Tinzyme Co., Limited

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T7 RNA Polymerase

Product Number: TR03

Shipping and Storage

Store at -20℃±5℃.

Component

Component	TR03
T7 RNA Polymerase	5KU

Description

As a biomacromolecule, mRNA can be synthesized at scale through in vitro transcription (IVT). The T7 promoter is currently one of the most efficient promoters for transcription, enabling rapid and straightforward production of large quantities of RNA molecules using T7 RNA polymerase. As a byproduct of IVT, double-stranded RNA (dsRNA) can be recognized by corresponding nucleic acid receptors, triggering innate immune inflammatory responses that severely impact the efficacy of mRNA vaccines. Strict control during production is essential. This product employs a T7 RNA polymerase engineered through molecular evolution, significantly reducing dsRNA levels in transcription products and lowering the immunogenicity of synthesized mRNA.

This product is a GMP-grade recombinant T7 RNA polymerase produced through large-scale fermentation using Escherichia coli. It is manufactured with pharmaceutical-grade raw materials and excipients, with strict control over host protein residues, nucleic acid residues, and other impurities. The production and quality management processes comply with GMP standards, ensuring traceability of the entire production process and all raw materials.

Application

1. Synthesize single stranded RNA for the preparation of mRNA vaccines and other applications.
2. Synthesize highly specific RNA probes.
3. Synthesize siRNA precursor.
4. Preparation of RNA splicing precursors.
5. Synthesize capped RNA using cap analogues.

Quality control

Project	Standard
Appearance	Clear liquid
Identification	Should be positive
Visible foreign matter	In compliance with regulations
pH	7.3-7.7
Activity	180-220U/μL
Purity	≥95%
Protein content	In compliance with regulations
Residual endonuclease	The degradation of substrates shall not exceed 10%
Residual exonuclease of nucleic acid	The degradation of substrates shall not exceed 10%
RNA enzyme residue	The degradation of substrates shall not exceed 10%
Bacterial endotoxin	< 5EU/mL
Exogenous DNA residue	≤ 100pg/mg
Residual bacterial protein	≤ 50ppm
Mycoplasma	Negative

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Heavy metal	≤ 10ppm
Microbial Limit	The total number of aerobic bacteria should not exceed 1cfu/10mL, and the total number of mold and yeast should not exceed 1cfu/10mL

Produce according to the following specifications

1. ISO 9001:2015, certified facility.
2. Good Manufacturing Practice for Drugs (current version) and its appendices.
3. Quality control of raw materials and excipients used in the production of biological products in the Chinese Pharmacopoeia (General Rule 0232).
4. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products It is an excipient used in cell therapy, gene therapy, and tissue engineering products.
5. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products Biogenic raw materials used for the production of cell or gene therapy drugs.

Storage buffer solution

50mM Trizma base; 100mM NaCl; 1mM EDTA; 20mM β-ME; 50% (v/v) Glycerol; 0.1% Triton X-100; pH 7.5.

Source

E.coli carrying bacteriophage T7 RNA polymerase gene

Features

It exhibits high specificity for the T7 promoter.

Definition of active units

The enzyme amount required to incorporate 1nmol of [^{3}H] GMP into an acid insoluble precipitate within 1 hour at 37℃ and pH 8.0 is defined as 1 active unit.

Template preparation

Linearized plasmids, PCR products, or synthesized DNA fragments with T7 promoter can be used as in vitro transcription templates for T7 RNA polymerase, which can be dissolved in TE buffer or RNase free water.

1. Plasmid template (recommended to add 1μg linearized plasmid as template for each reaction) plasmids with T7 promoter can be used as transcription templates. The linearization and purity of plasmids can affect transcription yield and RNA integrity. Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain RNA of a specific length, the plasmid must be completely linearized. Linearized plasmids should ensure that the double stranded end is flat or the 5 'end is a protruding structure.
2. PCR product template (recommended to add 0.1μg~1μg as template for each reaction) PCR products with T7 promoter can be used as in vitro transcription templates. Add the T7 promoter to the 5 'end of the upstream primer of the sense chain during PCR amplification of the template. The PCR product is purified and used as a template.
3. The synthesized DNA template (recommended to add 0.1μg~0.5μg as a template for each reaction) can also serve as a template for in vitro transcription of DNA fragments containing the T7 promoter.

Protocol

1. In vitro transcription

1.1. After melting each component, mix them evenly and briefly centrifuge to collect them at the bottom of the tube. Store them on ice for later use.

1.2. Add the following components at room temperature:

Note: After calculating the system, first add water, buffer, and NTP, and finally add the template and enzyme.

Transcription system:

Component	
10×Transcription Buffer, GMP Grade	2μL
ATP/GTP/CTP/UTP Mix	7.5mM (Na salt NTP) -10mM (Tris salt NTP)
Template DNA	500ng-1μg
T7 RNA Polymerase	50-200U
RNase Free Water	Up to 20μL

Co transcriptional system:

Component	
10×Transcription Buffer, GMP Grade	2μL
ATP/GTP/CTP/UTP Mix	7.5mM (Na salt NTP) -10mM (Tris salt NTP)
Cap1 GAG	7.5mM (Na salt NTP) -10mM (Tris salt NTP)
Template DNA	500ng-1μg
T7 RNA Polymerase	50-200U
RNase Free Water	Up to 20μL

Note: Due to the salt type of NTP affecting the yield of transcription and co transcription, it is recommended to choose a final concentration of 7.5mM for NTP reaction using Na salt, which can achieve a yield of 100-160μg; If Tris salt is used for NTP, it is recommended to choose a final concentration of 10mM for the reaction, and the yield can reach 180-220μg.

RNase inhibitor can be added to the reaction system at a concentration of 1U/μL to prevent RNase contamination.

The template DNA should be RNaseA Free and of high purity, with a recommended OD260/280 of 1.8-2.0.

1.3. Gently mix the components with a pipette and collect them by centrifugation briefly. Incubate at 37℃ for 2-3 hours.

To avoid the impact of evaporation on the reaction system, it is recommended to conduct the reaction in a PCR instrument. The reaction time can be adjusted appropriately according to the size of the product fragment. For example, when synthesizing RNA smaller than 0.3kb, the reaction can be extended to 4 hours or longer. Overnight reaction for 16 hours will not affect the quality of the product.

1.4. Add 2-4U of DNase I to the reaction system, incubate at 37℃ for 15 minutes, and digest the transcribed DNA template. (Optional)

Compared to the product RNA, the content of template DNA is very low and generally 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 needs to be diluted with RNase free water before detection.

2. Product purification

2.1. Purification of lithium chloride

2.1.1. Add 30μL of Lithium Chloride Preparation Solution (7.5M Lithium Chloride, 50mM EDTA) and 30μL of RNase Free Water (note: effective precipitation cannot be obtained by this method when RNA is less than 300nt or concentration is less than 100ng/μL) to a volume of 20μL. The precipitation effect is best when the RNA concentration is greater than 400ng/μL. When the concentration of transcription products is low, between 100-400ng/μL, there is no need to dilute with water, and 30μL Lithium Chloride Precipitation Solution can be directly used for precipitation;

2.1.2. Mix well and place at -20℃ for at least 30 minutes.

2.1.3. Centrifuge at 12000rpm for 15 minutes, remove the supernatant, and collect the precipitate.

2.1.4. Wash three times with pre cooled 70% ethanol.

2.1.5. Detection after RNase Free Water reconstitution.

2.2. Column purification

Column purification can remove proteins and free nucleotides.

Dilute the product to 100μL by adding 80μL RNase free water before purification, and then purify according to the column purification instructions.

Due to the high RNA production, in order to avoid exceeding the carrying capacity of the binding column, please estimate the required number of columns.

2.3. Magnetic bead purification

Magnetic bead purification can remove proteins and free nucleotides.

Purify according to the instructions for magnetic bead purification.

2.4. Phenol/chloroform purification method

Phenol/chloroform extraction can remove proteins and most free nucleotides.

2.4.1. Dilute the product to 180μL by adding 160μL of RNase free water.

2.4.2. Add 20μL of 3M sodium acetate (pH 5.2) to the diluted product and mix thoroughly with a pipette.

2.4.3. Add 200μL of phenol/chloroform mixture (1:1) for extraction, centrifuge at 10000 rpm for 5 minutes at room temperature, and transfer the upper solution (aqueous phase) to a new RNase free EP tube.

2.4.4. Add chloroform with an equal volume to water for extraction twice, and collect the upper aqueous phase.

2.4.5. Add 2 times the volume of anhydrous ethanol and mix well. Incubate at -20℃ for at least 30 minutes and centrifuge at 15000 rpm at 4℃ for 15 minutes.

2.4.6. Discard the supernatant and add 500μL of pre cooled 70% ethanol to wash the RNA precipitate. Centrifuge at 15000 rpm at 4℃ and discard the supernatant.

2.4.7. Open the lid and dry for 2 minutes. Add 20-50μL of RNase free water or other buffer solution to dissolve the RNA precipitate.

2.4.8. Store at -70℃.

RNA quantification

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

Dye method: RiboGreen dye is used for RNA quantification, and free nucleotides do not affect quantification. It can accurately quantify RNA in purified or unpurified reaction products.

Note

1. Template efficiency and incubation time: The yield of different templates may vary depending on their sequence, structure, length, purity, as well as the sequence and length of specific RNA polymerase promoters. Pollutants that affect transcription yield include ribonucleases or contaminants such as phenol, trace metals, and SDS.
2. Optimized reaction: The recommended reaction conditions can be applied to the in vitro transcription of most templates, but for some templates, the yield can be improved by extending the reaction time (4-hour overnight reaction) and increasing the amount of template used.
3. Maintain RNase free environment: use RNase free tubes and pipettes; Gloves should be worn when handling kit components or samples containing RNA, and gloves should be changed frequently, especially when in contact with potential sources of RNase contamination such as door handles, pens, pencils, and human skin. When not in use, all reagents should be sealed properly. During the incubation process, seal all test tubes containing RNA.
4. When configuring the reaction system, 0.5μL of RNase inhibitor can be added.
5. Due to the presence of spermidine in the 10 × Translation Buffer, it will form a precipitate with the template DNA at low temperatures. When preparing the reaction solution, it needs to be done at room temperature. Adjust the order of component addition, calculate the system, add water, buffer, and NTP first, and finally add the template and enzyme.

FAQ

1. **How to choose restriction endonucleases when linearizing plasmid templates?**

Plasmids with promoters can serve as transcription templates, and the linearization and purity of plasmids can affect transcription yield and RNA integrity. Due to the lack of effective termination, circular plasmids can transcribe RNA products of different lengths. In order to obtain RNA of specific lengths, the plasmid must be completely linearized, and the linearized plasmid must ensure that the double stranded end is flat or the 5 'end is a protruding structure. Therefore, it is necessary to select type II restriction endonucleases that can produce prominent structures at the end or 5 'end, and the recognition site of the enzyme is a rare site.

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

The template DNA should be RNaseA Free and of high purity, with a recommended OD260/280 of 1.8-2.0.

3. **Do transcription templates need to be removed?**

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

4. **Low transcript production or transcription failure:**

Suggest creating a control group and an experimental group. If the yield of the control group is low, please contact nearshore protein technical support. If the yield of the control group experiment is normal but the yield of the experimental group is low, there may be a quality problem with the template itself that leads to the low yield. Please try the following solution to solve it:

4.1. The experimental template contains components that inhibit the reaction. It is recommended to purify the template again to determine the quantification and integrity of the template;

4.2. Regarding the issue with the experimental template sequence, it is recommended to extend the reaction time at 37℃, increase the amount of template input, or try other promoters and RNA polymerases.

5. **Low yield of short transcription products:**

When the transcription product is less than 0.3kb, prolonging the reaction time or increasing the template amount can increase RNA production.

6. **Product electrophoresis tailing phenomenon:**

6.1. The experimental operation process was contaminated by RNase;

6.2. DNA template contaminated by RNase;

Suggest re purifying the template DNA, and pay attention to RNase contamination control during all experimental processes.

7. **RNA product fragment larger than expected:**

The plasmid template is not fully linearized or has a prominent structure at the 3 'end of the sense strand. It is recommended to re linearize the plasmid template to ensure complete linearization. For linearized plasmids, please ensure that the double strand has a flat end or a prominent structure at the 5' end;

RNA has an incompletely denatured secondary structure, and the denatured gel is replaced 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 replacing the RNA polymerase;

8.2. Advanced structures are formed in the template, and it is recommended to try adding SSB protein;

8.3. RNase contamination.