mRNA Poly(A) Tailing Kit

Please read the datasheet carefully prior to use.

Cat. No. LA201

Version Number. Version 2.0

Storage: at -20 ℃ for one year

Description

This product catalyzes the conversion of ATP into AMP and incorporates into the 3′ end of RNA sequentially, which doesn't rely on the presence of templates, that is, adding Poly(A) to the 3′end of mRNA. Polyadenylation modification increases the stability of RNA in cells and enhances the efficiency of RNA expression after transfection or microinjection. This product has a very high tailing efficiency, and can be used in the modification of mRNA tailing after in vitro transcription (JT101) and capping (LC101), preparing for RNA samples used for cell transfection.

Applications

1.Adding Poly(A) tail to the 3' end of mRNA after in vitro transcription (T7 High Efficiency Transcription Kit, Cat.No. JT101) and capping (mRNA Capping Kit, Cat.No. LC101), which can stabilize the structure of 5' cap and enhance the stability and translation efficiency of mRNA in eukaryotic cells.

2. Adding Poly(A) tail for cDNA synthesis, providing binding sites for oligo-dT primer.

3. Label the 3′ ends of RNA by modified ATP or its analogs.

Kit Contents

Component	LA201-01 (25 rxns)	LA201-02 (100 rxns)
mRNA Poly(A) Tailing Enzymes	50 μl	200 μl
10×mRNA Poly(A) Tailing Buffer	80 μl	320 μl
RNase-free Water	1 ml	2×1 ml

Procedures (Adding poly(A) tail to RNA (≤ 10 μg) in 30 μl reaction system. 20 μl capped mRNA generated with mRNA Capping Kit (LC101) can be used in the tailing reaction below directly)

1. Add ≤10 μg RNA to a PCR tube, and prepare the reaction system according to the following table:

Reaction Component	Usage
mRNA	≤10 μg
10×mRNA Poly(A) Tailing Buffer	3 μl
mRNA Poly(A) Tailing Enzymes	0.5~2 μl
RNase-free Water	To 30 μl
Total Volume	30 μl

2. Incubate at 37°C for 30 minutes. Heat at 65°C for 10-20 minutes to terminate the reaction, or purify the reaction system directly.

Product Purification:

The prepared RNA products can be purified by magnetic beads-based reagent (EC501), column-based reagent (ER701), or by phenol/chloroform extraction or lithium chloride precipitation to remove proteins and free nucleotides. The purified RNA can be used for downstream experiments or stored at -80°C.

1. Magnetic beads-based purification

The magnetic bead-based method can remove proteins and free nucleotides. Refer to the instruction of *MagicPure*® RNA Beads (EC501) for the operation procedure.

2. Column-based purification

The column-based method can remove protein and free nucleotides. Add RNase-free Water to dilute the product to 100 μl before purification, and then follow the instruction of *EasyPure*® RNA Purification Kit (ER701) for purification. It is recommended to elute again to improve the recovery.

Note: The recovery rate of the column-based method is lower than magnetic bead-based method.

3. Phenol/chloroform purification

The method of Phenol/chloroform extraction can remove protein and most of the free nucleotides, and the purification steps are as follows:

1) Add RNase-free Water to dilute the product to 180 μl;

2) Add 20 μl of 3 M sodium acetate (pH 5.2) to the above diluted product, and pipet to mix thoroughly;

3) Add 200 μl of the mixture of phenol/chloroform (volume ratio 1:1) for extraction, centrifuge at 10,000 rpm for 5 minutes at room temperature, and transfer the upper aqueous phase solution to a new 1.5 ml centrifuge tube;

4) Add chloroform equal to the volume of water to extract twice, and collect the upper aqueous phase;

5) Add 2 times the volume of aqueous phase of absolute ethanol, mix well, keep still at -20°C for at least 30 minutes, centrifuge at 15,000 rpm for 15 minutes at 4°C, discard the supernatant;

6) Add 500 μl of pre-cooling 70% ethanol to wash the RNA precipitate, centrifuge at 15,000 rpm for 5 minutes at 4°C, and discard the supernatant;

7) Open the lid and dry for 2 minutes in an RNase-free environment, add 20-50 μl RNase-free Water to dissolve the RNA precipitate.

4. Lithium chloride precipitation

The method demands the RNA length must be greater than 300 nt, and the concentration must not be less than 100 ng/μL.

1) Add 45 μl lithium chloride solution (7.5 M LiCl, 50 mM EDTA) and 45 μl RNase-free Water to 30 μl reaction mixture, and pipet to mix thoroughly;

2) Place at -20°C for at least 30 minutes;

3) Centrifuge at 15,000 rpm for 15 minutes at 4°C, discard the supernatant, and collect the precipitate;

4) Add 500 μl pre-cooling 70% ethanol to wash the RNA precipitate, centrifuge at 15,000 rpm at 4°C, discard the supernatant, and wash three times;

5) Dissolve the RNA precipitate with RNase-free Water, store at -80°C after detection.

Notes

Avoid RNase contamination: Be sure to use RNase-free reagents and consumables, such as water, tips (with filters), and centrifuge tubes, and wear lab coats and gloves during the operation.

- Different experiments require different numbers of A tailing, and the length of added Poly (A) can be adjusted by changing the amount of enzyme, reaction time, and the concentration of RNA. Use this product to conduct tailing reaction for 10 μg mRNA (about 0.4 k), about 150 A bases can be added to the 3' end of the RNA by adding 2 μl of enzyme and react at 37°C for 30 minutes, and about 200 A bases can be added when the reaction time is 60 minutes generally.

  When using this product for the first time, it is recommended to explore the specific reaction conditions based on the data below and the expected length of Poly(A).

- Using this product, the amount of A bases is not equal for all RNAs under the same reaction system, and the number of added A bases is also related to the length of the RNA. For tailing for RNA with equal quality but different lengths, the number of added A to long fragments is greater than that of short fragments under the same reaction condition due to the different moles of RNA in the system (For example, the number of moles of RNA with 10 μg and 3k is less than of 0.4 k. Since long fragments have fewer free 3' ends, more A bases can be added).

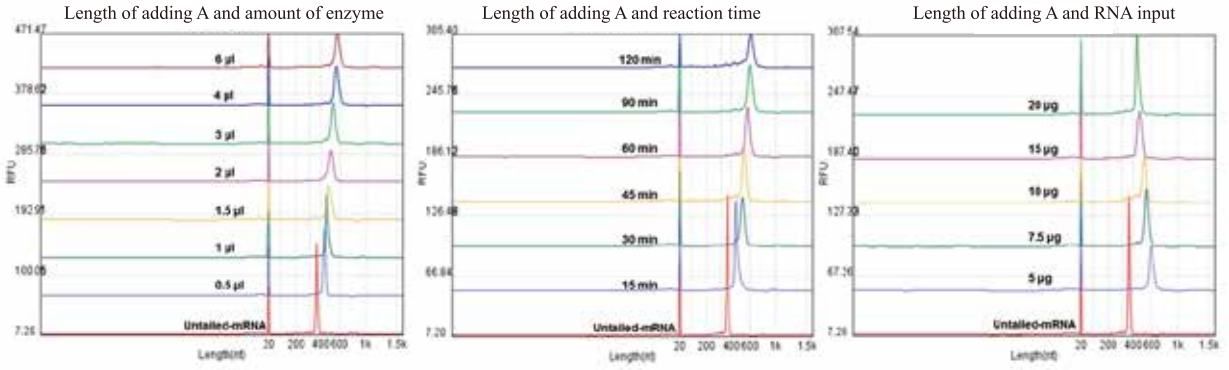
• After tailing reaction, if the RNA product needs to be used for cell transfection or microinjection, it must be purified.

• The reaction system can be scaled up or down according to the experimental needs, for example, the mRNA capped (100 μl capped mRNA) with mRNA Capping Kit (LC101) can be used in the following tailing reaction directly:

Reaction Component	Volume
mRNA	100 μl (≤50 μg)
10×mRNA Poly(A) Tailing Buffer	15 μl
mRNA Poly(A) Tailing Enzymes	2.5~10 μl
RNase-free Water	To 150 μl
Total Volume	150 μl

Reference Data

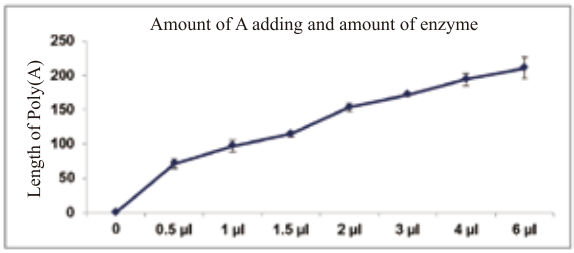
1. The relationship between the length of adding A and the amount of enzyme, reaction time and RNA input.

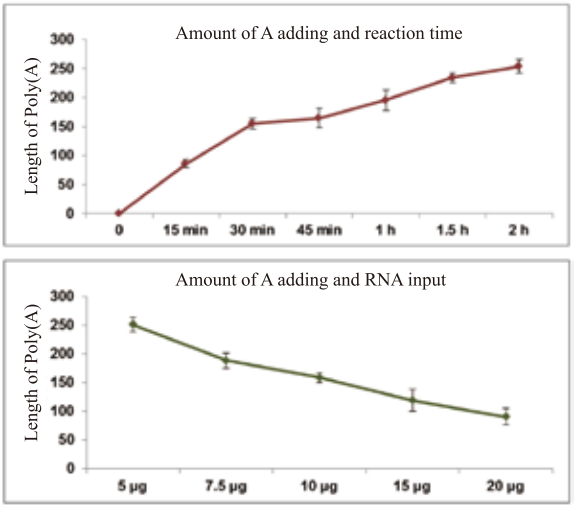


Picture 1. Result of the reaction performance (1)

Under the condition of the same reaction time (30 minutes) and the same RNA input (10 μg), the length of A tailing increased with the increase of the amount of enzyme; under the conditions of the same amount of enzyme (2 μl) and the same RNA input, the length of A tailing increased with the prolongation of the reaction time; under the condition of the same amount of enzyme (2 μl) and the same reaction time (30 minutes), the length of A tailing decreased with the increase of the concentration of RNA. In this data display, the length of mRNA is about 0.4 k before tailing.

2. The quantitative relationship between the number of adding A and the amount of enzyme, reaction time and RNA input.





Picture 2. Result of the reaction performance (2)

Common Problems and Solutions

1.Poly(A) tail is too long

If the obtained Poly(A) tail is longer than expected, the number of A adding can be reduced by reducing the amount of enzyme and shortening the reaction time, or it can be achieved by increasing the input of RNA to increase the concentration of RNA in the system or reducing the volume of the reaction system.

2.Poly(A) tail is too short

If the obtained Poly(A) tail is shorter than expected, the length of Poly(A) can be increased by increasing the amount of enzyme and prolonging the reaction time, or it can be achieved by reducing the input of RNA to reduce the concentration of the RNA in the system or increase the volume of the reaction system, or adding some additional ATP raw materials.

3. Failed tailing

The failure of the tailing reaction may be caused by the following reasons: ①The structure of the 3' end of the RNA is complex and easy to form secondary structures; ②The RNA substrate is degraded or contaminated; ③Enzyme is inactivated. Before the tailing reaction, appropriate heating (70°C for 3 minutes) can be used to remove the influence of the secondary structure, and the RNA can be re-prepared or purified again to ensure the integrity and purity of the substrate. Confirm whether the enzyme has been stored at -20°C and the enzyme should be placed on ice during the experimental operation.

Quality Control

Item	Standard	Method
Appearance	Transparent and clarified	Visual inspection
Purity	≥95%	SDS-PAGE or SEC-HPLC
Activity of exonuclease	Not detectable	Incubate 10 U enzyme with 1 μg Hela gDNA at 37°C for 16 hours
Activity of endonuclease	Not detectable	Incubate 10 U of enzyme with 1 μg pUC19 plasmid at 37°C for 16 hours
Activity of non-specific nuclease	Not detectable	Incubate 10 U of enzyme with 1 μg of 1 kb DNA Ladder at 37°C for 16 hours
RNase residue	Not detectable	Incubate 10 U enzyme with 1 μg Hela RNA/in vitro transcribed RNA at 37°C for 4 hours
Bacteria residue	Not detectable	Spread on the LB plate, incubate at 37 ° C for 2 days without formation of colony, or incubate in SOC liquid medium overnight, OD600 is not changed significantly
Bacterial endotoxin content	＜10 EU/mg	Chinese Pharmacopoeia 2020 Edition IV Gel Limit Test (General Rule 1143)
Residual DNA of *E.coli*	≤1 Copy/U	qPCR
Mycoplasma detection	Negative	Mycoplasma detection kit

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