invitrogen

MEGAscript™ T7 Transcription Kit Plus

USER GUIDE

Catalog Numbers A57622-25, A57622, A57623

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Revision C

For Research Use Only. Not for use in diagnostic procedures.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0030028 C (English)

Revision	Date	Description
C	18 September 2025	Added Cat. No. A57622-25.
B	20 August 2024	Updated the volumes for overage requirements for T7 Enzyme Mix and TheraPure™ 10X Reaction Buffer.
A.0	4 October 2023	Initial release.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The MEGAscript™ T7 Transcription Kit Plus contains all the reagents and buffers necessary for the *in vitro* synthesis of large amounts of RNA. Each 20 µL reaction will yield ≥100 µg RNA, and the scaled-up protocol described in this user guide can give ≥1 mg of RNA. This kit is suitable for full- or partial-modified nucleotide substitution to make modified RNA.

The MEGAscript™ kits are ultra-high yield *in vitro* transcription kits. The high yields are achieved by modifying typical transcription reaction conditions so that very high nucleotide concentrations can be effectively used. The kits contains *in vitro* transcription reaction components for 50 or 1000 20-µL reactions and a control template.

MEGAscript™ kits are intended for the synthesis of large amounts of unlabeled or low specific activity RNA for a variety of uses including *in vivo* or *in vitro* translation studies. The kits are not recommended for synthesis of high specific activity probes.

The MEGAscript™ T7 Transcription Kit Plus is supplied with a linearized control emGFP template (pMK-HA-emGFP) that contains a T7 RNA polymerase promoter with an AG initiation for co-transcriptional capping with the CleanCap™ Reagent AG. The emGFP coding sequence is flanked by the 5’- and 3’-untranslated regions (UTR) from the human alpha globin gene. The template does not contain a poly(A) tail; therefore, we recommend adding the tail if doing in vivo work with RNA made from this template. This user guide provides guidance on how to add the tail.

Contents and storage

Table 1 MEGAscript™ T7 Transcription Kit Plus (Cat. Nos. A57622-25, A57622, A57623)

Component	Amount			Storage [1]
	(25 reactions kit) Cat. No. A57622-25	(50 reactions kit) Cat. No. A57622	(1000 reactions kit) Cat. No. A57623	
Nuclease-free water[2]	1.95 mL	1.95 mL	40 mL	–20°C. Store in a non-frost-free freezer
T7 Enzyme Mix	50 µL	100 µL	2 × 1 mL	
TheraPure™ 10X Reaction Buffer[3]	500 µL	500 µL	5 × 500 µL	
TheraPure™ 100 mM ATP Solution	38 µL	75 µL	1.5 mL	
TheraPure™ 100 mM CTP Solution	38 µL	75 µL	1.5 mL	
TheraPure™100 mM GTP Solution	38 µL	75 µL	1.5 mL	
TheraPure™ 100 mM UTP Solution	38 µL	75 µL	1.5 mL	
TheraPure™ DNase 1 (2 U/ µL)	25 µL	50 µL	1 mL	
pMK-HA-GFP Control Template (0.5 µg/µL)	10 µL	10 µL	10 µL	
LiCl Precipitation Solution	750 µL	1.5 mL	30 mL	

[1] When using the kit, keep all reagents on ice except the 10X Reaction Buffer which should be at room temperature.

[2] Nuclease-free water can be stored at -20°C, 4°C, or room temperature.

[3] Minimize exposure to air as much as possible since this component contains dithiothreitol (DTT) which becomes oxidized when exposed to air. We recommend making aliquots of this component.

Required materials not supplied

- DNA template: The DNA template must have the T7 RNA polymerase promoter upstream of the sequence to be transcribed. The suggested template concentration is 0.1 – 0.5 µg/µL in water or TE (10 mM Tris-HCl (pH 7–8), 1 mM EDTA).
- If doing *in vivo* or *in vitro* translation studies with the RNA, a 7-methyl guanosine (m7G) cap at the 5’ end is required for translation. We recommend the CleanCap™ Reagent AG for co-transcriptional capping. See “Synthesize capped RNA transcripts with CleanCap™ Reagent AG” on page 9.
- (optional) For purification of the synthesized RNA, we recommend the MEGAclear™ Transcription Clean-Up Kit (AM1908).

2 Methods

Prepare template DNA

This kit is designed to function best with templates that code for RNA transcripts in the 0.9 to 10 kb range. The kit can be used to produce shorter RNA, but modify the reaction as described in Appendix A, “Troubleshooting”.

Linearized plasmid DNA or PCR products that contain a T7 RNA polymerase promoter can be used as templates for *in vitro* transcription with the MEGAscript™ T7 Transcription Kit Plus. For more information, see “T7 RNA polymerase promoter” on page 19.

See appendix for details on:

- “Plasmid templates” on page 18
- “PCR templates” on page 18
- “Template DNA features” on page 18

Transcription reaction assembly (standard 20 µL reaction)

1. Thaw the frozen reagents.
   a. Place the T7 Enzyme Mix on ice.
      It is stored in glycerol and will not be frozen at –20°C.
   b. Vortex the 10X Reaction Buffer, and the NTPs until they are completely in solution.
   c. Once thawed, store the NTPs on ice, but keep the 10X Reaction Buffer at room temperature while assembling the reaction. All reagents should be microfuged briefly before opening.

2. Assemble transcription reaction at room temperature

   The spermidine in the 10X Reaction Buffer can co-precipitate the template DNA if the reaction is assembled on ice. The following amounts are for a single 20 µL reaction. Add them in the order as listed. If several reactions will be performed, a master mix composed of the 10X Reaction Buffer and NTPs can be prepared. Reactions may be scaled up or down if desired. For a scaled up protocol to make 1 mg RNA, see "Large scale transcription reaction assembly for ≥1 mg RNA" on page 11.

Amount	Component	Final concentration
To 20 µL	Nuclease-free water	—
2 µL	10X Reaction Buffer	1X
1.5 µL	100 mM ATP Solution	7.5 mM
1.5 µL	100 mM CTP Solution	7.5 mM
1.5 µL	100 mM GTP Solution	7.5 mM
1.5 µL	100 mM UTP Solution	7.5 mM
0.5–1 µg	Linear template DNA[1]	25–50 ng/µL
2 µL	T7 Enzyme Mix	—

[1] Use ~0.5 µg PCR product template or ~1 µg linearized plasmid template.

3. Mix thoroughly.

   Gently flick the tube or pipette the mixture up and down gently, and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.

4. Incubate at 37°C, 2 hours.

   To achieve maximum yield, we recommend a 2-hour incubation, using a thermocycler set to 37°C with the lid at 70°C.

5. (optional) Add 1 µL DNase I, mix well and incubate 15 min at 37°C.

   The DNase I treatment removes the template DNA.

Recovery of RNA procedural guidelines

- We recommend the MEGAclear™ Transcription Clean-Up Kit for RNA purification.
  - This kit was developed specifically for purifying RNA from high yield *in vitro* transcription reactions. The quick and simple procedure removes nucleotides, short oligonucleotides less than 100 nucleotides in length, proteins, and salts from the RNA. The recovered RNA can be used for any application that requires high purity such as *in vivo* translation.
- Lithium chloride (LiCl) precipitation may be used to purify the RNA.
  - It can remove unincorporated nucleotides and most proteins. LiCl precipitation may not efficiently precipitate RNAs smaller than 300 nucleotides. The concentration of RNA should be at least 0.1 µg/µL for efficient precipitation.

See the next section for the protocol for RNA purification by LiCl precipitation.

Purify RNA by LiCl precipitation

This protocol is for purification of RNA from the standard 20-µL reaction. It can be scaled up for larger scale reactions (eg: 10-fold for a 200 µL reaction).

1. Bring the total reaction volume to 50 µL with nuclease-free water.
2. Precipitate the RNA by adding 30 µL of the provided LiCl Precipitation Solution.
3. Mix thoroughly. Chill for ≥30 minutes at –20°C.
4. Centrifuge at 4°C for 15 minutes at maximum speed to pellet the RNA.
5. Carefully remove the supernatant. Wash the pellet once with ~1 mL 70% ethanol, and re-centrifuge to maximize removal of unincorporated nucleotides.
6. Carefully remove the 70% ethanol, and resuspend the RNA in nuclease-free water, Invitrogen™ THE RNA Storage Solution, or TE buffer.

Synthesize capped RNA transcripts with CleanCap™ Reagent AG

The MEGAscript™ T7 Transcription Kit Plus is compatible for co-transcriptional capping with CleanCap™ Reagent AG to create capped RNA transcripts for *in vivo* or *in vitro* translation. We recommend using a T7 RNA polymerase promoter with an AG initiation sequence to achieve high yields and capping efficiencies with CleanCap™ Reagent AG. See “Template DNA features” on page 18.

1. For co-transcriptional capping with CleanCap™ Reagent AG and the MEGAscript™ T7 Transcription Kit Plus, combine the following:

Amount	Component	Final concentration
To 20 µL	Nuclease-free water	—
2 µL	10X Reaction Buffer	1X
1.5 µL	100 mM ATP Solution	7.5 mM
1.5 µL	100 mM CTP Solution	7.5 mM
1.5 µL	100 mM GTP Solution	7.5 mM
1.5 µL	100 mM UTP Solution	7.5 mM
1.2 µL	100 mM CleanCap™ Reagent AG[1]	6 mM
0.5–1 µg	Linear template DNA[2]	25–50 ng/µL
2 µL	T7 Enzyme Mix	—

[1] The cap:NTP ratio is 0.8:1. It is recommended to keep it at this ratio to achieve high capping efficiencies.

[2] Use ~0.5 µg PCR product template or ~1 µg linearized plasmid template.

2. Mix thoroughly.
    a. Gently flick the tube or pipette the mixture up and down gently, and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.

3. Incubate at 37°C, 2 hours.
    a. To achieve maximum yield, we recommend a 2-hour incubation, using a thermocycler set to 37°C with the lid at 70°C.

4. (optional) Add 1 µL DNase I, mix well and incubate 15 minutes at 37°C. The DNase I treatment removes the template DNA.

5. Perform RNA purification. The RNA can be purified using the MEGAclear™ kit or LiCl precipitation as described previously.

Modified nucleotides

Modified nucleotides can be added to the *in vitro* transcription reaction to produce modified RNAs. Compared to unmodified RNAs, modified RNAs can have higher translatability and lower innate immune activation *in vivo*. The MEGAscript™ T7 Transcription Kit Plus has been designed to allow full- or partial-modified nucleotide substitution. The use of modified nucleotides may impact RNA yields.

When doing a substitution, be sure that the final concentration of the NTP is 7.5 mM. For example, a 50% substitution of a modified UTP (modUTP) for UTP will have 3.75 mM modUTP and 3.75 mM UTP for a final concentration of 7.5 mM. Likewise, a full substitution of modUTP for UTP will have a final concentration of 7.5 mM modUTP.

Quantify reaction products

Methods to quantify reaction products include:

- “Yield quantitation by UV light absorbance” on page 17
- “Yield quantitation with Qubit™ or RiboGreen assays” on page 17
- “RNA assessment by agarose gel electrophoresis” on page 17
- “RNA assessment by Agilent™ Bioanalyzer™ system” on page 17

Large scale transcription reaction assembly for ≥1 mg RNA

1. Assemble transcription reaction at room temperature in a 1.5-mL microcentrifuge tube.

   The following table is a 10X scaled up reaction that can generate 1 mg RNA. Add the components in the order as listed.

Amount	Component	Final concentration
To 200 µL	Nuclease-free water	—
20 µL	10X Reaction Buffer	1X
15 µL	100 mM ATP Solution	7.5 mM
15 µL	100 mM CTP Solution	7.5 mM
15 µL	100 mM GTP Solution	7.5 mM
15 µL	100 mM UTP Solution	7.5 mM
5–10 µg	Linear template DNA[1]	25–50 ng/µL
20 µL	T7 Enzyme Mix	—

[1] Use ~0.5 µg PCR product template or ~1 µg linearized plasmid template.

2. Mix thoroughly.
   a. Gently flick the tube or pipette the mixture up and down gently, and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.

3. Incubate at 37°C, 2 hours.
   a. To achieve maximum yield, we recommend a 2-hour incubation, using a dry heat block set to 37°C.

4. (optional) Add 10 µL DNase I, mix well and incubate 15 minutes at 37°C.

5. Perform RNA purification.
   a. The RNA can be purified using the MEGAclear™ kit or LiCl precipitation as described previously. If using the MEGAclear™ kit, you will need to split the reaction and load onto 2 or 3 MEGAclear™ spin columns as the loading capacity per column is 0.5 µg RNA. If performing LiCl precipitation, be sure to scale up the protocol 10-fold.

Control reaction

The control template pMK-HA-emGFP is a linearized plasmid containing the emGFP coding sequence flanked by the human alpha globin 5'- and 3'- UTRs. It is under transcriptional control by the T7 RNA polymerase promoter with an AG initiation sequence for use with CleanCap™ Reagent AG. A MEGAscript™ T7 Transcription Kit Plus reaction with the control template and CleanCap™ Reagent AG will give ≥100 µg of a 0.9 kb RNA. This transcript encodes a ~27 kDa protein; however, the control template does not contain a poly(A) tail. We recommend adding the tail if doing *in vivo* work with RNA made from this template. The tail can be added enzymatically or by PCR. Both methods are described in the following sections.

Reaction setup with control template

Use 2 µL of pMK-HA-emGFP (1 ug) in a standard 20 µL reaction. See "Transcription reaction assembly (standard 20 µL reaction)" on page 7 or "Synthesize capped RNA transcripts with CleanCap™ Reagent AG" on page 9.

Enzymatically add poly(A) tail

A poly(A) tail can be enzymatically added to the HA-emGFP RNA in the *in vitro* transcription reaction mixture with the Invitrogen™ Poly A Tailing Kit by following these steps:

1. Add the tailing reagents in the order shown below to a 21 µL MEGAscript™ T7 reaction after the DNase I step.

Amount	Component
21 µL	MEGAscript™ T7 Transcription Kit Plus
35 µL	Nuclease-free water
20 µL	5X *E*-PAP Buffer
10 µL	25 mM $MnCl_2$
10 µL	10 mM ATP
4 µL	*E*-PAP

   Final volume is 100 µL.

2. Incubate at 37°C for 1 hour.

3. Perform RNA purification using the MEGAclear™ kit or LiCl precipitation.

4. The tailing reaction can by confirmed by agarose gel electrophoresis.

Create control template with poly(A) tail by PCR

A poly(A) tail can be added by PCR using a reverse primer with an overhanging polyT tract. The following forward and reverse primer sequences are required for adding the poly(A) tail to the control template.

Primer	Sequence
Forward primer	1 AGTAATACGA CTCACTATAA GGAGA
Reverse primer	1 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT 51 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTGCCGC CCACTCAGAC 101

1. The following is a suggested protocol for making the PCR template with Platinum™ SuperFi™ II Green PCR Master Mix.

Amount	Component
19 µL	Nuclease-free water
25 µL	2x Platinum™ SuperFi™ II Green PCR Master Mix
2.5 µL	10 µM Forward Primer
2.5 µL	10 µM Reverse Primer
1 µL	1 ng/uL pMK-HA-emGFP Control Template

Note: The pMK-HA-emGFP Control Template has been diluted from 500 ng/µL to 1 ng/µL. This can be done by mixing 2 µL of the control template with 8 µL nuclease-free water to make a 100 ng/µL solution. This solution can be serially diluted 1:10 into nuclease-free water two times to get a 1 ng/µL solution.

2. Incubate the PCR mixture in a thermocycler with the following program:

Cycle step	Temperature	Time	Cycle
Initial denaturation	98°C	30 seconds	1
Denaturation Annealing Extension	98°C 60°C 72°C	10 seconds 10 seconds 35 seconds	35
Final extension	72°C 4°C	5 minutes Hold	1

3. (optional) Digest plasmid by adding 1 µL Invitrogen™ Anza™ 10 DpnI and incubating at 37°C for 15 minutes.

4. Examine PCR product on an agarose gel to verify that it is unique and the expected size (~1 kb). We recommend the product to be purified by a PCR purification kit like PureLink™ PCR Purification Kit. Use 500 ng of the PCR product template for the *in vitro* transcription reaction. See “Transcription reaction assembly (standard 20 µL reaction)” on page 7 or “Synthesize capped RNA transcripts with CleanCap™ Reagent AG” on page 9

A

Troubleshooting

Observation	Possible cause	Recommended action
Control reaction is not working	If the yield of the control reaction is low, there may be a technical problem with the way the kit is being used. For example, the spermidine in the 10X Reaction Buffer may cause precipitation of the template DNA if it is not diluted by the other ingredients prior to adding the DNA. (This is the reason that the water is added first.)	Repeat the reaction, following the procedure carefully.
		If necessary, contact Technical Services.
Low yield of full-length RNA	If the reaction generates full-length RNA but low yields, it is possible that contaminants in the DNA template are inhibiting the RNA polymerase.	Additional purification of the DNA template may be required.
Low yield short transcript	This kit is designed to function best with transcription templates in the 0.9 to 10 kb range. Under these conditions, 1 µg of plasmid DNA template per 20 µL reaction gives maximal RNA yield. Increasing the incubation time, template, or polymerase concentration does not generally increase the yield of the reaction. However, with smaller templates, these parameters may require adjustment to maximize reaction yields.	Increase the reaction time. Increasing the incubation time is the easiest variable to change and should be tried first. Try increasing the incubation time to 4 or 6 hours. This allows each RNA polymerase molecule to engage in more initiation events.
		Increase the template concentration. Increasing the template concentration is the next variable that should be tested. This can be helpful because, with short templates, the initiation step of the transcription reaction is rate limiting. It is important to remember that 1 µg of a short template contains a much larger molar amount of DNA than 1 µg of a longer template.
Multiple transcription reaction products or transcripts of the wrong size.	If transcription reaction products run in a gel as more than one band, or as a single band smaller than expected, then the sample may have not been adequately denatured and is running aberrantly due to secondary structure.	To ensure that the RNA is completely denatured. See “RNA electrophoresis in agarose gel” on page 21.
	If the RNA has been completely denatured, there may be problems with premature termination by the polymerase. Possible causes of this are sequences which resemble the phage polymerase termination signals, stretches of a single nucleotide, and GC-rich templates.	The template will need to be optimized.

Observation	Possible cause	Recommended action
Multiple transcription reaction products or transcripts of the wrong size. *(continued)*	If the RNA transcript appears larger than the expected size, the plasmid DNA that is used for the template may not be completely digested. Even if small amounts of undigested circular plasmid DNA are present, T7 RNA Polymerase can produce large amounts of long transcripts.	Check the digestion of the plasmid for complete digestion compared to a sample of undigested plasmid. If undigested plasmid is present repeat the restriction digest.
	Alternatively, larger sized bands may be observed when the RNA is not completely denatured due to the presence of strong secondary structure.	To ensure that the RNA is completely denatured. See "RNA electrophoresis in agarose gel" on page 21.
RNA transcript smearing on gel	RNA appears degraded (eg: smeared) due to residual RNase present.	Digest the DNA prep with proteinase K (100–200 µg/mL) in the presence of 0.5% SDS for 30 minutes at 50°C. Follow this with phenol/chloroform extraction and DNA precipitation by ethanol. Resuspend the DNA template in nuclease-free water. The RNase Inhibitor that is present in the transcription reaction, can only inactivate trace RNase contamination. Large amounts of RNase contamination will compromise the size and amount of transcription products.

B

Quantification of reaction products

Yield quantitation by UV light absorbance

Reading the absorbance at 260 nm (A260) of RNA sample is the simplest way to determine yield, but any unincorporated nucleotides and/or template DNA in the mixture will contribute to the reading. Therefore, we recommend purification of the RNA product before measuring A260. The RNA yield can be calculated as follows:

A260 x 40 = µg/mL RNA

The Thermo Scientific™ NanoDrop™ microvolume spectrophotometers are a convenient and quick way to measure the A260 of the RNA sample.

Yield quantitation with Qubit™ or RiboGreen assays

Fluorescence-based assays for RNA quantitation is a convenient and sensitive way to measure RNA concentration. The Qubit™ RNA BR Assay Kit can be used to measure concentration. If you have a fluorometer or a fluorescence microplate reader, then the Invitrogen™ Quant-iT™ RiboGreen RNA Kit can be used. Follow the manufacturer's directions if performing either assay.

RNA assessment by agarose gel electrophoresis

The RNA can be evaluated by agarose gel electrophoresis to determine its quality and what percentage of the products are full-length. Because RNA forms secondary structures, we recommend using the Thermo Scientific™ RNA Gel Loading Dye (2x) to denature the secondary structures and allow the RNA to migrate according to size in a standard TAE agarose gel. See "RNA electrophoresis in agarose gel" on page 21.

RNA assessment by Agilent™ Bioanalyzer™ system

The RNA can be evaluated on an Agilent™ 2100 Bioanalyzer™ system using one of their RNA 6000 Nano™ kits to get an idea of RNA quality and what percentage of the products are full-length. Follow the manufacturer's instructions for using the Agilent™ 2100 Bioanalyzer™ system and the RNA 6000 Nano™ Kit.

C

DNA templates

Plasmid templates

DNA should be relatively free of contaminating proteins and RNA. We observe the greatest yields with very clean template preparations. Most commercially available plasmid preparation systems yield DNA that works well (eg: PureLink™ HiPure Plasmid Miniprep Kit or PureLink™ HiPure Plasmid Midiprep Kit)

Plasmid DNA must be linearized with a restriction enzyme downstream of the insert to be transcribed. Circular plasmid templates will generate extremely long, heterogeneous transcripts because RNA polymerases are very processive. It is worthwhile to examine the linearized template DNA on a gel to confirm that cleavage is complete. We recommend a restriction enzyme that gives a blunt cut (eg: *SmaI*) or a 5' overhang (eg: *EcoRI*).

The linearized DNA can be purified by a PCR purification kit like PureLink™ PCR Purification Kit or by DNA precipitation. See "DNA purification by phenol/chloroform extraction and ethanol precipitation" on page 21.

PCR templates

DNA generated by PCR can be transcribed directly from PCR if it contains a T7 RNA polymerase promoter upstream of the sequence to be transcribed. To achieve maximum yield, we recommend the PCR products to be purified by a PCR purification kit like PureLink™ PCR Purification Kit. The PCR products should be examined on an agarose gel before use as a template to verify that the products are unique and the expected size.

Template DNA features

If doing *in vivo* work with the synthesized RNA, we recommended that the template DNA has the following 4 features:

- T7 RNA polymerase promoter
- 5'- and 3'- untranslated regions (UTR)
- Coding sequence
- Poly(A) tail

T7 RNA polymerase promoter

Linearized plasmid DNA and PCR products that contain a T7 RNA polymerase promoter site can be used as templates for *in vitro* transcription. The following figure shows the recommended promoter sequence.

Figure 1 T7 RNA polymerase promoter sequence.
The underline shows the minimum promoter sequence needed for efficient transcription. The GG initiation sequence is in bold and is the first two bases of the RNA transcript.

If using CleanCap™ Reagent AG with the MEGAscript™ T7 Transcription Kit Plus, the promoter needs to have an AG initiation sequence to achieve high yields and capping efficiencies. Figure 2 shows the modified promoter sequence for use with CleanCap™ Reagent AG. Templates with the standard T7 RNA polymerase promoter in which have an GG initiation sequence can be changed into the AG initiation sequence by site-directed mutagenesis (eg: Thermo Scientific™ Phusion™ Site-Directed Mutagenesis Kit). Alternatively, a DNA template with the standard promoter can be easily changed to the modified promoter by performing PCR with a primer containing the base change to create a template with the AG initiation sequence.

Figure 2 Modified promoter sequence for use with the CleanCap™ Reagent AG
The underline shows the minimum promoter sequence needed for efficient transcription. The AG initiation sequence is in bold and is the first two bases of the RNA transcript.

5'- and 3'- untranslated region

A 5’- and 3’- UTR flanking the coding sequence can improve RNA translation and stability. The UTR’s from the human alpha or beta globins are commonly used and can give high translation of the RNA.

Coding sequence

The coding sequence should be free of any introns and contain a Kozak sequence (CACC) immediately upstream from the start codon (ATG) for translation of the RNA.

Poly(A) tail

A poly(A) tail is a long stretch of only adenine bases at the 3’ RNA end and greatly improves RNA translation and stability. It can be added enzymatically to the RNA by *E. coli* Poly(A) polymerase (eg: Poly A Tailing Kit). However, we recommend that the tail is included in the DNA template to achieve transcripts more uniform in length as the *E. coli* Poly(A) polymerase can give RNAs with variable tail lengths. We have also gotten better *in vivo* results when using a template containing a poly(A) tail.

A template with a poly(A) tail can be included in the plasmid DNA or can be created by PCR with a reverse primer containing an overhanging poly(T) tract. For an example, see"Create control template with poly(A) tail by PCR" on page 13. We recommend using templates with poly(A) tails that are 80 – 120 nucleotides long.

D Additional procedures

DNA purification by phenol/chloroform extraction and ethanol precipitation

1. Add one volume of phenol:chloroform:isoamyl alcohol (25:24:1) to your sample, and vortex or shake by hand thoroughly for approximately 20 seconds.
2. Centrifuge at room temperature for 5 minutes at 16,000 × *g*. Carefully remove the upper aqueous phase, and transfer the layer to a fresh tube. Be sure not to carry over any phenol during pipetting.
3. Add ammonium acetate and 100% ethanol to the sample as described:
   volume of 7.5 M ammonium acetate = 0.5 × volume of sample
   volume of 100% ethanol = 2.5 × (volume of sample + ammonium acetate)
4. Mix well and place the tube at -20°C overnight to precipitate the DNA from the sample.

   Note: If you wish to continue with the protocol, place the tube in dry ice or at -80°C for at least 1 hour.
5. Centrifuge the sample at 4°C for 30 minutes at 16,000 × *g* to pellet the DNA.
6. Carefully remove the supernatant without disturbing the DNA pellet.
7. Add 150 µL of 70% ethanol. Centrifuge the sample at 4°C for 2 minutes at 16,000 × *g*. Carefully remove the supernatant.
8. Repeat step 7 once. Remove as much of the remaining ethanol as possible
9. Dry the DNA pellet at room temperature for 5–10 minutes.
10. Resuspend the DNA pellet in TE Buffer.

RNA electrophoresis in agarose gel

RNA can be analyzed in a standard TAE agarose gel by following these steps:

1. Pour an agarose gel of the appropriate percentage for the size of the RNA transcript. We recommend 1% agarose for transcripts > 500 bases and 2.5% for those < 500 bases.
   a. For 100 mL of gel, add 100 mL 1X TAE buffer to 1 or 2.5 g of agarose.

b. Heat the solution in a microwave oven until bubbles appear and the agarose is completely dissolved.

c. Remove the flask carefully and swirl gently to mix the solution.

CAUTION! The microwaved solution may become superheated and foam over when agitated.

Allow it to cool to 50–60°C.

d. Add 5 µL of Thermo Scientific™ SYBR™ Safe DNA Gel Stain and swirl gently to mix the solution.

e. Pour the gel and allow it to set.

2. Prepare the RNA samples.
   a. Mix 2 µL of 25 ng/µL RNA sample with 8 µL of RNA Gel Loading Dye (2x). The excess dye will ensure that the RNA has been completely denatured.

   b. Heat the mixture at 70°C for 10 minutes.

   c. Immediately chill on ice for 5 minutes.

   d. Spin down prior to loading on a gel.

3. Load the samples and run the gel in 1X TAE buffer at ~5 volts/cm until the bromophenol blue dye has migrated ~3/4 of the gel. Examine the gel on an appropriate instrument (eg: an iBright™ CL1500 Imaging System).

   We recommend including a RNA ladder for RNA sizing quantification. The RNA ladder (eg: RiboRuler™ High Range RNA Ladder) should be prepared in the RNA Gel Loading Dye (2x).

E Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Cat. No.
MEGAclear™ Transcription Clean-Up Kit	AM1908
PureLink™ HiPure Plasmid Miniprep Kit	K210002
PureLink™ HiPure Plasmid Midiprep Kit	K210004
PureLink™ PCR Purification Kit	K310002
Phusion™ Site-Directed Mutagenesis Kit	F541
Poly A Tailing Kit	AM1350
THE RNA Storage Solution	AM7001
Qubit™ RNA BR Assay Kit	Q10210
Quant-iT™ RiboGreen™ RNA Assay Kit	R11490
RNA Gel Loading Dye (2x)	R0641
Platinum™ SuperFi™ Green PCR Master Mix	12369010
RNase*Zap*™ RNase Decontamination Solution	AM9780
RiboRuler™ Low Range RNA Ladder	SM1831
RiboRuler™ High Range RNA Ladder	SM1821
Lipofectamine™ MessengerMAX™ Transfection Reagent	LMRNA003
UltraPure™ Agarose	16500100
UltraPure™ DNA Typing Grade™ 50X TAE Buffer	24710030
SYBR™ Safe DNA Gel Stain	S33102
Owl™ A2 Large Gel Systems	A2-BP
GeneArt™ services	**website**
Custom DNA oligos	10336022
TheraPure™ GMP N1-Methylpseudo-UTP, 100 mM sodium solution	R0491SKB007

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

ISO certification

Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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