T7 RNAi Transcription Kit

TR102

Vazyme

Instruction for Use

Version 22.1

Contents

01/Product Description

T7 RNA polymerase can perform in vitro transcription of RNA from DNA templates containing T7 promoter using four NTPs as substrates. T7 RNAi Transcription Kit is an optimized version of T7 High Yield RNA Transcription Kit that is designed for transcription of double-stranded RNA. It can be used to transcribe 21 bp siRNA and long-fragment dsRNA. The purified transcripts 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 RNA.

02/Components

	Components	TR102-01(25 rxns)	TR102-02(50 rxns)
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 ddH_2O	5 ml	5 ml
	RNA Clean Beads	2 ml	4 ml

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

03/Storage

Store Box 1 at -30 ~ -15℃, and transport at ≤0℃.

Store Box 2 at 2 ~ 8℃. Adjust the shipping method according to the destination.

04/Applications

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.

05/Self-prepared Materials

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

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

06/Notes

1.Wear disposable gloves and mask during the experiment to avoid degradation of the product by RNase.

2.Please use RNase-free experimental consumables.

3.Ensure the template as a single fragment by electrophoresis before transcription.

07/Template Preparation

07-1/PCR Template

The long-stranded dsRNA can be amplified by 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 annealed into double strands in the ratio of 1:1. 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 is annealed to double strands after transcription. In general, the amount of transcripts yield of Scheme ① and Scheme ② is higher.

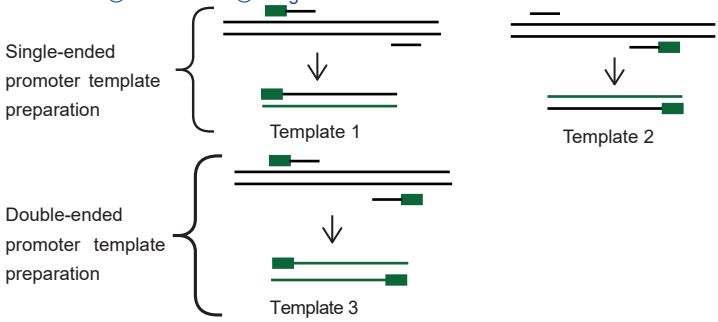


Figure 1. Schematic of PCR amplification of in vitro transcription templates

Experimental Procedure of dsRNA Transcription:

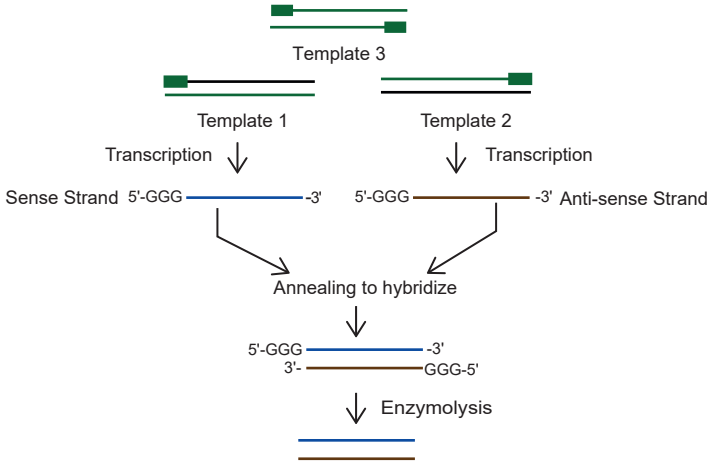


Fig 2. Experimental of dsRNA transcription

07-2/Template Synthesis

The transcription template of siRNA is short and can synthesis four single-stranded DNAs separately, and then annealed to obtain two double-stranded DNA templates according to method shown in Figure 3. Since the siRNA template fragment is short, and the binding efficiency of polymerase and the template is low, so it is recommended to add 6 bases (GATCAC) to the 5' end of the T7 promoter to promote the binding efficiency of the polymerase and the template during the template synthesis process.

▲ The two free bases at 3' end of siRNA will increase the binding efficiency of siRNA and mRNA. When the free base is UU, the inhibition effect on the target gene is the strongest. If the free base is GG, the RNase in the cell will degrade this structure, leading to the decrease of 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

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

b.Perform the following annealing procedure in PCR instrument:

Temperature	Time
95℃	2 min
95 ~ 22℃	0.1℃/sec
22℃	10 min

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

Experimental Procedure of siRNA Transcription:

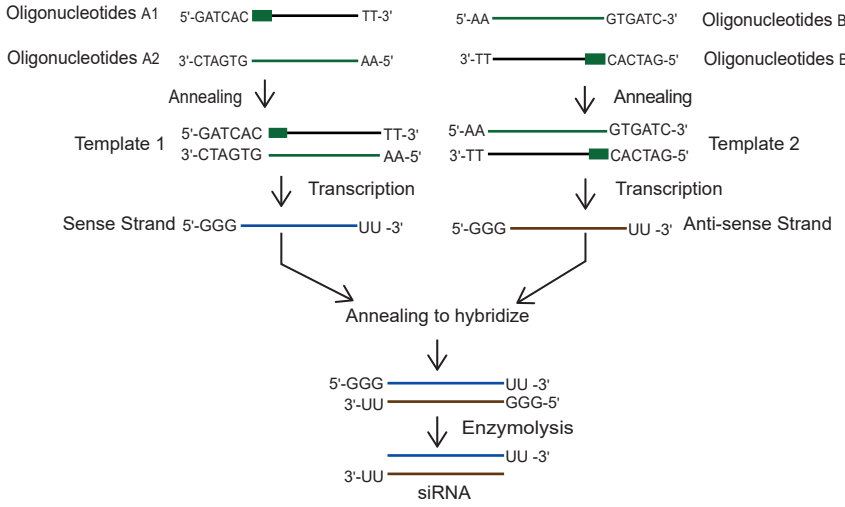


Fig 3. Experimental procedure of siRNA transcription

08/Experiment Process

1. Prepare the reaction system according to the following table (Take the mixed template transcription scheme as an example):

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 ddH_2O	up to 20 μl

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

2. Incubate at 37℃ for 2 h in 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℃ for 10 min after step 2, and then naturally cooled to anneal to dsRNA.

▲ In the same PCR tube, transcription products less than 800 bp will complement to each other to form dsRNA, and more than 800 bp 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 double enzyme digestion system according to the following table:

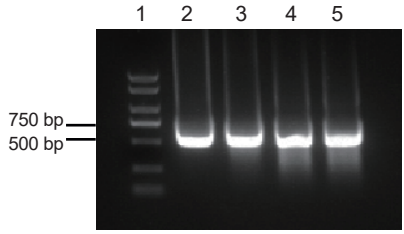
Components	Volume
Transcription Product	20 μl
RNase-free H_2O	17 μl
DNase I	1 μl
RNase T1 (10 U/μl)	2 μl
Total	40 μl

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

6. Incubate at 37℃ for 30 min.

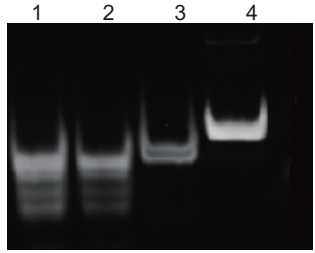
7. Detect the transcripts by electrophoresis.

Long-fragment dsRNA can be detected by agarose gel electrophoresis, and siRNA should be detected by polyacrylamide gel (PAGE) electrophoresis. Due to the small size of the siRNA, the transcripts 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)

Fig 4. 2% agarose gel electropherogram results of 500 bp dsRNA



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

Fig 5. 12% PAGE electrophoresis results of siRNA

09/Product Purification

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

▲ Magnetic beads 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 ddH_2O (self-prepared).

1. Take the RNA Clean Beads out of 4℃ and equilibrate for 30 min at room temperature. Please invert or vortex before use.
2. Add 80 μl magnetic beads 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 isopropanol and mix thoroughly.
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 min, and carefully remove the supernatant after the solution is clarified. Please be careful not to stir the beads when sucking the supernatant.

5. Keep the PCR tube on the magnetic stand and add 200 μl 80% ethanol prepared freshly. Be careful not to stir the beads, incubate for 30 sec at room temperature, and carefully remove the supernatant. Repeat this step once.
6. Open the lip and dry the magnetic beads with air for 5 - 10 min.
   ▲ Dry until there is no gleaming water on the surface of the magnetic beads, excessive drying will affect the elution of RNA.
7. Remove the PCR tube from the magnetic stand, add 40 μl RNase-free ddH_2O, use pipette to rinse the magnetic beads on the tube wall, mix thoroughly, and incubate for 3 min at room temperature.
8. Place the PCR tube on the magnetic stand, and carefully remove the supernatant to new RNase-free EP tube after the solution is clarified. Do not suck the beads.
   ▲ In order to avoid the influence of magnetic beads on subsequent experiments, when transferring the product, please reserve 1 - 2 μl solution to prevent absorption of the magnetic beads.
9. Measure the absorbance of the product at A_{260}, determine its concentration, and store the purified product at -20℃.

10/siRNA Interference Experiment

In this experiment, 293T cells were cotransfected 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 transfection reagent.

▲ In the RNA interference experiment, the state of cells, the ratio of transfection reagent to RNA, the concentration of RNA, the interference efficiency of RNA, the transfection efficiency and toxicity of the transfection reagent will affect the final interference results. If exclusion of the above conditions, the interference is still not successful, 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.

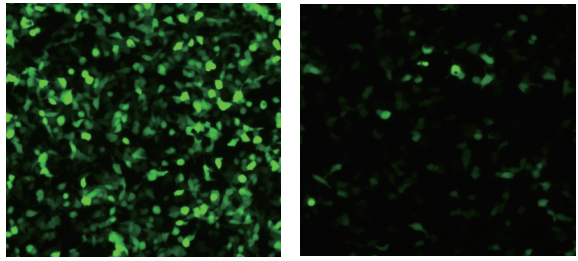


Fig 6. Image of siRNA interference with the expression of green fluorescent protein
The left image shows 293T cells cotransfected with GFP plasmid and Negative control GFP siRNA for 24 h; The right image shows 293T cells cotransfected with GFP plasmid and Positive GFP siRNA for 24 h.

11/FAQ & Troubleshooting

◇dsRNA experimental design

There are three schemes for the experimental design of dsRNA. Please select the transcription scheme according to experimental required, and refer to 07-1/PCR Template for template preparation. If Scheme ① and Scheme ② are selected, the input amount of two templates should be transcribed in the 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 strand design

Since siRNA has relatively short transcription template, it is usually use chemical synthesis method to synthesize four single-stranded DNA templates, and then anneal to form transcription template. The 3' end of the siRNA has two free bases, and it is recommended to add two bases of T at the 3' end of the template strand when designing the template. The template strand of the siRNA comprises of 6 base enhancer, 20 bases T7 promoter, 19 - 21 bases target sequence, and two free bases T. The structure is as follows:

Enhancer	T7 promoter	Specific sequence
GATCAC	TAATACGACTCACTATAGGG	$X_{19\text{-}21}$TT

◇Low yield of transcripts

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

① The template contains component that inhibits the reaction;

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

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

a.Purify the template and accurately quantify the template;

b.Increase the input amount of template;

c.Extending the reaction time at 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 and the enzyme is low, such as the yield of siRNA synthesis is lower than that of single- and double-stranded RNA of other lengths. It is recommended to increase the reaction time or increase the input amount of template to increase RNA yield.

◇Electrophoresis tailing

If there is tailing phenomenon in electrophoresis process, the reason may be that the input amount of template 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 electrophoresis shows that product band is smaller than expected, possibly reasons are as follows:

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

② The high GC content in the template forms termination structure similar to the stem-loop. Different RNA polymerases recognize different termination sequences. If the template contains termination structure, it is recommended to try different RNA polymerases or redesign the template sequence.

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