CELLSCRIPT™
RNA for Translation in Cells

Monomethylated RNA Cap Analog

Cat. No. **C-C61025**

INTRODUCTION

5'-terminal caps are involved in mRNA processing, stability and initiation of protein synthesis.[1] Uncapped RNA transfected or injected into cells is rapidly degraded by cellular RNases.[2,3] Therefore, RNA prepared for microinjection into oocytes or for transfection into eukaryotic cells should be capped. *In vitro*, capped mRNAs are translated more efficiently than uncapped transcripts in rabbit reticulocyte lysate or wheat germ translation systems.[4] Transcripts synthesized for use in *in vitro* RNA splicing experiments may also need to be capped.[5]

CELLSCRIPT's Monomethylated RNA Cap Analog m^7G[5']ppp[5']G (Figure 1) can be used in cotranscriptional capping reactions, in conjunction with any *in vitro* transcription kit not containing premixed NTPs. A protocol for the synthesis of Cap Analog-capped RNA using components of a T7-Scribe™ IVT Kit (CELLSCRIPT) is provided. This reaction will yield up to 45 μg of total RNA with ~80% capping efficiency in a 2 hour reaction.

CELLSCRIPT also offers the Anti-Reverse Cap Analog (ARCA), $m_2^{7,3'\text{-}O}$G[5']ppp[5']G, which contains a 3'-O-methyl group on the m^7G nucleotide. ARCA can only be incorporated in the correct orientation at the 5' end of the RNA during an *in vitro* transcription/capping reaction.[6-8] This is not true for the monomethylated cap analog (m^7G[5']ppp[5']G). Thus, ARCA incorporation results in the synthesis of capped RNA that is more efficiently translated *in vivo* than monomethylated cap analog. Additionally, CELLSCRIPT also offers the AmpliCap-Max™ T7 High Yield Message Maker Kit and the Message-MAX™ T7 ARCA-Capped Message Transcription Kit, both of which yield up to 60 μg of total RNA with ~80% capping efficiency in a 30 minute reaction. The AmpliCap-Max Kit uses the monomethylated RNA Cap Analog and the MessageMAX Kit uses the anti-reverse cap analog.

MATERIALS

Materials Supplied

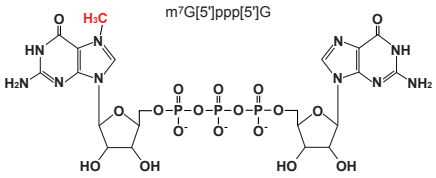
Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

Monomethylated RNA Cap Analog (20 mM Solution)		
Component		Volume
Monomethylated Cap Analog m^7G[5']ppp[5']G	1,250 nmoles Cat. # C-C61025	62.5 μl

Monomethylated Cap Analog absorbance maximum at 254 nm.

Inquire about custom kit sizes at 608-442-6484 or sales@cellscript.com.

Figure 1. Monomethylated RNA Cap Analog Structure.



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Materials Required, but not Supplied

- A DNA template for transcription of your RNA of interest
- *In vitro* transcription reaction components or kit
- Materials or kits for purification of the RNA product
  (For suggestions, see Section C "Purification of the Transcription Product")
- RNase-free TE Buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA)

SPECIFICATIONS

Storage Buffers

Monomethylated RNA Cap Analog is provided in sterile deionized water (pH 7.0).

Functional Testing

Monomethylated RNA Cap Analog is functionally tested for capping in an *in vitro* transcription/capping reaction. Greater than 50% of the RNA synthesized must be capped, based on relative band intensities of capped and uncapped transcripts following separation on a PAGE/urea gel.

Contaminating Activity Assays

Monomethylated RNA Cap Analog is free of detectable RNase and DNase activities.

BEFORE YOU START: IMPORTANT TIPS FOR CAP ANALOG CAPPING

♦ **Percentage of Capped RNA**:

The protocol provided below, will produce an RNA mixture where ~80% of the RNA will contain a Cap Analog-derived cap. This is because Cap Analog is present in the reaction in a 4:1 molar ratio compared to GTP. Users can customize the percentage of capped RNA produced in the reaction by altering the Cap Analog to GTP molar ratio in the reaction. Higher ratios will yield less total RNA but contain a higher percentage of capped RNA. Lower ratios will yield more total RNA but contain a lower percentage of capped RNA.

PROCEDURE

A. Synthesis of Cotranscriptionally-Capped RNA

1. This protocol uses components of a T7-Scribe IVT Kit (CELLSCRIPT).

   Set up the transcription reaction **at room temperature** by adding the reagents **in the order indicated below**:

T7-Scribe-based Cap Analog Capping Reaction	
Component	Amount
RNase-Free Water	x µl
Linearized template DNA with T7 RNAP promoter	1 µg
10X T7-Scribe Transcription Buffer	2 µl
100 mM ATP	1.5 µl
100 mM CTP or 5mCTP	1.5 µl
100 mM UTP or ΨTP	1.5 µl
30 mM GTP diluted from 100 mM stock	1 µl
20 mM Monomethylated RNA Cap Analog	6 µl
100 mM Dithiothreitol	2 µl
ScriptGuard™ RNase Inhibitor	0.5 µl
T7-Scribe Enzyme Solution	2 µl
Total Reaction Volume	20 µl

! Assemble transcription reactions at room temperature in the order indicated at left. Assembly of transcription reactions at <22°C or in an alternate order, can result in the formation of an insoluble precipitate.

10X T7-Scribe Transcription Buffer stored at –70°C may result in the formation of a white precipitate. To dissolve it, heat the tube at 37°C for 5 minutes and mix thoroughly.

One microgram of DNA template is recommended for most reactions. If the DNA template is <0.19 µg/µl, concentrate it, then resuspend in the appropriate amount of RNase-Free Water.

2. Incubate at 37°C for 2 hours.

B. DNase I Treatment of a Cotranscriptional Capping Reaction (Optional)

1. DNase I treatment can be used to remove the DNA template if necessary for subsequent applications.

Standard DNase I Treatment of IVT/Capping Reaction	
Component	Amount
IVT/Capping Reaction (from Step A)	20 µl
RNase-Free DNase I	1 µl
Total Reaction Volume	21 µl

2. Incubate for 15 minutes at 37°C.

3. Proceed to RNA Purification.

C. Purification of the Transcription Product

Purify the RNA using your preferred method. The method chosen should remove residual proteins and unincorporated NTPs from the RNA. Several options are listed below. RNA can be stored at –20°C or –70°C. For long-term storage, RNA can be stored as an ethanol pellet.

I) **Ammonium Acetate Precipitation**: Selectively precipitates RNA, while leaving most of the protein, DNA and unincorporated NTPs in the supernatant. Note: for this method, the RNA to be purified must be >100 bases in size.

1) Add one volume of 5 M ammonium acetate (21 µl for the standard reaction), mix well.
2) Incubate for 15 minutes on ice.
3) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
4) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
5) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
6) Allow pellet to dry, then resuspend in RNase-Free Water, TE or other suitable buffer.
7) While usually unnecessary, steps 1-6 may be repeated a second time for even cleaner RNA.
8) Allow the pellet to dry, then resuspend in 30-50 µl of RNase-Free Water for quantitation. **Do not resuspend the RNA in an EDTA-containing solution** if the RNA will later be enzymatically converted to Cap1 RNA (e.g., with CELLSCRIPT's ScriptCap™ 2'-O-Methyltransferase Kit).
9) Quantitate the RNA by spectrophotometry or fluorimetry. If desired, adjust the concentration of the RNA with RNase-Free Water. The RNA can now be frozen and stored at –20°C or –70°C.

II) **Organic Extraction / Ammonium Acetate Precipitation**: Removes all proteins and selectively precipitates RNA, while leaving most of the DNA and unincorporated NTPs in the supernatant. Note: for this method, the RNA to be purified must be >100 bases in size.

1) Adjust reaction volume to 50 µl total using RNase-Free Water (add 29 µl to the reaction).
2) Add one volume (50 µl) of TE-saturated phenol/chloroform. Vortex vigorously for 10 seconds.
3) Spin in a microcentrifuge at >10,000 x g for 5 minutes to separate the phases.
4) Remove the aqueous (upper) phase with a pipette and transfer to a clean tube.
5) Add one volume (50 µl) of 5 M ammonium acetate, mix well then incubate for 15 minutes on ice.
6) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
7) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
8) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
9) Allow the pellet to dry, then resuspend in 30-50 µl of RNase-Free Water for quantitation. **Do not resuspend the RNA in an EDTA-containing solution** if the RNA will later be enzymatically converted to Cap1 RNA (e.g., with CELLSCRIPT's ScriptCap™ 2'-O-Methyltransferase Kit).
10) Quantitate the RNA by spectrophotometry or fluorimetry. If desired, adjust the concentration of the RNA with RNase-Free Water. The RNA can now be frozen and stored at –20°C or –70°C.

Continued on next page.

III) **Organic Extraction / Chromatography / Ethanol Precipitation**: Removes all proteins, digested DNA, and unincorporated NTPs from the RNA.

1) Adjust reaction volume to 50 µl total using RNase-Free Water (add 29 µl to the reaction).
2) Add one volume (50 µl) of TE-saturated phenol/chloroform. Vortex vigorously for 10 seconds.
3) Spin in a microcentrifuge at >10,000 x g for 5 minutes to separate the phases.
4) Remove the aqueous (upper) phase with a pipette and transfer to a clean tube.
5) Remove digested DNA and unincorporated NTPs by spin column chromatography.[9] For commercially-available columns, follow the manufacturer's instructions for this step. Recover the RNA in 50-100 µl.
6) Add one-tenth volume (5-10 µl) of 3 M sodium acetate and 2.5 volumes (125-250 µl) of 95% ethanol to the tube, mix well.
7) Incubate for 15 minutes on ice.
8) Pellet the RNA by centrifugation at >10,000 x g for 15 minutes at 4°C.
9) Remove the supernatant with a pipette and gently rinse the pellet with 70% ethanol.
10) Remove the 70% ethanol with a pipette without disturbing the RNA pellet.
11) Allow the pellet to dry, then resuspend in 30-50 µl of RNase-Free Water for quantitation. **Do not resuspend the RNA in an EDTA-containing solution** if the RNA will later be enzymatically converted to Cap1 RNA (e.g., with CELLSCRIPT's ScriptCap™ 2'-O-Methyltransferase Kit).
12) Quantitate the RNA by spectrophotometry or fluorimetry. If desired, adjust the concentration of the RNA with RNase-Free Water. The RNA can now be frozen and stored at –20°C or –70°C.

IV) **RNA-Binding Purification Column**: Several options are available commercially from multiple vendors. Follow the manufacture's recommended protocol.

1) Follow the manufacture's recommended protocol.
2) The final resuspension of RNA should be in 30-50 µl of RNase-Free Water for quantitation. **Do not resuspend the RNA in an EDTA-containing solution** if the RNA will later be enzymatically converted to Cap1 RNA (e.g., with CELLSCRIPT's ScriptCap™ 2'-O-Methyltransferase Kit).
3) Quantitate the RNA by spectrophotometry or fluorimetry. If desired, adjust the concentration of the RNA with RNase-Free Water. The RNA can now be frozen and stored at –20°C or –70°C.

RELATED PRODUCTS

– AmpliCap™ SP6 High Yield Message Maker Kit
– AmpliCap-Max™ T7 High Yield Message Maker Kit
– Anti-Reverse Cap Analog (ARCA)
– A-Plus™ Poly(A) Polymerase Tailing Kit
– INCOGNITO™ SP6 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
– INCOGNITO™ T7 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 ARCA 5mC- & Ψ-RNA Transcription Kit
– MessageMAX™ T7 ARCA-Capped Message Transcription Kit
– mScript™ mRNA Production System
– ScriptCap™ 2'-O-Methyltransferase Kit
– ScriptCap™ m^7G Capping System
– ScriptGuard™ RNase Inhibitor
– SP6-Scribe™ Standard RNA IVT Kit
– T7-Scribe™ Standard RNA IVT Kit
– T7 & SP6 Phage RNA Polymerases

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The performance of this product is guaranteed for one year from the date of purchase.

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