T7 High Yield RNA Transcription kit

TR101

Version 9.1

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Introduction

The T7 High Yield Transcription Kit is an optimized system for high yield in vitro transcription of RNA from DNA templates containing T7 RNA Polymerase promoter. The kit contains T7 RNA Polymerase which can synthesize RNA quickly and easily from the downstream of the T7 promoter, and obtains a large amount of RNA. Modified nucleotide can be added to the system to generate biotin or dye-labeled RNA.

This kit can yield 150 - 200 μg of RNA with a template input of 0.5 μg. The RNA yield can be widely used in many downstream applications such as RNA structure and function studies, RNase protection, probe hybridization, RNAi, microinjection and in vitro translation.

Package Information

Components	TR101-01 50 rxn	TR101-02 100 rxn
T7 RNA Polymerase Mix	100 μl	200 μl
10 × Reaction Buffer	100 μl	200 μl
ATP Solution	100 μl	200 μl
UTP Solution	100 μl	200 μl
GTP Solution	100 μl	200 μl
CTP Solution	100 μl	200 μl
DNase I	50 μl	100 μl
Control Template (0.5 μg/μl)	10 μl	20 μl
RNase-free H_2O	1 ml	2 x 1 ml

Storage

Store all the components at -20℃.

Application

In vitro transcription of RNA.

User Prepared

1. Template: linearized plasmid with a T7 RNA polymerase promoter sequence, PCR product or synthetic DNA fragment.
2. Purification: phenol, chloroform, sodium acetate, ethanol; or RNA purification column; RNase-free H_2O.
3. Other: RNase-free EP tube, pipette tip; PCR instrument.

Template Preparation

A linearized plasmid contained a double-stranded T7 promoter, PCR product or synthetic DNA fragment can be used as an in vitro transcription template for the T7 High Yield RNA Transcription Kit. The template can be dissolved in TE buffer or RNase-free H_2O at a recommended concentration of 0.5 μg/ul.

1. Plasmid

A plasmid with a T7 promoter can be used as a template, and the linearization and purity of the plasmid affects the yield and the integrity of RNA. Since the circular plasmid can't be effectively terminated, it will generate RNA of different lengths. In order to obtain RNA of a specific length, the plasmid must be completely linearized, and the double strand should be ensure to be blunt or the 5' end of the coding strand is abrupt.

Out structure (B strand in Figure 1). It is recommended to put 1 μg of linearized plasmid as a template for each reaction.

▲ After linearization of the plasmid, it is recommended to purify it before used as a template to avoid the affects of RNase, protein, RNA and salt residues on the system.

2. PCR Product Template

The PCR product with the T7 promoter can be used as an in vitro transcription template. The T7 promoter (TAATACGACTCAC-TATAGGG) was added to the 5' end of the upstream primer of the sense strand when the template was amplified by PCR. The PCR product can be used directly as a template without purification, but it will result in higher yield of RNA after purification.

▲ When the PCR product is used as a transcription template. It is necessary to confirm the unity of the product by electrophoresis. It is recommended to input 0.1 - 0.5 μg of template in each reaction system.

3. Synthetic DNA Template

Synthetic DNA fragments with the T7 promoter can also be used as templates for in vitro transcription.

▲ It is recommended to input 0.1 - 0.5 μg of template in each reaction system.

Protocol

1. Transcription Scheme

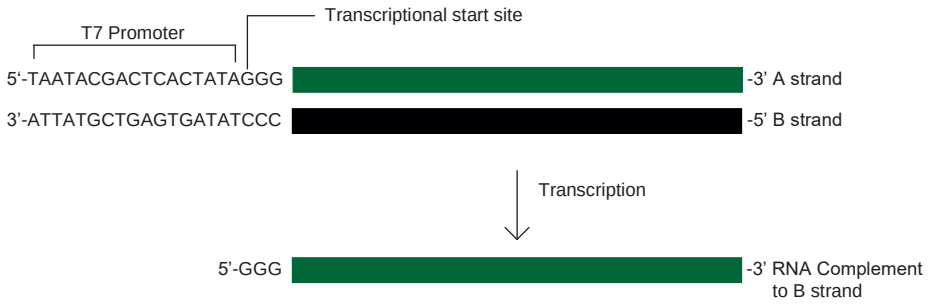

Fig 1. RNA Transcription Scheme

2. In Vitro Transcription

The transcription scheme of RNA is shown in Figure 1. Please refer to the DNA in the figure to design transcription template. This kit does not provide a modified NTP and hat-like structure.

▲Please wear gloves and use nuclease-free EP tubes and reagents before the in vitro transcription reaction to avoid RNase contamination.

1. Shake the components except T7 RNA Polymerase Mix, centrifuge briefly to collect them to the bottom of the tube and store them on ice.

2. Select the three reaction system A, B, and C according to the desired product type. The recommended template input is 0.1 - 1μg.

A. Non-modified RNA System

Prepare the reaction system according to the following table:

Components	Volume
10 × Reaction Buffer	2 μl
ATP Solution	2 μl
GTP Solution	2 μl
UTP Solution	2 μl
CTP Solution	2 μl
DNA Template	x μl
T7 RNA Polymerase Mix	2 μl
RNase-free H_2O	Up to 20 μl

B. Modified RNA System

Prepare the reaction system according to the following table:

Components	Volume
10 × Reaction Buffer	2 μl
ATP Solution	2 μl
GTP Solution	2 μl
CTP Solution	2 μl
UTP Solution	1.5 μl
Modified UTP (10 mM)	5 μl
DNA Template	x μl
T7 RNA Polymerase Mix	2 μl
RNase-free H_2O	Up to 20 μl

▲The system uses Modified UTP as an example. If other Modified NTP substrates are used, please refer to the UTP Solution and Modified UTP ratio to prepare the reactants.

C. Capped RNA System

Prepare the reaction system according to the following table:

Components	Volume
10 × Reaction Buffer	2 μl
ATP Solution	2 μl
CTP Solution	2 μl
UTP Solution	2 μl
GTP Solution	0.4 μl
m7G (5') ppp (5') G (50 mM)	2.4 μl
DNA Template	x μl
T7 RNA Polymerase Mix	2 μl
RNase-free H_2O	Up to 20 μl

▲ The system uses m7G (5') ppp (5') G as an example. If other hat structures are used, please refer to the ratio of GTP Solution and hat structure to prepare the reaction system.

4. Mix the components gently with a pipette and collect by brief centrifugation and incubate for 2 h at 37℃.

▲ To avoid the evaporation, it is recommended to carry out the reaction in a PCR instrument. The reaction time can be appropriately 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 longer, and even 16 h overnight does not affect the quality of the product.

5. Add 1 μl of DNase I to the reaction system and incubate for 15 min at 37℃ to digest the DNA template. (optional)

▲ The template DNA content is very low compare with the product RNA, generally it not need to be removed.

6. The synthesized RNA can be used in downstream experiments after electrophoresis analysis and purification.

▲ The product concentration is extremely high and needs to be diluted with RNase-free H_2O before testing.

3. Products Purification

Non-modified RNA can be purified by column or phenol/chloroform extraction; modified RNA is recommended to use column purification method; for product fragment size requirements, it is recommended to use gelation recovery and purification.

▲ Please use RNase-free H_2O to prepare reagents and use RNase-free EP tubes.

1. Phenol/chloroform purification method

Phenol/chloroform extraction removes proteins and most free nucleotides.

a. Dilute the product to 180 μl by adding 160 μl of RNase-free H_2O.

b. Add 20 μl of 3 M sodium acetate (pH 5.2) to the diluted solution and mix well with a pipette.

c. Add 200 μl of phenol/chloroform mixture (1:1) for extraction, centrifuge at 10,000 rpm for 5 min at room temperature, and transfer the upper layer solution (aqueous phase) to a new RNase-free EP tube.

d. Add chloroform in the same volume as water, extract twice, and collect the upper aqueous phase.

e. Add 2 volumes of absolute ethanol and mix well, incubate at -20℃ for at least 30 min, and centrifuge at 15000 rpm for 15 min at 4℃.

f. Discard the supernatant and wash the RNA pellet with 500 µl of pre-cooled 70% ethanol, centrifuge at 15,000 rpm at 4℃, and discard the supernatant.

g. Open the lid for 2 min, add 20 µl - 50 µl RNase-free H_2O or other buffer to dissolve the RNA pellet.

h. Store at -80℃.

2. Column purification

Column purification removes proteins and free nucleotides.

The product was diluted to 100 µl by adding 80 µl of RNase-free H_2O before purification, and purified according to the column purification instructions.

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

4. RNA Quantification

1. Ultraviolet absorption method: Free nucleotides will affect the accuracy of quantification. RNA purification should be performed before using this method.
2. Dye method: RNA quantified with RiboGreen dye, free nucleotides does not affect quantification, which allows accurate quantification of RNA in purified or unpurified reaction products.

5. Data Reference

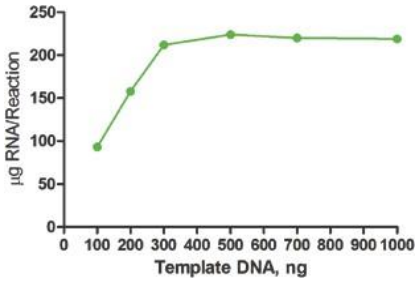

Fig 3. Relationship between the input and yield of 1.4 kb template

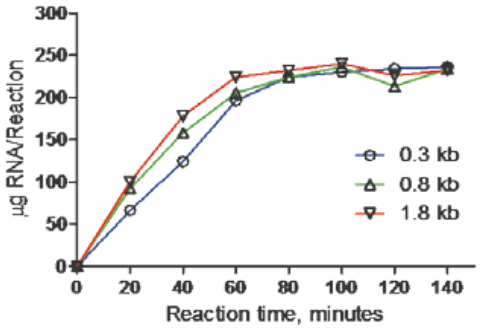

Fig 4. Relationship between reaction time and yield

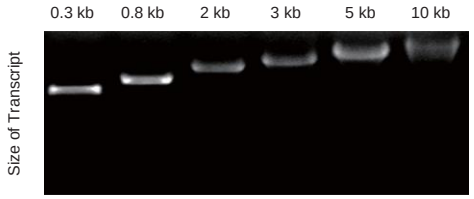

Fig 5. Electropherograms of different length transcripts

FAQs and solutions

◇ Low transcript production

The template quality was closely related to the yield. if the yield of the experimental group was significantly lower than that of the control group. Possible reasons are : ① There were inhibitory components in the experimental template; ② The reason come from template itself.

It is recommended to set up a control group and an experimental group. If the control group has low yield, please consult Novozan technical support. If the output of the control group is normal but the yield of the experimental group is low, it indicates that the experimental template itself causes the production to be low. Please try the following solutions:

a. Repurify the template; b. Determine the template quantification and its integrity; c. Extend the reaction time at 37℃; d. Increase the amount of template input; e. Try other promoters and RNA polymerase.

◇ Short fragment transcript yield is low

Short transcription initiation fragments inhibit the reaction, and when the transcript is less than 0.3 kb, prolonging the reaction time or increasing the amount of template can increase RNA production. Overnight reaction (16 h) or use of 2 μg of template can maximize RNA production.

◇ Product electrophoresis tailing phenomenon

The possible reasons for a tailing phenomenon during electrophoresis are: ① The experimental procedure is contaminated by RNase; ② The DNA template is contaminated by RNase.

The RNase inhibitors in the system can only inhibit traces of RNase residues. It is recommended to repurify the template DNA, and using RNase-free tips and EP tubes during the experiment, wearing disposable latex gloves and masks, all reagents should be prepared with RNase-free H_2O.

◇ The RNA product fragment is larger than expected

If the electrophoresis shows that the product band is larger than expected, the reason may be: ① the plasmid template may not be fully linearized; ② the 3' end of the sense strand is a prominent structure; ③ RNA has an incompletely denatured secondary structure.

It is recommended to confirm the template structure and change the electrophoresis method from agarose gel to denatured gel to detect the RNA product.

◇ RNA product fragments are smaller than expected

If the electrophoresis shows that the product band is smaller than expected, the reason may be: ① the template sequence contains a termination sequence similar to T7 RNA polymerase; ② the template forms a high-level structure due to its high GC content; ③ RNase residue.

Different polymerases recognize different termination sequences, and if the template contains a termination structure, it is recommended to try other RNA polymerases. If the GC content of template is high, it is recommended to add the SSB protein to improve transcription efficiency. If the band appears to be inconsistent with expected, please run the denatured gel to detect the product.