HyperScribe™ T7 siRNA Synthesis Kit

Product description

The HyperScribe™T7 siRNA Synthesis Kit is designed for the rapid production of double-stranded RNA (dsRNA) in vitro. Short siRNA and long dsRNA were synthesized in vitro by using T7 RNA polymerase with linear double-stranded DNA containing T7 Promoter sequence as template and NTPs as substrates. In addition, this kit contains RNase T1, which can specifically cut off the 3 'phosphoric acid of the guanylate of single-stranded RNA. The resulting double-stranded RNA transcripts can be purified for subsequent RNAi related experiments such as lipid nanoparticle (LNP) delivery and microinjection.

Composition and storage conditions

Components \ Size		25 rxns	50 rxns	Storage
Box 1	T7 RNA polymerase Mix	50 μL	100 μL	-20°C
	10 × Reaction Buffer	50 μL	100 μL	-20°C
	10 × Annealing Buffer	250 μL	500 μL	-20°C
	NTP Mix	200 μL	400 μL	-20°C
	DNase I	25 μL	50 μL	-20°C
	RNase T1 (100 U/μL)	25 μL	50 μL	-20°C
	RNase T1 Dilution Buffer	300 μL	600 μL	-20°C
	Control Template	5 μL	10 μL	-20°C
Box 2	RNA Clean Beads	2 mL	4 mL	4°C
	RNase-free H_2O	5 mL	10 mL	4°C

Shipping: Box1: Dry Ice; Box2: Blue Ice **Shelf life:** 2 years

***Note:** The Control Template provided is a 500 bp double-stranded DNA PCR product containing a T7 promoter at both ends, at a

concentration of 0.5ug/uL.

Experimental operation

1. Prepare the DNA template

(1) PCR products, linearized plasmid DNA, cDNA, or oligonucleotides can all be used as templates for in vitro transcription. If long-chain dsRNA is transcribed, the transcription template can be obtained by PCR amplification with a forward primer with T7 promoter (TAATACGACTCACTATA), as shown in Figure 1.

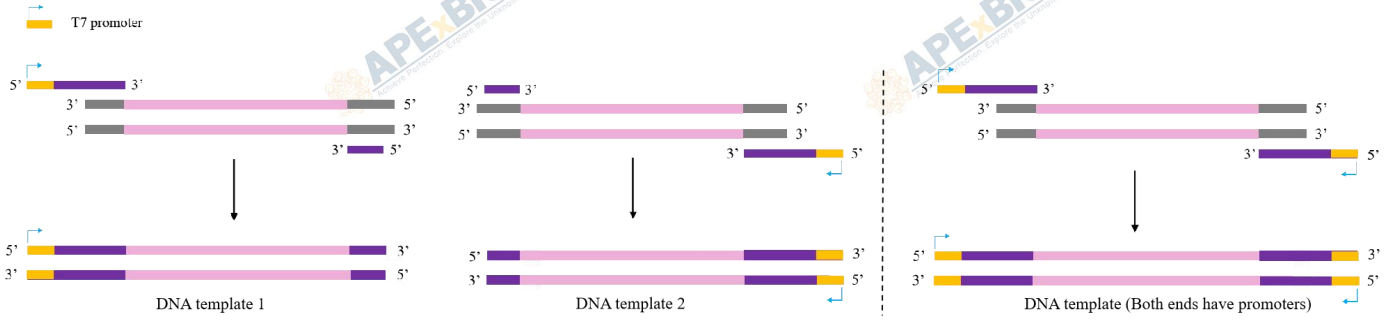

Figure 1 In vitro transcription template amplified by PCR

***Note:** PCR amplification products can be directly used as templates without purification, but higher RNA output can be obtained by transcription after template purification.

(2) If siRNA is transcribed, four single-stranded DNA can be synthesized separately, and then two double-stranded DNA templates can be obtained by annealing, as shown in Figure 2. Due to the short siRNA template fragment, the binding efficiency of polymerase to the template is low, so six bases (GATCAC) can be added to the 5' end of the T7 promoter to promote the binding of enzyme to the template.

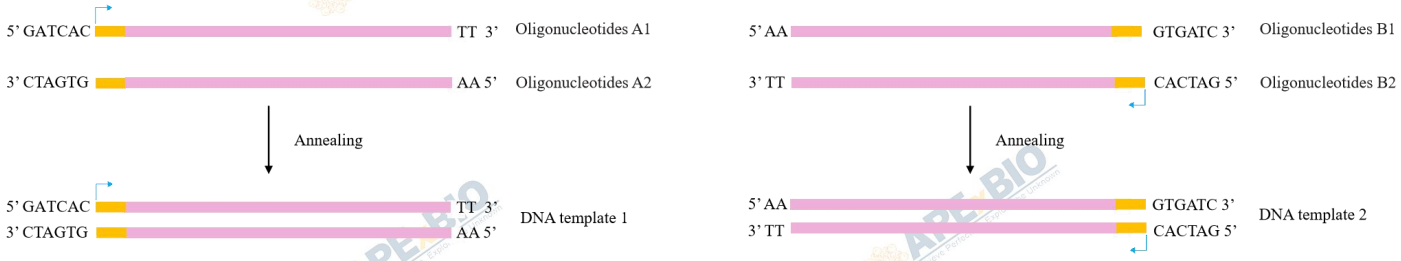

Figure 2 Oligonucleotides as in vitro transcriptional templates

a) The four synthesized Oligonucleotides are annealed into two transcription templates according to the following system:

Reagent	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
Total Reaction Volume	20 μL

***Note:** The 3' terminal of siRNA with two free bases can improve 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, resulting in the decrease of siRNA activity.

b) Perform the following annealing procedure in the PCR apparatus:

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

2. In vitro transcription of siRNA synthesis

(1) The corresponding components are thawed, and then the in vitro transcription reaction system is prepared on the ice with reference to the following table:

Reagent	Volume	Final Concentration
NTP Mix	8 μL	
10 × Reaction Buffer	2 μL	1×
T7 RNA polymerase Mix	2 μL	
DNA Template 1	1-4 μL	
DNA Template 2	1-4 μL	
RNase-free H_2O	Up to 20 μL	
Total Reaction Volume	20 μL	

***Note:** There are three transcription schemes: 1) DNA Template1 and 2 are transcribed in two PCR tubes respectively, and the products are annealed 1:1 into double strands;2) DNA Template1 and 2 are mixed in the same PCR tube and then annealed into double strands; 3) A template with promoters on both ends is transcribed and annealed into a double strand. In general, the first two transcription products are higher

in quantity. In addition, the recommended amount of DNA Template 1 and 2 should be 1:1, 0.5 μg each.

(2) Gently mix the reaction system (gentle suction or slight vortex), and then instantaneously centrifuge to collect residual liquid from the tube wall.

(3) Incubate at 37 ℃ for 2 h.

***Note:** The reaction time can be adjusted appropriately according to the size of the product fragment, if the synthesis of RNA less than 300 bp, the reaction can be extended to 4 h or more, and the overnight reaction will not affect the quality of the product.

(4) If the double chain length is＞800 bp, it needs to incubate at 72 ℃ for 10 min after the reaction at 37 ℃, and then natural cooling and annealing to form dsRNA.

***Note:** In the same PCR tube, the transcription products less than 800 bp will complement each other to form dsRNA after reaction, while those longer than 800 bp need to be annealed to form dsRNA. If two templates are transcribed in different PCR tubes, the two products need to be mixed and annealed after the end of the reaction.

3. Degradation of DNA template and single-stranded RNA

(1) Dilute 100 U/μL RNase T1 to 10 U/μL by using RNase T1 Dilution Buffer.

***Note:** RNase T1 specifically degrades the three G bases of the 5 'end of single-stranded RNA. Diluted RNase T1 should be used as soon as possible and should not be stored.

(2) Thaw the corresponding components, and then prepare the enzyme digestion system on the ice with reference to the following table:

Reagent	Volume	Final Concentration
Transcription Product	20 μL	
RNase-free H_2O	17 μL	
DNase I	1 μL	
RNase T1 (10 U/μL)	2 μL	0.5 U/μL
Total Reaction Volume	40 μL	

(3) Gently mix the reaction system (gentle suction or slight vortex), and then instantaneously centrifuge to collect residual liquid from the tube wall.

(4) Incubation at 37 ℃ for 30 min to digest the DNA template and single-stranded RNA.

4. dsRNA purification

(1) The RNA Clean Beads are removed from the storage environment at 4℃ and equilibrated at room temperature for 30 min.

***Note:** Before using the magnetic bead, please reverse or slightly scroll to make the magnetic bead completely and evenly suspended in the tube.

(2) Add 80 μL magnetic bead solution to the system after the above degradation reaction, and use a pipette to blow the solution fully mixed.

***Note:** If the transcription product is less than 100 bp, an additional 200 μL of isopropyl alcohol should be added and thoroughly mixed.

(3) Stand at room temperature for 8 min, allowing the RNA to fully combine with the magnetic beads.

(4) Place the PCR tube on the magnetic frame for about 5 minutes, and carefully remove the supernatant with the pipette, taking care to avoid touching the magnetic beads.

(5) Keep the PCR tube always on the magnetic frame, add 200 μL of newly prepared 80% ethanol. Incubate at room temperature for 30 s, and carefully remove the supernatant, taking care to avoid touching the magnetic beads.

(6) Repeat step (5) once to remove all the liquid in the tube.

(7) Remove the PCR tube from the magnetic frame, open the tube cover and dry the magnetic beads at room temperature for 5-10 min to make the residual ethanol fully volatilize.

***Note:** Dry at room temperature until there is no water on the surface of the magnetic beads. Excessive drying will reduce the elution efficiency of RNA.

(8) Add 40 μL RNase-free H_2O, gently blow the magnetic beads with pipette. Then stand at room temperature for 3 min to elute, avoiding bubbles during operation.

(9) Place the PCR tube on the magnetic frame. After the magnetic beads were completely absorbed by the magnetic force and the solution is clarified,the supernatant is carefully transferred to the new RNase-free PCR tube with pipette. Taking care to avoid touching the magnetic beads.

***Note:** In order to avoid the influence of magnetic beads on subsequent experiments, when transferring the supernatant, please reserve 1-2 μL of solution to prevent absorption of the magnetic beads.

(10) The concentration of the product is determined by A260 absorption value, and the quality is detected by RNA gel electrophoresis.

(11) Conduct subsequent RNAi interference experiments or store the obtained dsRNA at -20℃.

Notes

1. Strict attention should be paid to avoid RNase contamination during the experimental operation.
2. Be sure to mix and equilibrate to room temperature before using the beads. Avoid over-drying when opening the lid of the beads, as cracking of the beads indicates that the beads are over-drying, and the RNA elution efficiency will be reduced.
3. This product is for scientific use only.

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