T7 High Yield RNA Synthesis Kit

REF: EG24104S

Storage Condition

-20℃

Components

Component	Amount (50 rxns)
T7 Enzyme Mix	100 µl
10× T7 Transcription Buffer	100 µl
ATP (100mM)	100 µl
UTP (100mM)	100 µl
GTP (100mM)	100 µl
CTP (100mM)	100 µl
Control Template (0.5 µg/µl)	10 µl
DNase I, RNase-free (1 U/µl)	100 µl
Nuclease-Free Water	1 ml

Description

The T7 High Yield RNA Synthesis Kit is designed for the large-scale synthesis of RNA through in vitro transcription using T7 RNA polymerase and DNA templates containing a T7 promoter. It is suitable for both long and short RNA transcripts. The T7 Enzyme Mix included RNase inhibitor and inorganic pyrophosphatase. Additionally, DNase I, RNase-free, is provided to eliminate template DNA after the transcription reaction. Using this kit, 1 µg of linearized double-stranded DNA template can yield at least 150 µg of RNA. The synthesized RNA is applicable for a wide range of downstream applications, including in vitro translation, RNA structure and function studies, RNase protection assays, probe hybridization, RNA interference, and more.

Transcription scheme

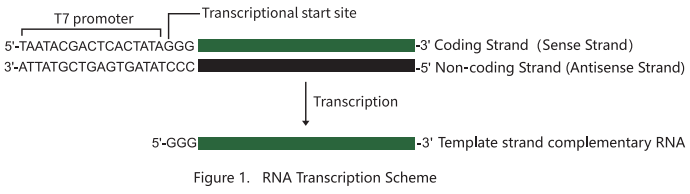

Figure 1. RNA Transcription Scheme

Figure 1. Non-cotranscribed T7 promoter RNA transcription scheme

Protocol

1. DNA template preparation

A linearized plasmid contained a double-stranded T7 promoter or PCR product can be used as an in vitro transcription template for the T7 High Yield RNA Synthesis Kit. The template can be dissolved in TE buffer or RNase-free Water.

T7 promoter sequence: TAATACGACTCACTATAN*

Note: N* represents the first base of the RNA transcript, which is typically guanine(G). However, if co-transcriptional capping is performed, it is determined by the cap analogs.

(1) Plasmid template

The target DNA is inserted into a plasmid vector containing a T7 promoter, then treated with a restriction enzyme and purified after full linearization.

Note1: Since terminators cannot guarantee 100% termination of transcription, circular plasmids may yield RNA transcripts of varying lengths. To produce RNA transcript of a defined length, plasmid DNA must be completely linearized with a restriction enzyme downstream of the insert to be transcribed.
Note2: The selected restriction enzyme for plasmid linearization should possess a cleavage site immediately downstream of the coding strand, while ensuring there are no recognition sites within the coding strand. we recommend selecting restriction enzymes that generate blunt ends or 5´-overhangs.
Note3: To avoid interference from proteins, salt ions, and other contaminants in the transcription system, it is recommended to purify the linearized plasmid before using it as a template for in vitro transcription.
Note4: Residual RNase A introduced during the extraction of plasmid DNA can significantly affect the quality of transcribed RNA. Therefore, it is advisable to use high-purity, RNase-free templates with an A260/A280 ratio ranging from 1.8 to 2.0.

(2) PCR templates

PCR products containing a T7 promoter can serve as templates for in vitro transcription. Initially, the T7 promoter sequence is added to the 5' end of the forward primer upstream of the coding strand. Subsequently, a high-fidelity polymerase is used to amplify the DNA template containing the T7 promoter, followed by transcription. Though PCR mixture can be used directly, better yields will be obtained with purified PCR products.

Note1: PCR products should be examined on an agarose gel to estimate concentration and to confirm amplicon size prior to its use as a template. It is recommended that 2~5 µl of PCR products can be used in a 20 µl in vitro transcription reaction.
Note2: To achieve higher yields of high-quality RNA, it is recommended to purify the PCR product prior to using it as a template for in vitro transcription.

2. In vitro transcription

(1) According to the required product type, the following three reaction systems are selected for reaction solution addition.

① Standard RNA Synthesis

Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10× T7 Transcription Buffer	2 µl	1×
T7 Enzyme Mix	2 µl	-
Template DNA	1 µg	50 ng/µl
ATP/CTP/GTP/UTP (100 mM each)[a,b]	2 µl each	10 mM each
Nuclease-Free Water	up to 20 µl	-

a. It is recommended to add Nuclease-Free Water first, followed by ATP/CTP/GTP/UTP.
b. Modified NTPs can be used to replace the corresponding unmodified NTPs at the same molar concentration. For modified UTP, it is advisable to use N1-Methyl-Pseudo-UTP (100 mM) (REF: EG21921) and Pseudo-UTP (100 mM) (REF: EG21922).

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② Capped RNA Synthesis

Prepare the following reaction mixture on ice:

Reagent	Amount	Final Concentration
10× T7 Transcription Buffer	2 μl	1×
T7 Enzyme Mix	2 μl	-
Template DNA	1 μg	50 ng/μl
ATP/CTP/GTP/UTP (100 mM each)	2 μl each	10 mM each
Cap1 Analogue (100 mM)[c]	1.6 μl	8 mM
Nuclease-Free Water	up to 20 μl	-

c. The recommended ratio of cap analog to each NTP is 4:5 which is appropriate for CleanCap series cap analogs. For cap analogs, it is recommended to use the Cap1 analog AG (Catalog No.: EG22909). When using cap analogs with other structures, please refer to the cap analog's instructions to determine the appropriate ratio of cap analog to GTP. The ratio may be adjusted based on capping efficiency; however, it is advisable to maintain the total final concentration of both components at 10 mM.

③ Non-radiolabeled RNA transcription system in vitro

Reagent	Amount	Final Concentration
10× T7 Transcription Buffer	2 μl	1×
T7 Enzyme Mix	2 μl	-
Template DNA	1 μg	50 ng/μl
ATP/CTP/GTP (100 mM each)	2 μl each	10 mM each
UTP (100 mM)	1.5 μl	7.5 mM
Modified UTP (50 mM)[d]	1 μl	2.5 mM
Nuclease-Free Water	up to 20 μl	-

d. This system is suitable for biotin-modified UTP, fluorescein-modified UTP, digoxigenin-modified UTP, and aminoallyl-modified UTP. Modified ribonucleotides reduce transcription efficiency; therefore, lower transcription yields should be expected as compared to transcription using unmodified UTP.
Note1: Transcription efficiency varies significantly depending on the template sequence. For initial experiments, it is recommended to adhere to the suggested template quantity and subsequently optimize the system accordingly. The template quantity can be adjusted within the range of 0.5 μg to 2 μg.

(2) Mix the components gently with a pipette and collect by brief centrifugation and incubate for 2 h at 37℃ . If the RNA is less than 100 nt, the reaction can be extended to 3~16 h.

(3) After completing the reaction, add 2 μl of DNase I, RNase-free per μg of template DNA to the product and incubate at 37℃ for 15 minutes to eliminate the DNA template.

(4) Post-transcription RNA is recommended to be purified using magnetic beads or column-based methods. Alternatively, phenol/chloroform or lithium chloride purification may also be used. The purified RNA can be used for downstream experiments or stored at -80℃ for use.

3. Control template transcription (not included in T package)

The control template is a linear DNA fragment containing a T7 promoter, and the transcribed product is approximately 4000 nt. In the recommended standard in vitro transcription reaction system, at least 150 μg of RNA can be obtained from 1 μg of control template DNA after a 2-hour reaction at 37℃ .

4. Product purification

Transcribed RNA can be purified using magnetic bead-based methods, column purification, phenol/chloroform extraction, and lithium chloride precipitation to remove proteins and free nucleotides. After purification, the RNA can be analyzed by electrophoresis and subsequently used for downstream experiments or stored at -80℃ for use.

(1) Magnetic bead purification method

Purify according to the instructions of magnetic beads.

(2) Column purification

The product was diluted to 100 μl by adding 80 μl of RNase-free ddH_2O before purification, and purified according to the column purification instructions.

(3) Phenol/chloroform purification method

① Add 115 μl of Nuclease-Free Water and 15 μl of 3 M sodium acetate (pH 5.2) to the 20 μl reaction mixture and mix thoroughly.

② Add 150 μl of phenol/chloroform mixture (1:1) for extraction, centrifuge at maximum speed (≥12,000 rpm) for 5 min at room temperature, and transfer the upper layer solution (aqueous phase) to a new RNase-free EP tube.

Note: Do not suck into the middle layer when transferring supernatant.

③ Add chloroform in the same volume as water, extract twice, and collect the upper aqueous phase and transfer it to a new RNase-free EP tube.

④ Add 2 volumes of absolute ethanol and mix well, incubate at -20 ℃ for at least 30 min. Centrifuge at maximum speed (≥12,000 rpm) for 15 min at 4℃ and collect the precipitate.

⑤ Discard the supernatant and wash the RNA pellet with 500 μl of pre-cooled 70% ethanol, Centrifuge at maximum speed (≥12,000 rpm) for 15 min at 4℃ , collect the precipitate.

⑥ Add 20 μl RNase-free ddH_2O to dissolve the RNA pellet. Store at -80℃ .

(4) Lithium chloride precipitation method

When using the lithium chloride precipitation method, the RNA length should be at least 100 nt, and the concentration should not be lower than 100 ng/μl.

① Add 30 μl of Nuclease-Free Water and 30 μl of 7.5 M lithium chloride to the 20 μl reaction mixture.

② After thorough mixing, incubate the mixture at -20 ℃ for at least 30 minutes. Subsequently, centrifuge at maximum speed (≥12,000 rpm) at 4℃ for 15 minutes to collect the precipitate.

③ Add 500 μl of ice-cold 70% ethanol to wash the RNA precipitate. Centrifuge at maximum speed (≥12,000 rpm) at 4℃ for 5 minutes and collect the precipitate.

④ Dissolve the RNA precipitate in 20 μl of Nuclease-Free Water. Store the purified RNA solution at -80℃ .

5. 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-specific fluorescent dyes or related kits can be used for RNA quantification. This method allows accurate quantification of RNA in purified or unpurified reaction products.

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6. RNA detection

(1) Gel electrophoresis

To assess the length and quality of the transcripts, it is essential to analyze the transcription products using electrophoresis on an appropriate non-denaturing or denaturing agarose or polyacrylamide gel. Denaturing electrophoresis reduces the formation of RNA secondary structures. Usually, RNA typically migrates as a single band of the correct size.

Transcript length	Electrophoretic gel is recommended
>500 nt	1% agarose gel
100~500 nt	2% agarose gel or 4~5% urea-denaturing polyacrylamide gel
50~100 nt	10% to 15% urea-denaturing polyacrylamide gel
<50 nt	20% urea-denaturing polyacrylamide gel

Note1: Both the electrophoresis buffer and gel should be prepared fresh before use. For urea-denaturing polyacrylamide gel electrophoresis, it is recommended to use 0.5× TBE (REF: CP17202) as the electrophoresis buffer.
Note2: Transcribed RNA can be diluted with Nuclease-Free Water before electrophoresis. A recommended loading amount for electrophoresis is 0.05~1 μg.
Note3: After adding RNA Loading Buffer, the samples can be treated at 65℃ for 5~10 minutes to reduce the formation of RNA secondary structures.
Note4: Safe Red DNA Stain (REF: CP18106) can be used to visualize RNA bands after electrophoresis. For polyacrylamide gels, it is recommended to use a soaking method to observe the bands.

(2) Capillary electrophoresis

Capillary electrophoresis offers a precise, digital assessment of RNA sample integrity, purity, or degradation. Compared to gel-based methods, this technique requires a smaller amount of RNA and provides higher sensitivity.

FAQ & Troubleshooting

FAQ & Troubleshooting	Possible Reason	Solution
Low transcript production	There were inhibitory components in the experimental template, or the template had poor purity or incorrect concentration.	If the output of the control group is normal，Please repurify the template and determine the template quantification and its integrity。 If the control group has low yield, please contact us (web: www. best-enzymes.com).
Short fragment transcript yield is low	Short transcription initiation fragments inhibit the reaction.	When the transcript is 100 nt, it is recommended to either prolonging the reaction time or increasing the amount of template to 2 μg.
RNA product fragments are smaller than expected	The template sequence contains a termination sequence similar to T7 RNA polymerase.	It is suggested that lowering the reaction temperature to 30℃ may increase the proportion of full-length transcripts, but the yield will decrease.
	The template forms a secondary structure due to its high GC content.	It is recommended to increase the reaction temperature to 42℃ or use single-strand binding (SSB) proteins to increase transcription efficiency.
The RNA product fragment is larger than expected	The plasmid template may not be fully linearized.	It is recommended to further digest the plasmid template DNA or re-optimize the digestion reaction system for plasmid template DNA preparation.
	RNA has an incompletely denatured secondary structure.	Increasing the concentration of denaturants or raising the denaturation temperature to 70℃ after adding RNA Loading Buffers.
Electrophoresis tailing phenomenon	The experimental procedure is contaminated by RNase.	It is recommended to use a dedicated workspace for transcription reactions, employing RNA-specific reagents and pipettes. During experiments, it is recommended to use RNase-free tips and EP tubes, wearing disposable latex gloves and masks, all reagents should be prepared with RNase-free water. Keep reagent containers tightly closed when not in use. Ensure tubes are securely sealed during the reaction process.
	The DNA template is contaminated by RNase	RNase contamination in the template may also result in RNA products smaller than expected. Therefore, it is recommended to repurify the template DNA and dissolve it with Nuclease-Free Water or DEPC-treated water.

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