T7 High Efficiency Transcription Kit

Please read the manual carefully before use.

Cat. No. JT101

Storage: at -20℃ for one year

Description

This kit is designed to efficiently transcribe the DNA sequence at the downstream of the T7 promoter which is contained in supercoiled plasmid DNA or linear DNA as templates, with T7 RNA Polymerase in an optimized in vitro transcription reaction mix. It is suitable for the preparation of high-concentration RNA with a length of more than 6000 nt. Using 1 μg of DNA template, 150~280 μg of RNA can be generated in a 20 μl reaction mix (if milligram-level RNA products are required, the reaction mix can be scaled up in parallel). The prepared RNA can be used for in *vitro* translation, RNase protection assays, RNA shearing, and hybridization probe labeling.

Kit Contents

Component	JT101-01 (25 rxns)	JT101-02 (100 rxns)
T7 Transcription Enzyme Mix	50 μl	200 μl
5×T7 Transcription Reaction Buffer	100 μl	400 μl
ATP (100 mM)	50 μl	200 μl
GTP (100 mM)	50 μl	200 μl
CTP (100 mM)	50 μl	200 μl
UTP (100 mM)	50 μl	200 μl
DNase I (1 unit/μl)	50 μl	200 μl
500 mM EDTA (pH 8.0)	25 μl	100 μl
RNase-free Water	1 ml	5 ml
Transcription Control Template (0.5 μg/μl)	10 μl	40 μl

RNA Synthesis

Principle of T7 RNA Polymerase Transcription

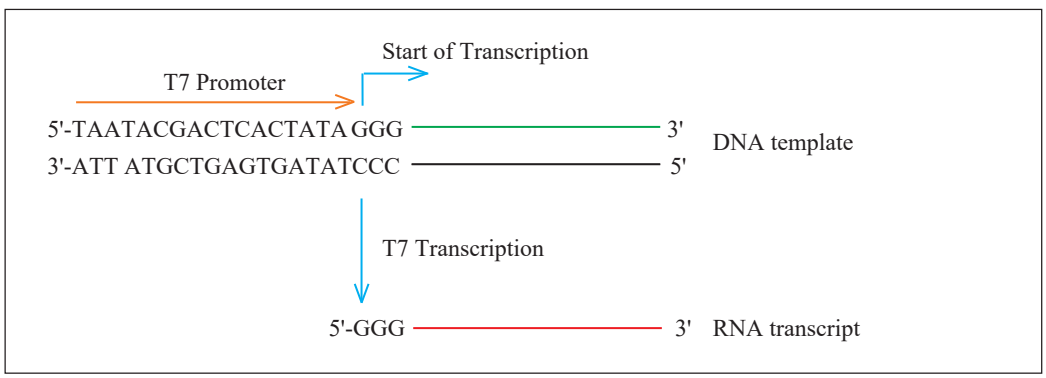

Figure 1. Principle of T7 RNA Polymerase Transcription

Template Preparation

1. Supercoiled Plasmid DNA

The supercoiled plasmid needs to have a T7 promoter and a terminator that can effectively terminate transcription. The termination efficiency of the T7 terminator of common vectors is less than 70%, and the transcription reaction will produce more transcripts that read through the terminator. To terminate transcription, it is recommended to use the transcription terminators consisting of the following sequences, or to use restriction enzymes creating linearized DNA at the ends of transcription (see term 2 for details)

Transcription template

T7 Promoter ——————————— Terminator

T7 Promoter: 5'-TAATACGACTCACTATAGGG$^{\#}$-3' #: G/A

Terminator: 5' TTCCATCTGTTTTCTTATCTGTTCTTTCATCTGTTCTTTTATCTGTTTGTTT 3'

2. Linearized DNA

Generally, it is a plasmid digested with restriction endonucleases or a PCR product. When linear DNA is used as a transcription template, 3' overhangs should be avoided, so restriction endonucleases that form 5' overhangs or blunt end products are generally selected. The digestion product or PCR product needs to be purified to remove protein, salt ions, etc. to obtain high-quality DNA as a template.

3. Template Amount

4. The reaction system recommended by this kit is suitable for reactions with most templates. 1 μg of template input can generate 150-280 μg of RNA in 2 hours. The table below summarizes the correlation between template input and product using this kit.

Template Amount	RNA Yield
2 μg	170~320 μg
1 μg	150~280 μg
500 ng	100~180 μg
200 ng	40~80 μg
100 ng	15~40 μg
50 ng	10~20 μg
10 ng	4~8 μg
1 ng	2~6 μg

However, specific yield will vary depending on the length, purity, sequence, and structure of different templates. For most transcription reactions, higher yield can be obtained by increasing template input and prolonging reaction time.

*Note: This table is suitable for most transcription reactions, but for transcription of short fragments <500 nt, it is suggested to refer to the following data reference 1-3.

Notes

• Avoid RNase contamination: Be sure to use RNase-free water, pipette tips (including filter cartridges) and microcentrifuge tubes, and wear lab coats and gloves during operation.

• Please prepare the reaction at room temperature: The 5×7 Transcription Reaction Buffer of this kit has a high salt concentration at low temperature, which leads to salt precipitation, affecting the template DNA and enzyme activity. Therefore, when preparing the reaction mix, except for the enzyme and NTP, which should be temporarily stored on ice, other components should be kept at room temperature, and the system should be prepared at room temperature.

• Pay attention to the order of sample addition and pre-calculate the volume. Add each reaction component in strict accordance with the following sequence: Water→Buffer→NTP→DNA template→Enzyme. The template and enzyme must be added last.

• For the transcription of short fragments <500 nt, it is recommended to use 2 μg of the template. For ultra-short fragments of about 100 nt, it is recommended to use 2 μg of template and extend the reaction time to more than 6 hours. The maximum yield can be obtained by an overnight 16-hour reaction.

• For transcription times of 6 hours or more, use 2 μg of the template and increase the final NTP concentration to 10 mM.

Operation procedures

1. Except for the T7 Enzyme Mix, the other components are briefly centrifuged and collected at the bottom of the tube.

2. Prepare transcription reactions:

Component	Volume	Final Concentration
Template	1 ng~2 μg	NA
5×T7 Transcription Reaction Buffer	4 μl	1×
A/G/C/UTP	1.6 μl each	8 mM each
T7 Transcription Enzyme Mix	2 μl	NA
RNase-free Water	Variable	NA
Total Volume	20 μl	20 μl

*Note: Pre-calculate the volume. Add each reaction component in strict accordance with the following sequence: Water→Buffer→NTP→DNA template→Enzyme. The template and enzyme must be added last.

3. Gently mix the components with a pipette and centrifuge briefly to collect at the bottom of the tube. Incubate at 37°C for 2 hours.

*Note: To avoid volatilization of the reaction solution due to prolonged transcription, it is recommended to perform the reaction in a PCR machine and set the heated lid to 65°C. The amount of template and incubation time can be adjusted appropriately.

4. Digestion of DNA template: After the reaction, add 2 μl of DNase I to react at 37°C for 30 minutes. At the end of the reaction, add 1 μl of 500 mM EDTA (pH 8.0) to stop the reaction (the subsequent purification should be carried out immediately after adding EDTA). Or directly perform the purification step without adding EDTA after digestion.

5. Product purification: Please refer to the manual of *EasyPure*® RNA Purification Kit (ER701) or *MagicPure*® RNA Beads (EC501).
6. Transcript quantification and detection:
(1) The RNA concentration was determined by a UV spectrophotometer. As the product concentration was extremely high, it is recommended to dilute before measuring.
(2) 100~1000 nt RNA products are recommended to be detected by 6% acrylamide, 7M denaturing gel, with 1×TBE Buffer as the electrophoresis buffer.
• 10×TBE Buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20mM EDTA.
• Gel preparation method: Per 10 ml, urea 4.2 g, RNase-free Water 4.4ml, 40% (acrylamide: methylenebisacrylamide=19:1) acrylamide 1.5 ml, 10×TBE Buffer 1 ml, 10% AP 100 µl, TEMED 10 µl. AP and TEMED are added after the urea was completely dissolved.
(3) 500~6000 nt RNA products are recommended to be detected by 1% formaldehyde agarose denaturing gel, with 1×MOPS Buffer as the electrophoresis buffe.
• 10×MOPS Buffer: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA.
• Gel preparation method: Per 100 ml, weigh 1 g of agarose and add it to 72 ml of RNase-free Water. After heating and melting, add 10 ml of 10×MOPS Buffer. When the solution is cooled to 50~60°C, add 18 ml of formaldehyde (37%). Mix well, and pour the gel.
(4) For electrophoresis detection, take 0.2-1 µg RNA, and dilute it to 5 µl with RNase-free Water. Add an equal volume of 2× RNA Loading Buffer (GH201), and mix wel., Incubate at 70°C for 10 minutes, and then put it in ice bath for 2 minutes. Load all samples. Gelstain or EB staining is used for observation after electrophoresis. RNA Maker is processed in the same way as RNA samples (or refer to the manufacturer's instruction manual).

Data References

1. The relationship between transcription time and yield: Take a 20 µl reaction volume with 1 µg template added as an example, and the results are as follows:

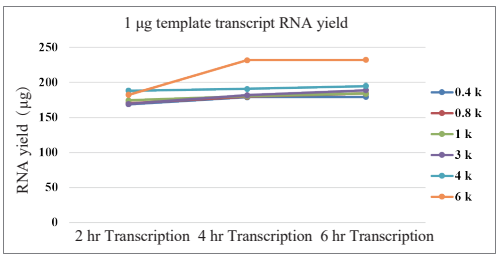

Figure 2. The relationship between the transcription yield and time of RNAs of different lengths (400nt short fragments are 2 µg template transcription results)

2. The relationship between template input and yield: Take a 20 µl reaction volume for 2 hours of transcription as an example, and the results are as follows:

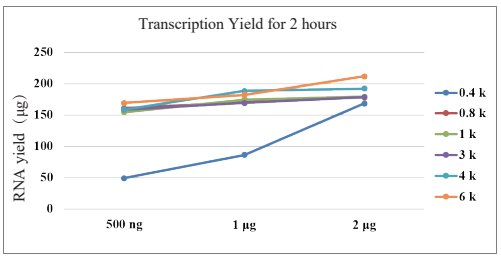

Figure 3. The relationship between the yield of transcribed RNA of different lengths and the amount of template input

3. The relationship between the input amount, transcription time and yield of short fragment templates with a length of less than 300 nt. Take a 20 µl reaction volume as an example, and the results are as follows:

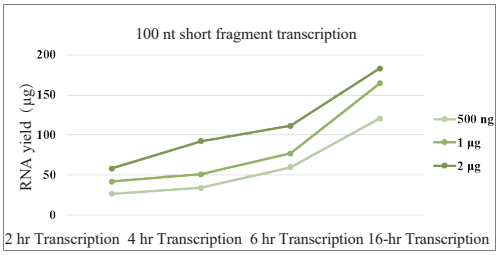

Figure 4. The relationship between the RNA transcription yield and the amount of template input and transcription time for RNAs less than 300 nt in length

FA&Q

1. Low transcription yield or transcription failure

Template quality is closely related to RNA yield, and it is recommended to set up a control group. If the yield of the control group is low, please contact TransGen customer service for guidance on the reaction system. If the output of the control group is normal while the output of the experimental group is low, there may be quality problems in the template of the experimental group. It is recommended to try the following solutions: ①The template may contain components that inhibit the reaction. Please repurify the template. The template DNA should be RNase-free and at high concentration; ②Determine template quantity and its integrity; ③ Extend transcription reaction time; ④Increase template input; ⑤Try other promoters and RNA polymerases.

2. Tailing appeas in transcript electrophoresi

It may be caused by: ①RNase contamination during electrophoresis experiment; ②Transcription template contaminated with RNase. It is recommended to re-prepare the template DNA. The consumables and reagents used in the experiment should be of RNase-free grade. Wear a mask and disposable latex gloves during the experimental operation. Pay attention to RNase contamination control.

3. Transcript length longer than expected

If electrophoresis shows that the product band is longer than expected, it may be caused by the following reasons: ①Inefficient terminators do not terminate transcription or templates are not fully linearized; ②The 3' end of the sense strand has a protruding structure; ③The product RNA has an incompletely denatured secondary structure.

The following solutions are suggested: ①Check whether the template is completely linearized, and ensure that the linearization is complete; ②Select a suitable restriction enzyme to ensure that the 5' end of the sense strand is overhanging or the double stranded DNA is blunt-ended; ③Check template structure and detect RNA product with denaturing gel.

4. Transcript length shorter than expected

If electrophoresis shows that the product band is shorter than expected, it may be caused by the following reasons: ①The template sequence contains a transcription terminator sequence similar to T7 RNA polymerase, resulting in premature termination of transcription; ②The template sequence has high GC content to form higher-order structures; ③RNase contamination.

If the template contains a terminator sequence, it is recommended to replace the RNA polymerase. If the template GC content is high, try adding SSB protein to increase yield and transcript length.

5. Low transcription yield of short fragments

If the transcription fragment is too short, the reaction will be inhibited. When the transcription length is less than 500 nt, please prolong the reaction time and increase the amount of template input. It is recommended to use 2 μg of template. For the transcription of 100 nt super-short fragments, it is recommended to use 2 μg of template to react for at least 6 hours, 16 hours of overnight transcription for maximum yield.

Quality Control

Term	Standard	Method
Appearance	Transparent and clear	Visual inspection
Purity	95%	SDS-PAGE or SEC-HPLC
Endonuclease Residues	Not detected	4 μl Enzyme/ 20 μl Buffer and 1 μg PUC19 plasmid areincubated at 37°C for 16 hours
Exonuclease Residues	Not detected	4 μl Enzyme/ 20 μl Buffer and 1 μg Hela gDNA are incubated at 37°C for 16 hours
Nonspecific Nuclease Activity	Not detected	4 μl Enzyme/20 μl Buffer and 1 μg 1 kb DNA Ladder are incubated at 37°C for 16 hours
RNase Residues	Not detected	4 μl Enzyme/20 μl Buffer and 1 μg Hela RNA/ in vitro transcribed RNA are incubated at 37°C for 4 hours
Bacterial Residues	Not detected	No colony formation after plated in LB medium and cultured at 37°C for 2 days, or no significant change in OD_{600} detected after overnight culture in SOC liquid medium
Bacterial Endotoxin Content	<10 EU/mg	Chinese Pharmacopoeia 2020 Edition Part 4 Gel Limit Experiment (General Chapter 1143)
Host DNA Residues	≤1 copy/U	Real-Time PCR
Mycoplasma Detection	Negative	Mycoplasma Detection Kit

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