CELLSCRIPT™
RNA for Translation in Cells

# ScriptCap™ $m^7G$ Capping System

Cat. No. **C-SCCE0625**

## INTRODUCTION

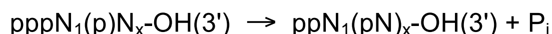
ScriptCap™ $m^7G$ Capping System catalyzes *in vitro* addition of a cap nucleotide to the 5' terminus of primary RNA that has a 5'-triphosphate group, such as RNA obtained from an *in vitro* transcription reaction. A "cap nucleotide" or "cap" is a guanine nucleoside that is joined via its 5' carbon to 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:

$$m^7G(5')ppp(5')N_1(pN)_x\text{-}OH(3')$$

where $m^7G$ 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 $N_1(pN)_x\text{-}OH(3')$ represents the primary RNA transcript, of which $N_1$ is the most 5' nucleotide.

The ScriptCap $m^7G$ Capping System sequentially catalyzes three different enzymatic reactions:

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

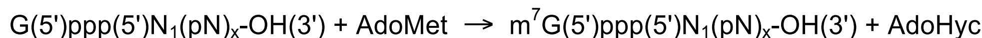


$$pppN_1(p)N_x\text{-}OH(3') \rightarrow ppN_1(pN)_x\text{-}OH(3') + P_i$$

(2) RNA guanyltransferase joins GTP to the 5' diphosphate of the most 5' nucleotide ($N_1$) of the RNA.

$$ppN_1(pN)_x\text{-}OH(3') + GTP \rightarrow G(5')ppp(5')N_1(pN)_x\text{-}OH(3') + PP_i$$

(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.



$$G(5')ppp(5')N_1(pN)_x\text{-}OH(3') + AdoMet \rightarrow m^7G(5')ppp(5')N_1(pN)_x\text{-}OH(3') + AdoHyc$$

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 $m^7G$ 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 $m^7G$ Capping System. In addition to having a 7-methyl-G cap nucleotide ($m^7G$), Cap 1 RNA also has a 2'-O-methyl group on the 5'-penultimate ($N_1$) nucleotide, which can further increase *in vivo* translation efficiency of the mRNA.[1]

A standard ScriptCap $m^7G$ 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 $m^7G$ Capping System reaction can be added directly to a CELLSCRIPT A-Plus™ Poly(A) Polymerase reaction for poly(A)-tailing of the 3' ends of the capped RNA.

The ScriptCap $m^7G$ Capping System offers an alternative to making capped RNA by co-transcriptional capping during an *in vitro* transcription (IVT) reaction in which a dinucleotide cap analog (e.g., $m^7G(5')ppp(5')G$) 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 $m^7G$ 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



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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

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

| **ScriptCap™ $m^7G$ Capping System Contents** (25 reactions) | |
|---|---|
| Kit Component | Reagent Volume |
| ScriptCap™ Capping Enzyme, 10 U/µl (red cap)<br>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<br>0.5 M Tris-HCl, pH 8.0, 60 mM KCl and 12.5 mM $MgCl_2$ | 250 µl |
| 10 mM GTP Solution | 250 µl |
| 20 mM *S*-adenosyl-methionine (SAM) | 30 µl |
| ScriptGuard™ RNase Inhibitor, 40 U/µl<br>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 |

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

### Materials Required, but not Supplied

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

## SPECIFICATIONS

### Unit Definitions

One ScriptCap $m^7G$ 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 $m^7G$ 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 $m^7G$ Capping System are free of detectable RNase and DNase activities.

## BEFORE YOU START: IMPORTANT TIPS FOR OPTIMAL CAPPING

♦ **SAM**:

For each reaction series, make a fresh dilution in RNase-Free Water from the 20 mM SAM stock to 2 mM (a 20X working stock solution). Dilute enough for the number of intended reactions plus one. Diluted SAM should stored at –20°C and can be used for up to one month post dilution.

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 $m^7G$ 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 0 structure, simply omit the ScriptCap 2'-O-Methyltransferase from 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 (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 RNA 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 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 |
| *In vitro* 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.

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 |

Do not include the ScriptCap Capping Enzyme in this mix.

Keep the thawed stock and diluted SAM solutions 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 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. |
| | Some RNAs form stable structures (e.g., homo-dimers, 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). |
| **White precipitate in reaction buffer** | 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
– INCOGNITO™ SP6 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
– INCOGNITO™ T7 Ψ-RNA Transcription Kit
– ScriptCap™ 2'-O-Methyltransferase Kit
– ScriptGuard™ RNase Inhibitor
– SP6-Scribe™ Standard RNA IVT Kit
– T7-FlashScribe™ Transcription Kit
– T7-Scribe™ Standard RNA IVT Kit

## 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.

## 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:

| **Standard ScriptCap $m^7G$ Capping Reaction** (step 1) | |
|---|---|
| Component | Amount |
| RNase-Free Water | x µl |
| *In vitro* 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.

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 $m^7G$ 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 |

Do not include the ScriptCap Capping Enzyme in this mix.

Keep the thawed stock and diluted SAM solutions 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 $m^7G$ 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 reaction (sold separately).

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

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