CELLSCRIPT™
RNA for Translation in Cells

Anti-Reverse Cap Analog (ARCA)

Cat. No. **C-C50110**

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] Because Anti-Reverse Cap Analog (ARCA), $m_2^{7,3'-O}$G[5']ppp[5']G, contains a 3'-O-methyl group on the m^7G nucleotide (Figure 1), ARCA can only be incorporated in the correct orientation at the 5'-end of the RNA during an *in vitro* transcription/capping reaction.[4,5,6] This is not true for the standard 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 standard cap analog.

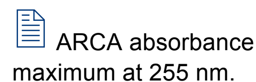
CELLSCRIPT's ARCA can be used in a cotranscriptional capping reaction, in conjunction with any *in vitro* transcription kit not containing premixed NTPs. A protocol for the synthesis of ARCA-capped RNA using components of a T7-Scribe™ IVT Kit (CELLSCRIPT) is provided. This reaction will yield up to 45 µg of total RNA with ~80% capping efficiency in a 2 hour reaction. CELLSCRIPT also offers the Message-MAX™ T7 ARCA-Capped Message Transcription Kit, which yields up to 60 µg of total RNA with ~80% capping efficiency in a 30 minute reaction.

MATERIALS

Materials Supplied

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

ARCA Cap Analog Contents (500 nmol)	
Component	Volume
$m_2^{7,3'-O}$G[5']ppp[5']G, ARCA 20 mM Solution in sterile deionized water (pH 7.0).	25 µl


ARCA absorbance maximum at 255 nm.

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

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

Functional Testing

ARCA 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

ARCA is free of detectable RNase and DNase activities.

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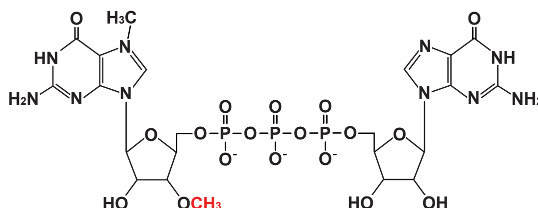
Figure 1. ARCA Structure.



BEFORE YOU START: IMPORTANT TIPS FOR ARCA CAPPING

♦ **Percentage of Capped RNA**:

The protocol provided below, will produce an RNA mixture where ~80% of the RNA will contain an ARCA-derived cap. This is because ARCA 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 ARCA 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 ARCA-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 ARCA 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 ARCA	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.

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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) **<u>Ammonium Acetate Precipitation</u>**: 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.

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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.

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.[7] 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

– 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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