# T7 RNAi Transcription Kit

**TR102**

Version 9.1

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## Introduction

The T7 RNA ploymerase can perform in vitro transcription of RNA from DNA templates containing T7 RNA Polymerase promoter using four NTP substrates. T7 RNAi Transcription Kit is an optimized version of T7 High Yield RNA Transcription Kit that designed for transcription of double-stranded RNA. It can also be used to transcribe 21 bp siRNA and long fragment dsRNA. The purified transcript can be used for RNAi experiments mediated by cationic liposome, calcium phosphate coprecipitation, electroporation, DEAE-dextran and microinjection. In general, one reaction produces 20 - 80 μg of RNA.

## Package Information

| | Components | TR101-01 50 rxn | TR101-02 100 rxn |
|---|---|---|---|
| **Box 1** | T7 Enzyme Mix | 50 μl | 100 μl |
| | 10 × Transcription Buffer | 50 μl | 100 μl |
| | 10 × Annealing Buffer | 250 μl | 500 μl |
| | NTP Mix | 200 μl | 400 μl |
| | DNase I | 25 μl | 50 μl |
| | RNase T1 (100 U/μl) | 25 μl | 50 μl |
| | RNase T1 Dilution Buffer | 300 μl | 600 μl |
| | Control Template* | 5 μl | 10 μl |
| **Box 2** | RNase-free $H_2O$ | 5 ml | 5 ml |
| | RNA Clean Beads | 2 ml | 4 ml |

*The Control Template provided by this kit is a 500 bp PCR product containing the T7 promoter of both ends at a concentration of 0.5 μg/μl.

## Storage

Store Box 1 at -20℃, and Store Box 2 at 4℃.

## Application

This kit is suitable for in vitro transcription of siRNA and long-chain dsRNA. For transcription of single-stranded RNA, T7 High Yield RNA Transcription Kit (Vazyme #TR101) is recommended.

## User Prepared

Template: a linearized plasmid with a T7 promoter sequence, a PCR product or a chemically synthesized DNA fragment.

Other: RNase-free EP tube, pipette tip; PCR instrument; magnetic stand; anhydrous ethanol.

## Notes

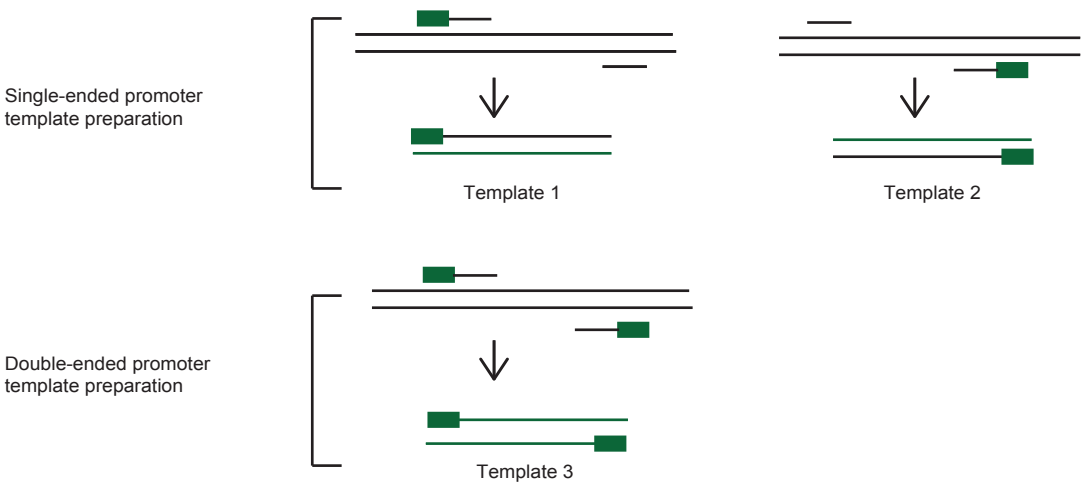
1. Wear disposable gloves and a mask when testing to avoid degradation of the product by RNase.
2. Please use RNase-free experimental consumables.
3. Electrophoresis to ensure the template as a single fragment before transcription.

## Template Preparation

### 1. PCR Product Template

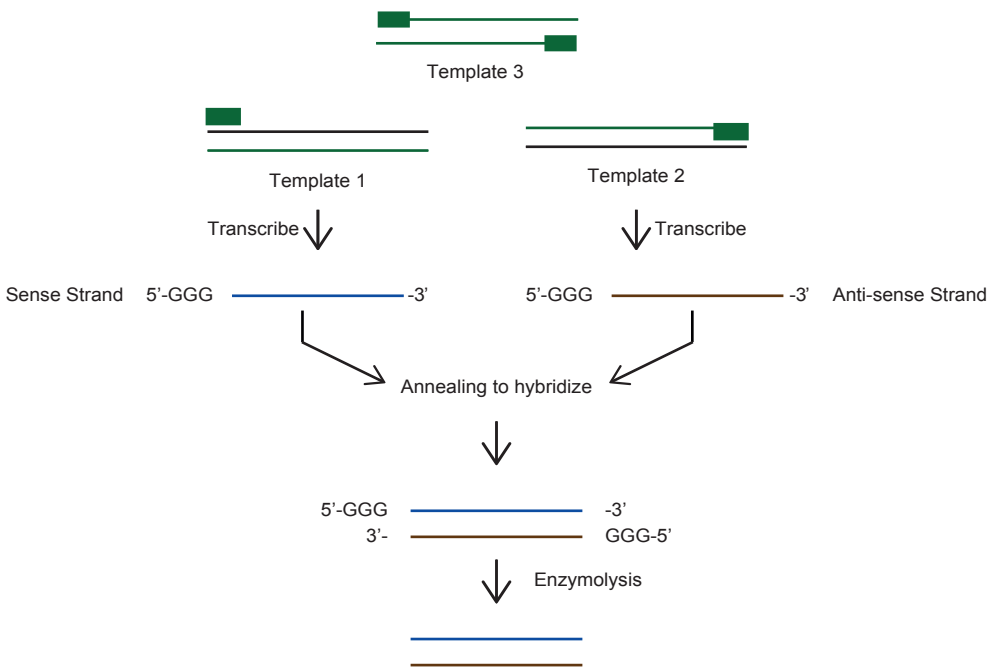
The long-stranded dsRNA can be amplified with a specific primer with a T7 promoter (TAATACGACTCACTATAGGG) at the 5' end. The scheme is shown in Figure 1 below. The green box indicates the T7 promoter and the green line indicates the transcription template strand. The transcription initiation site is located at the GGG (underlined) position of the T7 promoter TAATACGACTCACTATAGGG. The PCR product can be used as a template without purification, but it will increase RNA yield after purification. The recommended template loading amount is 0.5 μg.

▲ There are three transcription schemes: ① Template 1 and template 2 are transcribed in two PCR tubes and then the product is 1:1 annealed into double strands. ② Mix Template 1 and template 2 in the same PCR tube and transcribed into double strands; ③ template 3 with a double-ended promoter was annealed to double strands after transcription. In general, the amount of transcripts yield of Schemes 1 and 2 is higher.




Picture 1. Schematic diagram of PCR amplification of in vitro transcription templates

### dsRNA Transcriptional Experimental Procedure:




Picture 2. Experimental procedure of dsRNA transcription

### 2. Template Synthesis

The transcript template of siRNA is short, four single-stranded DNAs can be separately synthesized, and then annealed as shown in FIG. 3 to obtain two double-stranded DNA templates. Since the siRNA template fragment is short, the efficiency of polymerase binds to the template is low. So it is recommended to add six bases of GATCAC to the 5' end of the T7 promoter to promote the binding of the enzyme to the template during the template synthesis process.

▲The two free bases contained at 3' end of siRNA will increase the binding efficiency of siRNA to mRNA.The free base of UU has the strongest inhibitory effect on the target gene. If the free base is GG, it will be degraded by intracellular RNase, which results in a decrease in siRNA activity.

a. Annealing into two transcription templates as follows:

| Components | Volume |
|---|---|
| RNase-free $H_2O$ | 14 μl |
| Oligonucleotides A1 (or B1) 100 μM | 2 μl |
| Oligonucleotides A2 (or B2) 100 μM | 2 μl |
| 10 × Annealing Buffer | 2 μl |

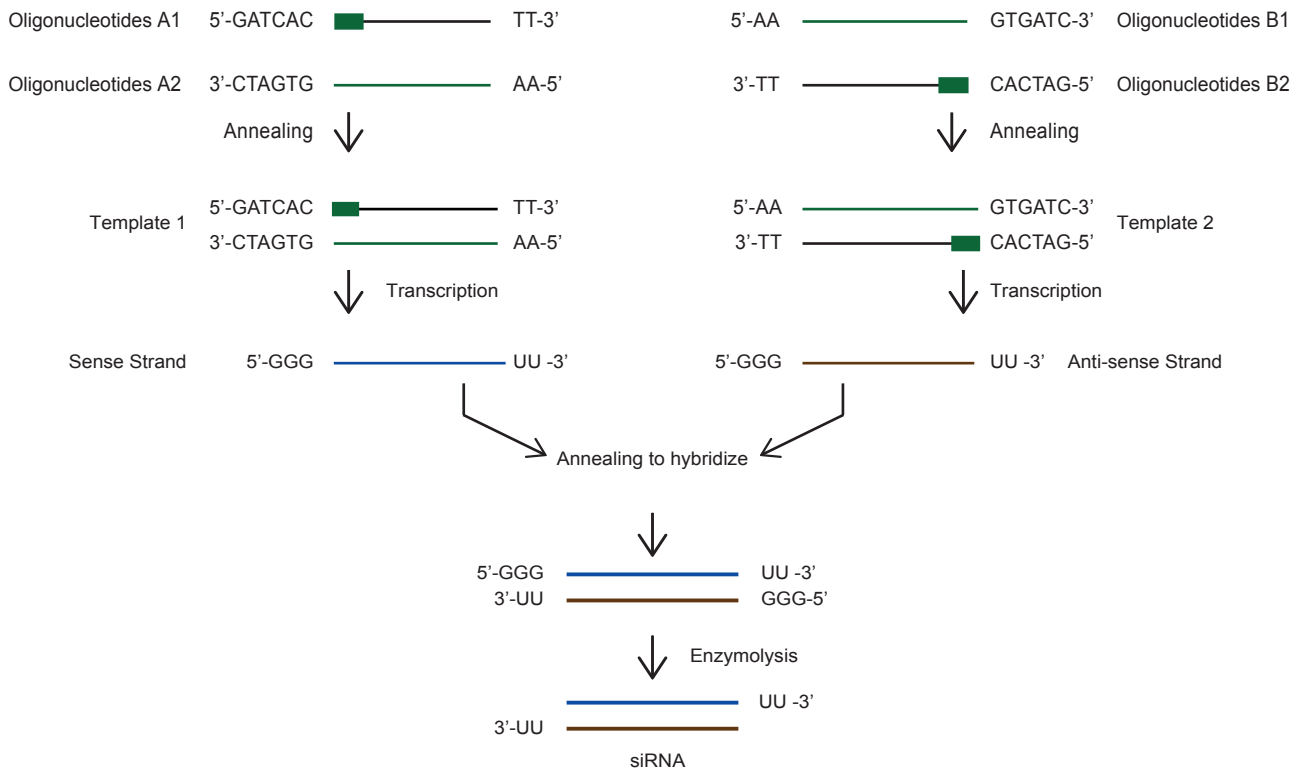
▲Templates A1 and A2, and templates B1 and B2 must be complementary to each other (as shown in Figure 3).

b. Perform the following annealing procedure in a PCR instrument:

| Temperature | Time |
|---|---|
| 95℃ | 2 min |
| 95 - 22℃ | 0.1℃/sec |
| 22℃ | 10 min |

▲Two 10 μM DNA templates were obtained after annealing, and the template can be directly transcribed in vitro.

**siRNA Transcriptional Experimental Procedure:**




Picture 3. Experimental procedure of siRNA transcription

## Protocol

1. Prepare the reaction system according to the following table:

| Components | Volume |
|---|---|
| NTP Mix | 8 μl |
| 10 × Transcription Buffer | 2 μl |
| T7 Enzyme Mix | 2 μl |
| DNA Template 1 | 1 - 4 μl |
| DNA Template 1 | 1 - 4 μl |
| RNase-free $H_2O$ | Up to 20 μl |

▲Mix template 1 and template 2 in a ratio of 1:1. The recommended input amount is 0.5 μg each. Mix gently by pipetting, and centrifuge the reagent to the bottom of the tube.

2. Incubate at 37˚C for 2 hours in a PCR instrument.

▲The reaction time can be adjusted according to the size of the product fragment. For example, if the RNA is less than 0.3 Kb, the reaction can be extended to 4 h or more, and the overnight reaction does not affect the quality of the product.

3. For the length of the double strand larger than 800 bp, incubate at 72˚C for 10 min after after step 2, and then naturally cooled to anneal to dsRNA.

▲Transcription products less than 800 bp will complement to each other to form dsRNA, and more than 800 bp will need to be annealed to form dsRNA. If two templates are separately transcribed in different PCR tubes, the two products need to be mixed for annealing to form dsRNA.

4. Dilute 100 U/μl RNase T1 to 10 U/μl with RNase T1 Dilution Buffer.

▲RNase T1 specifically degrades single-stranded RNA and 3 G bases at the 5' end. The diluted RNase T1 should be used as soon as possible and should not be stored.

5. Prepare a double enzyme digestion system according to the following table:

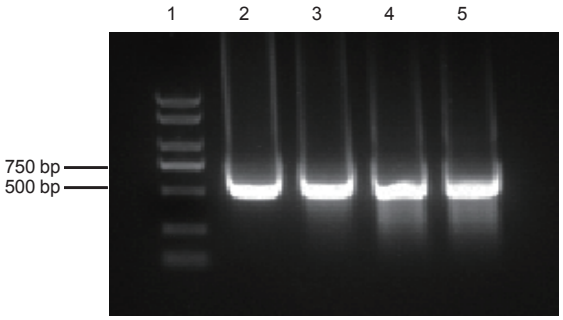
| Components | Volume |
|---|---|
| Transcription Product | 20 μl |
| RNase-free $H_2O$ | 17 μl |
| DNase I | 1 μl |
| RNase T1 (10 U/μl) | 2 μl |
| Total | 40 μl |

▲Mix the solution gently by pipetting, and briefly centrifuge to collect the reagent to the bottom of the tube.

6. Incubate at 37˚C for 30 min.

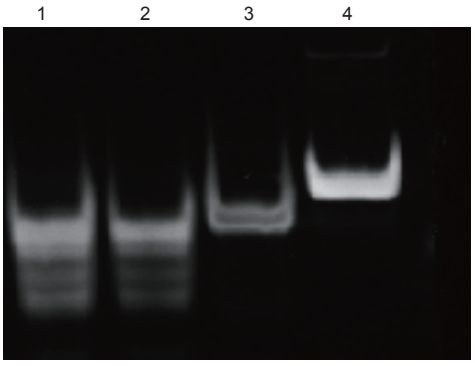
7. Detect the transcripts by electrophoresis.

▲Long fragment dsRNA can be detected by agarose gel electrophoresis, and siRNA was detected by polyacrylamide gel (PAGE) electrophoresis. Due to the small size of the siRNA, the transcription product will have dispersion.




1: DL2,000 Plus DNA Maker;
2 and 4: products before and after enzymatic hydrolysis of 500 bp dsRNA, respectively (Scheme 2);
3 and 5: products before and after enzymatic hydrolysis of 500 bp dsRNA, respectively (Scheme 3)

Figure 4. 2% agarose gel electropherogram of a 500 bp dsRNA




1: Transcript of siRNA;
2: Double enzyme digestion product of siRNA transcript;
3: Single chain template;
4: Double-strand annealing template.

Figure 5. 12% PAGE electrophoresis results of siRNA.

## Introduction

The RNA product can be purified by the methods of magnetic beads, column, phenol/chloroform extraction or gel recovery. This kit recommends the magnetic beads purification methods.

▲Magnetic bead purification can quickly and efficiently remove proteins, free nucleic acids and salts. The 80% ethanol used for purification needs to be prepared with RNase-free $H_2O$ (prepared by user).

1. Take the RNA Clean Beads out of 4˚C and equilibrate for 30 min at room temperature. Please invert or vortex before use.
2. Add 80 µl of the magnetic bead solution to the transcript and pipette 10 times or more to mix the solution thoroughly.

▲ If fragment length of the transcript is less than 100 bp, add 200 µl of isopropanol and mix well.

3. Incubate for 8 min at room temperature to allow RNA to bind to the beads.
4. Place the PCR tube on the magnetic stand for about 5 minutes. And carefully remove the supernatant after the solution is clarified. Please be careful not to stir the beads when drawing the supernatant.
5. Keep the PCR tube on the magnetic stand and add 200 µl of freshly prepared 80% ethanol. Be careful not to agitate the beads, incubate for 30 sec at room temperature, and carefully remove the supernatant. Repeat this step once.
6. Open the lip to let the magnetic beads air dry for 5 - 10 min.

▲ Dry to the surface of the magnetic beads without water, excessive drying will affect the elution of RNA.

7. Remove the PCR tube from the magnetic stand, add 40 µl of RNase-free $H_2O$, pipette the beads on the tube wall, mix well, and incubate for 3 min at room temperature.
8. Place the PCR tube on the magnetic stand. After the solution is clarified, carefully transfer the supernatant to a new RNase-free EP tube. Do not pick up the beads.

▲In order to avoid the influence of magnetic beads on subsequent experiments, when transferring the product, please reserve 1 - 2 µl of the solution to prevent the magnetic beads from being absorbed.

9. Measure the absorbance of the product at A260, determine its concentration, and store the purified product at -20˚C.

## siRNA Interference Experiment

In this experiment, 293T cells were co-transfected by plasmid with green fluorescent protein (GFP) sequence and 21 bp GFP siRNA to interfere with the expression of fluorescent protein, and ExFect2000 Transfection Reagent (Vazyme #T202) was used as a transfection reagent.

▲In the RNA interference experiment, there are a lot of influencing factors: the state of the cells, the ratio of the transfection reagent to the RNA, the concentration of the RNA, the interference efficiency of the RNA, the transfection efficiency and toxicity of the transfection reagent, etc. All the above factors affect the final interference result. If the interference from the above conditions is unsuccessful, the RNA sequence needs to be redesigned. Negative Control GFP siRNA has the same base composition as Positive GFP siRNA, but has no gene targeting function.

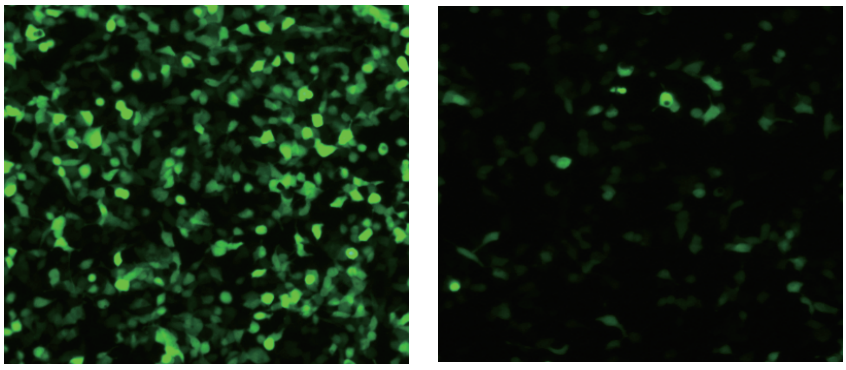


Figure 6. siRNA interferes with the expression of green fluorescent protein.
The left panel shows 293T cells co-transfected with GFP plasmid and Negative control GFP siRNA for 24 h;
The right panel shows 293T cells co-transfected with GFP plasmid and Positive GFP siRNA for 24 h.

## FAQs and Solutions

◇dsRNA experimental design

There are three options for the experimental design of dsRNA. Please select the transcription scheme accordingly. And refer to step 07-1/PCR template for template preparation. If options 1 and 2 are selected, the input amount of two templates should in a ratio of 1:1. Otherwise, the amount of the sense strand and the anti-sense strand RNA are not equal, and more single-stranded RNA will remain after annealing.

◇siRNA template chain design

Since siRNA has a relatively short transcription template, it is usually use chemical synthesis method to synthesize four single-stranded DNA templates, and then anneal to form a transcription template. The 3' end of the siRNA carries two free bases, and it is recommended to add two bases of A at the 3' end of the template strand when designing the template. The template strand of the siRNA comprises of a 6 base enhancer, a 20 base T7 promoter, a 19 - 21 base target sequence, and two free bases A. The structure is as follows:

| Enhancer | T7 promoter | Specific sequence |
|---|---|---|
| GATCAC | TAATACGACTCACTATAGGG | X19-21AA |

◇Low yield of transcripts

In general, each reaction can yield 20 - 80 μg of RNA. If the experimental group yield is low, the possible reasons are as follows:

1. The template contains a component that inhibits the reaction;

2. The amount of template input, template length and template structure are closely related to the yield.

It is recommended to set up a control group use the Control Template provided in the kit. If the control group has low yield, please consult Vazyme's technical support; if the control group has normal yield but the experimental group yield is low, indicating that the experimental template itself causes low yield, please try the following solutions:

a. Purify the template and accurately quantify the template;

b. Increase the amount of template input;

c. Extending the reaction time of 37℃;

d. Redesign the template.

◇Transcript yield of short fragment template is low

The template fragment is short, and the binding efficiency of the template to the enzyme is low, such as the synthesis of siRNA is lower than that of other lengths of RNA. It is recommended to increase the reaction time or increase the input amount of template to increase RNA production.

◇Electrophoresis tailing

If there is a tailing phenomenon in the electrophoresis process, the reason may be that the template input amount is large or the two templates are not input in the ratio of 1:1, which leads to incomplete enzymatic hydrolysis of the template or single-stranded RNA. It is recommended to extend the double enzymatic hydrolysis time appropriately. Templates and single-stranded RNA are extremely low in total transcripts and generally do not affect subsequent experiments.

◇The length of RNA product is smaller than expected

If the electrophoresis shows that the product band is smaller than expected, possibly due to:

① Template sequence contains a termination sequence similar to T7 RNA polymerase;

② The high GC content in the template forms a termination structure similar to the stem ring.

Different RNA polymerases recognize different termination sequences. If the template contains a termination structure, it is recommended to try different RNA polymerases or redesign the template sequence.