**Tinzyme Co., Limited**

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# T7 RNA Transcription Enzyme Mix

**Product Number: TM01**

## Shipping and Storage

-20℃

## Description

As a biological macromolecule, mRNA can be synthesized on a large scale by in vitro transcription (IVT). T7 promoter is one of the promoters with the highest transcription efficiency. Therefore, T7 RNA Polymerase can be used for in vitro transcription to obtain more synthetic products. T7 RNA Transcription Enzyme Mix has been optimized by a series of transcription systems. One reaction can transcribe up to 150-200μg of RNA, and the synthesized RNA can be used downstream in many aspects such as mRNA vaccine preparation, RNA structure and function research, RNase protection, probe hybridization, RNAi, microinjection and in vitro translation application.

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.

This product has passed the HALAL certification.

| Element | Standard |
|---|---|
| Appearance | Clear and transparent solution |
| Visible Particles | Meet the specification |
| pH | 7.5-8.5 |
| Performance | 1μl enzyme mix can be used to transcribe no less than 150μg RNA |
| Endonuclease Residues | The degradation of substrate was ≤10% |
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| RNase Residues | The degradation of substrate was ≤10% |
| Bacterial Endotoxins | <10EU/mg |
| Exogenous DNA Residues | ≤100pg/mg |
| Host-cell Protein Residues | ≤50ppm |
| Mycoplasma | Negative |
| Heavy Metals | ≤10ppm |
| Microbial Limit | Total aerobic microbial count≤1cfu/10ml, total yeasts and molds count≤1cfu/10ml |

## 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.

## Application

1. Single stranded RNA synthesis
2. RNA probe synthesis.

3. siRNA precursor synthesis
4. Precursor for RNA splicing preparation
5. Capped RNA synthesis.

## Packaging

| Components | Volume |
|---|---|
| T7 RNA Transcription Enzyme mix, GMP Grade | 50μl |
| T7 RNA Transcription Enzyme mix, GMP Grade | 1ml |
| T7 RNA Transcription Enzyme mix, GMP Grade | 10ml |

## Note

1. Template efficiency and incubation time:
   This kit can generate 150-200μg of RNA with 1μg of template input, however, the yield of different templates will vary depending on the sequence, structure, length, purity of the template and the sequence and length of the specific RNA polymerase promoter. Contaminants that affect transcript yield include RNases or contaminants such as phenol, trace metals, and SDS.
2. Optimized reaction:
   The recommended reaction conditions are suitable for in vitro transcription of most templates, however, for some templates, the yield can be improved by increasing the reaction time (4 hours-overnight reaction) and increasing the amount of template.
3. The amount of templates:
   The table below summarizes our experience in regulating the amount of templates. Results may vary depending on the template used, and extending the reaction time to 4-6 hours increases the yield of RNA.

| The amount of templates | The yield of RNA |
|---|---|
| 1000ng (1μg) | 130-160μg |
| 500ng (0.5μg) | 110-130μg |
| 100ng (0.1μg) | 30-50μg |
| 50ng (0.05μg) | 15-25μg |
| 10ng (0.01μg) | 10-20μg |
| 1ng (0.001μg) | 3-8μg |

4. Maintain RNase-free environment:
   Use RNase-free tubes and pipettes;
   Gloves should be worn and changed frequently when handling kit components or samples containing RNA, especially after exposure to potential sources of RNase contamination such as doorknobs, pens, pencils, and human skin.
   All reagents should be sealed when not in use. During incubation, all tubes containing RNA were sealed.
5. Since the 10×Transcription Buffer contains spermidine, which may bind nucleic acid and, generate insoluble complex at low temperature, it is recommended not adding template DNA and enzyme until the last step

## Template Preparation

Linearized plasmids with double-stranded T7 promoter, PCR products or synthetic DNA fragments can be used as templates for in vitro transcription of T7 High Yield RNA Transcription Kit, and the templates can be dissolved in TE buffer or RNase-free Water.

1. Plasmid template (recommended to add 1μg of linearized plasmid to each reaction as template)
   Plasmids with T7 promoter can be used as transcription templates. The linearization and purity of plasmids will affect the yield of transcription and the integrity of RNA. Due to the lack of effective termination, circular plasmids will transcribe RNA products of different lengths. To obtain RNA of a certain length, the plasmid must be fully linearized, and the linearized plasmid

must ensure that the duplex is blunt-ended or 5' -terminal protruding.

2. PCR product template (recommended to add 0.1μg~1μg to each reaction as template)
PCR products with T7 promoter can be used as templates for in vitro transcription. The T7 promoter was added to the 5' end of the upstream primer of the sense strand when PCR amplifying the template. The PCR product was purified and used as a template.

3. Synthesized DNA template (recommended to add 0.1μg~0.5μg to each reaction as template)
Synthetic DNA fragments with T7 promoters can also be used as templates for in vitro transcription.

## Protocol

**1. In Vitro Transcription**

1.1. Mix the components well except T7 RNA Transcription Enzyme mix, GMP Grade, centrifuge briefly to collect the liquid at the bottom of the tube, and put it on ice for later use.

**Note: Please don't add template DNA and enzyme until the last step.**

1.2. Add the following components:

**1.2.1. Unmodified RNA transcription system**

| Components | Quantity |
|---|---|
| 10×Transcription Buffer, GMP Grade | 2μl |
| ATP/GTP/CTP/UTP (100mM) | 1.5μl for each |
| Template DNA | 100ng-1μg |
| T7 RNA Transcription Enzyme mix, GMP Grade | 1μl |
| RNase Free Water | Up to 20μl |

**1.2.2. Modified RNA transcription system**

| Components | Quantity |
|---|---|
| 10×Transcription Buffer, GMP Grade | 2μl |
| Modified ATP/GTP/CTP/UTP (100mM) | 1.5μl for each |
| Template DNA | 100ng-1μg |
| T7 RNA Transcription Enzyme mix, GMP Grade | 1μl |
| RNase Free Water | Up to 20μl |

Modified NTP such as pUTP, 5-Me-CTP, N1-Me-pUTP, 5-OMe-UTP, etc.

**1.2.3. Co-transcription system**

| Components | Quantity |
|---|---|
| 10×Transcription Buffer, GMP Grade | 2μl |
| Modified ATP/GTP/CTP/UTP (100mM) | 1.5-2μl for each |
| CAP1 GAG (100mM) | 1.5-2μl |
| Template DNA | 100ng-1μg |
| T7 RNA Transcription Enzyme mix, GMP Grade | 1μl |
| RNase Free Water | Up to 20μl |

Taking CAP1 GAG as an example, if using other hat structures, please refer to the recommended proportion of hat structures to prepare the reaction system.

1.3. Gently mix the components with a pipette, collect by centrifugation briefly, and incubate at 37°C for 3 h.
▲In order to avoid the influence of evaporation on the reaction system, it is recommended to carry out the reaction in a PCR machine. The reaction time can be appropriately adjusted according to the length of the product fragments. For example, if RNA less than 0.3 kb is synthesized, the reaction can be extended to 4 h or longer, and the 16 h overnight reaction will not affect thequality of the product.

1.4. Add 2-4U of DNase I Recombinant GMP grade (GMP-DI05) to the reaction system, incubate at 37°C for 15 min, and digest the transcribed DNA template (optional).

▲Compared with product RNA, the content of template DNA is very low. Generally, it does not need to be removed. It can also be digested with DNase I.

1.5. The synthesized RNA can be used for downstream experiments after electrophoresis analysis and purification.

▲The product concentration is extremely high, and it needs to be diluted with RNase-free Water before detection.

**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 10,000rpm 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.

▲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 (7.5M Lithium Chloride, 50 mM EDTA) and 30μl RNase Free Water to 20μl product RNA (Note: 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. When the concentration of the transcription product is low, at 100 400ng/μl, it does not need to be diluted with water and precipitates directly with 30μl Lithium Chloride Precipitation Solution.).

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. How to choose a restriction endonuclease when linearizing a plasmid template?

A plasmid with a promoter can be used as a transcription template. The linearization and purity of the plasmid will affect the yield of transcription and the integrity of the RNA. Since the circular plasmid has no effective termination, RNA products of different lengths will be transcribed. In order to obtain RNA of a specific length, the plasmid must be completely linearized, and the linearized plasmid must ensure that the double strand is blunt-ended or 5' -terminal protruding. Therefore, it is necessary to select a class II restriction endonuclease that can produce a blunt end or 5' -terminal protruding, and the recognition site of the enzyme is a rare site.

2. Is there a requirement for the purity of the transcription template?

   Template DNA should be RNase A-Free and high purity, and the recommended OD260/280 is 1.8~2.0.

3. Does the transcription template have to be removed?

   It is best to add DNase I to remove the template after transcription is complete.

4. Low transcript yield or transcription failure:

   Suggest creating a control group and an experimental group. If the production of the control group is low, please contact us. If the control group's experimental yield is normal but the experimental group's yield is low, there may be quality issues with the template itself leading to low yield. Please try the following solutions:

   4.1. There are components that inhibit the reaction in the experimental template. It is recommended to repurify the template to determine the quantification and integrity of the template;

   4.2. For the problem of the experimental template sequence, it is recommended to extend the reaction time at 37°C, increase the amount of template input, or try other promoters and RNA polymerases.

5. Low yield of short fragment transcripts:

   When the transcript is less than 0.3kb, prolonging the reaction time or increasing the amount of template can improve the RNAyield.

6. Product electrophoresis tailing phenomenon:

   6.1. The experimental operation process is contaminated by RNase;

   6.2. DNA template is contaminated with RNase;

   It is recommended to repurify the template DNA, and pay attention to RNase contamination control in all experimental procedures.

7. The RNA product fragment is larger than expected:

   The plasmid template is not completely linearized or the 3' end of the sense strand has an overhang structure. It is recommended to re-linearize the plasmid template to ensure that the plasmid is completely linearized and the linearized plasmid must be blunt-ended or 5' -terminal protruding;

   RNA has incomplete denatured secondary structure, replace the denaturing gel to detect RNA products.

8. The RNA product fragment is smaller than expected:

   8.1. The template sequence includes a termination sequence similar to T7 RNA polymerase, which leads to premature termination of transcription. It is recommended to try to replace the RNA polymerase;

   8.2. Advanced structure is formed in the template, it is recommended to add SSB protein;

   8.3. RNase contamination.

## Related products

| Product Number | Product Name |
|---|---|
| M062 | Vaccinia Capping Enzyme |
| GMP-RI01 | RNase Inhibitor, GMP Grade |
| M072 | mRNA Cap 2´O Methyltransferase |
| GMP-DI05 | DNase I Recombinant GMP grade |
| M012 | Poly(A) Polymerase |
| M036 | Pyrophosphatase, Inorganic (yeast) (ppase) |
| TR01 | T7 RNA Polymerase |

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