Product Description

Developed independently by BioReady Biotech, the T7 High-Efficiency Transcription Kit is optimized for transcription reaction systems across different templates and nucleotide types. This kit enables efficient production of high yields of RNA from DNA templates in vitro.Using T7 promoter-specific RNA polymerase, the kit transcribes high-yield single-stranded RNA from plasmids or PCR products containing the T7 promoter sequence, using natural or modified nucleotides as substrates. By including Cap0, Cap1, or other cap analogs in the reaction, capped full-length mRNA can be synthesized directly via co-transcription.

The kit recommends a 20 µL in vitro transcription system. With 1 µg of plasmid template or an equivalent amount of PCR product template, RNA yield exceeds 200 µg using either natural or modified nucleotides. Through process design and optimization, the kit supports gram-scale RNA production.The transcribed RNA can be used for RNA structural and functional analysis. Uncapped single-stranded RNA is prone to degradation, downstream processing is recommended based on project requirements,circular RNA should be circularized promptly, linear RNA can be capped using the Vaccinia Capping Enzyme GMP-grade (GMP-BP-E05) and mRNA Cap 2´-O-Methyltransferase GMP-grade(GMP-BP-E06), mRNA capped co-transcriptionally is ready for functional studies.

Components

Components	Cat. No.	Volume
ATP Solution(100 mM)	BP-AS-01	100 µL
GTP Solution(100 mM)	BP-AS-02	100 µL
CTP Solution(100 mM)	BP-AS-03	100 µL
UTP Solution(100 mM)	BP-AS-04	100 µL
T7 RNA Polymerase	BP-E01	100 µL
RNase Inhibitor(40 U/µL)	BP-E02	100 µL
Inorganic Pyrophosphatase(0.1 U/µL)	BP-E03	100 µL
10×Transcription Buffer	BP-AS-08	100 µL
Control Template+	BP-AS-09	5 µL
Precipitation Reagent	BP-AS-10	1 mL
DNase I (2 U/µL)	BP-E04	50 µL
10×DNase I Reaction Buffer	BP-AS-13	100 µL
RNase-free ddH_2O	BP-AS-11	1 mL

*The control template+ is for verification of in vitro transcription only and is not intended for downstream applications.

Storage

Store at -20±5°C.

Materials

Consumables,RNase-free PCR tubes, RNase-free pipette tips, RNA purification magnetic beads (if needed)
Templates,Plasmid or PCR product containing the T7 promoter.
Reagent,Absolute ethanol.

Protocol

1. Template Preparation

1.1 Plasmid Template

Plasmid templates must be free of protein and RNA contamination. Commercially prepared industrial-or clinical-grade plasmids generally perform well with this kit.

1.2 Plasmid Linearization

Plasmid DNA must be linearized with a restriction enzyme downstream of the target gene for efficient transcription. Even trace amounts of circular plasmid lead to long heterogeneous RNA transcripts. Verify complete linearization by gel electrophoresis after purification.

1.3 Linearized Product Recovery

Terminate the linearization reaction by adding 1/20 volume of 0.5 M EDTA, 1/10 volume of 3 M sodium acetate or 5 M ammonium acetate, and 2 volumes of absolute ethanol. Mix well, precipitate at -20°C for 1 h or overnight. Centrifuge at

≥12,000 rpm for 15 min at 4°C to pellet DNA. Discard supernatant, briefly centrifuge for 5-10 s, and carefully remove residual liquid. Air-dry completely in a clean bench to eliminate ethanol, which inhibits downstream enzymes. Dissolve the pellet in RNase-free ddH_2O to approximately 1 μg/μL.

2. In Vitro Transcription

2.1 Reagent Preparation

Place T7 RNA Polymerase, RNase Inhibitor, and Inorganic Pyrophosphatase on ice immediately upon removal. Thaw ATP Solution, GTP Solution, CTP Solution, and UTP Solution at room temperature, then transfer to ice. Vortex 10× Transcription Buffer until fully dissolved, keep at room temperature during reaction setup.

Components	Volume
RNase-free ddH_2O	To 20 μL
ATP Solution(100 mM)	2 μL
CTP Solution(100 mM)	2 μL
GTP Solution(100 mM)	2 μL
UTP Solution(100 mM)	2 μL
10×Transcription Buffer	2 μL
RNase Inhibitor(40 U/μL)	1 μL
Inorganic Pyrophosphatase(0.1 U/μL)	1 μL
T7 RNA Polymerase	2 μL
Template+	1 μg

2.3 Mix gently by pipetting or flicking the tube, briefly centrifuge to collect the reaction at the bottom.

2.4 Incubate at 37°C for at least 2 h in a heated-lid thermal cycler. For small RNA (≤300 bp), extend to 3 h for sufficient yield. Reaction time up to 16 h does not compromise RNA quality.

2.5 Template DNA Digestion, add 1 μL DNase I and 2 μL 10× DNase I Reaction Buffer. Incubate at 37°C for 15 min to digest the DNA template.

3. RNA Purification and Recovery

3.1 Add equal volumes of ice-cold RNase-free ddH_2O and ice-cold Precipitation Reagent, mix gently by pipetting .

3.2 Place at -20°C for at least 30 min.

3.3 Centrifuge at 15,000 rpm for 20 min at 4°C.

3.4 Carefully remove supernatant, wash pellet with 500 μL 70% ethanol. Centrifuge at 15,000 rpm for 15 min at 4°C.

3.5 Remove ethanol thoroughly. Resuspend RNA in an appropriate buffer for downstream applications. Determine concentration and store at -80°C.

Notes

1. Ensure no ethanol remains in PCR or plasmid templates. Open lids in a clean bench to evaporate ethanol completely before dissolving DNA.
2. In vitro transcription is highly sensitive to RNase contamination. Use only RNase-free tips and tubes. Prepare reactions in an RNase-free environment (e.g decontaminated clean bench). Avoid opening RNA vessels outside the clean bench.
3. Follow the order of addition strictly. Use 10× Transcription Buffer at room temperature. Add DNA template last to avoid co-precipitation with spermidine at low temperatures, which reduces yield.
4. Mix thoroughly before use and avoid repeated freeze-thaw cycles.