CELLSCRIPT™
RNA for Translation in Cells

ScriptCap™ 2'-O-Methyltransferase Kit

Cat. No. **C-SCMT0625**

INTRODUCTION

The ScriptCap™ 2'-O-Methyltransferase Kit prepares cap 1-RNA from any source of cap 0-RNA. In part, cap 1 methylation serves, to increase the translation efficiency of the mRNA.[1] ScriptCap™ 2'-O-Methyltransferase transfers a methyl group from the donor molecule *S*-adenosyl-methionine (SAM) to the 2'-O position of the penultimate nucleotide of a cap 0 RNA (m^7G[5']ppp[5']NpN...) to synthesize RNA with a cap 1 structure (m^7Gppp[$m^{2'-O}$]NpNpN...). The cap 0 RNA can be produced by enzymatically capping uncapped RNA using the ScriptCap™ m^7G Capping System or by *in vitro* transcription of a DNA template in the presence of a dinucleotide cap analog (e.g., $m_2^{7,3'-O}$GpppG; e.g., using a MessageMAX™ T7 ARCA-Capped Message Transcription Kit). Cap 1 RNA can also be synthesized from uncapped RNA in a single reaction mixture that contains both the ScriptCap m^7G Capping System and ScriptCap 2'-O-Methyltransferase plus SAM.

One ScriptCap 2'-O-Methyltransferase Kit reaction methylates 60 µg of 5'-Cap 0 capped RNA.

MATERIALS

Materials Supplied

Do not store in a frost-free freezer. Do not store at –70°C.

ScriptCap™ 2'-O-Methyltransferase Kit Contents (25 reactions)	
Kit Component	Reagent Volume
ScriptCap™ 2'-O-Methyltransferase, (blue cap) 100 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
20 mM *S*-adenosyl-methionine (SAM)	65 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

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

Materials Required, but not Supplied

- Cap 0 RNA substrate
- Materials or kits for purification of the RNA product

726 Post Road, Madison, WI 53713 • 888-826-5586 (U.S. only) • (608) 442-6484 • Fax (608) 442-6187

SPECIFICATIONS

Unit Definition

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.

Contaminating Activity Assays

All components of the ScriptCap 2'-O-Methyltransferase Kit are free of detectable RNase and DNase activities.

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.

- **Cap 0 RNA Source**:

  RNA should be purified and resuspended in RNase-Free Water. **Do not resuspend the RNA in an EDTA-containing solution**.

  a) Cap 0-RNA produced using the capping enzyme-based ScriptCap m^7G Capping System (sold separately): Directly add the ScriptCap 2'-O-Methyltransferase to the ScriptCap m^7G Capping System reaction either simultaneously or sequentially without prior reaction clean-up.

  b) Cap 0-RNA generated using a dinucleotide cap analog in an *in vitro* transcription reaction: Cleanup the RNA prior to treatment with ScriptCap 2'-O-Methyltransferase. Purify the RNA by your preferred method. The method chosen should remove residual proteins and unincorporated nucleotides from the RNA.

- **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'-penultimate nucleotide to the ScriptCap 2'-O-Methyltransferase. In order to increase the enzymatic efficiency on such RNAs, use longer or hotter heat denaturation conditions than those noted in the protocol. Times and temperatures required will vary.

- **Poly(A)-Tails**:

  If the cap 1-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 (see the A-Plus Poly(A) Polymerase product literature for details). Capped and tailed RNA must be purified prior to use in RNA transfection experiments.

PROCEDURE

A. Synthesis of Cap 1-RNA from Cap 0-RNA

1. The protocol below was designed for use with 50-60 μg of cap 0-RNA. Combine the following reagents:

Standard ScriptCap 2'-O-Methyltransferase Kit Reaction (step 1)	
Component	Amount
RNase-Free Water	x μl
Cap 0-RNA, 50-60 μg	≤81 μl
Total Volume	81 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 2'-O-Methyltransferase Kit Reaction (step 3)	
Component	Amount
10X ScriptCap Capping Buffer	10 μl
20 mM SAM	2.5 μl
ScriptGuard RNase Inhibitor	2.5 μl
ScriptCap 2'-O-Methyltransferase, 400 Units	4 μl
Total Volume	19 μl

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.

! Keep the thawed stock of SAM on ice.

4. Combine the Cap 0 RNA Solution from Step 1 with the Cocktail from Step 3.

Standard ScriptCap 2'-O-Methyltransferase Kit Reaction (step 4)	
Component	Amount
Heat-denatured cap 0-RNA (from step 1)	81 μl
Cocktailed reaction components (from step 3)	19 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 Cap 1 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). Purification of poly(A)-tailed Cap 1 RNA is recommended prior to use for RNA transfection.

B. Simultaneous Capping and 2'-O-Methylation to Synthesize Cap 1 RNA from Uncapped RNA

1. You need to purchase the ScriptCap m^{7}G Capping System in addition to ScriptCap 2'-O-Methyltransferase in order to synthesize cap 1 RNA from uncapped RNA using the following protocol. This protocol was designed for use with 50-60 μg of uncapped RNA.

   Combine the following reagents:

Simultaneous Capping and 2'-O-Methylation (step 1)	
Component	Amount
RNase-Free Water	x μl
In vitro transcribed uncapped RNA, 50-60 μg	≤67 μl
Total Volume	67 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.

Simultaneous Capping and 2'-O-Methylation (step 3)	
Component	Amount
10X ScriptCap Capping Buffer	10 μl
10 mM GTP *	10 μl
20 mM SAM	2.5 μl
ScriptGuard RNase Inhibitor	2.5 μl
ScriptCap 2'-O-Methyltransferase (100 U/μl)	4 μl
Total Volume	29 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.

Simultaneous Capping and 2'-O-Methylation (step 4)	
Component	Amount
Cocktailed reaction components (from step 3)	29 μl
ScriptCap Capping Enzyme (10 U/μl)*	4 μl
Heat-denatured RNA (from step 1)	67 μl
Total Reaction Volume	100 μl

* The 10 mM GTP in Step 3 and ScriptCap Capping Enzyme in Step 4 are components of the ScriptCap m^{7}G Capping System.

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 Cap 1 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). Purification of poly(A)-tailed Cap 1 RNA is recommended prior to use for RNA transfection.

TROUBLESHOOTING

Symptom	Solution
Low capping or 2'-O-methylation efficiency	RNA to be treated with ScriptCap 2'-O-Methyltransferase should be purified and resuspended in RNase-free water. Do not resuspend the RNA in an EDTA-containing solution.
	Prior to 2'-O-methylation, purify the input RNA using a method that removes residual proteins, contaminants and unincorporated nucleotides and/or cap analogs.
	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.
	Increase the 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.

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
– INCOGNITO™ T7 ARCA 5mC- & Ψ-RNA Transcription Kit
– MessageMAX™ T7 ARCA-Capped Message Transcription Kit
– ScriptCap™ m^7G Capping System
– ScriptGuard™ RNase Inhibitor
– SP6-Scribe™ Standard RNA IVT Kit
– T7 mScript™ Standard mRNA Production System
– T7-FlashScribe™ Transcription Kit
– T7-Scribe™ Standard RNA IVT Kit

REFERENCE

1. Kuge, H. et al., (1998) Nucl. Acids Res. 26, 3208.

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

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