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CELLSCRIPT™
RNA for Translation in Cells

ScriptCap™ Cap 1 Capping System

Cat. Nos. **C-SCCS1710 and C-SCCS2250**
Art.-Nr. Biozym: 150365 and 150366

INTRODUCTION

The ScriptCap™ Cap 1 Capping System provides both ScriptCap Capping Enzyme and ScriptCap 2'-O-Methyltransferase allowing the user to produce either a Cap 0 or Cap 1 cap structure on the 5' end of *in vitro* transcribed (IVT) RNA. ScriptCap Capping Enzyme catalyzes *in vitro* addition of a cap nucleotide to the 5' terminus of primary RNA that has a 5'-triphosphate group. 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 the 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 N7-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 $N_1(pN)_x$-OH(3') represents the primary RNA transcript, of which N_1 is the most 5' nucleotide.

The ScriptCap Capping Enzyme 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 (SAM or 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 built by ScriptCap Capping Enzyme has a “cap 0” structure. Cap 0 RNA can be converted to a "cap 1" structure *in vitro* by the simultaneous use of ScriptCap 2'-O-Methyltransferase in the capping reaction together with the ScriptCap Capping Enzyme.

ScriptCap 2'-O-Methyltransferase prepares Cap 1-RNA from Cap 0-RNA by transferring a methyl group from the donor molecule SAM to the 2'-O position of the penultimate nucleotide of a cap 0 RNA to synthesize RNA with a cap 1 structure.

$$m^7GpppN_1(pN)_x\text{-}OH(3') + AdoMet \rightarrow m^7Gppp[m^{2'\text{-}O}]N_1(pN)_x\text{-}OH(3') + AdoHyc$$

Cap 0 RNA Cap 1 RNA

Cap 1 RNA can further increase *in vivo* translation efficiency of the mRNA as well as to help mark the mRNA as "self RNA" relative to intracellular immuno-surveillance mechanisms.[1,2]

A standard ScriptCap Cap 1 Capping System reaction will cap approximately 60 µg of RNA, but reactions can be scaled up or down to accommodate the user’s needs.

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Capped RNA from an ScriptCap Cap 1 Capping System reaction can be added directly to an A-Plus™ Poly(A) Polymerase reaction (CELLSCRIPT), without prior cleanup, for poly(A)-tailing of the 3' ends of the capped RNA allowing for convenient synthesis of mRNA.

The ScriptCap Cap 1 Capping System offers an alternative to making capped RNA by co-transcriptional capping during an IVT reaction in which a dinucleotide cap analog (e.g., m^7GpppG) is included in place of a portion of the GTP.[3] Provided that the 5' terminus of the RNA is not structured, the capping efficiency using the ScriptCap Cap 1 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%.[4,5] 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.

The ScriptCap Cap 1 Capping System improves upon co-transcriptional capping methods by ensuring virtually **100% transcript capping**, all caps in the **proper orientation** and the ability to produce **large amounts** of capped RNA at a **reasonable cost**.

REFERENCES

1. Kuge, H. et al., (1998) Nucl. Acids Res. 26, 3208.
2. Schlee, M. and Hartmann, G., (2016) Nat. Rev. Immunol. 16, 566.
3. Konarska, M.M. et al., (1984) Cell 38, 731.
4. Jemielity, J. et al., (2003) RNA 9, 1108.
5. Grudzien, E. et al., (2004) RNA 10, 1479.

RELATED PRODUCTS

- A-Plus™ Poly(A) Polymerase Tailing Kit
- INCOGNITO™ T7 Ψ-RNA Transcription Kit
- INCOGNITO™ SP6 Ψ-RNA Transcription Kit
- INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
- INCOGNITO™ T7 ARCA 5mC- & Ψ-RNA Transcription Kit
- MessageMAX™ T7 ARCA-Capped Message Transcription Kit
- ScriptCap™ 2'-O-Methyltransferase Kit
- ScriptCap™ m7G Capping System
- ScriptGuard™ RNase Inhibitor
- SP6-Scribe™ Standard RNA IVT Kit
- T7-FlashScribe™ Transcription Kit
- T7 mScript™ Standard mRNA Production System
- T7-Scribe™ Standard RNA IVT Kit

MATERIALS

Materials Supplied

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

ScriptCap™ Cap 1 Capping System Contents		
Kit Component	Reagent Volume	
	C-SCCS1710 10 Reactions	C-SCCS2250 50 Reactions
ScriptCap™ Capping Enzyme, 10 U/µl	40 µl	200 µl
ScriptCap™ 2'-O-Methyltransferase, 100 U/µl	40 µl	200 µl
10X ScriptCap™ Capping Buffer 0.5 M Tris-HCl, pH 8.0, 60 mM KCl and 12.5 mM $MgCl_2$	100 µl	500 µl
10 mM GTP Solution	100 µl	500 µl
20 mM *S*-adenosyl-methionine (SAM)	50 µl	250 µl
ScriptGuard™ RNase Inhibitor, 40 U/µl	25 µl	125 µl
RNase-Free Water	0.67 ml	3.35 ml

Materials Required, but not Supplied

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

SPECIFICATIONS

Storage Buffers

ScriptCap Capping Enzyme and ScriptCap 2'-O-Methyltransferase are provided in a 50% glycerol solution containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM Dithiothreitol (DTT), 0.1 mM EDTA and 0.1% Triton X-100®.

ScriptGuard RNase Inhibitor is provided in a 50% glycerol solution containing 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM DTT, 0.1 mM EDTA and 0.1% Triton X-100®.

Unit Definitions

One ScriptCap Cap 1 Capping System reaction produces 60 µg of 5'-Cap 0 or Cap 1 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.

One unit of ScriptCap 2'-O-Methyltransferase methylates one picomole of a control Cap 0 RNA in 1 hour at 37°C under standard assay conditions.

One unit of ScriptGuard RNase Inhibitor results in 50% inhibition of 5 ng of RNase A.

Contaminating Activity Assays

All components of the ScriptCap Cap 1 Capping System are free of detectable RNase and DNase activities.

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

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 Cap 1 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. In extreme cases, or when a heat denaturation step is not a viable option, reengineering of the 5' end sequence may be necessary to alleviate the secondary structure. This is often accomplished with a single point mutation within the first 5 bases of the transcript (non-coding region). Contact CELLSCRIPT Technical Services for suggestions and recommendations.

♦ **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 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 RNA transfection experiments.

♦ **Maintaining an RNase-Free Environment**:

Highly stable RNases are ubiquitous, including on human skin.

Creating an RNase-free work environment and maintaining RNase-free solutions is critical for successful work with RNA.

We strongly recommend to:

– Use RNase-free tubes and pipette tips.

– Always wear gloves when handling kit components or samples containing RNA and change gloves frequently, especially after touching potential sources of RNase contamination such as door knobs, pens, pencils and human skin. Do not touch any kit component or tube containing RNA with an ungloved hand.

– Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

PROCEDURE

A. Synthesis of Cap 1 RNA from Uncapped RNA

1. The protocol below was designed to build a cap 1 structure on 5' end of 50-60 µg of uncapped RNA. **If a cap 0 structure is desired**, replace the ScriptCap 2'-O-Methyltransferase in Step 3 with an equivalent volume of RNase-Free Water.

   Combine the following reagents:

Standard ScriptCap Cap 1 Capping Reaction (step 1)	
Component	Amount
RNase-Free Water	x µl
In vitro transcribed RNA, 50-60 µg	≤64.5 µl
Total Volume	64.5 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 Cap 1 Capping Reaction (step 3)	
Component	Amount
10X ScriptCap Capping Buffer	10 µl
10 mM GTP	10 µl
20 mM SAM	5 µl
ScriptGuard RNase Inhibitor	2.5 µl
ScriptCap 2'-O-Methyltransferase (100 U/µl)	4 µl
Total Volume	31.5 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.

If a cap 0 structure is desired, replace the ScriptCap 2'-O-Methyl-transferase with RNase-Free Water.

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 Cap 1 Capping Reaction (step 4)	
Component	Amount
Cocktailed reaction components (from step 3)	31.5 µl
ScriptCap Capping Enzyme (10 U/µl)	4 µl
Heat-denatured RNA (from step 1)	64.5 µl
Total Reaction Volume	100 µl

The efficiency of 2'-O-methylation can be lower if the RNA 5' end is structured. If your RNA is not completely 2'-O-methylated, we recommend increasing the incubation time to 2 hours. Also, since the concentration of methylation sites for a given mass is higher for small RNA than for large RNA, increase the reaction time for small RNA. For example, we suggest to increase the reaction time to 2 hours if your RNA is <730 nucleotides in length.

5. Incubate at 37°C for 30 minutes.
6. The Capped 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 or 2'-O-methylation 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 EDTAcontaining 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. 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., homodimers, hairpins) at the 5' end, limiting access by Capping Enzyme or 2'-O-Methyltransferase. 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.

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

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