Yeasen Biotechnology (Shanghai) Co., Ltd.
Hotline: 400-6111-883
E-mail: order@yeasen.com

HB221121

T7 High Yield RNA Synthesis Kit for Co-transcription

Product Information

Product Name	Catalog No.	Size
T7 High Yield RNA Synthesis Kit for Co-transcription	10673ES50	50 T
	10673ES60	100 T
	10673ES70	500 T

Product Description

T7 High Yield RNA Synthesis Kit for Co-transcription optimizes the transcription reaction system. The kit can synthesize the single-stranded RNA with cap1 efficiently by using T7 RNA polymerase, linear double-stranded DNA with the T7 promoter sequence as the template, NTPs, including GAG, as the substrates to control the DNA sequence downstream of the promoter. During transcription, modified nucleotides can be added to the system to prepare biotin or dye-labeled RNA. The kit can synthesize long transcripts and short transcripts, RNA can be produced 100-200 μg with 1 μg of DNA template. The kit uses the co-transcriptional capping strategy to produce the Cap1 RNA. Also modified UTP is substituted with N^1-Me-Pseudo UTP to decrease the immunogenicity.

The RNA synthesized by *in vitro* transcription can be used for various downstream applications, such as RNA structure and function research, RNase protection, probe hybridization, RNAi, microinjection and in vitro translation.

Contents

Contents No.	Name	Cat#/Specification		
		10673ES50 (50 T)	**10673ES60 (100 T)**	**10673ES70 (500 T)**
10673-A	T7 RNA Polymerase Mix	100 μL	200 μL	1000μL
10673-B	10×Transcription Buffer	100 μL	200 μL	1000μL
10673-C	ATP（100mM）	100 μL	200 μL	1000μL
10673-D	CTP（100mM）	100 μL	200 μL	1000μL
10673-E	GTP（100mM）	100 μL	200 μL	1000μL
10673-F	N^1-Me-Pseudo UTP（100mM）	100 μL	200 μL	1000μL
10673-G	Control DNA Template（500ng/μL）	10 μL	20 μL	100μL
10673-H	DNase I (2 U/μL)	50μL	100μL	500μL
10673-I	GAG(3’OMe)m7(3’OMeG)(5’)ppp(5’)(2’OMeA)pG (100 mM)	100 μL	200 μL	1000μL
10673-J	Lithium chloride solution	1500μL	3000μL	15ml
10673-K	RNase-free ddH_2O	2500μL	5000μL	25ml

Applications

In vitro RNA synthesis.

Shipping and Storage

Dry ice transportation. Store at -15℃ ～-25℃, valid for two years.

Notes:

1. Be careful not to mix RNase in the reaction system.
2. Experiment equipment (such as: pipette tip, product tube, etc.) should strictly use RNase-free products.

3. For your safety and health, please wear lab coats and disposable gloves.

4. For research use only!

Synthesis principle

Figure 1: In vitro RNA co-transcription process

Experimental methods

1. DNA template preparation

Linearized plasmids with double-stranded T7 promoters or PCR amplification products can be used as T7 High Yield RNA Synthesis Kit for Co-transcription kit in vitro transcription templates, which can be dissolved in TE buffer or RNase free H_2O.

T7 promoter sequence: TAATACGACTCACTATA**A***GG (Note: **A*** is the first base of RNA transcription).

A. Plasmid template

Insert the target DNA to the plasmid vector containing the T7 promoter, and then treated with restriction enzymes, purified after completely linearized.

Note: 1. The circular plasmids have no effective termination, RNA products of different lengths will be transcribed. In order to obtain a specific length RNA, the plasmid must be completely linearized.

2. The restriction enzyme selected for plasmid linearization needs to be on the right side of the promoter region, downstream of the inserted DNA fragment, and has no recognition site in the inserted DNA fragment. The restriction enzyme should be capable of forming 5' sticky ends or smooth ends.

3. In order to avoid the influence of protein and salt ions on the system, the plasmid is recommended to be purified when used as a template for in vitro transcription after linearization.

B. PCR product template

The PCR product with T7 promoter can be used as an in vitro transcription template. First, add the T7 promoter sequence (TAATACGA CTCACTATAAGG) to the 5' end of the upstream primer sense strand; next, the T7 promoter DNA template is amplified under the action of high fidelity enzyme; then transcription is performed. PCR products can be used directly as templates without purification, but higher RNA output will be obtained after purification.

Note: 1. The specificity and concentration of the PCR product must be confirmed by electrophoresis when used as a template. Put 2-5 µL of PCR product into the 20 µL reaction system.

2. In order to obtain more high-quality RNA, the PCR product should be recovered by gel and used as a template for in vitro transcription.

2. In vitro RNA co-transcription

A. Thawing reagents

Centrifuge the T7 RNA Polymerase Mix briefly and place on ice. Thaw 10× Transcription Buffer, ribonucleotides (ATP, CTP, GTP, N^1-Me-Pseudo UTP) and GAG, then centrifuge to the bottom of the tube, place 10× Transcription Buffer at room temperature, and place others on ice.

B. Assembly transcription reaction at room temperature

Prepare the reaction system according to the following system:

Components	Volume（μL）	Final concentration
RNase free H_2O	Up to 20	-
10×Transcription Buffer	2	1×
CTP / GTP/ ATP/ N^1-Me-Pseudo UTP (100 mM each)	2 each	10 mM each
GAG (100 mM)	2	10 mM
Template DNA	1 μg	-
T7 RNA Polymerase Mix	2	-

Note: 1. The reaction is configured at room temperature. Since 10× Transcription Buffer contains spermidine, the concentration of spermidine too high will cause DNA template precipitation at low temperature.
2. Short transcript (<100 nt), 2 μg template can be used, transcription time increased to 4-8 hs.
3. For long transcripts (>1000 nt), recommended to use linearized plasmid templates for transcription.
4. Perform the reaction in a PCR machine with the hot lid open to prevent the reaction solution from evaporating for a long time.
5. The reaction product may have a white precipitate. This is free pyrophosphate and magnesium ions produce the magnesium pyrophosphate in the reaction, won't affect the subsequent experiments. You can add some EDTA to clear it. If the addition of EDTA affects subsequent experiments, the supernatant can also be recovered by centrifugation.
6. The reagents and containers must without RNase contamination.

C. Incubate at 37°C for 3 hours

Mix the above reaction solution, briefly centrifuge to the bottom of the tube, and incubate at 37°C for 3hs. If the transcript length is less than 100 nt, increase the reaction time to 4-8 hs.

D. DNase I treatment (optional)

After the reaction is complete, add 1 μL of DNase I (RNase free) to each tube and incubate at 37°C for 15 mins to remove the template DNA.

3. Product purification

The transcribed RNA can be precipitated by lithium chloride solution in this kit, then remove proteins and free nucleotides. The purified RNA can be subjected to downstream experiments or stored at -80°C after electrophoresis detection. The lithium chloride precipitation method demands the RNA length must be greater than 300 nt, and the concentration must not be less than 100 ng/μL.

①Add 30 μL RNase free H_2O and 30 μL 7.5M lithium chloride to 20 μL reaction mixture.

②After mixing uniformly, place it at -20°C for at least 30 mins, centrifuge at 4°C for 15 mins at maximum speed, and collect the precipitate.

③Add 500 μL of ice-cold 70% ethanol to wash the RNA pellet.

④Dissolve the RNA pellet with 20 μL RNase free H_2O. The purified RNA solution is stored at -80°C.

4. RNA quantification

A. Ultraviolet absorption

Free nucleotides will affect the accuracy of quantification. Please purify RNA before using this method. The yield of RNA is determined by the A260 reading of the product. For single-stranded RNA, 1 A260 is equivalent to 40 μg/mL, so the yield of RNA can be calculated as follows: A260 x dilution factor x 40 = μg/mL RNA.

B. Dye method

Use RiboGreen dye to quantify RNA, free nucleotides won't affect quantification, purified or unpurified RNA in reaction products can be accurately quantified.

5. RNA size and quality detection

A. Agarose electrophoresis

In order to determine the size, integrity and quality of RNA, agarose gel electrophoresis or polyacrylamide gel electrophoresis is required for detection.

B. Agilent 2100 Bioanalyzer detection

Agilent 2100 Bioanalyzer can be used to evaluate the integrity and quality of RNA. It only requires a small amount of RNA for analysis. High-quality RNA should show obvious and sharp peaks on the electrogram.

Frequently Asked Questions:

1. Low transcript yield

The quality of template is closely related to the yield. The yield of experimental group is significantly lower than the control group. The possible reasons are: ① the experimental template contains inhibitory components; ② The template has something wrong.
Suggestions: ① Re-purify the template; ② Determine the template quantification and its integrity; ③ Extend the reaction time; ④ Increase the amount of template input; ⑤ Try other promoters and RNA polymerases.

2. Low yield of short transcripts

Short transcription initiation fragment will inhibit the reaction. When the transcription product is less than 100 nt, extending the reaction time to 4-8 hs or increasing the amount of template to 2 μg will increase RNA yield.

3. RNA transcription length is greater than expected

If the electrophoresis shows that the product band is larger than the expected size, the possible reasons: ①The plasmid template may not be completely linearized; ②The 3' end of the sense strand has a prominent structure; ③The RNA has a secondary structure that is not completely denatured.
Suggestions: ①Check whether the template is completely linearized, and if necessary, perform additional linearization; ②Select a suitable restriction enzyme to avoid 3' overhangs, or use Klenow Fragment /T4 DNA polymerase to complete the transcription before proceeding; ③Use denatured gel to detect RNA products.

4. RNA transcription length is less than expected

If the electrophoresis shows that the product band is smaller than the expected size, the possible reasons: ①The template contains a termination sequence similar to T7 RNA polymerase; ②The GC content in the template is high.
Suggestions: ① Lower the reaction temperature (for example, 30°C). Sometimes lowering the temperature can increase the transcription length, but it will reduce the yield. Or try different RNA polymerases for transcription; ②If the template GC content is high, use 42℃ to transcript, or add SSB to increase the yield and transcription length.

5. Electrophoresis tailing of transcription products

There is tailing phenomenon during electrophoresis. Possible reasons: ①Contaminated by RNase during experimental operation; ② Contaminated DNA template by RNase.
Suggestions: ①Use RNase-free pipette tips and EP tubes, wear disposable latex gloves and masks, and all reagents are prepared with RNase free H_2O. ②Re-purify the template DNA.