INSTRUCTION MANUAL

HiScribe™ T7 ARCA mRNA Kit (with tailing)

NEB #E2060S

20 reactions

Version 5.0_1/26

Table of Contents

Introduction 2

DNA Template Preparation 3

- Plasmid Templates 3
- PCR Templates 3

mRNA Synthesis Protocols 4

- Capped and Tailed mRNA Synthesis 4
- Capped and Tailed mRNA Synthesis with Modified Nucleotides 5

Purification of Synthesized RNA 6

- LiCl Precipitation 6
- Phenol:Chloroform Extraction and Ethanol Precipitation 6
- Spin Column Chromatography 7
- Gel Purification 7

Evaluation of Reaction Products 8

- Quantification by UV Light Absorbance 8
- Analysis of Transcription Products by Gel Electrophoresis 8
- mRNA Quality Analysis by Bioanalyzer or Capillary Electrophoresis 8

Troubleshooting 10

- Control Reaction 10
- Low Yield of Full-length RNA 10
- Addition of DTT 10
- Low Yield of Short Transcript 10
- RNA Transcript Smearing on Denaturing Gel 10
- RNA Transcript of Larger Size than Expected 10
- RNA Transcript of Smaller Size than Expected 10
- Tailing Length Control 10
- No Tailing or Partial Tailing 11
- mRNA not Functional 11

Ordering Information 12

Revision History 13

The HiScribe T7 ARCA mRNA Kit (with tailing) Includes:

All kit components should be stored at –20°C. Each kit contains sufficient reagents for 20 x 20 µl reactions. Each standard reaction yields up to 20 µg of capped mRNA from 1 µg control template.

ARCA/NTP Buffer Mix (2X)

T7 RNA Polymerase Mix

CLuc Control Template (0.25 µg/µl)

DNase I (RNase-free) (2 units/µl)

E. coli Poly(A) Polymerase

Poly(A) Polymerase Reaction Buffer (10X)

LiCl Solution (7.5 M LiCl, 10 mM EDTA)

Dithiothreitol (DTT) (0.1 M)

Required Materials Not Included:

DNA Template:	The DNA template must be linear and contain the T7 RNA Polymerase promoter with correct orientation in relation to target sequence to be transcribed.
Modified-NTP:	N1-Methyl-Pseudouridine-5′-Triphosphate (NEB #N0431) 5-Methyl-Cytidine-5′-Triphosphate (NEB #N0432) Pseudouridine-5′-Triphosphate (NEB #N0433) 5-Methoxy-Uridine-5′-Triphosphate (NEB# N0434) Biotin-, Fluorescein-, Digoxigenin-, or Aminoallyl-NTP
General:	37°C incubator or thermocycler, nuclease-free water
Purification:	Buffer- or water-saturated phenol/chloroform, ethanol and 3M sodium acetate, pH 5.2, or Monarch® RNA Spin Cleanup Kit (50 µg – NEB #T2040)
Gel Analysis:	Gels and running buffers, gel apparatus, power supply

Introduction

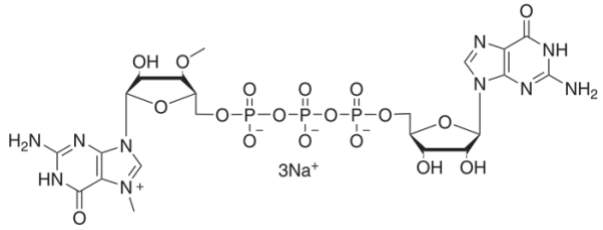
Most eukaryotic mRNAs require a 7-methyl guanosine (m7G) cap structure at the 5′ end and a poly(A) tail at the 3′ end to be efficiently translated. The HiScribe T7 ARCA mRNA Kit (with tailing) can be used to synthesize capped (cap-0) mRNAs with poly(A) tails of heterogenous length. The provided cap structure, Anti-Reverse Cap Analog (ARCA) (NEB #S1411), is incorporated co-transcriptionally by T7 RNA Polymerase. The kit also includes DNase I for DNA template removal, *E. coli* Poly(A) Polymerase for direct A-tailing without cleanup, and LiCl for quick mRNA purification.

Figure 1. Overview of mRNA synthesis workflow with the HiScribe T7 ARCA mRNA Kit

The kit is capable of partial incorporation of base-modified UTP and CTP (up to 50% each) without significantly affecting the mRNA yield. By using a DNA template encoding a poly(A) tail, capped and tailed modified mRNA can be synthesized in a single reaction in 30 minutes. mRNAs synthesized with the kit can be used for cell transfection, microinjection, *in vitro* translation and RNA vaccines.

The ARCA dinucleotide cap analog is incorporated into mRNA exclusively in the correct orientation due to a methyl group at the 3′ position of the m7G cap, generating capped mRNA that is more efficiently translated. Standard cap analogs can be incorporated in either orientation, resulting in only 50% of cap-0 mRNA that is functional in protein translation.

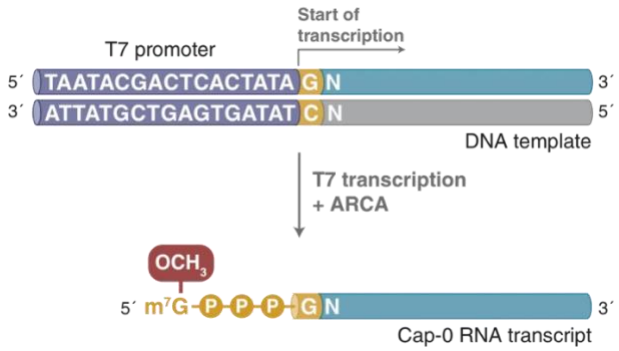
Figure 2. Structure of Anti-Reverse Cap Analog (ARCA, NEB #S1411)



DNA Template Preparation

Linearized plasmid DNA, PCR products or synthetic DNA oligonucleotides can be used as templates for *in vitro* transcription with the HiScribe T7 ARCA mRNA Kit, provided that they contain a double-stranded T7 promoter region upstream of the sequence to be transcribed. Figure 3 illustrates the minimal T7 promoter sequence and the start of transcription as well as a run-off, capped transcript after T7 transcription.

Figure 3. T7 RNA Polymerase co-transcriptionally caps RNA with anti-reverse cap analog (ARCA)



Plasmid Templates

Completely linearized plasmid template of highest purity is critical for successful use of the HiScribe T7 ARCA mRNA Kit (with tailing). Quality of the template DNA affects transcription yield and the integrity of mRNA synthesized. The highest transcription yield is achieved with the highest purity template. Plasmid purified by many laboratory methods can be successfully used, provided it contains mostly supercoiled form, and is free from contaminating RNase, protein, RNA, and salts.

To produce an mRNA transcript of defined length, plasmid DNA must be completely linearized with a restriction enzyme, downstream of the insert to be transcribed. In contrast, circular plasmid templates will generate long heterogeneous mRNA transcripts in higher quantities because of the high processivity of T7 RNA Polymerase. NEB has a large selection of restriction enzymes for this purpose; we recommend selecting restriction enzymes that generate blunt ends or 5′-overhangs.

After linearization, we recommend purifying the template DNA by phenol:chloroform extraction:

1. Extract DNA with an equal volume of 1:1 phenol:chloroform mixture, and repeat, if necessary.
2. Extract twice with an equal volume of chloroform to remove residual phenol.
3. Precipitate the DNA by adding 1/10th volume of 3 M sodium acetate, pH 5.2, and two volumes of ethanol. Incubate at –20°C for at least 30 minutes.
4. Pellet the DNA in a microcentrifuge for 15 minutes at top speed. Carefully remove the supernatant.
5. Rinse the pellet by adding 500 µl of 70% ethanol and centrifuging for 15 minutes at top speed. Carefully remove the supernatant.
6. Air dry the pellet and resuspend it in nuclease-free water at a concentration of 0.5–1 µg/µl.

PCR Templates

PCR products containing a T7 RNA Polymerase promoter in the correct orientation can be transcribed. We recommend using Q5® Hot Start High-Fidelity DNA Polymerase (NEB #M0493/M0494). Though PCR mixture can be used directly, better yields will be obtained with purified PCR products. PCR products can be purified according to the protocol for plasmid restriction digests above, or by using commercially available spin columns (we recommend the Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130). PCR products should be examined on an agarose gel to estimate concentration and to confirm amplicon size prior to its use as a template in the HiScribe T7 ARCA mRNA Kit. Depending on the PCR products, 0.1–0.5 µg of PCR fragments can be used in a 20 µl *in vitro* transcription reaction.

mRNA Synthesis Protocols

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Reactions are typically 20 µl but can be scaled up linearly as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR strip tubes.

Capped and Tailed mRNA Synthesis

The example protocol below will generate cap-0 mRNA with heterogenous poly(A) tails using canonical NTPs.

1. Thaw the necessary components at room temperature. Keep the T7 RNA Polymerase Mix on ice.
2. Mix and pulse-spin in a microfuge to collect the solutions to the bottom of the tubes.
3. Set up the reaction at room temperature in the order listed in the table below:

COMPONENTS	20 µl REACTION	FINAL AMOUNT
Nuclease-free Water	X µl	
2X ARCA/NTP Mix	10 µl	1 mM GTP, 4 mM ARCA, 1.25 mM CTP, 1.25 mM UTP, >1.25 mM ATP final
Template DNA	X µl	1 µg
DTT (0.1M)	1 µl	5 mM
T7 RNA Polymerase Mix	2 µl	

4. Mix thoroughly by pipetting and pulse-spin in a microfuge. Incubate at 37°C for 30-60 minutes in a dry air incubator or thermocycler to prevent evaporation. For reaction times of 60 minutes or less, a water bath or heating block may be used. The yield will not be compromised if the incubation temperature is within the range of 35–40°C.

   Reactions for short mRNA transcripts (< 0.3 kb) should be incubated for 60 minutes or longer. It is safe to incubate the reaction for 16 hours (overnight).

5. To remove template DNA, add 2 µl of DNase I (RNase-free) (NEB #M0303), mix, and incubate for 15 minutes at 37°C. Save 1 µl for gel analysis if desired. Proceed directly to the next step; do not heat the reaction or purify the RNA.

6. Set up the tailing reaction in the order listed in the table below. No extra ATP is necessary for the tailing reaction because the unpurified IVT product contains enough ATP.

COMPONENTS	50 µl REACTION*
Nuclease-free Water	20 µl
IVT Reaction (*from step 5)*	20 µl
10X Poly(A) Polymerase Reaction Buffer	5 µl
E. coli Poly(A) Polymerase	5 µl

* Standard tailing reaction volume is 50 µl, but average tail length is slightly longer with the same 5 µl volume of Poly(A) Polymerase in a 100 µl reaction volume for some transcripts.

7. Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 30 minutes. Save 1 µl for analysis if necessary.

   For very short RNA (< 0.3 kb), tailing time can be extended to 1 hour to achieve sufficient length.

   The 3´ end of RNA must be exposed for efficient tailing by E. coli Poly(A) Polymerase. If the 3´ end is buried inside the RNA structure, it will not be available for tailing. A 30 min tailing reaction will result in 150 nt or longer poly(A) tail for the majority of mRNAs. Due to the nature of the tailing reaction, tail length will vary depending on RNA sequence, structure, yield, length, etc.

7.Proceed with purification of synthesized RNA and/or evaluation of transcription products yield and/or length.

Capped and Tailed mRNA Synthesis with Modified Nucleotides

This protocol can be used to synthesize cap-0 mRNA containing partial substitutions of canonical nucleotides with base-modified nucleotides (not provided) from a DNA template containing the T7 RNA Polymerase promoter sequence immediately followed by guanosine. Up to 2.5 mM total base-modified UTP and/or CTP can be added into the transcription reaction without significantly impacting the mRNA yield. Modified GTP and ATP should not be used because they will interfere with capping and tailing efficiency. For complete modified nucleotide substitution to generate cap-1 mRNA, we recommended using the HiScribe T7 mRNA Kit with CleanCap® Reagent AG (NEB #E2080), in which the cap analog and all four nucleotides are supplied separately.

The example protocol below uses 1.25 mM N1-Methyl-Pseudo-UTP and 1.25 mM 5-Methyl-CTP to generate mRNA containing 50% 5mCTP and 50% N1-Methyl-Pseudo-UTP.

Protocol

1. Thaw the necessary components at room temperature. Keep the T7 RNA Polymerase Mix on ice.
2. Mix and pulse-spin in a microfuge to collect the solutions to the bottom of the tubes.
3. Set up the reaction at room temperature in the order listed in the table below:

COMPONENTS	20 µl REACTION	FINAL AMOUNT
Nuclease-free Water	X µl	
2X ARCA/NTP Mix	10 µl	1 mM GTP, 4 mM ARCA, 1.25 mM CTP, 1.25 mM UTP, >1.25 mM ATP final
10 mM N1-Methyl-Pseudo-UTP	2.5ul	1.25 mM
10 mM 5-Methyl-CTP	2.5ul	1.25 mM
Template DNA	X µl	1 µg
DTT (0.1M)	1 µl	5 mM
T7 RNA Polymerase Mix	2 µl	

4. Mix thoroughly by pipetting and pulse-spin in a microfuge. Incubate at 37°C for 30-60 minutes in a dry air incubator or thermocycler to prevent evaporation. For reaction times of 60 minutes or less, a water bath or heating block may be used. The yield will not be compromised if the incubation temperature is within the range of 35–40°C.

   Reactions for short mRNA transcripts (< 0.3 kb) should be incubated for 60 minutes or longer. It is safe to incubate the reaction for 16 hours (overnight).
5. To remove template DNA, add 2 µl of DNase I (RNase-free) (NEB #M0303), mix, and incubate for 15 minutes at 37°C. Save 1 µl for gel analysis if desired. Proceed directly to the next step; do not heat the reaction or purify the RNA.
6. Set up the tailing reaction in the order listed in the table below. No extra ATP is necessary for the tailing reaction because the unpurified IVT product contains enough ATP.

COMPONENTS	50 µl REACTION*
Nuclease-free Water	X µl
IVT Reaction (*from step 5)*	20 µl
10X Poly(A) Polymerase Reaction Buffer	5 µl
E. coli Poly(A) Polymerase	5 µl

* Standard tailing reaction volume is 50 µl, but average tail length is slightly longer in a 100 µl reaction volume for some transcripts.

7. Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 30 minutes. Save 1 µl for analysis if necessary.

   For very short RNA (< 0.3 kb), tailing time can be extended to 1 hour to achieve sufficient length.

   The 3′ end of RNA must be exposed for efficient tailing by E. coli Poly(A) Polymerase. If the 3′ end is buried inside the RNA structure, it will not be available for tailing. A 30 min tailing reaction will result in 150 nt or longer poly(A) tail for the majority of mRNAs. Due to the nature of the tailing reaction, tail length will vary depending on RNA sequence, structure, yield, length, etc.

7.Proceed with purification of synthesized RNA and/or evaluation of transcription products yield and/or length.

Purification of Synthesized RNA

In general, RNA synthesized by *in vitro* transcription can be purified by LiCl precipitation, phenol-chloroform extraction followed by ethanol precipitation, or by using a spin column-based method. If absolute full-length RNA is required, we recommend gel purification. For capped RNA, non-radioactively labeled RNA, or high specific activity radiolabeled RNA probes, spin column chromatography is the preferred method.

LiCl Precipitation

LiCl precipitation can be used for quick recovery of the synthesized RNA and is an effective method for removing the majority of unincorporated NTPs and enzymes. However, RNAs shorter than 300 bases or at concentrations lower than 0.1 mg/ml do not precipitate well. In such cases, other purification methods may be used.

Protocol

1. To the 50 µl reaction, add 25 µl LiCl solution and mix well.
2. Incubate at –20°C for 30 minutes.
3. Centrifuge at 4°C for 15 minutes at top speed to pellet the RNA.
4. Remove the supernatant carefully.
5. Rinse the pellet by adding 500 µl of cold 70% ethanol and centrifuge at 4°C for 10 minutes.
6. Remove the ethanol carefully. Spin the tube briefly to bring down any liquid on the wall.
7. Remove residual liquid carefully using a sharp tip (e.g., loading tip).
8. Air dry the pellet and resuspend the mRNA in 50–200 µl of 0.1 mM EDTA or a suitable RNA storage solution. Mix well.
9. Store the RNA at –20°C or below.

Phenol-chloroform Extraction and Ethanol Precipitation

For removal of proteins and most of the free nucleotides, phenol-chloroform extraction and ethanol precipitation of RNA transcripts is the preferred method.

Protocol

1. Adjust the reaction volume to 180 µl by adding nuclease-free water. Add 20 µl of 3 M sodium acetate (pH 5.2) or 20 µl of 5M ammonium acetate and mix thoroughly.
2. Extract with an equal volume of 1:1 phenol:chloroform mixture, followed by two extractions with chloroform. Collect the aqueous phase and transfer it to a new tube.
3. Precipitate the RNA by adding 2 volumes of ethanol. Incubate at –20°C for at least 30 minutes and collect the pellet by centrifugation.
4. Remove the supernatant carefully.
5. Rinse the pellet by adding 500 µl of cold 70% ethanol and centrifuge at 4°C for 10 minutes.
6. Remove the ethanol carefully. Spin the tube briefly to bring down any liquid on the wall.
7. Remove residual liquid carefully using a sharp tip (e.g., loading tip).
8. Air dry the pellet and resuspend the mRNA in 50–200 µl of 0.1 mM EDTA or a suitable RNA storage solution.
9. Store the mRNA at –20°C or below.

Spin Column Chromatography

Spin columns will remove unincorporated nucleotides, proteins, and salts. We recommend using a Monarch Spin RNA Cleanup Kit (50 µg capacity, NEB #T2040) with the Monarch Spin RNA Cleanup Kit Protocol.

Adjust the volume of the reaction mixture to 100 µl by adding nuclease-free water to the IVT product and mix well. Purify the RNA by following the manufacturer's instructions.

Gel Purification

Gel purification of mRNA tailed by *E. coli* Poly(A) Polymerase is not recommended because the tailed mRNA is heterogeneous in length and will result in a smear on the gel.

Evaluation of Reaction Products

Quantification by UV Light Absorbance

RNA concentration can be determined by measuring the ultraviolet light absorbance at 260 nm. However, any unincorporated nucleotides and template DNA in the mixture will affect the measurement, so must be removed before the RNA concentration can be quantified.

A Nanodrop spectrophotometer can directly read RNA concentrations from 10 ng/µl to 3000 ng/µl; it may be necessary to dilute your RNA prior to measurement. For single-stranded RNA, 1 A_{260} is equivalent to an RNA concentration of 40 µg/ml. The RNA concentration can be calculated as follows: A_{260} x dilution factor x 40 = __ µg/ml RNA

Analysis of Transcription Products by Gel Electrophoresis

To evaluate transcript length, integrity and quantity, an aliquot of the transcription reaction should be run on an appropriate denaturing agarose or polyacrylamide gel. Transcripts larger than 0.3 kb can be run on agarose gels, whereas denaturing polyacrylamide gels (5–15%) are necessary for smaller transcripts. The gels should be run under denaturing conditions to minimize formation of secondary structures by the transcript.

Sample preparation

1. Prepare denatured samples by mixing 100–200 ng RNA sample with 10-20 µl of RNA Loading Dye, 2X (NEB #B0363).
2. Denature the RNA sample and an aliquot of RNA marker by heating at 70°C for 10 minutes.
3. Pulse-spin prior to loading onto the gel.

Gel electrophoresis

4a. **Denaturing agarose gel:**

Load 100-200ng denatured RNA sample.

It is common practice to electrophorese RNA on a fully denaturing agarose gel, such as one containing formaldehyde. However, it is possible to run RNA on a native agarose gel and obtain suitable results. The use of native agarose gels eliminates problems associated with toxic chemicals and the difficulties encountered when staining and blotting formaldehyde gels.

4b. **Denaturing PAGE/Urea Gel:**

Load 20–100ng denatured RNA sample.

We recommend using commercially available premade gels and standard TBE gel running buffer (10X TBE buffer: 0.9 M Tris Base, 0.9 M Boric Acid, 20 mM EDTA).

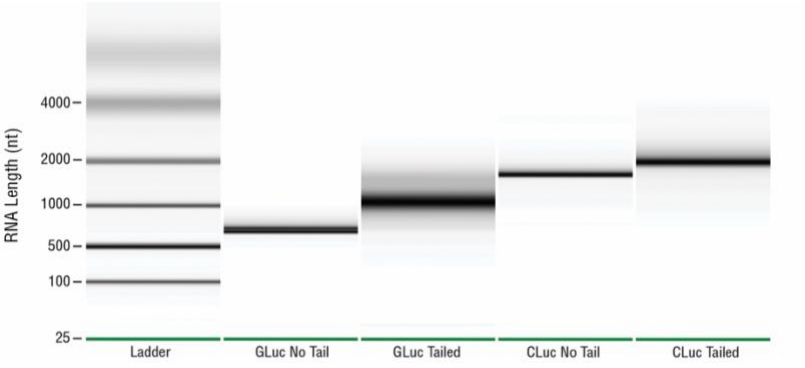
Visualize RNA

5. Stain the gel post-electrophoresis with SYBR® Gold (preferred) or ethidium bromide.

mRNA Quality Analysis by Bioanalyzer or Capillary Electrophoresis

Choose appropriate assay chips and follow the instructions carefully.

Figure 4. Analysis of mRNAs before and after tailing on Agilent 2100 Bioanalyzer



mRNA Functional Analysis by Cell Transfection

Cell transfection experiments show that CLuc mRNA synthesized with the kit is efficiently expressed in U2OS cells (Figure 5).

Figure 5. Analysis of CLuc mRNA before and after tailing on Agilent 2100 Bioanalyzer

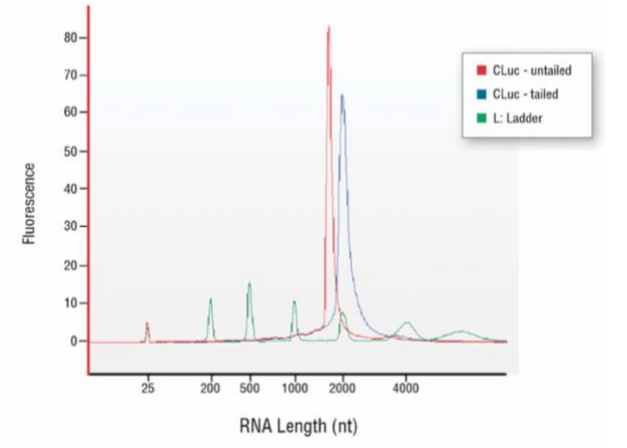
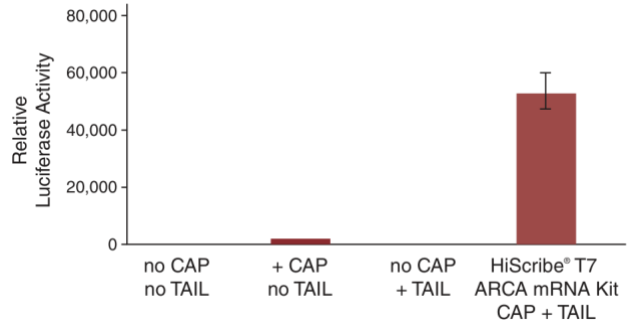


Figure 6. Both cap and tail are required for mRNA function in cell culture



Luciferase expression in U2OS cells. Purified Cypridina luciferase RNA produced as indicated was co-transfected into U2OS cells with purified Gaussia luciferase mRNA. mRNAs produced using the HiScribe T7 ARCA mRNA Kit (With Tailing) are 5´- capped and have 3´- poly(A) tails. After 16 hours incubation at 37°C, cell culture supernatants from each well were assayed for CLuc and GLuc activity and luminescence values were recorded. Relative luciferase activity was calculated using the equation:

Relative Luciferase Activity = [CLuc activity(x)/GLuc activity(x)] / [CLuc activity(no CAPno TAIL) /GLuc activity(no CAP no TAIL)]

Data are presented as mean +/- SEM of two or more independent experiments.

Troubleshooting

Control Reaction

The CLuc control template DNA is a linearized plasmid containing the *Cypridina* luciferase gene under the transcriptional control of the T7 promoter. The size of the runoff transcript is 1.76 kb. The control reaction should yield ≥ 15 µg RNA transcript in 30 minutes.

If the control reaction is not working, there may be technical problems during reaction set up. Repeat the reaction by following the protocol carefully and supplement with DTT to a final concentration of 5 mM; take any precaution to avoid RNase contamination. Contact NEB for technical assistance.

The control plasmid sequence can be found within the DNA Sequences and Maps Tool under the name "pCMV-CLuc 2". The CLuc control template is generated by linearizing the plasmid with XbaI.

Low Yield of Full-length RNA

If the transcription reaction with your template generates full-length RNA, but the yield is significantly lower than expected, it is possible that contaminants in the DNA template are inhibiting the RNA polymerase, or the DNA concentration may be incorrect. Alternatively, additional purification of DNA template may be required. Phenol-chloroform extraction is recommended (see template DNA preparation section).

Addition of DTT

The RNA polymerase in the kit is sensitive to oxidation and could result in lower RNA yield over time due to repeated handling etc. Adding DTT to the reaction may help restore the kit performance in such cases. Adding DTT will not compromise the reaction in any situation.

Low Yield of Short Transcript

High yields of short transcripts (< 0.3 kb) are achieved by extending incubation time and increasing the amount of template. Incubation of reactions up to 16 hours (overnight) or using up to 2 µg of template will help to achieve maximum yield.

RNA Transcript Smearing on Denaturing Gel

If the RNA appears degraded (e.g. smeared) on a denaturing agarose or polyacrylamide gel, the DNA template may be contaminated with RNase. DNA templates contaminated with RNase can affect the length and yield of RNA synthesized (a smear below the expected transcript length). If the plasmid DNA template is contaminated with RNase, perform phenol/chloroform extraction, then ethanol precipitate and dissolve the DNA in nuclease-free water (see template DNA preparation section).

RNA Transcript of Larger Size than Expected

If the RNA transcript appears larger than expected on a denaturing gel, template plasmid DNA may be incompletely digested. Even small amounts of undigested circular DNA can produce large amounts of long transcripts. Check template for complete digestion, and if undigested plasmid is confirmed, repeat restriction enzyme digestion.

Larger size bands may also be observed when the RNA transcript is not completely denatured due to the presence of strong secondary structures.

RNA Transcript of Smaller Size than Expected

If denaturing gel analysis shows the presence of smaller bands than the expected size, it is most likely due to premature termination by the polymerase. Some sequences that resemble T7 RNA Polymerase termination signals will cause premature termination. Incubating the transcription reaction at lower temperatures, for example at 30°C, may increase the proportion of full-length transcript, however the yield will be decreased. For GC rich templates, or templates with secondary structures, incubation at 42°C may improve yield of full-length transcript.

Tailing Length Control

A standard 30 min tailing reaction can add a poly(A) tail at least 150 nt in length to an average size mRNA generated from the IVT reaction. Short RNA may require longer incubation time for sufficient tailing.

No Tailing or Partial Tailing

The 3′ end of the mRNA must be exposed for efficient tailing. T7 RNA Polymerase tends to generate 3′ end heterogeneity by adding extra untemplated bases, so a small percentage of the mRNA may adopt alternate structures which may not be suitable for tailing. The following tips may help with successful tailing.

- Run the whole mRNA synthesis workflow without freezing the RNA between steps.
- To avoid preferential tailing, pre-incubate tailing mix at 37°C for 3 minutes before adding Poly(A) Polymerase. Mix well immediately.
- Tailing reaction should be at 37–40°C. Lower temperatures are not recommended.
- If still no tailing, redesign the DNA template with different sequences at the 3′ end.

mRNA Not Functional

- Verify the mRNA is intact, capped, and tailed.
- Be sure the mRNA is clean and free from any inhibitors.
- Follow instructions carefully with appropriate controls.
- Verify the DNA template has the correct sequence.

Ordering Information

NEB #	PRODUCT	SIZE
E2060S	HiScribe T7 ARCA mRNA Kit (with tailing)	20 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
T2040S/L	Monarch Spin RNA Cleanup Kit (50 µg)	10/100 preps
T2030S/L	Monarch Spin RNA Cleanup Kit (10 µg)	10/100 preps
B0363S	RNA Loading Dye (2X)	4 x 1 ml
M0303S/L	DNase I (RNase-Free)	1,000/5,000 units
M0493S/L	Q5 Hot Start High-Fidelity DNA Polymerase	100/500 units
M0494S/L	Q5 Hot Start High-Fidelity 2X Master Mix	100/500 units
N0362S	ssRNA Ladder	25 gel lanes
N0364S	Low Range ssRNA Ladder	25 gel lanes
S1411S/L	3′-O-Me-m7G(5′)ppp(5′)G RNA Cap Structure Analog	1/5 µmol
M0366S	mRNA Cap 2′-O-Methyltransferase	2,000 units
M0276S/L	*E. coli* Poly(A) Polymerase	100/500 units
N0431S	N1-Methyl-Pseudouridine-5′-Triphosphate	0.1 ml
N0432S	5-Methyl-Cytidine-5′-Triphosphate	0.1 ml
N0433S	Pseudouridine-5′-Triphosphate	0.1 ml
N0434S	5-Methoxy-Uridine-5′-Triphosphate	0.1 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	2/15
1.1		10/16
2.0		1/19
3.0	Applied new manual format.	4/20
4.0	Updated to include addition of DTT. Updated location of control plasmid on page 7. Also updated table formatting and legal footer.	7/23
5.0	Reorganized and updated protocols for consistency. Removed "optional" note about DTT.	1/26