoroughly with RNAse-free water.
- Only use dedicated RNAse-free barrier filter pipette tips and RNAse-free water.
- Keep as much as possible your RNA sample on ice and store the purified RNA at -80°C.

2.2 General Protocol

This general protocol is **designed for the addition of approximately 200 A to 100 µg of RNA in 45 min at 37°C.**

Step 1: RNA denaturation (155µl)

Dilute 100µg of RNA in RNAse-free water for a total volume of 155µL.

Heat the RNA at 65°C for 5 minutes.
Immediately after, place the denatured RNA on ice.

Step 2: Reaction Mix preparation (45 µL)

Add sequentially the components described in the table below:

Polyadenylation buffer 10x	20 µl
10 mM ATP	20 µl
RNase inhibitor	2 µl
Poly(A) Polymerase (15 U)	3 µl
Total Volume	45 µl
Keep the reaction mix on ice	

Mix the heat-denatured RNA (Step 1) with reaction mix (Step 2).

Total Volume: 200µL.

Step 3: Proceed to Polyadenylation

Incubate the 200 µL reaction at 37°C for 45 min.

NOTE: Reaction conditions can be modified depending on each RNA characteristics and expected PolyA tail length by varying 1- the incubation time (from 15min to 2h max), 2- the quantity of Poly(A) polymerase (up to 60U).

Step 4: Reaction Stop

The Poly(A) polymerase reaction can be stopped by:

- Precipitation with LiCl/EDTA*.
- Immediately freezing the sample at -20 or -80°c after the reaction.
- Removing the enzyme with organic solvent extraction (such as phenol-chloroform extraction and salt/alcohol precipitation).
- Chelating Mg2+ with EDTA at a final concentration >11mM.

NOTE: Do not heat-denature the enzyme since heat can degrade RNA.

NOTE: The RNA must be purified before its subsequent use in translation experiment, transfection or microinjection *in vivo*.

Polyadenylated RNA can now be purified using your method of choice (organic extraction methods or commercially available RNA purification Kits).

* **We suggest the precipitation method as follow:**

- Precipitate the RNA by adding of Nuclease-free water and 1.5 volume of precipitation solution (Lithium Chloride 7.5M, EDTA 0.05M, pH8), for a 200µL reaction mix: add 300 µL of the respective solutions.

- Incubate over night at -20°C.

- Centrifuge the tubes at 16000*xg* at 4°C for 15 minutes.

- Carefully discard the supernatant without impairing the pellet.

- Wash the pellet with 500 µl of 70% ethanol.

- Centrifuge again at 16000xg at 4°C for 5 minutes.

- Remove the ethanol and allow the pellet to dry.

- Resuspend the pellet in Nuclease-free water or buffer of your choice.

2.3 Trouble shooting

The poly(A) tail is longer than expected:

- Reduce the incubation time.
- Use less unit of poly(A) polymerase in the reaction.
- Decrease the total reaction volume in order to increase the substrate concentration.

The poly(A) tail is shorter than expected:

- Increase the incubation time.
- Use more unit of poly(A) polymerase in the reaction.
- Increase the total reaction volume in order to decrease the substrate concentration.

No poly(A) tail after the reaction:

- Check the purity of the RNA: presence of impurity can inhibit the reaction. Proceed with purification of the RNA.
- Proceed with heat denaturation step since absence of polyadenylation can be due to secondary structure in 3' end of the RNA.
- Check storage conditions of components: the enzyme should be kept at -20°C and keep on ice when setting up a reaction. The ATP must not be expose to elevated temperature.

2.4 Performances characteristics

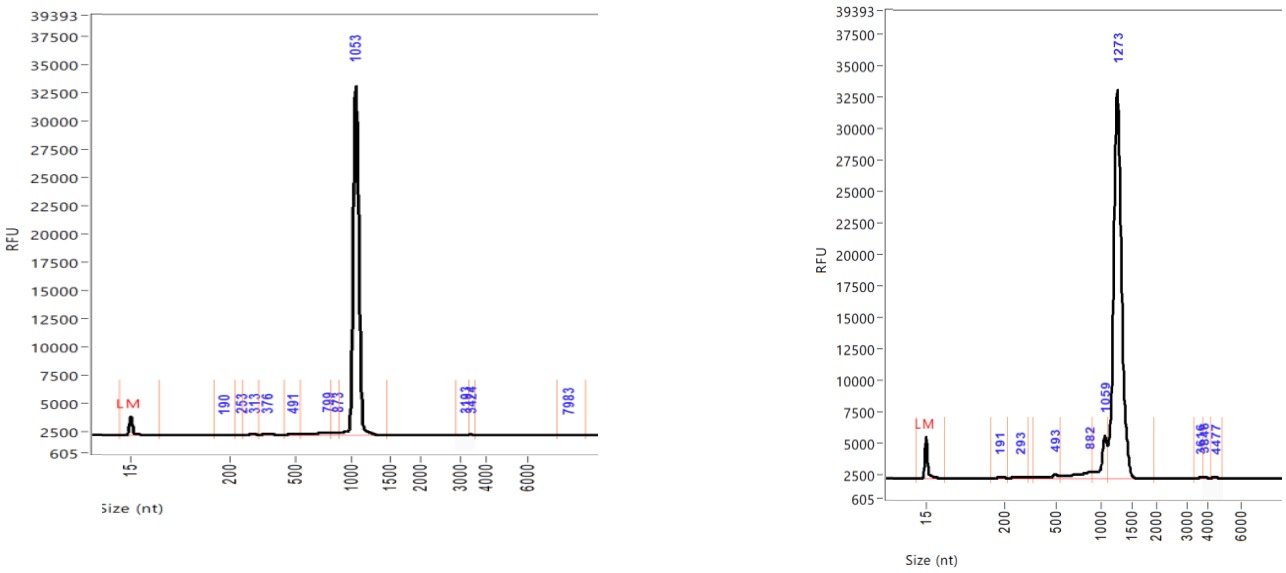

Fig. 1. Fragment Analyzer profiles of RNA before (A) and after (B) poly Adenylation in the recommended conditions, shows an increase in length of about 200 A.

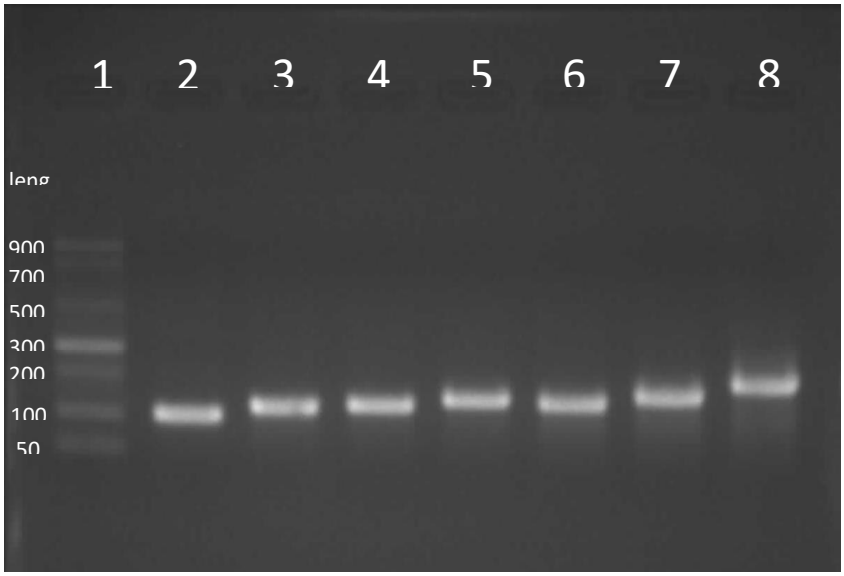

Fig.2. Agarose gel electrophoresis after polyAdenylation of a 918 nt mRNA

Line 1: SS RNA lader
Line 2 : Non polyadenylated 918 nt mRNA
Line 3 : 100 µg mRNA, 8u of poly(A) polymerase, 45 min at 37°C.
Line 4 : 100 µg mRNA, 15u of poly(A) polymerase, 45 min at 37°C.
Line 5 : 100 µg mRNA, 30u of poly(A) polymerase, 45 min at 37°C.
Line 6 : 100 µg mRNA, 8u of poly(A) polymerase, 2 hours at 37°C.
Line 7 : 100 µg mRNA, 15u of poly(A) polymerase, 2 hours at 37°C.
Line 8 : 100 µg mRNA, 30u of poly(A) polymerase, 2 hours at 37°C.

Additional products for your mRNA production and transfection experiments :

- **IVT Kit** for RNA production by *In Vitro* transcription
- **Capping Kit** for the *In Vitro* enzymatic capping of RNA
- **RmesFect** for mRNA transfection

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