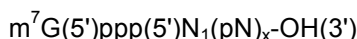


INTRODUCTION

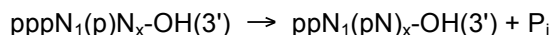
The ScriptCap™ m⁷G Capping System catalyzes *in vitro* addition of a cap nucleotide to the 5' terminus of primary RNA that has a 5'-triphosphate group, such as RNA obtained from an *in vitro* transcription (IVT) reaction. A "cap nucleotide" or "cap" is a guanine nucleoside that is joined via its 5' carbon to a triphosphate group that is, in turn, joined to the 5' carbon of the most 5' nucleotide of the primary mRNA transcript, and in most eukaryotes, the nitrogen at the 7 position of guanine in the cap nucleotide is methylated. Such a capped transcript can be represented as:



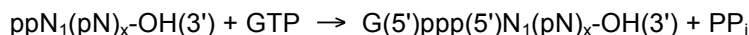
where m⁷G represents the 7-methylguanosine cap nucleoside, ppp represents the triphosphate bridge between the 5' carbons of the cap nucleoside and the first nucleotide of the primary RNA transcript, and N₁(pN)_x-OH(3') represents the primary RNA transcript, of which N₁ is the most 5' nucleotide.

The ScriptCap m⁷G Capping System sequentially catalyzes three different enzymatic reactions:

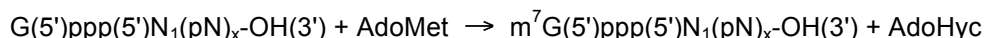
- (1) RNA triphosphatase cleaves the 5' triphosphate of RNA to a diphosphate.



- (2) RNA guanylyltransferase joins GTP to the 5' diphosphate of the most 5' nucleotide (N₁) of the RNA.



- (3) Guanine-7-methyltransferase, using S-adenosyl-methionine (AdoMet) as a co-factor, catalyzes methylation of the 7-nitrogen of guanine in the cap nucleotide.



This process, referred to as "capping," improves the stability and translation efficiency of the RNA compared to uncapped RNA. The capped RNA product from a ScriptCap m⁷G Capping System has a "Cap 0" structure. Cap 0 RNA can be converted to a "Cap 1" structure *in vitro* by using CELLSCRIPT's ScriptCap 2'-O-Methyltransferase in the capping reaction together with the ScriptCap m⁷G Capping System. In addition to having a 7-methyl-G cap nucleotide (m⁷G), Cap 1-RNA also has a 2'-O-methyl group on the 5'-penultimate (N₁) nucleotide, which can further increase *in vivo* translation efficiency of the mRNA.¹

A standard ScriptCap m⁷G Capping System reaction will cap approximately 60 µg of RNA, but reactions can be scaled up or down to accommodate the user's needs.

Capped RNA from an ScriptCap m⁷G Capping System reaction can be added directly to a CELLSCRIPT A-Plus™ Poly(A) Polymerase Tailing Kit reaction for poly(A) tailing of the 3' ends of the capped RNA.

The ScriptCap m⁷G Capping System offers an alternative to making capped RNA by co-transcriptional capping during an *in vitro* transcription reaction in which a dinucleotide cap analog (e.g., m⁷G(5')ppp(5')G) is included in place of a portion of the GTP.² Provided that the 5' terminus of the RNA is not structured, the capping efficiency using the ScriptCap m⁷G Capping System can approach 100%. In contrast, since the cap analog competes with GTP for initiation of transcription by the RNA polymerase, co-transcriptional capping efficiency is limited by the concentration of the cap analog and the ratio of its concentration to that of the GTP. Thus, the percentage of RNA that is capped using a cap analog in a transcription reaction is always less than 100%.^{3,4} The amount of capped RNA that can be made in a

co-transcriptional capping reaction using a cap analog is also limited by the need to reduce GTP concentrations to permit the cap analog to compete for initiation of transcription. On the other hand, co-transcriptional capping with a cap analog can be beneficial if the RNA to be capped has a highly structured 5' terminus. Contact CELLSCRIPT to discuss the options for your project.

MATERIALS

Materials Supplied

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

ScriptCap™ m ⁷ G Capping System Contents (25 reactions)	
Kit Component	Reagent Volume
ScriptCap™ Capping Enzyme, 10 U/μl in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA and 0.1% Triton® X-100.	100 μl
10X ScriptCap™ Capping Buffer 0.5 M Tris-HCl, pH 8.0, 60 mM KCl and 12.5 mM MgCl ₂	250 μl
10 mM GTP	250 μl
20 mM S-adenosyl-methionine (SAM)	30 μl
ScriptGuard™ RNase Inhibitor, 40 U/μl in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM DTT, 0.1 mM EDTA and 0.1% Triton X-100.	65 μl
RNase-Free Water	2 x 1.4 ml



Materials Required, but not Supplied

- IVT RNA
- Materials or kits for purification of the RNA product

SPECIFICATIONS

Unit Definitions

One ScriptCap m⁷G Capping System reaction produces 60 μg of 5'-Cap 0 capped RNA.

One unit of ScriptCap Capping Enzyme releases 1 nmole of inorganic phosphate from GTP in 10 minutes at 37°C under standard assay conditions.

Functional Testing

The ScriptCap m⁷G Capping System is functionally tested for mRNA triphosphatase, guanylyl-transferase and guanine-7-methyltransferase activities in multiple assays.

Contaminating Activity Assays

All components of the ScriptCap m⁷G Capping System are free of detectable RNase and DNase activities.



For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.

BEFORE YOU START: IMPORTANT TIPS FOR OPTIMAL CAPPING**◆ SAM:**

SAM slowly degrades over time at room temperature and above.
Keep thawed SAM solutions on ice at all times.

◆ RNA Source:

RNA produced in an *in vitro* transcription reaction should be purified and resuspended in RNase-Free Water prior to use in the ScriptCap m⁷G Capping System. **Do not resuspend the RNA in an EDTA-containing solution.**

◆ RNA Secondary Structure:

Some RNA transcripts can form stable secondary structures (homodimers and hairpins) involving the 5'-most nucleotides of the transcript severely limiting access of the 5'-most nucleotide to the ScriptCap Capping Enzyme. In order to increase the capping efficiency of such RNAs, use longer or hotter heat denaturation conditions than those noted in the protocol. Times and temperatures required will vary.

◆ Cap 0- vs. Cap 1-mRNA:

The difference between Cap 0- and Cap 1-mRNA is the addition of a methyl group at the 2'-O position of the penultimate (second from the 5' end) nucleotide of the transcript. This methylation is part of the natural capping process in higher eukaryotic cells and in some but not all cases improves *in vivo* translation versus the corresponding Cap 0-mRNA.

The ScriptCap Capping Enzyme and ScriptCap 2'-O-Methyltransferase work together to produce the Cap 1 structure. To obtain a Cap 1 structure, add the ScriptCap 2'-O-Methyltransferase (available separately) to the reaction. When using a new cell line or translation system, we recommend performing a comparison between Cap 0- and Cap 1-mRNA translation efficiencies to determine the optimal cap structure for that system.

◆ Poly(A) Tails:

If the capped RNA requires subsequent 3'-poly(A)-tailing, using CELLSCRIPT's A-Plus Poly(A) Polymerase Tailing Kit (sold separately) allows the user to skip RNA purification prior to poly(A)-tailing. Capped and tailed RNA must be purified prior to use in mRNA transfection experiments.

PROCEDURE

A. Synthesis of Cap 0 RNA from Uncapped RNA

1. The protocol below was designed for use with 50-60 µg of uncapped RNA. It builds a Cap 0 structure on the 5' end of uncapped RNA.

Combine the following reagents:

Standard ScriptCap m ⁷ G Capping Reaction (step 1)	
Component	Amount
RNase-Free Water	x µl
<i>In vitro</i> transcribed RNA, 50-60 µg	≤68.5 µl
Total Volume	68.5 µl



Heat-denaturation of the RNA is an optional step, but it is strongly recommended for RNAs which have not previously been characterized for their ease of enzymatic capping.

Important Only heat-denature the RNA and water components. **Do not** include any other reagent in this step.

2. Incubate at 65°C for 5-10 minutes, then transfer to ice.
3. While the heat-denatured RNA is cooling on ice, prepare a "Cocktail" of the following reaction components together in a separate tube.

Standard ScriptCap m ⁷ G Capping Reaction (step 3)	
Component	Amount
10X ScriptCap Capping Buffer	10 µl
10 mM GTP	10 µl
2 mM SAM (freshly diluted from 20 mM stock)	5 µl
ScriptGuard RNase Inhibitor	2.5 µl
Total Volume	27.5 µl

Important Do not include the ScriptCap Capping Enzyme in this mix.

Important Keep the thawed stock solution on ice.



A white precipitate may form in the 10X ScriptCap Capping Buffer upon storage. To dissolve it, heat the tube at 37°C for 5 minutes and mix thoroughly.

4. **Just prior to starting the reaction**, add the ScriptCap Capping Enzyme to the Cocktail from Step 3 and then combine this with the uncapped RNA solution from Step 1.

Standard ScriptCap m ⁷ G Capping Reaction (step 4)	
Component	Amount
Cocktailed reaction components (from step 3)	27.5 µl
ScriptCap Capping Enzyme (10 U/µl)	4 µl
Heat-denatured RNA (from step 1)	68.5 µl
Total Reaction Volume	100 µl

5. Incubate at 37°C for 30 minutes.
6. The Cap 0 RNA can now be purified, or it can be 3' polyadenylated without purification by adding the reaction mixture directly to an A-Plus Poly(A) Polymerase Tailing Kit reaction (sold separately).

TROUBLESHOOTING

Symptom	Solution
Low capping efficiency	RNA to be treated with ScriptCap Capping Enzyme should be purified and resuspended in RNase-free water. Do not resuspend the RNA in an EDTA-containing solution.
	Prior to capping, purify the input RNA using a method that removes residual proteins, contaminants and unincorporated nucleotides.
	Verify that ScriptGuard RNase Inhibitor was added to the reaction.
	SAM slowly degrades at room temperature and above. Keep SAM solutions on ice at all times. Be sure to use fresh dilutions of SAM which have always been stored on ice. Inefficient N7 methylation will result in inefficient capping.
	Increase the RNA heat-denaturation conditions used. For example, 65°C for 20 minutes, 75°C for 10 minutes, 85°C for 5 minutes, etc...
	Increase the capping reaction incubation time. For example, up to 3 hours at 37°C.
White precipitate in reaction buffer	Some RNAs form stable structures (e.g., homodimers, hairpins) at the 5' end, limiting access by Capping Enzyme. Analyze the sequence and increase the RNA denaturation temperature to above the T _m (e.g., to 65°C for 20 min, 75°C for 10 min, 85°C for 5 min). If the 5' end is highly structured, it might be necessary to modify the 5' end sequence using molecular biology techniques. Often this can be accomplished by making a single point mutation within the first 5 bases of the DNA template for the RNA transcript (non-coding region).
	Incubate the reaction buffer at 37°C for 5 minutes then mix thoroughly to dissolve the precipitate.
	Do not store the kit at –70°C.

RELATED PRODUCTS

- A-Plus™ Poly(A) Polymerase Tailing Kit
- EZ-QC™ mRNA Capping Efficiency Assay Kit
- EZ-QC™ XBG mRNA Capping Efficiency Assay Kit
- INCOGNITO™ T7-FlashScribe™ Ψ-RNA Transcription Kit
- INCOGNITO™ T7-FlashScribe™ N1meΨ-RNA Transcription Kit
- INCOGNITO™ T7 Ψ-RNA Transcription Kit
- INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
- ScriptCap™ 2'-O-Methyltransferase Kit
- ScriptCap™ Cap 1 Capping System
- T7-FlashScribe™ Transcription Kit V2
- T7-Scribe™ Standard RNA IVT Kit

TECHNICAL APPENDIX

A. Synthesis of Cap 0 RNA from Uncapped RNA with Minimal Amounts of ScriptCap Capping Enzyme

1. This protocol produces results comparable to those of the standard Cap 0 capping reaction (page 4), while minimizing the amount of enzyme required in the reaction.

Combine the following reagents:

ScriptCap m ⁷ G Capping Reaction (step 1)	
Component	Amount
RNase-Free Water	x µl
<i>In vitro</i> transcribed RNA, 50-60 µg	≤71.5 µl
Total Volume	71.5 µl



Heat-denaturation of the RNA is an optional step, but it is strongly recommended for RNAs which have not previously been characterized for their ease of enzymatic capping.

Important Only heat-denature the RNA and water components. **Do not** include any other reagent in this step.

2. Incubate at 65°C for 5-10 minutes, then transfer to ice.
3. While the heat-denatured RNA is cooling on ice, prepare a “Cocktail” of the following reaction components together in a separate tube.

Standard ScriptCap m ⁷ G Capping Reaction (step 2)	
Component	Amount
10X ScriptCap Capping Buffer	10 µl
10 mM GTP	10 µl
2 mM SAM (freshly diluted from 20 mM stock)	5 µl
ScriptGuard RNase Inhibitor	2.5 µl
Total Volume	27.5 µl

Important Do not include the ScriptCap Capping Enzyme in this mix.

Important Keep the thawed stock SAM solution on ice.



A white precipitate may form in the 10X ScriptCap Capping Buffer upon storage. To dissolve it, heat the tube at 37°C for 5 minutes and mix thoroughly.

4. **Just prior to starting the reaction**, add the ScriptCap Capping Enzyme to the Cocktail from Step 3 and then combine this with the uncapped RNA solution from Step 1.

Standard ScriptCap m ⁷ G Capping Reaction (step 4)	
Component	Amount
Cocktailed reaction components (from step 3)	27.5 µl
ScriptCap Capping Enzyme (10 U/µl)	1 µl
Heat-denatured RNA (from step 1)	71.5 µl
Total Reaction Volume	100 µl

5. Incubate at 37°C for 2 hours.
6. The Cap 0 RNA can now be purified, or it can be 3' polyadenylated without purification by adding the reaction mixture directly to an A-Plus Poly(A) Polymerase Tailing Kit reaction (sold separately).

REFERENCES

1. Kuge, H. et al., (1998) Nucl. Acids Res. 26, 3208.
2. Konarska, M.M. et al., (1984) Cell 38, 731.
3. Jemielity, J. et al., (2003) RNA 9, 1108.
4. Grudzien, E. et al., (2004) RNA 10, 1479.

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