Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Poly(A) Polymerase, GMP Grade

Product Number: GMP-M012 Animal-free Ampicillin-free

Shipping and Storage

-20℃.

Description

Poly(A) Polymerase does not depend on the presence of template, and can catalyze the sequential incorporation of ATP in the form of AMP into the 3´terminal of RNA, i.e., the addition of polyA tail to the 3´terminal of RNA. Poly (A) polymerase has A high tail adding efficiency and can add 20 to 200 A bases to the 3´terminal of RNA. Polyadenylation improves the stability of RNA in cells and enhances the expression efficiency of RNA after transfection or microinjection. Poly(A) tails can be used as primer binding sites for first-strand cDNA synthesis in some applications and can be used for end-labeling or quantification of miRNA.

The original enzymes of T7 RNA Transcription Enzyme Mix produced in E. coli. Our manufacturing processes are strictly controlled to ensure the end products free from host protein or nucleic acid contaminations and other impurities following the Pharmaceutical Manufacturing Guidelines. We guarantee the manufacturing and quality control comply with GMP regulation for tracking each and every step of the manufacturing process, including raw material sourcing.

Source	E. coli.
Storage buffer	20mM Tris-HCl; 300mM NaCl; 1mM DTT; 1mM EDTA; 0.1%TritonX-100; 50% (v/v) Glycerol, pH 8.0
Storage conditions	-20±5℃;
Activity definition	At 37℃, within 10 minutes, the amount of enzyme required that will incorporate 1 nmol AMP into RNA is defined as one unit of enzyme activity

Specification

Element	Standard
Appearance	Clear and transparent solution
Visible	Particles Meet the specification
pH	7.5-8.5
Activity	4.9KU/ml-5.1KU/ml
Endonuclease Residues	The degradation of substrate was ≤10%
Exonuclease Residues	The degradation of substrate was ≤10%
RNase Residues	ΔCt（Ct sample-Ct negative）≤0.5
Bacterial Endotoxins	<5EU/ml
Heavy Metal Residues	≤10ppm

Tip: ChP refers to the Pharmacopoeia of the People's Republic of China.

Complying to following regulations

1. ISO 9001:2015, certified facility.
2. GMP Appendix – Cellular therapeutic product National Medical Products Administration.
3. The Pandect of Genetic Therapeutic Product for Human Chinese Pharmacopoeia Commission.
4. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
5. USP Chapter <92>, Growth Factors and Cytokines Used in Cell Therapy Manufacturing
6. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

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Application

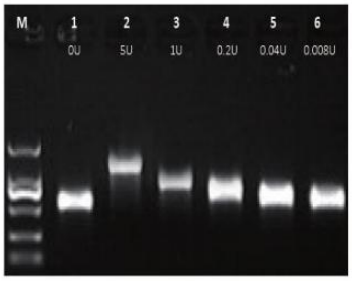
1. RNA 3´end labeling.
2. Adding Poly (A) tail to RNA for cloning or affinity purification. For example, adding miRNA to Poly (A) to provide oligo-dT primer binding site for cDNA synthesis.
3. Improve RNA translation efficiency in eukaryotic cells.

Packaging

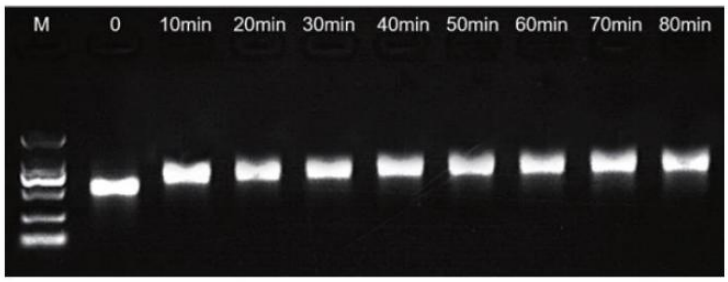
Components	Volume
Poly(A) Polymerase, GMP Grade (5U/μl)	20μl
Poly(A) Polymerase, GMP Grade (5U/μl)	1ml
Poly(A) Polymerase, GMP Grade (5U/μl)	10ml
Poly(A) Polymerase, GMP Grade (5U/μl)	50ml

Note

1. This enzyme can use RNA as substrate only.
2. This enzyme has high selectivity in adding AMP to the 3´ terminal of RNA, and does not add Poly (A) of the same length to all RNA molecules.
3. The enzyme requires Mg^{2+} and other bivalent cations to be active.
4. The length of RNA plus A tail is affected by enzyme amount, ATP and reaction time, and the amount of A required in different experiments will be different. The length of A addition can be adjusted by controlling reaction time and enzyme amount.



With the increase of enzyme amount, the length of tail increased with the same amount of RNA and reaction time.



With the extension of reaction time, the length of tail increased with the same amount of RNA and enzyme.

5. This enzyme can also react using M-MuLV reverse transcriptase reaction buffer.
6. EDTA inhibited the activity of this enzyme. If the reaction is stopped, EDTA can be added to the final concentration of 10mM, or the reaction system can be purified directly.
7. After tailing addition, RNA must be purified before transfection cells or microinjection. For specific purification methods, see the operation procedure.

Protocol

1. Tailing

1.1. Add the following components:

Components	Quantity
10×Poly(A) Polymerase Buffer, GMP Grade	2μl
ATP, GMP Grade (10 mM)	1μl
RNA	1-10μg
Poly(A) Polymerase, GMP Grade (5U/μl)	0.2~1μl

RNase Free Water	Up to 20μl

1.2. Gently mix the components with a pipette, collect by centrifugation briefly, and incubate at 37°C for 30-60min.

2. RNA Purification

2.1. Method 1: Phenol/chloroform purification method

Phenol/chloroform extraction removes proteins and most free nucleotides.

2.1.1. Add 160μl RNase-free Water to dilute the product to 180μl.

2.1.2. Add 20μl of 3M sodium acetate (pH 5.2) to the diluted product and mix well with a pipette.

2.1.3. Add 200μl of phenol/chloroform mixture (1:1) for extraction, centrifuge at 10,000 rpm for 5 min at room temperature, and transfer the upper layer solution (aqueous phase) to a new RNase-free EP tube.

2.1.4. Add the same volume of chloroform as water to extract twice, and collect the upper aqueous phase.

2.1.5. Add 2 volumes of absolute ethanol and mix well, incubate at -20°C for at least 30 minutes, and centrifuge at 15,000 rpm for 15 minutes at 4°C.

2.1.6. Discard the supernatant and add 500μl of pre-chilled 70% ethanol to wash the RNA pellet, centrifuge at 15,000 rpm at 4°C, and discard the supernatant.

2.1.7. Open the lid and dry for 2 min. Add 20-50μl RNase-free Water or other buffers to dissolve the RNA precipitate.

2.1.8. Store at -70°C.

2.2. Method 2: Column purification

Column purification can remove proteins and free nucleotides.

Add 80μl RNase-free Water to dilute the product to 100μl before purification, and then purify according to the column purification instructions.

Note:Due to the high RNA yield, in order to avoid exceeding the loading capacity of the binding column, please estimate the number of columns required.

2.3. Method 3: Magnetic beads purification

Magnetic beads purification can remove proteins and free nucleotides.

Purify according to the magnetic bead purification instructions.

2.4. Method 4: Lithium chloride purification

2.4.1. Add 30μl Lithium Chloride Precipitation Solution (7.5 M Lithium Chloride, 50 mM EDTA) and 30μl RNase Free Water to 20μl product RNA (The final concentration of Lithium Chloride Precipitation Solution is greater than 2.5M. Note: When RNA is less than 300nt or concentration is less than 100ng/μl, effective precipitation cannot be obtained by this method. The best precipitation effect was obtained when RNA concentration was greater than 400ng/μl).

2.4.2. After mixing, put it at -20℃ for at least 30 min.

2.4.3. Centrifuge at 12,000 rpm for 15 min, remove the supernatant, and collect the pellet.

2.4.4. Wash three times with pre-chilled 70% ethanol.

2.4.5. Detection after reconstitution in RNase Free Water.

3. RNA Quantification

Ultraviolet absorption method: Free nucleotides will affect the accuracy of quantification. Please perform RNA purification before using this method.

Dye method: RNA quantification is performed with RiboGreen dye, free nucleotides will not affect the quantification, and RNA in purified or unpurified reaction products can be accurately quantified.

FAQ

1. Poly(A) tail is longer than expected.

Appropriately shorten the reaction time.

Appropriately reduce the amount of enzyme in the reaction system.

Increase the concentration of RNA in the reaction system by introducing more RNA or decrease the volume of the reaction

system.

2. Poly(A) tail is shorter than expected.

Extend the reaction time appropriately.

Appropriately increase the amount of enzyme in the reaction system.

Reduce the concentration of RNA in the system by reducing the amount of RNA or increase the volume of the system.

3. Failed to tailing

The 3'end of RNA may be hidden in the secondary structure, which can be removed by proper heating.

RNA substrate is contaminated, RNA substrate purification can be performed again.

Enzyme inactivation, confirm whether the enzyme is stored at -20℃, keep the enzyme always on ice during experimental operation.

ATP degradation, confirm that ATP is stored at -20℃, keep the enzyme always on ice during the test operation.

Related Products

Product Number	Product Name
GMP-M072	mRNA Cap 2´ O Methyltransferase, GMP Grade
GMP-RI01	RNase Inhibitor, GMP Grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade (ppase)
GMP-DI05	DNase I Recombinant GMP grade
GMP-M062	Vaccinia Capping Enzyme, GMP Grade
GMP-T701	T7 RNA Polymerase, GMP Grade
D1331	dATP 100mM solution
D2331	dGTP 100mM solution
D3331	dCTP 100mM solution
D4331	dTTP 100mM solution