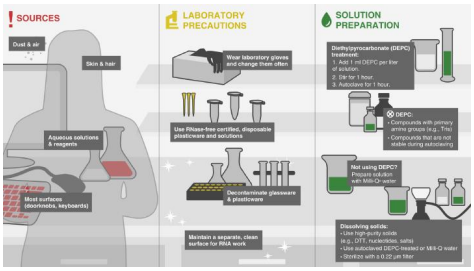
HiScribe™ T7 High Yield RNA Synthesis Kit

- Up to 180 µg of RNA per reaction from 1 µg of control template
- Enables full substitution of NTPs for labeling and incorporation of modified bases
- Linearized control template included for verification of RNA synthesis

1126 Product Citations

Featured Video



Avoiding RNase Contamination

Catalog #	Concentration	Size	List Price	Your Price	Quantity
E2040S	Not Applicable	50 reactions	$232.00	Sign In	0

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Product Information

The HiScribe T7 High Yield RNA Synthesis Kit is an extremely flexible system for *in vitro* transcription of RNA using T7 RNA Polymerase. The kit allows for synthesis many kinds of RNA including internally labeled and co-transcriptionally capped transcripts.

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

The kit contains sufficient reagents for 50 reactions of 20 µl each. Each standard reaction yields up to 180 µg of RNA from 1 µg control template. Each kit can yield up to 9 mg RNA. For ^{32}P labeling, the kit contains enough reagents for 100 reactions of 20 µl

each.

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.
- 3′-O-Me-m7G(5′)ppp(5′)G RNA Cap Structure Analog (NEB #S1411)
- m7G(5′)ppp(5′)G RNA Cap Structure Analog (NEB #S1404)
- m7G(5′)ppp(5′)A RNA Cap Structure Analog (NEB #S1405)
- G(5′)ppp(5′)A RNA Cap Structure Analog (NEB #S1406)
- G(5′)ppp(5′)G RNA Cap Structure Analog (NEB #S1407)
- Modified-NTP: Biotin-, Fluorescein-, Digoxigenin-, or Aminoallyl-NTP
- Labeling: [α-^{32}P] labeled ribonucleotide (800-6,000 Ci/mmol)
- General: 37°C incubator or PCR machine, nuclease-free water
- DNase I: DNase I (RNase-free) (NEB #M0303)
- Purification: Buffer- or water-saturated phenol/chloroform, ethanol and 3 M sodium acetate, pH 5.2, spin columns
- Gel Analysis: Gels and running buffers, gel apparatus, power supply

Figure 1. Transcription by T7 RNA Polymerase

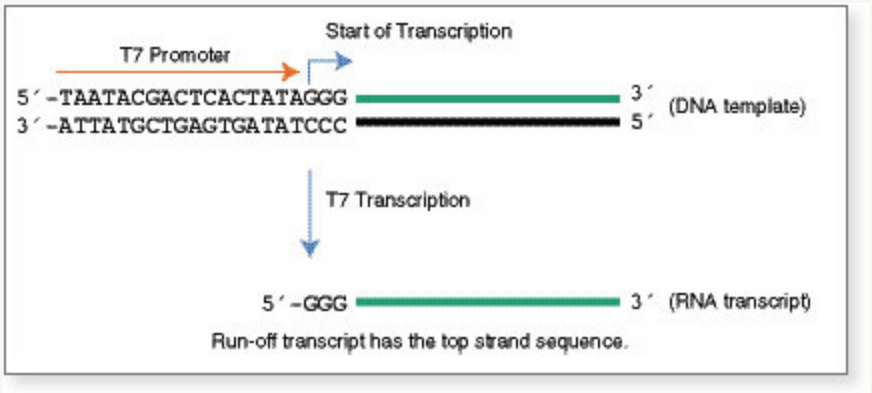
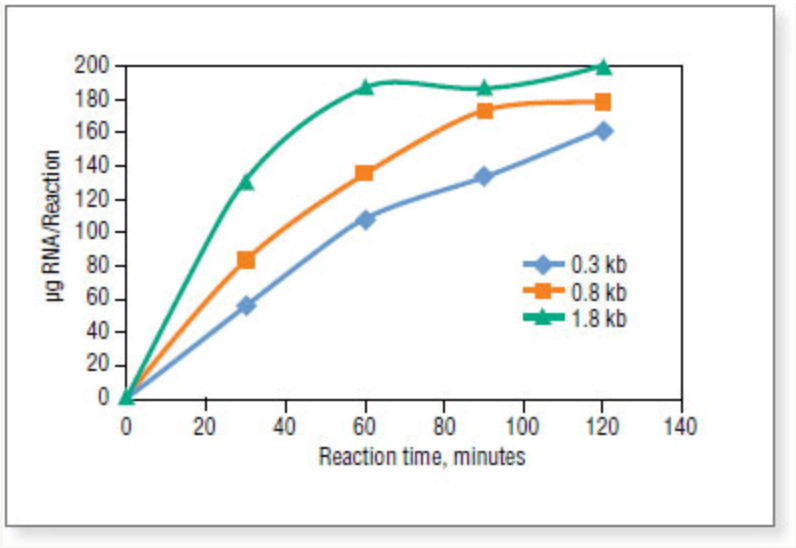
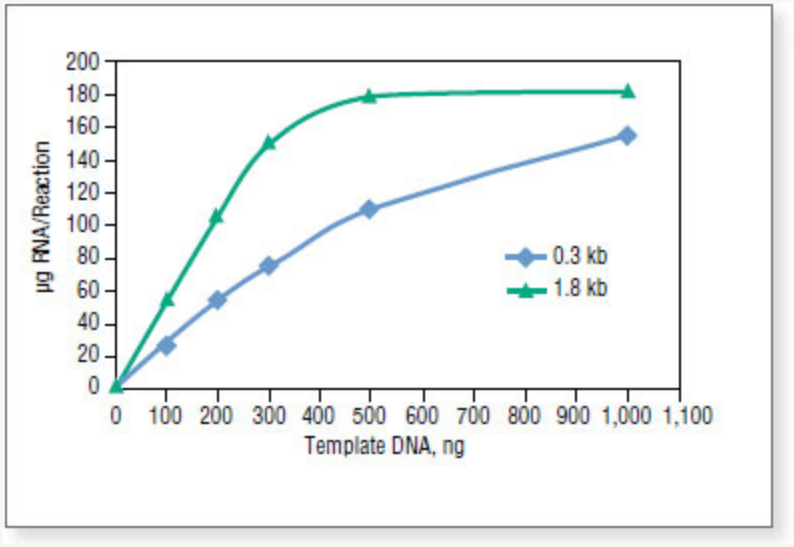


Figure 2. Time course of standard RNA synthesis from three DNA templates



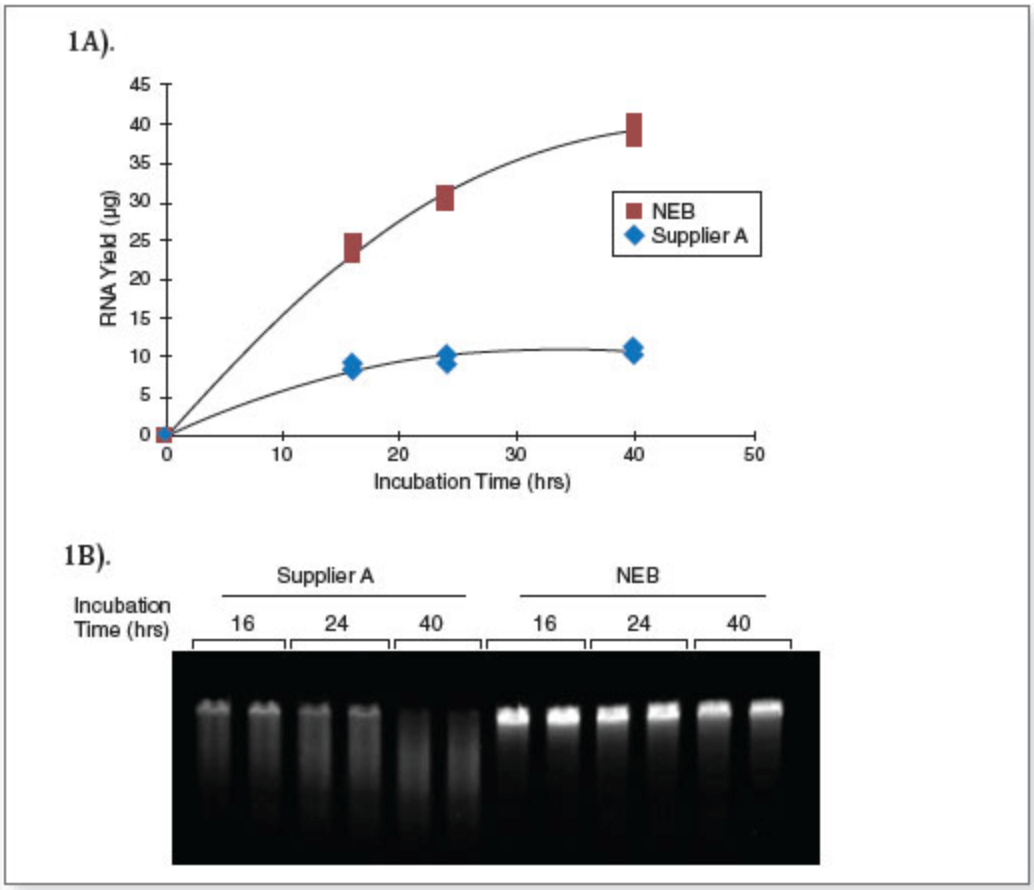
Reactions were incubated at 37°C in a PCR machine. Transcripts were purified by spin columns and quantified on NanoDrop™ Spectrophotometer.

Figure 3. Effect of template amount on RNA yield



Standard reactions were incubated at 37°Cin a PCR machine for 2 hours. Transcripts were purified by spin columns and quantified on NanoDrop™ Spectrophotometer.

Figure 4: Improved RNA yield and integrity from extended duration transcription reactions



reactions were assembled, in duplicate, according to the manufacturers' suggested protocols using 3 ng of dsDNA template encoding a 1.8 kb RNA, and incubated at 37°C for 16, 24 and 40 hours. At each time point, the corresponding tubes were transferred to -20°C to stop the reaction. Transcription reactions were column purified after the last time point.

(A) Transcript yield – After column purification, RNA concentration was measured using a NanoDrop spectrophotometer and total RNA yield was calculated. These data demonstrate that a substantially higher yield of RNA was synthesized using the HiScribe T7 High Yield RNA Synthesis Kit as compared to the competitor's kit.

(B) Transcript integrity – 150 ng of column purified RNA was run a 1.2% denaturing agarose gel, stained with ethidium bromide and visualized by UV fluorescence. The data demonstrate greatly improved transcript integrity after extended duration RNA synthesis reactions using the HiScribe T7 High Yield RNA Synthesis Kit as compared to the competitor's kit.

Kit Components

The following reagents are supplied with this product:

Show all

NEB #	Component Name	Component #	Stored at (°C)	Amount	Concentration

NEB #	Component Name	Component #	Stored at (°C)	Amount	Concentration
E2040S			**-20**		
	CTP	N0454AVIAL	-20	1 x 0.1 ml	100 mM
	FLuc Control Template	N0426AVIAL	-20	1 x 0.01 ml	0.5 µg/µl
	UTP	N0453AVIAL	-20	1 x 0.1 ml	100 mM
	ATP	N0451AVIAL	-20	1 x 0.1 ml	100 mM
	T7 RNA Polymerase Mix	M0255AVIAL	-20	1 x 0.1 ml	Not Applicable
	GTP	N0452AVIAL	-20	1 x 0.1 ml	100 mM
	10X T7 Reaction Buffer	B2041AVIAL	-20	1 x 0.1 ml	Not Applicable

Product Categories: RNA Synthesis Products

Applications: RNA Labeling, In vitro Synthesis (IVT)

Related Products

Companion Products

- RNA Loading Dye, (2X)
- RNase Inhibitor, Human Placenta
- RNase Inhibitor, Murine
- DNase I (RNase-free)
- Q5® Hot Start High-Fidelity DNA Polymerase
- ssRNA Ladder
- Low Range ssRNA Ladder
- 3´-0-Me-m^{7}G(5')ppp(5')G RNA Cap Structure Analog
- m^{7}G(5')ppp(5')A RNA Cap Structure Analog
- G(5')ppp(5')A RNA Cap Structure Analog
- G(5')ppp(5')G RNA Cap Structure Analog
- m^{7}G(5')ppp(5')G RNA Cap Structure Analog
- Vaccinia Capping System
- mRNA Cap 2 O Methyltransferase
- *E. coli* Poly(A) Polymerase

- Ribonucleotide Solution Mix
- Ribonucleotide Solution Set

Protocols, Manuals & Usage

Protocols

1. DNA Template Preparation (E2040)
2. RNA Synthesis with Modified Nucleotides (E2040)
3. Purification of Synthesized RNA (E2040)
4. Standard RNA Synthesis (E2040)
5. Capped RNA Synthesis (E2040)
6. High Specific Activity Radiolabeled RNA Probe Synthesis (E2040)
7. Evaluation of Reaction Products (E2040)
8. Poly(A) Tailing of RNA using E. coli Poly(A) Polymerase (NEB# M0276)
9. Protocol for Co-transcriptional capping using CleanCap® Reagent AG from TriLink and HiScribe™ T7 High Yield RNA Synthesis Kit from New England Biolabs®

Manuals

The Product Manual includes details for how to use the product, as well as details of its formulation and quality controls.

manualE2040

FAQs & Troubleshooting

FAQs

1. Can I use the Monarch RNA Cleanup Kits to cleanup my in vitro transcription (IVT) reaction?
2. How can I improve on a low yield of RNA from the transcription reaction?

Troubleshooting

- **Control Reaction**
  The FLuc control template DNA is a linearized plasmid containing the firefly luciferase gene under the transcriptional control of T7 promoter. The size of the runoff transcript is 1.8 kb. The control reaction should yield ≥ 150 µg RNA transcript in 2 hours.

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

  The control plasmid sequence can be found here. The FLuc control template is generated by linearizing the plasmid with StuI.

- **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 denaturing agarose or polyacrylamide gel, DNA template is 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, 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 which 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.

  If premature termination of transcription is found in high specific activity radiolabeled RNA probe synthesis, increase the

concentration of “limiting NTP”. Additional “cold” 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 p

Tech Tips

It is important to mix each component well before setting up reactions.

Make sure reactions are thoroughly mixed.

We recommend incubating the reactions in a dry air incubator or in a PCR machine.

Citations & Technical Literature

Citations

Product Citation Tool

Additional Citations

- Lee, NC., Larionov, V., Kouprina, N. (2015) Highly efficient CRISPR/Cas9-mediated TAR cloning of genes and chromosomal loci from complex genomes in yeast *Nucleic Acids Res*; 43(8), e55. PubMedID: 25690893
- Jaitin, DA., Kenigsberg, E., Keren-Shaul, H., Elefant, N., Paul, F., Zaretsky, I., Mildner, A., Cohen, N., Jung, S., Tanay, A. and Amit, I. (2014) Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science*; 343, 776-779. PubMedID: 24531970

Quality, Safety & Legal

Quality Control Assays

Quality Control tests are performed on each new lot of NEB product to meet the specifications designated for it. Specifications and individual lot data from the tests that are performed for this particular product can be found and downloaded on the Product Specification Sheet, Certificate of Analysis, data card or product manual. Further information regarding NEB product quality can be found here.

Specifications & Change Notifications

Specifications

The Specification sheet is a document that includes the storage temperature, shelf life and the specifications designated for the product. The following file naming structure is used to name these document files: [Product Number]_[Size]_[Version]

E2040S_v1

Certificate of Analysis

The Certificate of Analysis (COA) is a signed document that includes the storage temperature, expiration date and quality controls for an individual lot. The following file naming structure is used to name these document files: [Product Number]_[Size]_[Version]_[Lot Number]

E2040S_v1_0241803	E2040S_v1_10012595	E2040S_v1_10013491
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E2040S_v1_10105808	E2040S_v1_10109077	E2040S_v1_10114318

Safety Data Sheets

The following is a list of Safety Data Sheet (SDS) that apply to this product to help you use it safely.

CTP	FLuc Control Template	UTP
ATP	T7 RNA Polymerase Mix	GTP
10X T7 Reaction Buffer		

Legal and Disclaimers

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