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HiScribe® SP6 RNA Synthesis Kit

NEB #E2070S

50 reactions

Version 2.0_7/20

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The HiScribe SP6 RNA Synthesis Kit Includes:

All kit components should be stored at –20°C. Each kit contains sufficient reagents for 50 reactions of 25 µl each. Each standard reaction yields ≥ 80 µg of unmodified RNA from 1 µg control template.

SP6 RNA Polymerase Mix
SP6 Reaction Buffer (10X)
ATP (Tris) (50 mM)
GTP (Tris) (50 mM)
UTP (Tris) (50 mM)
CTP (Tris) (50 mM)
SP6 Control Template (0.25 µg/µl)
DNase I (RNase-free) (2 units/µl)
LiCl Solution

Required Materials Not Included:

DNA Template:	The DNA template must be linear and contain the SP6 RNA Polymerase promoter with the correct orientation in relation to the target sequence to be transcribed
General:	Thermocycler or 37°C dry air incubator, microcentrifuge, nuclease-free water, nuclease-free tubes and tips
Purification:	Phenol, chloroform, ethanol, 3M Sodium Acetate, pH 5.2 or 5 M Ammonium Acetate, spin columns, equipment for RNA quantitation
Gel Analysis:	Gels, running buffers, loading dye, nucleic acid ladders, gel apparatus, power supply

Optional Materials:

Cap Analogs:	NEB #S1411, #S1404, #S1405, #S1406, #S1407
Modified NTPs:	Biotin-, Fluorescein-, Digoxigenin-, Aminoallyl-
Labeling:	[α-^{32}P] labeled ribonucleotide (800-6,000 Ci/mmol), storage phosphor screen

Introduction

The HiScribe SP6 RNA Synthesis Kit is designed for the *in vitro* transcription of RNA using SP6 RNA Polymerase. This kit is suitable for synthesis of high yield RNA transcripts and for incorporation of cap analogs (not included) or modified nucleotides (not included) to obtain capped, biotin-labeled or dye-labeled RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA for use as probes or targets.

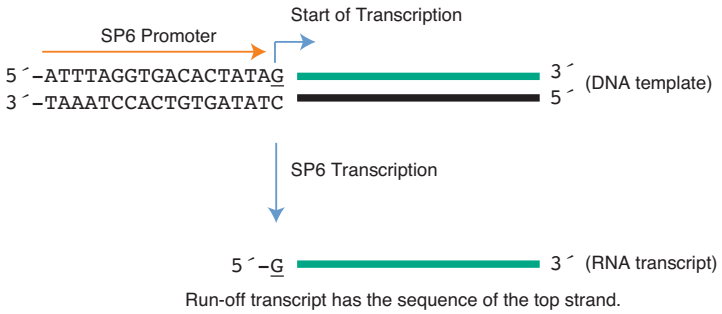
RNA synthesized from this kit is suitable for many applications including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection or gel shift assays, hybridization-based blots, anti-sense RNA or RNAi experiments, microarray analysis, microinjection, sgRNA synthesis and *in vitro* translation studies.

This kit contains sufficient reagents for 50 reactions of 25 µl each. Each standard reaction yields ≥ 80 µg of RNA from 1 µg SP6 Control Template DNA. Each kit can yield ≥ 4 mg of RNA.

DNA Template Preparation

Linearized plasmid DNA, PCR products or synthetic DNA oligonucleotides can be used as templates for *in vitro* transcription with the HiScribe SP6 RNA Synthesis Kit provided that they contain a double-stranded SP6 promoter region upstream of the sequence to be transcribed. Figure 1 illustrates the minimal SP6 promoter sequence as well as the run-off transcript after SP6 transcription.

Figure 1. Transcription by SP6 RNA Polymerase



Plasmid Templates

Completely linearized plasmid template of the highest purity is critical for successful use of the HiScribe SP6 RNA Synthesis Kit. The quality of the template DNA affects the yield and integrity of the RNA as the highest yield is achieved with the highest purity template. Plasmids purified by many different laboratory methods can be successfully used, provided the preparations contain mostly supercoiled DNA and are free from contaminating RNases, protein, RNA and salts.

To produce RNA transcripts of a defined length, plasmid DNA must be completely linearized with a restriction enzyme downstream of the insert to be transcribed. Circular plasmid templates will generate long, heterogeneous RNA transcripts in higher quantities due to the high processivity of SP6 RNA polymerase. NEB has a large selection of restriction enzymes; we recommend selecting restriction enzymes that generate blunt ends or 5′-overhangs.

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

1. Extract the DNA with an equal volume of 1:1 phenol:chloroform, repeat as necessary.
2. Extract twice with an equal volume of chloroform to remove residual phenol.
3. Precipitate the DNA by adding 1/10th volume 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. Wash the pellet by adding 500 µl of 70% ethanol and centrifuge 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 the SP6 RNA Polymerase promoter in the correct orientation can be transcribed with the HiScribe SP6 RNA Synthesis Kit. Though the PCR products can be used directly in a synthesis reaction, better yields can be obtained with purified PCR products (Monarch® PCR & DNA Cleanup Kit, NEB #T1030). Templates should be examined on an agarose gel to estimate concentration and confirm amplicon size. In general, 0.1 – 0.5 µg of PCR template can be used in a 25 µl *in vitro* transcription reaction.

Synthetic DNA Oligonucleotides

Synthetic DNA oligos, which are either entirely double-stranded or mostly single-stranded with a double-stranded SP6 promoter sequence, can be used in the HiScribe SP6 RNA Synthesis Kit, though yields may be variable depending on sequence and purity of the oligonucleotides. In general, use of single-stranded templates with a double-stranded promoter results in lower yields.

RNA Synthesis Protocols

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
Reactions are typically 25 µl but can be scaled up as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR tubes.

Standard RNA Synthesis

1. Thaw necessary kit components, mix and pulse spin in a microfuge to collect solutions at the bottom of the tubes. Keep on ice.
2. If you are planning to set up many reactions, it is convenient to prepare a master mix by combining equal volumes of the SP6 Reaction Buffer (10X) and each of the four ribonucleotide (NTP) solutions. Use 12.5 µl of master mix per reaction.
3. Assemble the reaction at room temperature in the following order:

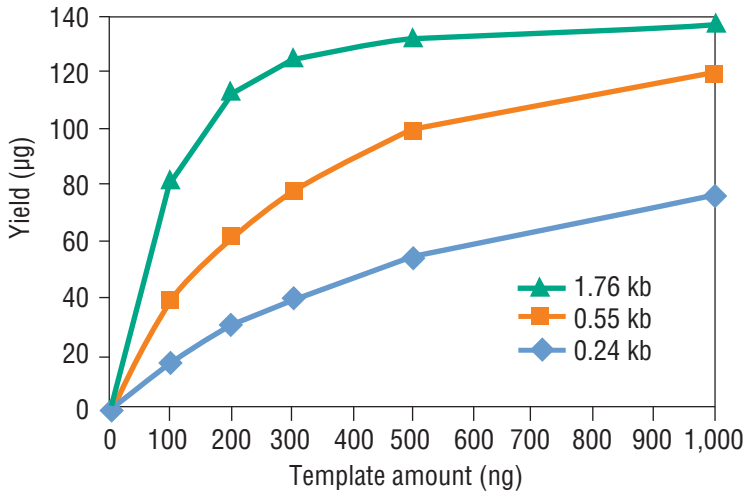
REAGENT	AMOUNT	FINAL CONCENTRATION
Nuclease-free water	X µl	
SP6 Reaction Buffer (10X)	2.5 µl	
ATP (Tris), 50 mM	2.5 µl	5 mM final
GTP (Tris), 50 mM	2.5 µl	5 mM final
UTP (Tris), 50 mM	2.5 µl	5 mM final
CTP (Tris), 50 mM	2.5 µl	5 mM final
Template DNA	X µl	1 µg
SP6 RNA Polymerase Mix	2.5 µl	
Total Reaction Volume	25 µl	

4. Mix thoroughly and pulse-spin in a microfuge. Incubate at 37°C for 30 min2 hours.

For reaction times of 60 minutes or less, a water bath or heat block may be used. For reaction times longer than 60 minutes we recommend using a dry air incubator or thermocycler to prevent evaporation/condensation.

5. Optional: DNase treatment to remove DNA template. Standard reactions normally generate large amounts of RNA at high concentrations. As a result, the reaction mixture may be quite viscous. It is easier to perform DNase treatment after the reaction mixture is diluted with water. To remove template DNA, add 25 µl nuclease-free water to each 25 µl reaction followed by 2 µl of DNase I (RNase-free). Mix and incubate for 15 minutes at 37°C.
6. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.

Figure 2. Effect of template amount on RNA yield.



Standard reactions with linearized plasmid DNA templates were incubated at 37°C in a PCR machine for 2 hours. Transcripts were treated with DNase I, purified by LiCl precipitation and quantified using a NanoDrop® Spectrophotometer.

Capped RNA Synthesis

The recommended ratio of cap analog to GTP is 4:1. Cap analogs are sold separately. Please refer to the ordering information section or www.neb.com for more information.

1. Thaw necessary kit components, mix and pulse-spin in a microfuge to collect solutions at the bottom of the tubes. Keep on ice.
2. Make a 10 mM GTP solution by diluting an aliquot of 50 mM GTP 1:5 with nuclease-free water.
3. Prepare the cap analog at 40 mM concentration.
4. Assemble the reaction at room temperature in the following order:

REAGENT	AMOUNT	FINAL CONCENTRATION
Nuclease-free water	X µl	
SP6 Reaction Buffer (10X)	2.5 µl	
ATP (Tris), 50 mM	2.5 µl	5 mM final
UTP (Tris), 50 mM	2.5 µl	5 mM final
CTP (Tris), 50 mM	2.5 µl	5 mM final
GTP (Tris), 50 mM	2.5 µl	1 mM final
Cap Analog (40 mM)	2.5 µl	4 mM final
Template DNA	X µl	1 µg
SP6 RNA Polymerase Mix	2.5 µl	
Total Reaction Volume	25 µl	

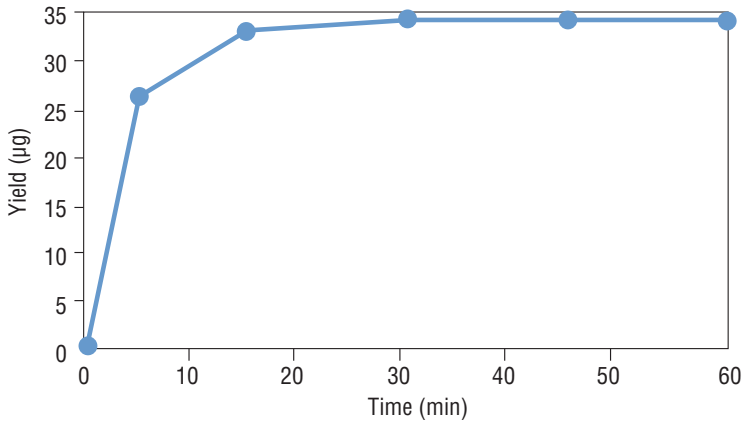
5. Mix thoroughly, pulse-spin in microfuge and incubate at 37°C for 30 minutes.

Table 1. Effect of cap analog:GTP ratios on RNA yield.

CAP ANALOG: GTP RATIO	CONCENTRATION OF CAP ANALOG:GTP (mM)	RNA YIELD (µg)	% CAPPED RNA
0:1	0:5	100	0
1:1	2.5:2.5	70	50
2:1	3.3:1.7	50	67
4:1	4:1	30	80
8:1	4.4:0.56	5–15	89

6. Optional: To remove the DNA template, add 25 µl of water and 2 µl of DNase I (RNase-free), mix and incubate at 37°C for 15 minutes.
7. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.

Figure 3. Time course of capped RNA synthesis.



SP6 control template (1.76 kb) was transcribed in the presence of 4 mM ARCA at 37°C in a PCR machine over one hour. Transcripts were treated with DNase I, purified by LiCl precipitation and quantified using a NanoDrop Spectrophotometer.

RNA Synthesis with Modified Nucleotides

Dye-labeled, Biotin-labeled or other modified NTPs are not supplied with this kit. The recommended molar ratio of modified NTP [Biotin-, Fluorescein-, Digoxigenin-, Aminoallyl-NTPs (or others)] to standard NTP is 1:3 or 1:2. The following reaction set up assumes that modified UTP is used.

1. Thaw necessary kit components, mix and pulse-spin in microfuge to collect solutions at the bottom of the tubes. Keep on ice.
2. Assemble the reaction at room temperature in the following order:

REAGENT	AMOUNT	FINAL CONCENTRATION
Nuclease-free water	X µl	
SP6 Reaction Buffer (10X)	2.5 µl	
ATP (Tris), 50 mM	2.5 µl	5 mM final
GTP (Tris), 50 mM	2.5 µl	5 mM final
CTP (Tris), 50 mM	2.5 µl	5 mM final
UTP (Tris), 50 mM	1.75 µl	3.5 mM final
Modified UTP (10 mM)	3.75 µl	1.5 mM final
Template DNA	X µl	1 µg
SP6 RNA Polymerase Mix	2.5 µl	
Total Reaction Volume	25 µl	

Note that the ratio of UTP:modified UTP can be adjusted to meet specific needs. The total amount of UTP can be lowered if higher RNA yield is not necessary.

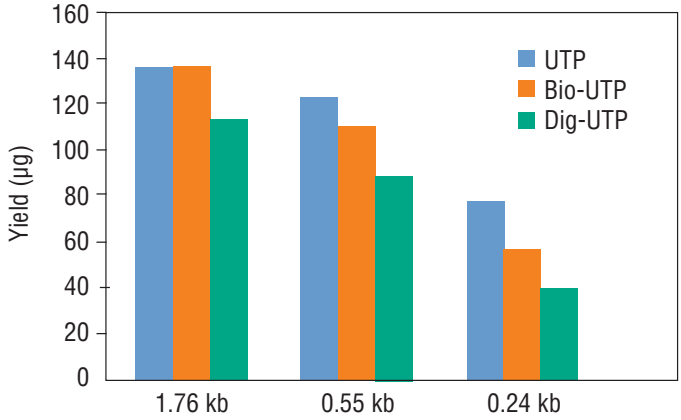
3. Mix thoroughly, pulse-spin in a microfuge and incubate at 37°C for 2 hours. For short transcripts, incubate at 37°C for 4–16 hours.

   Modified ribonucleotides reduce transcription efficiency, therefore lower yields should be expected as compared to transcription using unmodified NTPs (Figure 4). In general, Biotin-NTP and Aminoallyl-NTP have less of an effect on yields, while lower yields can be expected for transcription reactions containing Fluorescein-NTP, Cy-NTP or Digoxigenin-NTP. In addition, transcripts containing modified ribonucleotides have reduced electrophoretic mobility due to higher molecular weight.

4. Optional: To remove the DNA template, add 25 µl of water and 2 µl of DNase I (RNase-free), mix and incubate at 37°C for 15 minutes.

5. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.

Figure 4. Effect of modified NTPs on RNA yield.



RNA synthesis reactions using linearized plasmid DNA templates were performed in the presence of unmodified nucleotides or modified nucleotides (Biotin-UTP, Digoxigenin-UTP) at the ratio of 2.3:1 (UTP:modified UTP). Reactions were incubated at 37°C in a PCR machine for 2 hours. Transcripts were treated with DNase I, purified by LiCl precipitation and quantified using a NanoDrop Spectrophotometer.

High Specific Activity Radiolabeled RNA Probe Synthesis

The HiScribe SP6 RNA Synthesis Kit can be used to synthesize high specific activity radiolabeled RNA probes by following the modified protocol below. More than 50% of the label can be incorporated in a 10 minute reaction. The labeled RNA probes have a specific activity of approximately 10^8 cpm/µg.

1. Choosing ^{32}P labeled nucleotide:
   We recommend using [α-^{32}P] UTP or CTP at 800-6000 Ci/mmol and ³ 10 mCi/ml for the synthesis of RNA labeled probes. We do not recommend using radiolabeled ATP or GTP since less label is generally incorporated. RNA labeled with [α-^{32}P] ATP or GTP appears to be more subject to decomposition during storage.
2. Thaw the necessary kit components, mix and pulse-spin in microfuge to collect solutions to the bottom of the tubes. Keep on ice.
3. Dilute 50 mM UTP to 50 µM UTP in two steps as described below if [α-^{32}P] UTP is used.
   a. Prepare 200 µl 1 mM UTP by combining 4 µl 50 mM UTP and 196 µl nuclease-free water. Extra 1 mM UTP can be stored at –20°C for future use.
   b. Prepare 100 µl 50 µM UTP by combining 5 µl 1 mM UTP and 95 µl nuclease-free water.
4. Prepare master mix. For accurate pipetting we recommend preparing 20 µl master mix, which is enough for 4 labeling reactions. The master mix contains reaction buffer, ATP, GTP and CTP.

REAGENT	AMOUNT
Nuclease-free water	9 µl
SP6 Reaction Buffer (10X)	5 µl
ATP (Tris), 50 mM	2 µl
GTP (Tris), 50 mM	2 µl
CTP (Tris), 50 mM	2 µl
Total Reaction Volume	20 µl

   Use 4 µl master mix for each labeling reaction. Extra master mix can be stored at –20°C for future use.

5. Assemble the reaction at room temperature in the following order:

REAGENT	AMOUNT	FINAL CONCENTRATION
Nuclease-free water	X µl	
Master Mix (A, G, C, Buffer)	5 µl	
UTP (Tris) 50 µM	3 µl	
[a-32P] UTP	2 µl	
Template DNA	2 µl	
SP6 RNA Polymerase Mix		
Total Reaction Volume	20 µl	

The labeled NTP is present at a limiting concentration and is therefore referred to as the “limiting nucleotide”. The “limiting nucleotide” is a mixture of both the labeled and unlabeled form of that specific NTP. There is a trade-off between synthesis of high specific activity probe and synthesis of full-length probe. The higher the concentration of the “limiting nucleotide”, the higher the proportion of full-length transcripts, but if the unlabeled nucleotide is used to increase the “limiting nucleotide” concentration, it will lower the specific activity of the transcript. For most labeling reactions, 4-8 µM of the “limiting nucleotide" is necessary for full-length probe synthesis with high specific activity. The template sequence will also affect the specific activity. For example, if the transcript contains more UTP then more ^{32}P-UTP will be incorporated resulting in a higher specific activity. For longer RNA transcripts > 1 kb it may be necessary to increase the concentration of the unlabeled “limiting nucleotide” to increase the proportion of full-length transcript, however the improvement in yield of full-length transcript will reduce the specific activity of the probe. We recommend increasing the concentration of the unlabeled “limiting nucleotide” to 20 µM.

Table 2. Concentration of [α-^{32}P] NTP in a transcription reaction.

SPECIFIC ACTIVITY (Ci/mmol)	CONCENTRATION (mCi/ml)	VOLUME USED PER 25 µl REACTION	CONCENTRATION IN 25 µl REACTION
800	10	1 µl	0.5 µM
800	20	1 µl	1 µM
800	40	1 µl	2 µM
3,000	10	1 µl	0.13 µM
3,000	20	1 µl	0.27 µM
3,000	40	1 µl	0.53 µM
6,000	40	1 µl	0.27 µM

6. Mix thoroughly, pulse-spin in a microfuge and incubate for 10 minutes. Incubation temperature is not crucial for labeling efficiency, room temperature to 40°C can be used.
7. Optional: To remove template DNA, add 25 µl of water and 2 µl of DNase I (RNase-free), mix and incubate for 15 minutes at 37°C.
8. Proceed with purification of synthesized RNA or analysis of transcription products by gel electrophoresis.

Figure 5. Generation of high specific activity ^{32}P-labeled RNA probes.

Labeling reactions were incubated for 10 minutes at room temperature. A small portion of each reaction was run on a 6% PAGE urea gel followed by exposure of the gel to a Storage Phosphor Screen (GE).

Purification of Synthesized RNA

In general, synthesized RNA can be purified by LiCl precipitation, phenol:chloroform extraction followed by ethanol precipitation, or by using a spin column method. If absolute full length RNA is required, we recommend gel purification.

LiCl Precipitation

The kit includes LiCl solution for rapid recovery of the synthesized RNA. LiCl is effective in removing the majority of unincorporated NTPs and enzymes. However, RNAs shorter than 300 nucleotides or at a concentration lower than 0.1 mg/ml do not precipitate well. In such cases, other purification methods may be used. LiCl purified RNA is suitable for cap addition with NEB's Vaccinia Capping System (NEB #M2080) and Poly(A) tailing with NEB's Poly(A) Polymerase (NEB #M0276).

1. Adjust the reaction volume to 50 µl by adding nuclease-free water.
2. Add 25 µl LiCl solution and mix well.

3. Incubate at –20°C for 30 minutes.
4. Centrifuge at 4°C for 15 minutes at top speed to pellet RNA.
5. Remove the supernatant and rinse the pellet with 500 µl of ice cold 70% ethanol.
6. Resuspend the RNA in 50 µl of 0.1 mM EDTA. Store the RNA at –20°C or below.

Phenol:chloroform Extraction and Ethanol Precipitation

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

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 5 M 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 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 and rinse the pellet with 500 µl of ice cold 70% ethanol.
5. Resuspend the RNA in 50 µl 0.1 mM EDTA. Store the RNA at –20°C or below.

Spin Column Purification

Spin columns will remove unincorporated nucleotides, proteins and salts. Adjust the volume of the reaction to 100 µl by adding nuclease-free water and mix well. Purify the RNA by following the spin column manufacturer's instructions. Each reaction produces ≥ 80 µg of RNA, which may exceed the column capacity, thus requiring additional columns.

Gel Purification

When high purity RNA transcripts are desired (RNA probes for footprinting assays or RNase protection assays), we recommend gel purification of the transcription product.

Evaluation of Reaction Products

Quantification by UV Light Absorbance

RNA concentration can be easily determined by measuring the ultraviolet light absorbance at 260 nm wavelength, however any unincorporated nucleotides and template DNA in the mixture will affect the reading. Free nucleotides from the transcription reaction must be removed before RNA concentration can be determined. A 1:200 dilution of a sample of the purified RNA should give an absorbance reading in the linear range of the spectrophotometer. RNA dilution may not be necessary if using a NanoDrop Spectrophotometer, which can read RNA concentrations from 10 ng/µl to 3000 ng/µl directly.

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 concentration, 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 and transcripts up to ~1 kb in length. The gels should be run under denaturing conditions to minimize formation of secondary structure from the transcript.

1. Preparation of denaturing gels
   a. Denaturing agarose gels:
      To make a 100 ml 1% denaturing agarose gel, add 1 gram agarose powder to 72 ml nuclease-free water. Melt the agarose and add 10 ml 10X MOPS buffer. Then, in a fume hood, add 18 ml fresh formaldehyde (37%), mix well. Pour the gel.
   b. Denaturing PAGE/Urea Gel:
      5–15% PAGE/Urea gel. We recommend using commercially available premade gels. Use standard TBE gel running buffer.
      10X TBE buffer: 0.9 M Tris Base, 0.9 M Boric acid, 20 mM EDTA
2. Gel electrophoresis of non-radiolabeled RNA
   a. Mix 0.2–1 µg RNA sample with an equal volume of RNA Loading Dye (2X) (NEB #B0363).
   b. Denature the RNA samples and an aliquot of RNA marker by heating at 65–70°C for 5–10 minutes.
   c. Pulse-spin prior to loading the RNA onto the gel.

d. Visualize RNA by staining the gel with ethidium bromide. If using an alternative dye such as SYBR® Gold, please refer to the manufacturer's instructions to determine how much RNA to load.

3. Gel electrophoresis of radiolabeled RNA
   a. Mix an aliquot of labeled RNA with an equal volume of RNA Loading Dye (2X) (NEB #B0363).
   b. Denature the RNA sample by heating at 65–70°C for 5–10 minutes.
   c. Pulse-spin prior to loading the RNA onto the gel.
   d. Visualize the RNA by autoradiography.

Agarose gels should be dried before exposing to X-ray film. Thin (< 1 mm) polyacrylamide gels can be transferred to filter paper or left on the glass running plate and covered with plastic wrap and exposed to X-ray film or a Storage Phosphor Screen when ^{32}P is used. Exposure time will vary depending on the specific activity of the RNA probe and method of detection used.

Troubleshooting

Control Reaction

The SP6 control template is a linearized plasmid containing the *Cypridina* luciferase (CLuc) gene under the transcriptional control of the SP6 promoter. The size of the run-off transcript is 1.76 kb. The control reaction should yield ³ 80 µg of RNA in 2 hours.

If the control reaction is not working, there may be technical problems during the reaction set up. Repeat the reaction by following the protocol carefully; take every precaution to avoid RNase contamination. Contact NEB for technical assistance.

The control plasmid pCLuc-SP6 sequence can be found at www.neb.com within the DNA Sequences and Maps Tool. The pCLuc-SP6 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).

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 is most likely 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 followed by ethanol precipitation. 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 circular DNA can produce large amounts of long transcripts. Check the template for complete digestion on an agarose gel compared to an uncut plasmid sample. If undigested plasmid is confirmed, repeat the 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 structure.

RNA Transcript of Smaller Size than Expected

If denaturing gel analysis shows the presence of smaller bands than expected, it is most likely due to premature termination by the polymerase. Some sequences which resemble the SP6 RNA Polymerase termination signal may cause premature termination. For GC-rich templates, or templates with secondary structure, incubation at 42°C may improve the yield of full-length transcript.

If premature termination of transcription is found in high specific activity radiolabeled RNA probe synthesis, increase the concentration of the “limiting nucleotide”. Additional unlabeled limiting NTP can be added to the reaction to increase the proportion of full-length transcript, however the improvement in yield of full-length product will compromise the specific activity of the probe.

Ordering Information

NEB #	PRODUCT	SIZE
E2070S	HiScribe SP6 RNA Synthesis Kit	50 reactions

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
E2040S	HiScribe T7 High Yield RNA Synthesis Kit	50 reactions
E2050S	HiScribe T7 Quick High Yield RNA Synthesis Kit	50 reactions
E2065S	HiScribe T7 ARCA mRNA Kit	20 reactions
E2060S	HiScribe T7 ARCA mRNA Kit (with tailing)	20 reactions
B0363S	RNA Loading Dye (2X)	4 x 1 ml
M0307S/L	RNase Inhibitor, Human Placenta	2,000/10,000 units
M0314S/L	RNase Inhibitor, Murine	3,000/15,000 units
M0303S/L	DNase I (RNase-Free)	1,000/5,000 units
M0493S/L	Q5 Hot Start High-Fidelity DNA Polymerase	100/500 units
T1030S/L	Monarch PCR & DNA Cleanup Kit	50/250 preps
N0362S	ssRNA Ladder	25 gel lanes
N0364S	Low Range ssRNA Ladder	25 gel lanes
S1411S/L	3′-O-Me-m^{7}G(5′)ppp(5′)G RNA Cap Structure Analog	1/5 µmol
S1405S/L	m^{7}G(5′)ppp(5′)A RNA Cap Structure Analog	1/5 µmol
S1406S/L	G(5′)ppp(5′)A RNA Cap Structure Analog	1/5 µmol
S1407S/L	G(5′)ppp(5′)G RNA Cap Structure Analog	1/5 µmol
S1404S/L	m^{7}G(5′)ppp(5′)G RNA Cap Structure Analog	1/5 µmol
M2080S	Vaccinia Capping System	400 units
M0366S	mRNA Cap 2′-O-Methyltransferase	2,000 units
M0276S/L	*E. coli* Poly(A) Polymerase	100/500 units
N0450S/L	Ribonucleotide Solution Set	10/50 µmol of
M0378S	T3 RNA Polymerase	5,000 units
M0251S/L	T7 RNA Polymerase	5,000/25,000 units
M0207S/L	SP6 RNA Polymerase	2,000/10,000 units

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	12/16
2.0	New format applied	7/20

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