Product Description

Co-transcriptional capping is a method for introducing cap structures during in vitro transcription, primarily used to enhance mRNA stability and translation efficiency. The mRNA co-transcriptional synthesis kit utilizes T7 RNA polymerase to transcribe DNA sequences downstream of a T7 promoter, using linear double-stranded DNA containing the T7 promoter sequence and an AG initiation sequence as the template, NTPs as substrates, and cap analogs. This process efficiently synthesizes single-stranded RNA with a 5'-m7G Cap1 structure without requiring additional enzymatic capping steps. It can also be used to prepare biotin- or dye-labeled RNA using modified nucleotides as substrates. This kit synthesizes both long and short transcripts via co-transcriptional methods, providing nucleotides including ATP, CTP, GTP, UTP, and the modified N1-Me-pUTP, as well as the cap analog Cap AG(3'Acm) (specific structural formula shown in Figure 1 below). An input of 1 μg of template DNA can yield 150-200 μg of Cap1 RNA. The RNA synthesized by transcription using this kit is suitable for various downstream applications, such as RNA structure and function studies, RNase protection assays, probe hybridization, RNAi, microinjection, and in vitro translation.

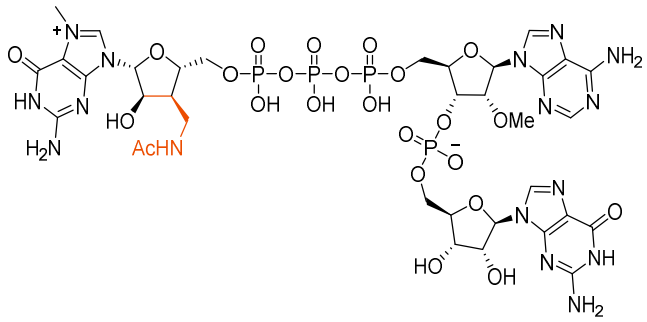

Figure 1 Structure of the cap analog Cap AG(3'Acm)

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
N1-Me-pUTP Solution(100 mM)	BP-AS-07	100μL
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
DNase I (2 U/μL)	BP-E04	100μL
Cap AG(3'Acm)(100mM)	BP-AS-38	100μL
10×Co-transcription Buffer	BP-AS-31	100μL
Precipitation Reagent	BP-AS-10	1 mL
RNase-free ddH_2O	BP-AS-11	1 mL

Storage

Store at −20±5℃.

Materials

Consumables, Nuclease-free PCR tubes, nuclease-free pipette tips, RNA purification and recovery magnetic beads (if necessary), etc.

Template, Linearized plasmid template with T7 promoter and AG start sequence, PCR product template.

Reagents, Anhydrous ethanol.

Protocol

1. Template Preparation

1.1 Plasmid Template

The plasmid template must be free from protein and RNA contamination. Most commercially prepared industrial-grade or clinical-grade plasmids perform well in mRNA co-transcription synthesis kits.

1.2 Plasmid Linearization

Plasmid DNA must be linearized with a restriction enzyme downstream of the target gene for transcription. Even trace amounts of circular plasmid can generate extremely long, heterogeneous RNA transcripts. It is often necessary to check the recovered linearized template DNA on a gel to confirm complete digestion.

1.3 Recovery of Linearized Product

Terminate the linearization reaction under the following conditions,

1/20 volume of 0.5 M EDTA

1/10 volume of 3 M sodium acetate or 5 M ammonium acetate

2× volume of absolute ethanol

Mix thoroughly and precipitate at -20℃ for 1 hour or overnight. Subsequently, pellet the DNA by centrifugation at >12,000 rpm for 15 minutes. Remove the supernatant, centrifuge again for 1 minute, and carefully aspirate any residual liquid using a 10 μL pipette. In a clean bench, dry the pellet thoroughly to allow complete evaporation of ethanol, avoiding inhibition of downstream enzymatic activity. Finally, dissolve the precipitate in RNase-free ddH_2O to a concentration of approximately 1 μg/μL.

2. In Vitro Transcriptio

2.1 Immediately place T7 RNA Polymerase, RNase Inhibitor, and Inorganic Pyrophosphatase on ice after removal. Thaw Cap AG(3'Acm), ATP Solution, GTP Solution, CTP Solution, UTP Solution, or N1-Me-pUTP Solution at room temperature and immediately transfer to ice. Thaw the 10× Co-transcription Buffer at room temperature. Vortex and briefly centrifuge all components to collect them at the bottom of the tubes. Prepare the transcription reaction mixture at room temperature.

2.2 Add each component sequentially according to the table below:

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 or N1-Me-pUTP Solution(100 mM)	2 μL
Cap AG(3'Acm)(100 mM)	2μL
10×Co-transcription Buffer	2 μL
RNase Inhibitor(40 U/μL)	1 μL
Inorganic Pyrophosphatase(0.1 U/μL)	1 μL
T7 RNA Polymerase	2 μL
DNA Template	1 μg

2.3 Mixing

Gently flick the tube or pipette the mixture up and down, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

2.4 Transcription

Using equipment with a heated lid, incubate at 37℃ for at least 2 hours. For in vitro transcription of short RNA fragments (≤300 bp), it is recommended to extend the reaction time to 3 hours to ensure sufficient yield. Extending the in vitro transcription time within a certain range (up to 16 hours) does not affect the quality of the product.

2.5 Template DNA Digestion

Add 2 μL of DNase I to the reaction system and continue incubation at 37℃ for 15 minutes to digest the DNA template.

3. Purification and Recovery

3.1 Add 2× the reaction volume of RNase-free ddH_2O (pre-chilled) and mix gently by pipetting.

3.2 Add 2× the reaction volume of Precipitation Reagent (stored at -20℃) and mix gently by pipetting.

3.3 Precipitate at -20℃ for at least 1 hour.

3.4 Centrifuge at 4℃, 15,000 rpm for 20 minutes.

3.5 Carefully remove the supernatant. Wash the pellet with 500 µL of 70% ethanol, then centrifuge at 4℃, 15,000 rpm for 15 minutes.

3.6 Carefully remove the 70% ethanol. Resuspend the RNA in an appropriate solution or buffer according to the requirements of downstream experiments. Measure the RNA concentration and store at -80℃.

Notes

1. When using either PCR product templates or linearized plasmid templates, it is essential to ensure that the template contains no residual ethanol. To achieve this, when dissolving the DNA precipitate, leave the tube cap open to allow complete evaporation of ethanol. This step can be performed in a clean bench.
2. The in vitro transcription reaction is highly sensitive to RNase. Strict precautions must be taken to avoid RNase contamination in the reaction system. Use only RNase-free consumables, such as pipette tips and microcentrifuge tubes. The reaction mixture should be prepared in an RNase-free environment whenever possible; a cleaned clean bench can be used for this operation. Additionally, avoid opening tubes containing synthesized RNA outside the clean bench area to prevent RNase contamination.
3. During system preparation, strictly follow the sample addition order specified in the manual. The 10× Co-transcription Buffer contains spermidine and should be used after returning to room temperature. DNA is prone to co-precipitate with spermidine at low temperatures, which can affect transcription yield. Therefore, the DNA template should be the last component added during system preparation.
4. Mix thoroughly before use and avoid repeated freeze-thaw cycles.