TransCap T7 Co-transcription Kit with CAP GAG

Please read the manual carefully before use.

Cat. No. JT103

Version No. Version 1.0

Storage: at -18°C or below for one year

Description

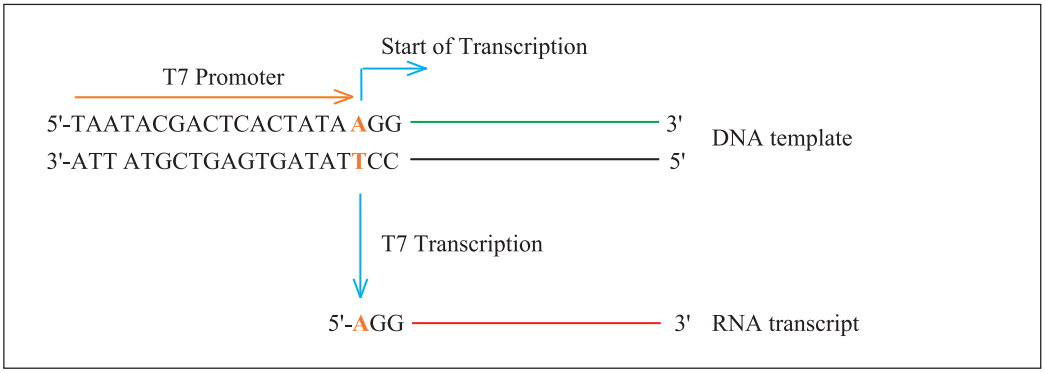
This kit uses T7 RNA polymerase to perform in vitro transcription of linear double-stranded DNA containing a T7 promoter sequence, with NTPs as substrates. The sequence downstream of the T7 promoter is transcribed, while the cap analog CAP GAG (ENE) is co-transcriptionally incorporated into the 5' end of the mRNA, generating single-stranded RNA with a 5'-m7G Cap1 structure. RNA with a Cap1 structure exhibits enhanced stability, thereby protecting the RNA from degradation in cells. This kit utilizes modified nucleotides (N1-me-Pseudo UTP) in place of conventional UTP. These modified nucleotides effectively reduce the immunogenicity of mRNA and suppress innate immune activation, resulting in in vitro transcribed mRNA with improved nuclease stability and translation efficiency. This makes the mRNA highly valuable for applications in medicine, therapeutics, and diagnostics.

A single reaction (20 µl system) using this kit can yield 120–160 µg of RNA product, and the system is scalable for high-level RNA production. The synthesized RNA can be used for in vitro translation, RNase protection assays, RNA splicing assay, and probe labeling, among other applications.

Kit Contents

Component	JT103-01 (25 rxns)	JT103-02 (100 rxns)
T7 Transcription Enzyme Mix	50 µl	200 µl
5×T7 Transcription Reaction Buffer	100 µl	400 µl
ATP (100 mM)	30 µl	120 µl
GTP (100 mM)	25 µl	100 µl
CTP (100 mM)	25 µl	100 µl
N1-me-Pseudo UTP (100 mM)	25 µl	100 µl
CAP GAG (ENE)	20 µl	80 µl
DNase I (1 unit/µL)	50 µl	200 µl
500 mM EDTA (pH 8.0)	25 µl	100 µl
RNase-free Water	1 ml	4×1 ml
Transcription Control Template (0.5 µg/µl，1kb)	10 µl	40 µl

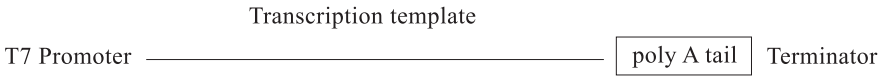
Principle of Co-Transcriptional Capping in RNA Synthesis



Template Preparation

1. Supercoiled Plasmid DNA Template

The supercoiled plasmid DNA template should contain a T7 promoter (with the RNA transcription start sequence: AGG). It is recommended to include a 120 bp poly-A tail sequence and an effective terminator to ensure efficient transcription termination. The termination efficiency of the commonly used T7 terminator in many standard vectors is less than 70%, which can lead to significant read-through transcription beyond the intended stop point. Therefore, it is recommended to use a terminator composed of the following sequence to ensure efficient and precise termination:



T7 Promoter: 5'-TAATACGACTCACTATAA*GG-3' (*： Transcription starts with A)

Terminator: 5'-TTCCATCTGTTTTCTTATCTGTTCTTTCATCTGTTCTTTTATCTGTTTGTTT 3'

2. Linear DNA Template

The linear DNA Template typically refers to plasmid DNA digested with restriction enzymes or PCR-amplified products. When using linear DNA as a transcription template, it is important to avoid 3′-overhangs. Therefore, restriction enzymes that generate 5′-overhangs or blunt ends are generally preferred.

If a 3′-overhang cannot be avoided, the template DNA should be treated with T4 DNA Polymerase prior to transcription to convert the ends to blunt. Digested or PCR-amplified products must be purified to remove proteins, salt ions, and other contaminants to obtain high-quality DNA for use as a template.

3. Template Amount

The reaction system recommended in this kit is suitable for most templates. An input of 1 μg of template DNA typically yields approximately 120~160 μg of RNA. However, the actual yield may vary depending on factors such as the template's length, purity, sequence, and secondary structure. For most transcription reactions, higher yields can be achieved by increasing the amount of template input and/or extending the reaction time.

Notes

- Avoid RNase Contamination: Always use RNase-free water, pipette tips, and microcentrifuge tubes. Wear a lab coat and gloves throughout the procedure.
- Prepare the Reaction at Room Temperature: The 5× T7 Transcription Reaction Buffer in this kit has a high salt concentration that can lead to salt precipitation at low temperatures, potentially affecting template DNA and enzyme activity. Therefore, when preparing the reaction mix, keep all components except the enzyme, NTPs and CAP GAG (ENE) at room temperature. The enzyme, NTPs and CAP GAG (ENE) may be kept on ice. Assemble the reaction at room temperature.
- Follow the Order of Addition: Pre-calculate all volumes and strictly adhere to the following order when adding components: Water → Buffer → NTPs →CAP GAG (ENE)→ DNA Template → Enzyme. The template and enzyme must always be added last.
- For Short Transcripts (<500 nt): It is recommended to use 2 μg of template. For very short fragments (~100 nt), use 2 μg of template and extend the reaction time to 6 hours or more. An overnight incubation of 16 hours can yield the maximum product.
- For Extended Transcription Times (≥6 hours): When reactions are run for 6 hours or longer, use 2 μg of template and increase the NTP concentration to 10 mM.

Operation procedures

1. Briefly centrifuge all components except the T7 Enzyme Mix to collect them at the bottom of the tubes.
2. Prepare the transcription reaction mixture as follows:

Component	Volume
DNA Template	500 ng~2 μg
5× T7 Transcription Reaction Buffer	4 μl
ATP	1.2 μl
GTP	1 μl
CTP	1 μl
N1-me-Pseudo UTP	1 μl
CAP GAG (ENE)	0.8 μl
T7 Transcription Enzyme Mix	2 μl
RNase-free Water	Variable
Total Volume	20 μl

* Note: Pre-calculate the total volume. Add nuclease-free water first, and strictly follow this order for adding reaction components: Water → Buffer → NTPs →CAP GAG (ENE)→ DNA Template → Enzyme.

3. Mix the components gently by pipetting, briefly centrifuge to collect the liquid at the bottom of the tube, and incubate at 37°C for 2 hours.

* Note: To prevent evaporation during prolonged transcription reactions, it is recommended to perform the reaction in a PCR machine with the heated lid set to 65°C. Template amount and incubation time can be adjusted as needed.

4. DNA Template Digestion: After the transcription reaction is complete, add 2 μL of DNase I and incubate at 37°C for 30 minutes. Following digestion, add 1 μL of 500 mM EDTA (pH 8.0) to stop the reaction (if EDTA is added, proceed immediately to the purification step). Alternatively, purification can be performed directly after digestion without adding EDTA.
5. Product Purification: Please follow the instructions provided with the *EasyPure*® RNA Purification Kit (ER701) or *MagicPure*® RNA Beads (EC501) for RNA purification.
6. Quantification and Analysis of Transcription Product:

① Quantification: Measure RNA concentration using a UV spectrophotometer. The product concentration is typically very high, so it is recommended to dilute the sample before measurement.

② Detection for RNA products (100–1000 nt): It is recommended to analyze on a 6% polyacrylamide, 7 M urea denaturing gel, using 1×TBE Buffer as the electrophoresis buffer.

- 10×TBE Buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA.
- Gel Preparation (per 10 mL): urea 4.2 g, RNase-free Water 4.4ml, 40% (acrylamide: methylenebisacrylamide=19:1) acrylamide 1.5 ml, 10×TBE Buffer 1 ml, 10% AP 100 μl, TEMED 10 μl. AP and TEMED are added after the urea was completely dissolved.

③ Detection for RNA products (500–6000 nt): It is recommended to analyze on a 1% formaldehyde-agarose denaturing gel, using 1×MOPS Buffer as the electrophoresis buffer.

- 10×MOPS Buffer: 0.4 M MOPS (pH 7.0), 0.1 M Sodium Acetate, 10 mM EDTA.
- Gel Preparation (per 100 mL): Add 1 g of agarose to 72 mL of RNase-free Water, heat to dissolve, then add 10 mL of 10×MOPS Buffer. Once cooled to 50–60°C, add 18 mL of formaldehyde (37%), mix well, and pour the gel.

④ Electrophoresis Sample Preparation: Dilute 0.2–1 μg of RNA to 5 μL with RNase-free Water, add an equal volume of 2×RNA Loading Buffer, mix well, incubate at 70°C for 10 minutes, and place on ice for 2 minutes. Load the entire sample. After electrophoresis, stain with Gelstain or Ethidium Bromide (EB) for visualization. Treat the RNA Marker the same way as the RNA samples (or refer to the supplier's instructions).

Reference Data

Fig 1. Synthesis of 800 nt mRNA using the co-transcriptional capping kit. A 20 µl reaction system was supplemented with 500 ng of DNA template, and mRNA yield was measured at different time points. The results showed that mRNA yield peaked at 120 minutes (2 hours) of reaction time. Actual yields may vary depending on specific experimental conditions. This figure is for reference only.

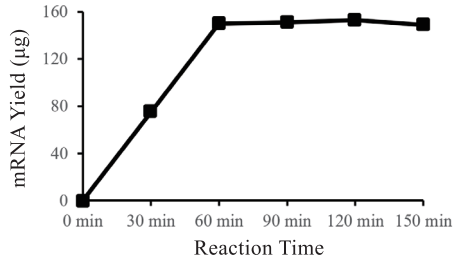


Fig 1. Time-course accumulation curve of mRNA synthesis

Fig 2. Capping of mRNA using cap analog and analysis of capping efficiency by high-performance liquid chromatography (HPLC). Table 1. The results showed a capping efficiency of 99%.

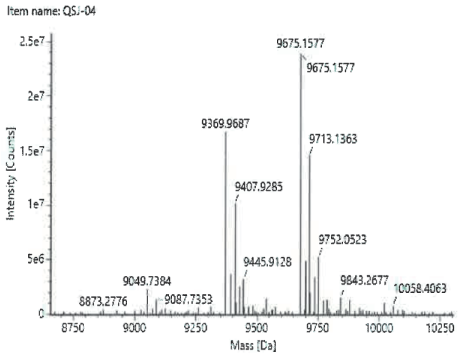


Fig 2. Analysis of mRNA capping efficiency

Table 1. Deconvolution results of sample components and analysis of capping efficiency

Name	Modifiers	Response	Observed mass (Da)	Expected mass (Da)	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	%Cap1
uncapped_monp	/	285962	9221.7516	9221.6159	135.70	14.70	2.90	
Cap1	/	23882538	9675.1577	9674.8543	303.40	31.40	2.90	
Cap1	+K (1)	14576915	9713.1363	9712.9447	191.60	19.70	2.90	99.37
Cap1	+Na (1)	4869230	9697.1357	9696.8362	299.50	30.90	2.90	
Cap1	DIEA	255152	9804.5693	9804.0975	471.80	48.10	2.90	
Cap1	HFIP	1534122	9843.2677	9842.8921	375.60	38.20	2.90	

Troubleshooting

1. Low Yield or Transcription Failure

   Template quality is closely related to RNA yield. It is recommended to set up a control group. If the control reaction yield is low, please contact Beijing TransGen Biotech Customer Service for guidance on reaction setup. If the control yield is normal but the experimental sample yield is low, the issue may lie with the quality of the experimental template. Please try the following solutions:

   ① The template may contain inhibitors. Re-purify the template DNA to ensure it is RNase-free and of high purity. ② Verify template quantity and integrity. ③ Extend the transcription reaction time. ④ Increase the amount of template input. ⑤ Try using a different promoter and RNA polymerase.

2. Smearing of Transcription Product in Electrophoresis

   Possible causes include:

   ① RNase contamination during the procedure. ② RNase contamination of the transcription template.

   Recommended solutions: Re-prepare the template DNA. Ensure all consumables and reagents are RNase-free. Wear a mask and disposable latex gloves during the procedure, paying strict attention to RNase contamination control.

3. Transcription Product is Longer than Expected

   If electrophoresis shows a band larger than expected, possible causes include:

   ① Incomplete linearization of the template. ② A 3'-overhang structure on the sense strand of the template. ③ Incomplete denaturation of secondary structures in the RNA product.

   Recommended solutions:

   ① Check if the template is completely linearized; ensure complete linearization. ② Select a suitable restriction enzyme to ensure the sense strand has a 5'-overhang or the DNA ends are blunt. ③ Check the template structure and analyze the RNA product on a denaturing gel.

4. Transcription Product is Shorter than Expected

   If electrophoresis shows a band smaller than expected, possible reasons are:

   ① The template sequence contains sequences resembling T7 RNA polymerase termination signals, causing premature termination. ② High GC content in the template sequence leads to complex secondary structures. ③ RNase contamination.

   If the template contains termination-like sequences, consider optimizing the RNA sequence. If the template has high GC content, try adding SSB protein to improve yield and full-length transcription.

5. Low Yield for Short Transcripts

   Very short transcripts can inhibit the reaction. For transcripts <500 nt, extend the reaction time and increase the template input. It is recommended to use 2 μg of template. For very short fragments (e.g., ~100 nt), use 2 μg of template and incubate for at least 6 hours. Overnight transcription for 16 hours can yield the maximum product.

6. Intended Use

   This kit is intended for research use only.

For research use only, not for clinical diagnosis.

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