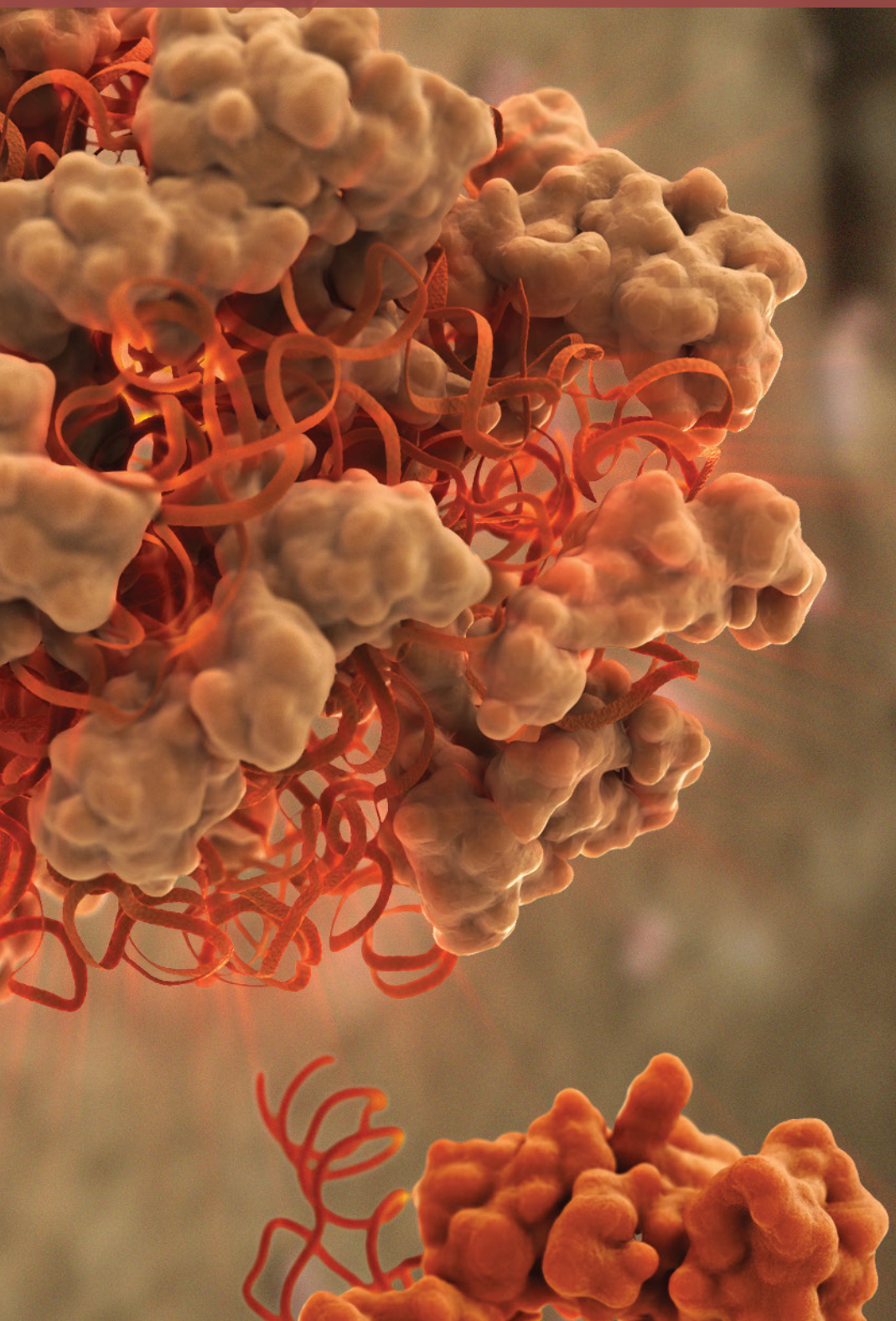
Now includes
RNase R, RNase 4 & Modified Ribonucleotides

RNA Synthesis

FROM TEMPLATE TO TRANSCRIPT



NEW ENGLAND Biolabs®

RNA Synthesis:

Tools to Take You from Template to Transcript

For almost 50 years, NEB® has been a world leader in the discovery and production of reagents for the life science industry. New England Biolabs' enzymology expertise allows us to supply reagents for the synthesis of high-quality RNA – from template generation and transcription, to capping, tailing, and cleanup after synthesis. These products are designed and manufactured by building upon decades of molecular biology experience, so that you can be confident they will work for your application.

In vitro synthesis of single-stranded RNA molecules is a widely used laboratory procedure that is critical to RNA research, as well as to RNA biopharmaceuticals. This technique is versatile in that it allows the researcher to tailor synthesis and introduce modifications to produce a transcript. Downstream applications include biochemical and molecular characterization of RNA for RNA-protein interactions and structural analyses, generation of RNA aptamers, synthesis of functional mRNAs for expression, and generation of small RNAs for alteration of gene expression (e.g., guide RNAs, RNAi). Furthermore, the use of *in vitro* synthesized RNA has been instrumental in the development of RNA vaccines and CRISPR/Cas9 genome editing tools, generation of pluripotent stem cells, screening of RNA inhibitors, as well as development of RNA amplification-based diagnostics.

High-yield robust reactions require optimization of each reaction component. NEB offers six *in vitro* RNA synthesis kits, all of which have been optimized to generate reproducible yields of quality RNA. Additionally, individual components can be purchased for *in vitro* transcription (IVT) and mRNA capping.

NEB's portfolio of research-grade and GMP-grade* reagents enables bench-scale to commercial-scale mRNA manufacturing. Our optimized HiScribe kits enable convenient *in vitro* transcription (IVT) workflows. When it is time to scale up and optimize reaction components, our standalone reagents are readily available in formats matching our GMP-grade offering, enabling a seamless transition to large-scale therapeutic mRNA manufacturing.

Learn more about products for your RNA-related workflow, including mRNA synthesis at **NEBrna.com**

* *"GMP-grade" is a branding term NEB uses to describe reagents manufactured or finished at our Rowley, MA facility, where we utilize procedures and process controls to manufacture reagents under more rigorous conditions to achieve more stringent product specifications, and in compliance with ISO 9001 and ISO 13485 quality management system standards. NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor do we manufacture products in compliance with all of the Current Good Manufacturing Practice regulations.*

mRNA synthesis workflow example & available NEB products

TEMPLATE GENERATION	*IN VITRO* TRANSCRIPTION	RNA CAPPING	POLY(A) TAILING	RNA PURIFICATION
GMP* COMING SOON Q5® Hot Start High-Fidelity DNA Polymerase	HiScribe® T7 mRNA Kit with CleanCap® Reagent AG		*E. coli* Poly(A) Polymerase	Monarch® Spin RNA Cleanup Kit (10 µg)
	HiScribe T7 ARCA mRNA Synthesis Kit (with tailing)			
GMP* phi29 DNA Polymerase	HiScribe T7 ARCA mRNA Synthesis Kit			Monarch Spin RNA Cleanup Kit (50 µg)
GMP* TelN Protelomerase	GMP* HiScribe T7 High Yield RNA Synthesis Components	GMP* Faustovirus Capping Enzyme		Monarch Spin RNA Cleanup Kit (500 µg)
GMP* dNTP solution mixes		GMP* Vaccinia Capping System		
GMP* BspQI*	HiScribe T7 Quick High Yield RNA Synthesis Kit	GMP* mRNA Cap 2´-O-Methyltranferase		Lithium Chloride
GMP* NEBuffer™ 4	HiScribe SP6 High Yield RNA Synthesis Kit	ARCA and other mRNA cap analogs		
DNA Assembly: • NEBuilder HiFi DNA Assembly • Golden Gate Assembly	T3 & SP6 RNA Polymerases	GMP* S-Adenosylmethionine (SAM)		
	GMP* T7 RNA Polymerase			
	Hi-T7 RNA Polymerase			
	Companion Products			**Companion Products**
	GMP* RNase inhibitor (Murine)			Monarch Buffer BX
	RNase Inhibitor (Human Placental)			Monarch Buffer WX
	GMP* Pyrophosphatase, Inorganic (*E. coli*)			Nuclease-free Water
	Pyrophosphatase, Inorganic (Yeast)			
	GMP* DNase I (RNase-free)			
	DNase I-XT			
	GMP* NTPs			
	Modified NTPs			

GMP* = available in GMP-grade

* NEB can offer large-scale preparations of restriction enzymes using Recombinant Albumin (BSA-free)

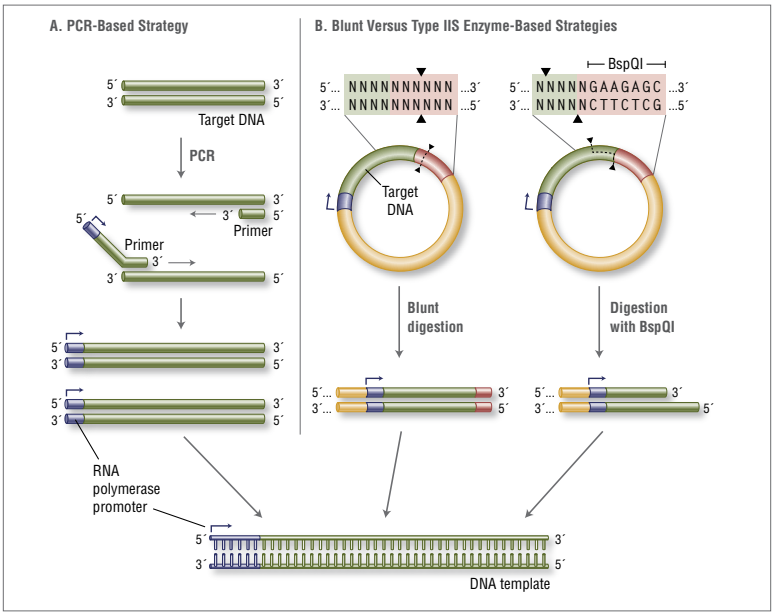
Template Generation

Effective *in vitro* transcription starts with high-quality template. The quality of the template DNA affects transcription efficiency, as well as the integrity of the synthesized RNA. Yield is highly dependent upon template purity. Any purification method may be used, as long as the product is free of contaminating RNase, protein and salts.

Whether your template is linearized plasmid or PCR-amplified DNA, NEB can supply the reagents for high-quality template generation that help you maximize RNA synthesis yields.

- Ensure that your template is error-free by choosing a robust, high-fidelity DNA polymerase for amplification, such as Q5® High-Fidelity DNA Polymerase (NEB #M0491). Reduce non-specific amplification by choosing a hot start formulation, such as Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493). PCR product should be purified and analyzed to estimate concentration, and to confirm amplicon size prior to its use as template in the transcription reaction.
- When using plasmid DNA, it is important to completely linearize the input DNA downstream of the insert with an appropriate restriction enzyme, such as SapI (NEB #R0569) or BspQI (NEB #R0712). The use of type IIS enzymes generates scarless templates, as shown in the figure below. This ensures that the synthesis reaction produces an RNA transcript of defined length, and may help prevent 'template switching' products. NEB offers a number of suitable restriction enzymes for this purpose.
- If you need to introduce changes to your template, the Q5 Site-Directed Mutagenesis Kit (NEB #E0554) enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours.

Figure 1. Transcription Template Generation



(A) PCR can be used to amplify target DNA prior to transcription. A polymerase promoter can be introduced via the upstream primer. Choose a high-fidelity polymerase to ensure that your template is error free.

(B) When using plasmid DNA as a template, linearize with an enzyme that produces blunt or 5´-overhanging ends. Using a type IIS restriction enzyme (e.g., BspQI) allows RNA synthesis with no additional 3´-nucleotide sequence from the restriction site.

RECOMMENDED PRODUCTS

NEB Restriction Enzymes

- Visit NEBRestrictionEnzymes.com for the full list

Q5 and Q5 Hot Start High-Fidelity DNA Polymerases (NEB #M0491 & #M0493)

- ~ 280X fidelity of *Taq* DNA Polymerase
- High specificity and robust yields with minimal optimization
- Superior performance for a broad range of amplicons (from high AT to high GC)
- Hot start formulation allows room temperature reaction setup and reduces non-specific amplification

Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130)

- Fast and simple purification in 5 minutes
- Highly pure DNA eluted in as little as 5 µl

Q5 Site-Directed Mutagenesis Kit (NEB #E0554)

- Rapid site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours

TOOLS & RESOURCES

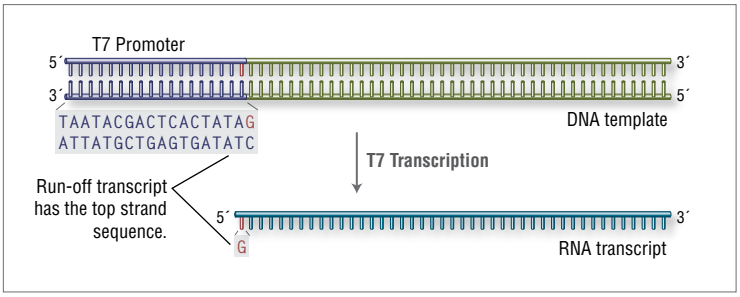
Visit NEBPCRPolymerases.com & NEBRestrictionEnzymes.com to find:

- FAQs and troubleshooting
- Interactive tools to help with experimental design
- Online tutorials for setting up a PCR or RE reaction

In vitro Transcription

In vitro RNA synthesis requires DNA template, enzymes, nucleotides and buffer components. High-yield robust reactions require optimization of each reaction component. NEB offers six *in vitro* RNA synthesis kits, all of which have been rigorously formulated to provide reproducible high yields of quality RNA.

Figure 2. T7 Transcription

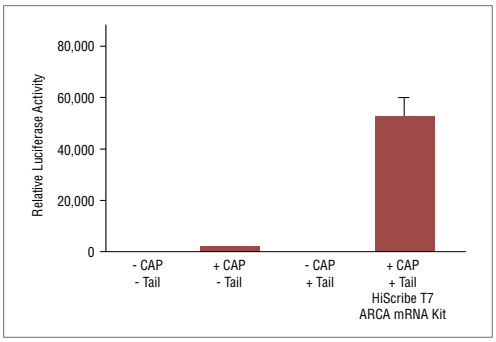


A transcription template contains a A T7 promoter sequence followed by the sequence of interest. The T7 promoter is required for transcription to occur. The sequence of the transcript is the same as the top strand of the DNA template and initiates from G residues encoded in the optimal T7 RNA polymerase promoter.

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 mRNA Kit with CleanCap Reagent AG (NEB #E2080) provides the highest yields of mRNA with a natural Cap-1 structure for a variety of applications. Tailing can be accomplished co-transcriptionally by using a DNA template encoding a poly(A) stretch or enzymatically using *E. coli* Poly(A) Polymerase (NEB #M0276). Additionally, The HiScribe T7 ARCA mRNA Synthesis Kit with Tailing (NEB #E2060) is available for co-transcriptional incorporation of Anti-Reverse Cap Analog, ARCA, using T7 RNA Polymerase resulting in a Cap-0 structure. A poly(A) tail is then added by *E. coli* Poly(A) Polymerase. A separate version of the kit (NEB #E2065), without *E. coli* Poly(A) Polymerase, is available. These kits also include DNase I and LiCl for DNA template removal and quick mRNA purification.

The HiScribe T7 High Yield RNA Synthesis Kit (NEB #E2040) delivers robust RNA synthesis for a wide range of template sizes (Figure 4). Flexible protocols ensure that performance is maintained, even under demanding conditions, such as extended reaction time using very low amounts of template (Figure 6). Protocols are included for partial or complete incorporation of modified or labeled nucleotides in the transcript body, and cap analogs at the RNA 5´ end.

Figure 3. Both Cap and Tail are Required for mRNA Function in Cell Culture

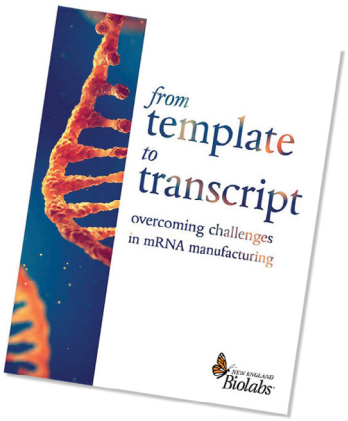


Luciferase expression in U2OS cells. Purified Cypridina luciferase RNA produced as indicated was co-transfected into U2OS cells with purified Gaussia luciferase mRNA. mRNAs produced using the HiScribe T7 ARCA mRNA Kit (with Tailing) are 5´-capped and have 3´ poly(A) tails. After 16 hours incubation at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

RECOMMENDED PRODUCTS

HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080)

- Streamlined workflow with single-step co-transcriptional capping
- CleanCap Reagent AG trinucleotide cap technology results in a natural Cap-1 structure, maximizing translatability and minimizing immune response from synthetic mRNA
- High capping efficiency
- Optimized for high yields
- Suitable for full or partial modified nucleotide substitution



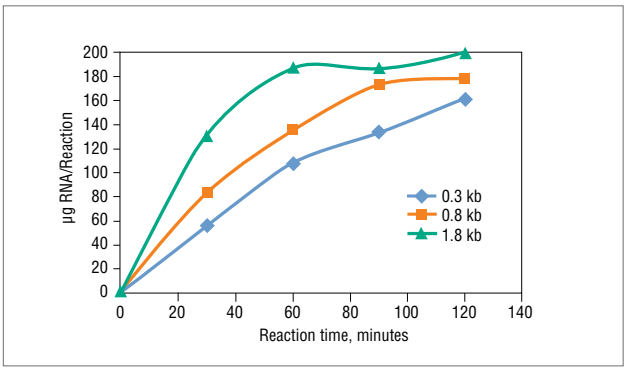
Download our eBook to learn how to overcome challenges with mRNA manufacturing

neb.com/forms/ebook-template-to-transcript

The HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050) utilizes a master mix format, allowing for faster reaction setup. All that is required is the addition of two master mix reagents to your DNA template and water, reducing pipetting errors. DNase I and lithium chloride are included for DNA template removal and quick RNA purification. Alternatively, our Monarch RNA Cleanup Kits can be used for quick cleanup of transcripts (see page 11).

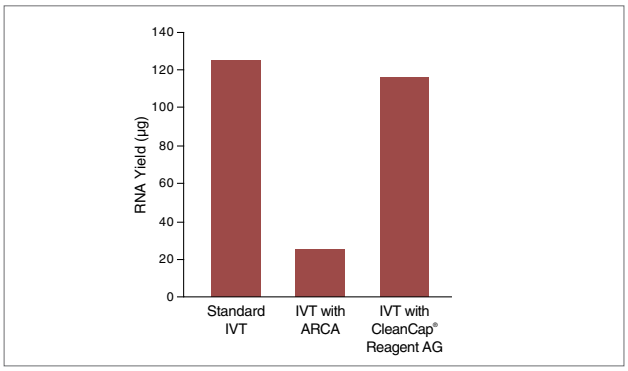
The HiScribe SP6 RNA Synthesis Kit (NEB #E2070) is designed for the *in vitro* transcription of RNA using SP6 RNA Polymerase. This kit is suitable for synthesis of high yield RNA transcripts and for incorporation of cap analogs (not included) or modified nucleotides (not included) to obtain capped, biotin-labeled or dye-labeled RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA for use as probes or targets.

Figure 4. Robust RNA Synthesis from a Variety of Template Sizes using the HiScribe T7 High Yield RNA Synthesis Kit



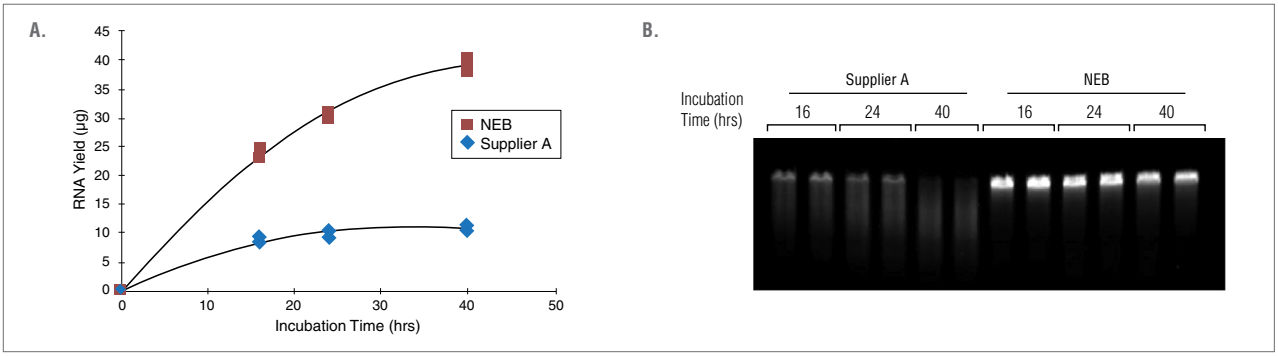
Time course of standard RNA synthesis from three DNA templates of different sizes using HiScribe T7 High Yield RNA Synthesis Kit. Reactions were assembled according to the protocol and incubated at 37°C for the indicated time. Transcripts were purified over spin columns and quantified on a NanoDrop™ Spectrophotometer to calculate reaction yield.

Figure 5. Comparison of RNA Yields from *In Vitro* Transcription Reactions with no cap analog, ARCA, or CleanCap Reagent AG



All reactions were performed with 5 mM CTP, 5 mM UTP and 6 mM ATP. Standard IVT reactions included 5 mM GTP and no cap analog. ARCA reactions contained a 4:1 ratio of ARCA:GTP (4 mM:1 mM). IVT with CleanCap Reagent AG contained 5 mM GTP and 4 mM CleanCap Reagent AG and was performed as described below (Standard mRNA Synthesis). Reactions were incubated for 2 hours at 37°C, purified and quantified by NanoDrop.

Figure 6. Improved RNA Yield and Integrity from Low Template Amount and Extended Duration Transcription Reactions using the HiScribe T7 High Yield RNA Synthesis Kit



Reactions were assembled, in duplicate, according to the manufacturer's 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.

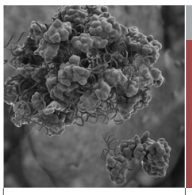
(A) Transcript yield – After column purification, RNA concentration was measured using a NanoDrop spectrophotometer and total RNA yield was calculated. These data demonstrate that a substantially higher yield of RNA was synthesized using the HiScribe T7 High Yield RNA Synthesis Kit as compared to the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

In vitro Transcription (Cont'd)

Recommended HiScribe RNA Synthesis Kits by Application

The HiScribe High Yield RNA Synthesis Kits are ideal for numerous downstream applications. Use the guide below to determine which kit is best suited for your application.



		T7 KITS					SP6 KITS
	APPLICATION	**HiScribe T7 High Yield RNA Synthesis Kit** (#E2040)	**HiScribe T7 Quick High Yield RNA Synthesis Kit** (#E2050)	**HiScribe T7 ARCA mRNA Kit** (#E2065)	**HiScribe T7 ARCA mRNA** (with Tailing) (#E2060)	**HiScribe T7 mRNA Kit with CleanCap Reagent AG** (#E2080)	**HiScribe SP6 RNA Synthesis Kit** (#E2070)
Probe labeling	Fluorescent labeling: FAM, Cyanine (Cy) dyes, etc. • Fluorescent *in situ* hybridization (FISH)	✔	✔				✔
	Non-fluorescent labeling: Biotin, Digoxigenin • *In situ* hybridization • Blot hybridization with secondary detection • Microarray	✔	✔				✔
	High specific activity radiolabeling • Blot hybridization • RNase protection	✔					✔
mRNA & RNA for transfection	Streamlined mRNA synthesis with ARCA co-transcriptional capping and enzymatic poly(A) tailing • Transfection • Microinjection • *In vitro* translation				✔		
	Streamlined ARCA capped RNA synthesis • Template encoded poly(A) tails • Non polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation			✔			
	Co-transcriptional capping with alternate cap analogs • Transfection • Microinjection • *In vitro* translation		✔				✔
	Co-transcriptional capping with CleanCap Reagent AG • Transfection • Microinjection • *In vitro* translation					✔	
	Post-transcriptional capping with Faustovirus Capping Enzyme or Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	✔	✔				✔
	Complete substitution of NTPs: 5-mC, pseudouridine, etc.	✔				✔	✔
	Partial substitution of NTPs: 5-mC, pseudouridine, etc.	✔	✔	✔	✔	✔	✔
	Unmodified RNA		✔				✔
	Hairpins, short RNA, dsRNA • Gene knockdown	✔	✔				✔
Structure, function, & binding studies	Complete substitution of NTPs • Aptamer selection • Isotopic labeling	✔					✔
	Partial substitution of one or more NTPs • Aptamer selection • Structure determination	✔	✔				✔
	Unmodified RNA • SELEX • Structure determination	✔	✔				✔

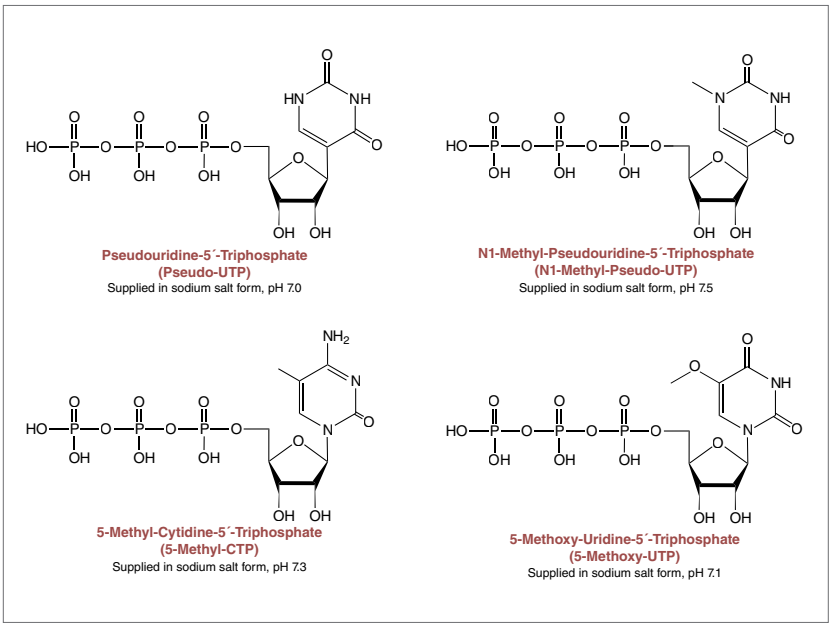
mRNA Modifications: Modified Ribonucleotides

mRNA has emerged as a potent modality for therapeutics. Incorporation of chemically modified ribonucleotides can improve the utility of mRNA by increasing stability, reducing immunogenicity, and enhancing translatability.

Four key modified nucleotides to advance your RNA work are now available from NEB.:

- No detectable RNase, DNase, phosphatase or protease contaminating activities.
- ≥95% purity, as determined by HPLC.
- Optimal activity and stability for up to 24 months.
- Drag and drop compatibility with HiScribe RNA Synthesis Kits.

Figure 7. Modified Ribonucleotides



RECOMMENDED PRODUCTS

N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP) (NEB #N0431)

5-Methyl-Cytidine-5'-Triphosphate (5-Methyl-CTP) (NEB #N0432)

Pseudouridine-5'-Triphosphate (Pseudo-UTP) (NEB #N0433)

5-Methoxy-Uridine-5'-Triphosphate (5-Methoxy-UTP) (NEB #N0434)

- NTPs are alo available as Ribonucleotide Solution Set (**NEB #N0450**) or Ribonucleotide Solution Mix (**NEB #N0466**).

Featured Technical Note

Scan here to download our technical note

Getting ready to scale up your RNA Synthesis?

Visit the Application note portal on **www.neb.com/application-notes** to download our technical note:

"Scaling of High-Yield *in vitro* Transcription Reactions for Linear Increase of RNA Production"

For a generalized set of recommendations for synthesizing high yields of RNA.

mRNA Modifications: Capping

Capping and tailing are key steps in producing active synthetic mRNA for use in functional studies; these modifications prevent degradation and facilitate translation in eukaryotic cells.

RNA Capping

Most eukaryotic cellular mRNAs are modified at their 5´ends by the addition of a 7-methyl guanosine (m^7G) residue in a 5´ → 5´ triphosphate linkage to the first encoded nucleotide of the transcript. The mRNA cap structure engages critical translation factors to recruit ribosomes to mRNAs, promoting translation.

Cap structures can be added to *in vitro* transcripts in two ways:

- After transcription by using capping enzymes, GTP and S-adenosyl methionine (SAM)
- During transcription by including cap analogs

RECOMMENDED PRODUCTS

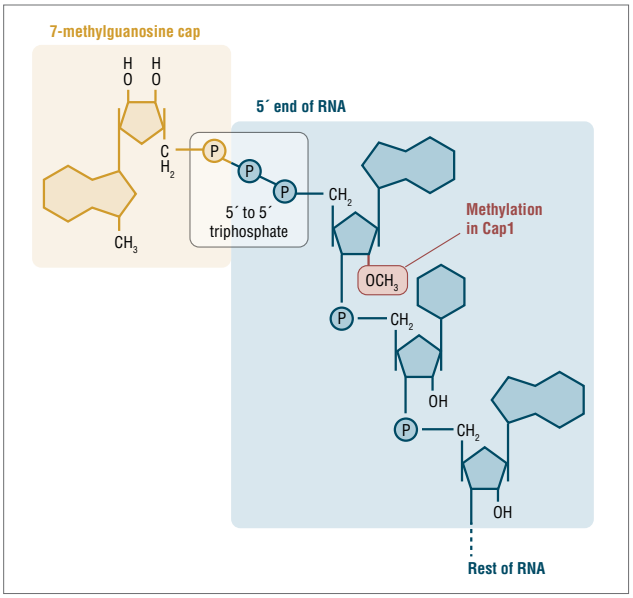
Faustovirus Capping Enzyme (NEB #M2081)

- Experience improved capping efficiency, even on difficult substrates
- Achieve robust capping with less enzyme
- Set up reactions under a broad temperature range, for added flexibility

mRNA Cap 2´-O-Methyltransferase (NEB #M0366)

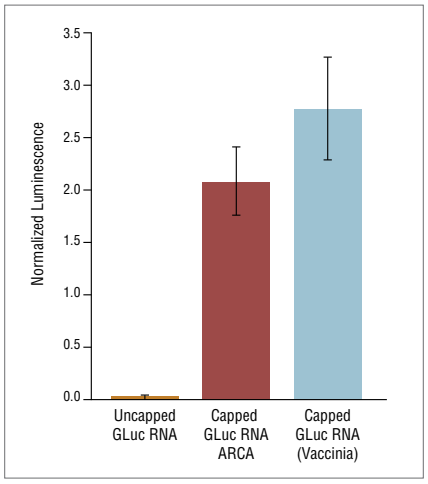
- Enhances translation of mRNA
- Improves mRNA expression *in vivo*

Figure 8. 5´ Cap Structure



Schematic representation of mRNA 5´ cap structure indicating the 7-methylguanosine, shown in yellow, and the 5´ end of the mRNA, shown in blue. The 2´-O-methyl group present in Cap-1 structures is shown in red.

Figure 9. GLuc Expression



Purified Cap-0 and uncapped GLuc mRNA were transfected into HeLa cells and incubated overnight (16 hrs.) at 37°C. Cell culture supernatants from each well were assayed for GLuc and CLuc activity and luminescence values were recorded. The GLuc luminescence values were normalized to the luminescence values of Cap-0 CLuc RNA.

Post-transcriptional Enzymatic mRNA Capping

Highest efficiency mRNA capping is achieved using the Faustovirus Capping Enzyme (NEB #M2081). This system has three enzymatic activities (RNA triphosphatase, guanylyltransferase, guanine methyltransferase); all are necessary for the addition of a complete Cap-0 structure, $m^7Gppp5´N$. *In vitro* transcripts can be capped in less than one hour in the presence of the capping enzyme, reaction buffer, GTP and the methyl donor, SAM. All capped structures are added in the proper orientation for recognition by the translational machinery, unlike co-transcriptional addition of some cap analogs (2).

ADVANTAGES

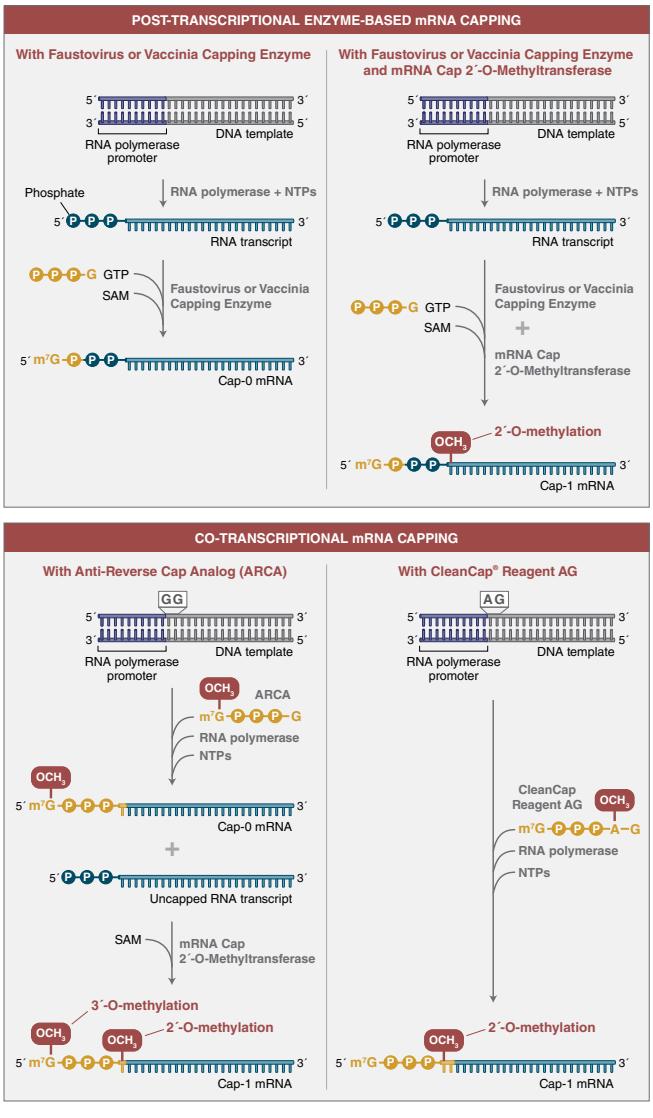
- Natural caps
- Cap-0 to Cap-1 possible in same reaction using 2´-O-Methyltransferase

Co-transcriptional Capping with Dinucleotide Cap Analogs

Anti-Reverse Cap Analog (ARCA) [3´-0-Me-m^{7}G(5´)ppp(5´)G RNA Cap Structure Analog, (NEB #S1411)] is the preferred cap analog for co-transcriptional capping. Transcription with ARCA produces 100% translatable capped transcripts, because it can only incorporate in the 'correct' orientation, where the N7-methylguanosine is at the terminus [m^{7}G(5´)pppG-RNA] (3,4).

In contrast, the standard cap analog [m^{7}G(5´)ppp(5´)G RNA Cap Structure Analog (NEB #S1404)] can be incorporated in either orientation [m^{7}G(5´)pppG-RNA] or [G(5´)pppm^{7}G-RNA], resulting in a mixture of transcripts (3,5). mRNAs with cap analog incorporated in the incorrect orientation are not efficiently translated, resulting in lower protein yields (2). The RNA products are a mixture of 5´-capped and 5´-triphosphorylated transcripts. This may necessitate purification or treatment with a phosphatase in order to avoid unintended immune stimulation by 5´-triphosphorylated RNA.

Figure 10. Schematic representation of alternative mRA synthesis workflows



Enzyme-based capping (top) is performed after in vitro transcription using 5´-triphosphate RNA, GTP, and S-adenosyl- methionine (SAM). Cap-0 mRNA can be converted to Cap-1 mRNA using mRNA cap 2´-O-methyltransferase (MTase) and SAM in a subsequent or concurrent reaction. The methyl group transferred by the MTase to the 2´-O of the first nucleotide of the transcript is indicated in red. Conversion of ~100% of 5´-triphosphorylated transcripts to capped mRNA is routinely achievable using enzyme-based capping.

Co-transcriptional capping (bottom) uses an mRNA cap analog, shown in yellow, in the transcription reaction. For ARCA (anti reverse cap analog) (left), the cap analog is incorporated as the first nucleotide of the transcript. ARCA contains an additional 3´-O-methyl group on the 7-methylguanosine to ensure incorporation in the correct orientation. The 3´-O-methyl modification does not occur in natural mRNA caps. Compared to reactions not containing cap analog, transcription yields are lower. ARCA-capped mRNA can be converted to Cap-1 mRNA using mRNA cap 2´-O-MTase and SAM in a subsequent reaction. CleanCap Reagent AG (right) uses a trinucleotide cap analog that requires a modified template initiation sequence. A natural Cap-1 structure is accomplished in a single reaction.

RECOMMENDED PRODUCTS

HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080)

- Streamlined workflow with single-step co-transcriptional capping
- CleanCap Reagent AG trinucleotide cap technology results in a natural Cap-1 structure, maximizing translatability and minimizing immune response from synthetic mRNA
- High capping efficiency
- Optimized for high yields
- Suitable for full or partial modified nucleotide substitution

HiScribe T7 ARCA mRNA Synthesis Kit (with tailing – NEB #E2060) (without tailing – NEB #E2065)

- Faster workflow takes you from IVT and capping to purification in 2 hours
- Enables incorporation of modified bases
- High efficiencies with correctly-oriented ARCA caps
- All-inclusive kit provides reagents for twice the reactions than competitors' kits

mRNA Modifications: Capping (Cont'd)

Cap-1 Modification and Co-transcriptional Trinucleotide Capping

The Cap-1 structure has been reported to enhance mRNA translation efficiency (6) and hence may help improve expression in mRNA transfection and in microinjection experiments.

Cap-0 transcripts can be enzymatically converted to cap-1 *in vitro*. mRNA Cap 2´-O-Methyltransferase (NEB #M0366) adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (Cap-0) resulting in a Cap-1 structure. Alternatively, Cap-1 mRNA can be synthesized co-transcriptionally with a trinucleotide cap analog such as CleanCap Reagent AG. The use of CleanCap reagent AG results in significant advantages over traditional dinucleotide co-transcriptional capping. CleanCap Reagent AG is a trinucleotide with a 5´-m7G joined by a 5´-5´ triphosphate linkage to an AG sequence. The adenine has a methyl group on the 2´-O position. The incorporation of this trinucleotide in the beginning of a transcript results in a Cap-1 structure.

Figure 11. Molecular structure of CleanCap Reagent AG

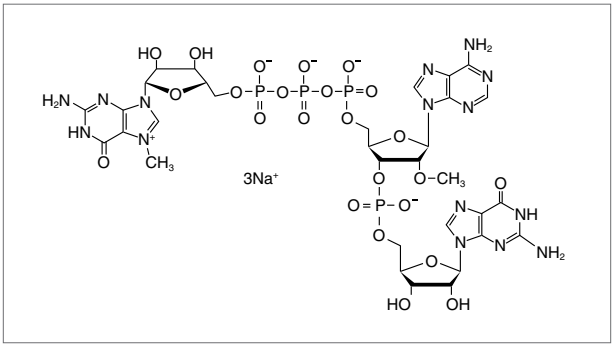
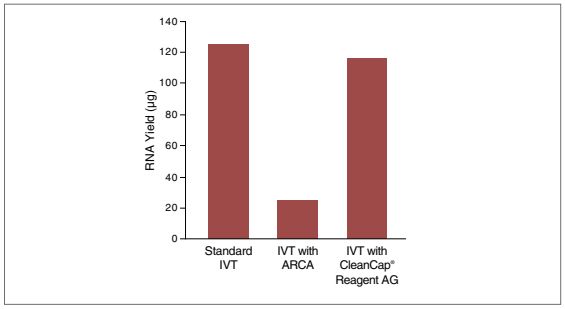


Figure 12. Comparison of RNA Yields from *In Vitro* Transcription Reactions with no cap analog, ARCA, or CleanCap Reagent AG



RNA Cap Analog Selection Chart

The 5´ terminal m7G cap present on most eukaryotic mRNAs promotes translation, *in vitro*, at the initiation level. For most RNAs, the cap structure increases stability, decreases susceptibility to exonuclease degradation, and promotes the formation of mRNA initiation complexes. Certain prokaryotic mRNAs with 5´ terminal cap structures are translated as efficiently as eukaryotic mRNA in a eukaryotic cell-free protein synthesizing system. Splicing of certain eukaryotic substrate RNAs has also been observed to require a cap structure.

PRODUCT	APPLICATION
HiScribe T7 mRNA Kit with CleanCap Reagent AG* *kit only	• High yield • Natural Cap-1 structure • Produces 100% translatable transcripts • Highest efficiency capping
Anti-Reverse Cap Analog 3´-O-Me-m^{7}G(5´) ppp(5´)G	• Produces 100% translatable capped transcripts • Co-transcriptional capping with T7 (NEB #M0251), Hi-T7 (NEB #M0658), SP6 (NEB #M0207) and T3 RNA polymerases (NEB #M0378) • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection
Standard Cap Analog m^{7}G(5´)ppp(5´)G	• Co-transcriptional capping with T7, Hi-T7, SP6 and T3 RNA polymerases • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection

PRODUCT	APPLICATION
Unmethylated Cap Analog G (5´)ppp(5´)G	• Co-transcriptional capping with T7, Hi-T7, SP6 and T3 RNA polymerases • Synthesis of unmethylated G capped RNA
Methylated Cap Analog for A +1 sites m^{7}G(5´)ppp(5´)A	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection
Unmethylated Cap Analog for A +1 sites G(5´)ppp(5´)A	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of unmethylated G capped RNA • Synthesis of A capped RNA

mRNA Modifications: Tailing

Poly(A) Tailing

Poly(A) tailing of RNA is another key step that has been found to increase the stability and translation efficiency of transfected *in vitro*-transcribed RNA. *In vivo*, poly(A) tails recruit poly(A) binding proteins to mRNAs, conferring stability by inhibiting exonucleases. In addition, poly(A) binding protein interactions with the cellular translation machinery figure prominently in the determination of translation efficiency.

Obtaining *in vitro* transcripts with 3´- poly(A) tails can be achieved in 2 ways:

- By enzymatic polyadenylation after transcription
- By encoding a poly(A) stretch in the transcription template

Poly(A) tails can be added after transcription using *E. coli* Poly(A) Polymerase (NEB #M0276) and ATP. Poly(A) tail addition is template independent in this case.

Poly(A) stretches in transcription templates can be encoded in plasmid templates. However, it is advisable to carefully design the template so that restriction enzyme linearization of the plasmid yields a template that encodes a poly(A) tail with no extra nucleotides. We recommend placing a BspQI (NEB #R0712) site immediately downstream of the poly(A) stretch to achieve this.

Alternatively, a poly(A) stretch can be added during PCR-based generation of transcription templates. In this approach, a reverse primer that contains a poly(dT) (e.g., dT120) stretch at the 5´ end is used (7).

RECOMMENDED PRODUCTS

E. coli Poly(A) Polymerase (NEB #M0276)

- Enhances translation of RNA transfected into eukaryotic cells

HiScribe T7 ARCA mRNA Synthesis Kit (with tailing) (NEB #E2060)

- Poly(A) Polymerase included for enzymatic polyadenylation

HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080)

- For template-encoded poly(A) tailing

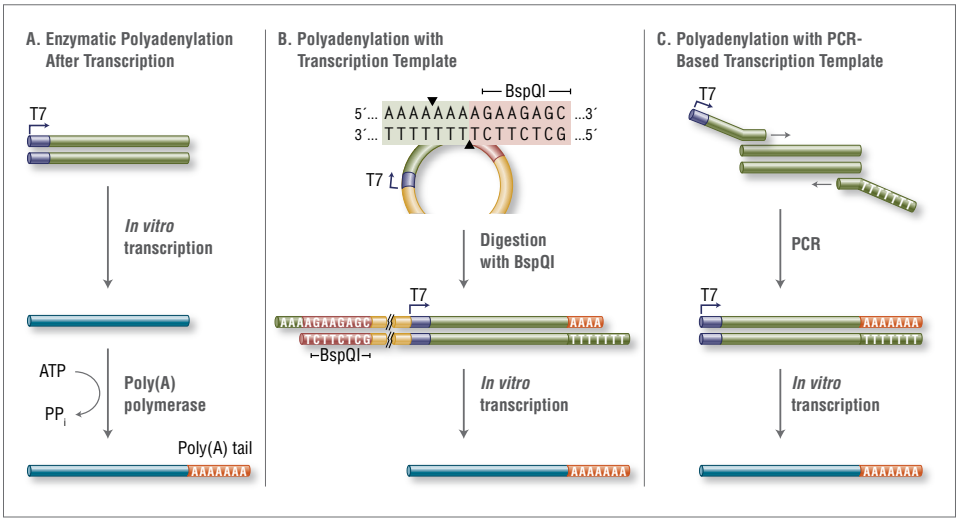
ADVANTAGES

- No additional steps with template-encoded poly(A) tails
- No introduction of poly(A) tracts into templates required for enzymatic polyadenylation with *E. coli* Poly(A) Polymerase

DISADVANTAGES

- Template generation can be difficult when using template-encoded poly(A) tails
- Enzymatic polyadenylation with *E. coli* Poly(A) Polymerase requires an additional step and produces a range of A-tail lengths

Figure 13. Polyadenylation Strategies



Polyadenylation can be done several ways: (A) enzymatically after transcription, (B) digestion of desired template with BspQI followed by transcription, or (C) by adding the tail to a template via PCR.

mRNA Modifications: Template Removal

Removing the template DNA after IVT can greatly simplify downstream processing. Enzymatic digestion of template DNA is the method of choice due to its simple implementation. NEB supplies DNase I (RNase-free) (NEB #M0303) and DNase I-XT (NEB #M0570) that are highly effective in digesting template DNA after IVT. While DNase I (RNase-free) is inhibited by salt concentrations >50 mM, DNase I-XT, which has been engineered by NEB scientists for high salt tolerance, exhibits optimal activity between 50-100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. This increased salt tolerance enables DNase I digestion without buffer adjustment, further simplifying the workflow. After DNase I digestion, the IVT reactions can be subjected to downstream processing to remove IVT reagents and enzymes and DNase I from the synthetic mRNA. NEB's Monarch Spin RNA Cleanup Kits (NEB #T2030, T2040, T2050) are designed for the purification of 10–500 µg of IVT RNA in a simple and quick spin-column format.

RECOMMENDED PRODUCTS

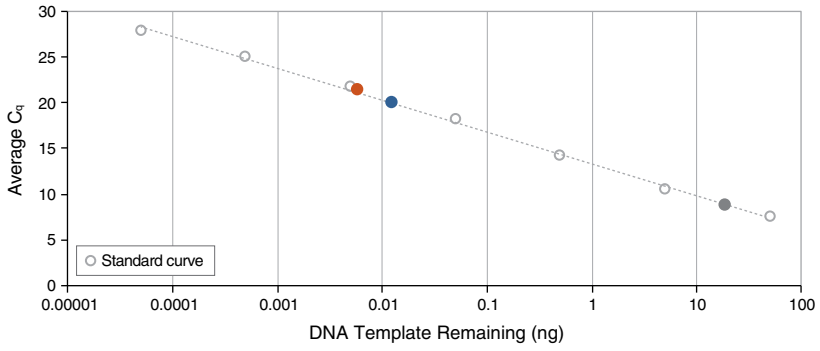
DNase I-XT (NEB #M0570)

- Degredation of DNA templates in transcription reaction

DNase I (RNase-free) (NEB #M0303)

- Ideal for removal of contaminating genomic DNA from RNA samples

Figure 14. DNase I-XT (#M0570) removes more DNA from IVT reactions and RNA preparations.



Sample	Average C_q	DNA Template Remaining (ng)	Residual DNA (%)	Fold DNA Reduction
No DNase	8.83	18.76	–	–
TURBO DNase (2U)	20.14	0.012	0.064	~1,500
DNase I-XT (2U) (NEB #M0570)	21.26	0.006	0.032	~3,000

In vitro transcription reactions (20 µl) were treated with 1) no DNase I; 2) 2 units TURBO® DNase or 3) 2 units DNase I-XT for 15 minutes at 37°C. Each sample was then purified using the Monarch RNA Cleanup Kit (500 µg, NEB #T2050) and eluted in nuclease-free water (50 µl). The level of residual DNA contamination was quantified by real-time PCR using the Luna Universal Probe qPCR Master Mix (NEB M3004). Average Cq (quantification cycle) values for each sample were compared to a standard curve (gray) to determine the percent of residual, PCR-amplifiable DNA. Both TURBO DNase and DNase I-XT require no dilution of the IVT reaction prior to DNase digestion, however, more DNA template is removed from an IVT reaction and undetectable by qPCR when treated with DNase I-XT.

mRNA Modifications: RNA Analysis

Tandem liquid chromatography-mass spectrometry (LC-MS) is a direct detection method that can provide reproducible and accurate identification, as well as qualitative and quantitative analysis of RNA. LC-MS allows for direct analysis of mRNA without amplification or conversion into DNA (8). Modifications that can be challenging for sequence-based methods (such as m1-pseudouridine, 5´ capping and 3´ poly(A) tailing) can be detected and, in some cases, quantified using LC-MS analysis (9).

For the analysis of global nucleoside content, the Nucleoside Digestion Mix (NEB #M0649) digests RNA molecules into nucleosides from which RNA modifications can be accurately identified and quantified.

Figure 15. RNase 4 cuts at U/A and U/G

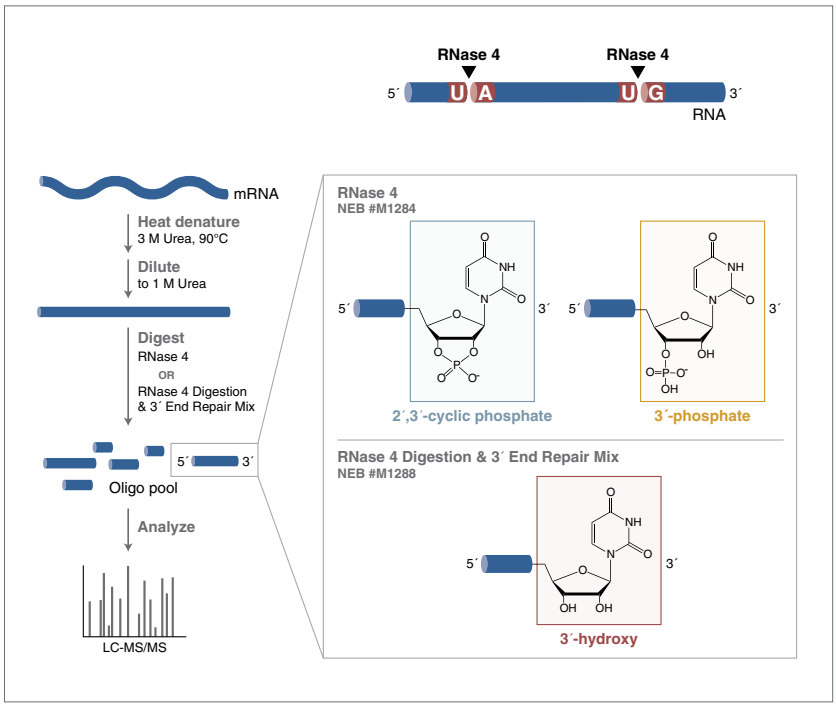
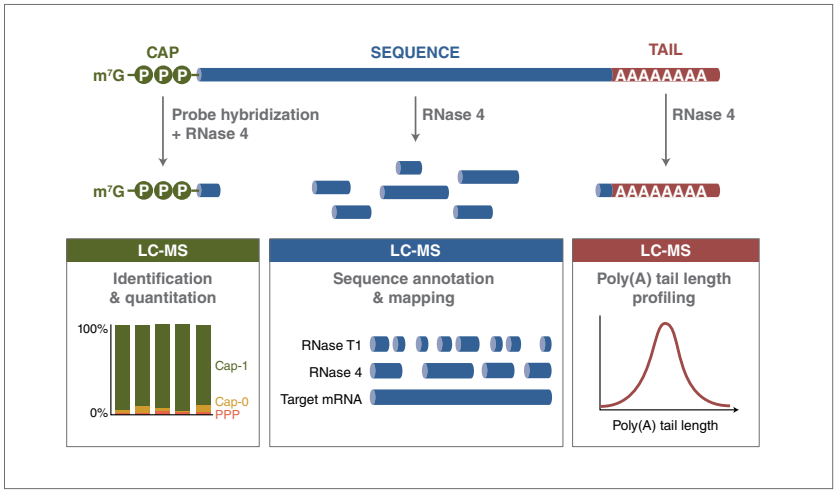


Figure 16. RNase 4 enables comprehensive mRNA analyis by LC-MS/MS



RECOMMENDED PRODUCTS

RNase 4 Digestion and 3' End Repair Mix (NEB #M1288)

- RNase 4 Digestion and 3´End Repair Mix is a coformulation of RNase 4 (cut sites: U/A and U/G) and T4 Polynucleotide Kinase that simplifies RNase 4 digestion products into a pool of U-ending RNA oligonucleotides with homogeneous 3´-hydroxyl termini.

RNase 4 (NEB #M1284)

- RNase 4 is a single-stranded endoribonuclease that cleaves RNA at uridine-purine (U/R) dinucleotide sites.

Nucleoside Digestion Mix (NEB #M0649)

- Digests RNA molecules into nucleosides from which RNA modifications can be accurately identified and quantified.

TOOLS AND RESOURCES

- For mRNA 5´-cap analysis, use stand-alone RNase 4 with our protocol for DNA Probe-Directed Analysis of mRNA 5´Cap Structures https://www.neb.com/en-us/protocols/2024/03/04/rnase-4-neb-m1284-dna-probe-directed-analysis-of-mrna-5-cap-structures

Schematic of the digestion workflow with RNase 4 or RNase 4 Digestion and 3´ End Repair Mix. The RNA sample is first heat denatured at 90°C in the presence of 3 M urea (user supplied) for 10 minutes. After dilution to 1 M urea, RNase 4 digestion reactions are performed in 1X NEBuffer r1.1 provided as a 10X stock solution. The resulting oligonucleotide pool is directly analyzed by LC–MS/MS. Boxes show the 3´ end chemistry of product oligonucleotides after RNA digestion with RNase 4 (gray; NEB #M1284) or RNase 4 Digestion and 3´ End Repair Mix (orange; NEB #M1288).

Cap: Relative populations of 5´ mRNA ends are identified and measured with the aid of a complementary DNA probe that blocks cleavage by RNase 4. Isolation of the cleaved product prior to LC–MS/MS analysis can be performed using a probe that contains an affinity tag. RNase 4 enables predictable, site-specific generation of 5´ end products with a simple experimental design.

Sequence: To verify the mRNA sequence and its modification status, samples are fully digested with RNase 4 to produce a series of defined oligonucleotides in sizes that are amenable to LC–MS/MS analysis. A coverage map can be generated by aligning the observed oligonucleotides to the reference sequence. One or more additional RNases (e.g., RNase T1) can be used in parallel digestions to increase the total coverage of mRNA.

Tail: RNase 4 can be used to assess the mRNA 3´ end by cutting and releasing the poly(A) tail for length profiling.

Circular RNA

Circular RNA (circRNA) has emerged as a promising therapeutic modality where enhanced durability and protein expression is desired. Circular RNAs lack a 3' poly(A) tail and 5' cap structure and are more resistant to exonucleases.

RECOMMENDED PRODUCT

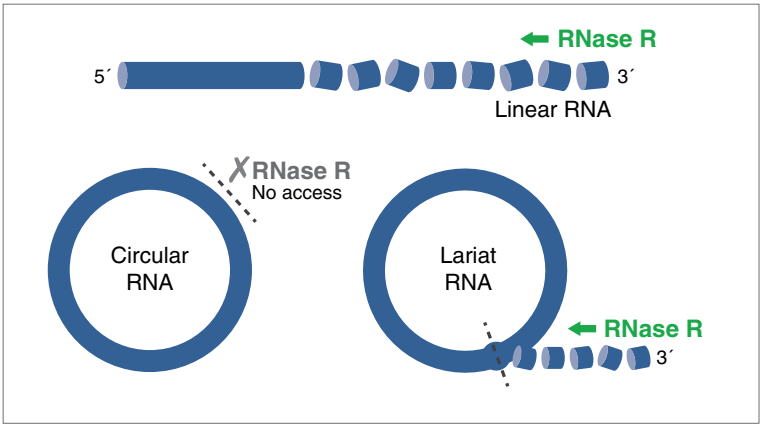
RNase R
(NEB #M0100)

- IHighly processive 3´ to 5´ exoribonuclease
- Digests nearly all linear RNAs with an accessible 3´ end, including rRNA and mRNA
- Enriches for circular RNAs and lariat RNAs from total RNA preps
- Requires magnesium for activity
- Inactivated with the addition of excess EDTA

COMPANION PRODUCTS

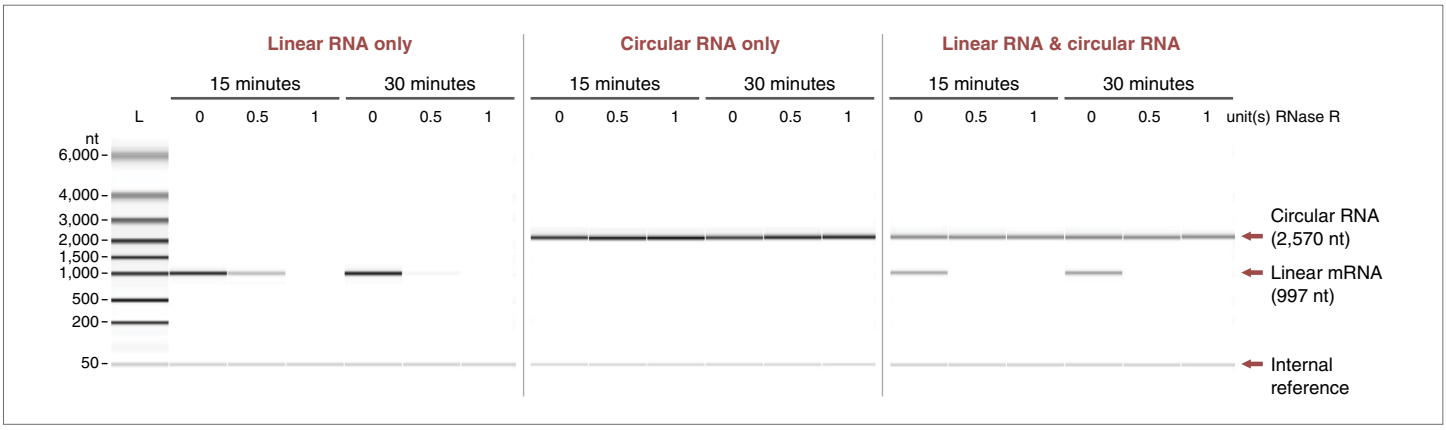
T4 RNA Ligase 1
(NEB #M0204)

Figure 17. RNase R digests linear RNA with an accessible 3´ end



RNase R digests linear RNA with an accessible 3´ end and can be used to enrich for circular and lariat RNA. RNase R can be used to digest linear RNAs. Circular RNAs are closed RNA molecules that are resistant to RNase R digestion. Lariat RNAs have a looped structure with a single-stranded RNA region at the 3´ end. RNase R can digest the linear RNA at the 3´ end, but stops at the branch point of the lariat, preserving the looped RNA.

Figure 18. RNase R efficiently degrades linear RNA and leaves circular RNA intact



NEB® RNase R efficiently degrades linear RNA and leaves circular RNA intact. Adding 1 unit of NEB RNase R per 1 µg of RNA sample containing only linear RNA, only circular RNA, or both linear and circular RNA results in circular RNA maintenance and linear RNA digestion. This reaction ran for 15 or 30 minutes at 37°C in 1X RNase R Reaction Buffer.

Purification after RNA Synthesis

Purification of RNAs from enzymatic synthesis or modification reactions is essential before use. After RNA synthesis by *in vitro* transcription, unincorporated nucleotides, short aborted transcripts, enzymes and buffer components must be removed before using the RNA for downstream applications including transfection, microinjection or RNP formation. Complete removal of small molecule and enzyme reaction components is also necessary after protocols such as RNA labeling, DNase I treatment, Proteinase K treatment, and mRNA capping.

RNA can be purified in various ways, including phenol/chloroform extraction and ethanol precipitation, lithium chloride precipitation, or by gel purification. Silica-based columns are a popular and user-friendly method for fast RNA cleanup. Column-based cleanup methods also provide an easy way to concentrate purified RNA by using low elution volumes. NEB is proud to offer a family of high performance and easy to use RNA cleanup kits for all your RNA workflows.

The Monarch Spin RNA Cleanup Kits provide a fast and simple column-based solution for RNA cleanup and concentration after *in vitro* transcription or other reactions. For convenience and flexibility, Monarch Spin RNA Cleanup Kits are available in three different binding capacities: 10 µg, 50 µg and 500 µg, each containing unique columns designed to prevent buffer retention, ensure no carryover of contaminants and to prevent elution of silica particles, a common annoyance in RNA cleanup applications. The simple bind-wash-elute protocol can be completed in minutes and is simplified by the inclusion of a single wash buffer.

Specifications:

MONARCH RNA CLEANUP KIT	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity	10 µg	50 µg	500 µg
RNA Size Range	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery	70–100%		
Elution Volume	6–20 µl	20–50 µl	50–100 µl
Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time
Common Downstream Applications	RT-PCR, RNA library prep for NGS, small RNA library prep for NGS, RNA labeling	RT-PCR, RNA library prep for NGS, formation of RNP complexes for genome editing, microinjection, RNA labeling, transfection	RT-PCR, RNA library prep for NGS, RNA labeling, RNAi, microinjection, transfection

RECOMMENDED PRODUCTS

Monarch Spin RNA Cleanup Kit (10 µg) (NEB #T2030)

Monarch Spin RNA Cleanup Kit (50 µg) (NEB #T2040)

Monarch Spin RNA Cleanup Kit (500 µg) (NEB #T2050)

ADVANTAGES

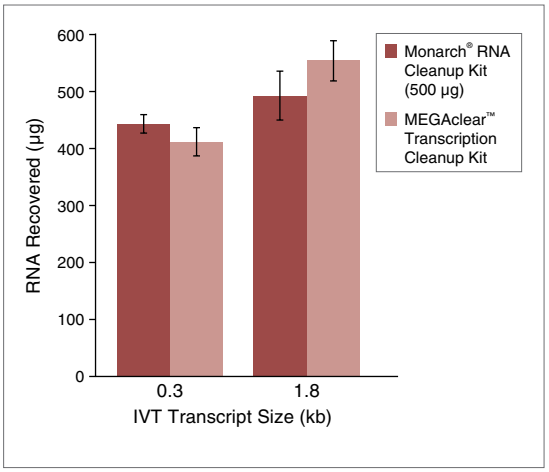
- Three binding capacities for versatility in any applications
- Clean up RNA in 5–10 minutes with simple bind/wash/elute protocol, using a single wash buffer
- Prevent buffer carryover and elution of silica particles with optimized column design
- High yields – 70-100% recovery
- High purity – $A_{260/280}$ and $A_{260/230}$ ≥ 1.8
- Bind up to 500 µg of RNA (NEB #T2050)
- Elute in as little as 6 µl (NEB #T2030)
- Enjoy the flexibility of columns and buffers available separately (see page 12)

TOOLS AND RESOURCES

Visit **www.neb.com/MonarchRNACleanup** to find:

- Protocol and optimization tips videos
- Performance data
- Troubleshooting guide for RNA cleanup, also shown on page 17

Figure 19. The Monarch Spin RNA Cleanup Kit (500 µg) cleans up large-scale *in vitro* transcription reactions and generates yields consistent with other large-scale RNA cleanup kits



0.3 kb and 1.8 kb RNAs were transcribed using the HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050). Following DNase I treatment (4 U DNase I, 37°C, 15 min), transcription reactions were pooled and 200 µl were cleaned up using either the NEB Monarch Spin RNA Cleanup Kit (500 µg, dark red) or the MEGAClear Transcription Clean-Up Kit (Thermo Fisher Scientific). In vitro transcribed RNA was eluted twice with 100 µl of nuclease-free water following a 5-minute on-column incubation (room temperature for Monarch and 65° C for MEGAClear). Recovery of the synthesized RNA transcript was calculated from the resulting A_{260} as measured using a Trinean DropSense™ 16. The Monarch Spin RNA Cleanup Kit (500 µg, NEB #T2050) produces similar RNA yields as the MEGAClear Kit for large-scale in vitro transcription reactions, without high temperature elution.

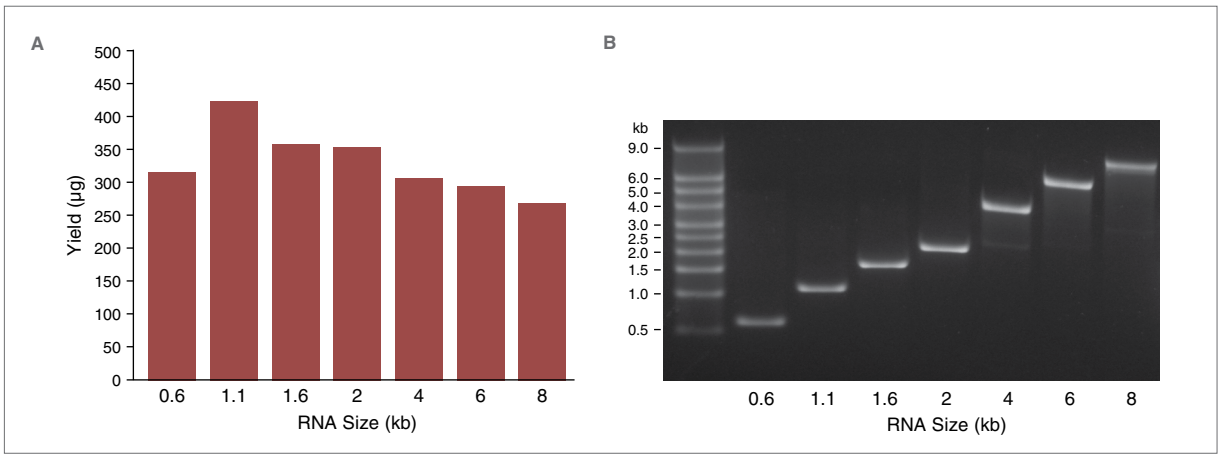
COMPANION PRODUCTS

Monarch Spin Columns S1A (10 µg)
(NEB #T2037)

Monarch Spin Columns S2A (50 µg)
(NEB #T2047)

Spin Columns S2B (500 µg)
(NEB #T2057)

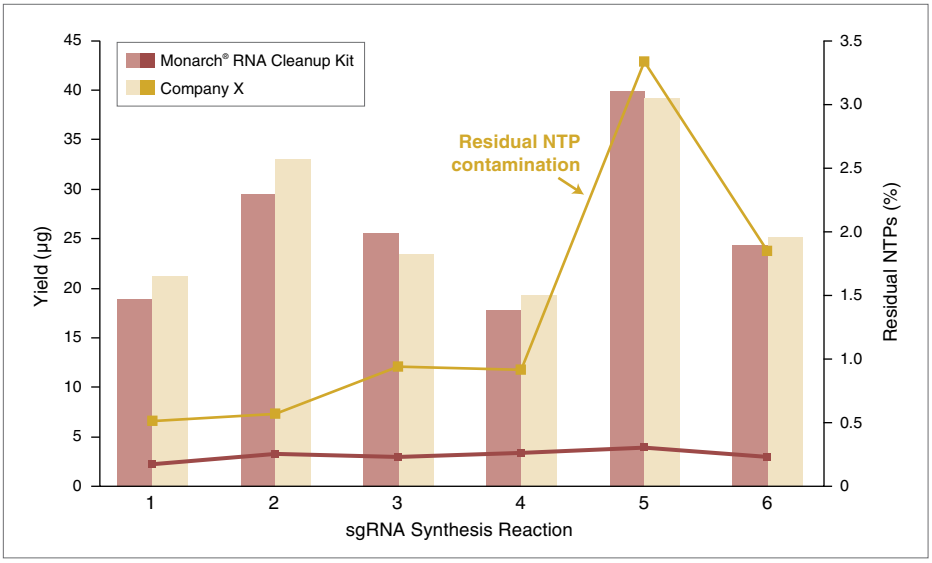
Figure 20. The Monarch RNA Cleanup Kit (500 µg) is suitable for cleaning up large quantities (>250 µg) of RNA from *in vitro* transcription reactions



A. RNA transcripts of varying sizes (0.6-8 kb) were synthesized using the HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050). 40 µl of each in vitro transcription (IVT) reaction was cleaned up using the Monarch RNA Cleanup Kit (500 µg) (NEB #T2050). RNA yields were calculated from the resulting A_{260}, measured using a Nanodrop™ spectrophotometer and ranged from 268–425 µg of RNA per IVT reaction.

B. RNA integrity (200 ng/lane) was assessed on a 1% agarose-TBE gel stained with SYBR® Gold.

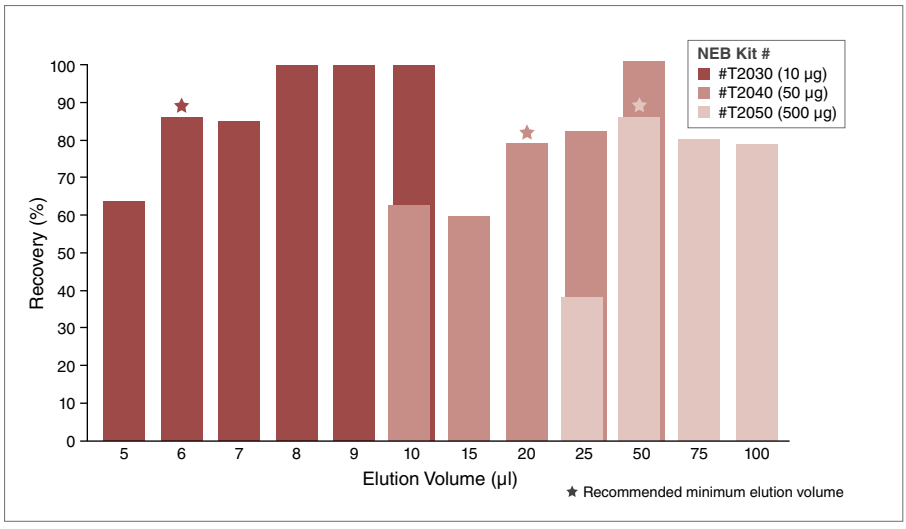
Figure 21. The Monarch Spin RNA Cleanup Kit (50 µg) produces sgRNA yields consistent with other competitor RNA cleanup kits and with lower residual NTP contamination



Six different sgRNA synthesis reactions from the EnGen® sgRNA Synthesis Kit, S. pyogenes (NEB #E3322) were cleaned up using either the Monarch Spin RNA Cleanup Kit (50 µg, NEB #T2040) or a competitor kit (according to manufacturer's recommendations) and eluted in 50 µl nuclease-free water. sgRNA yield was calculated from the resulting A_{260}, measured using a Trinean DropSense 16. The Monarch Spin RNA Cleanup Kit produced sgRNA yields consistent with other commercially available RNA cleanup kits.

Following cleanup, residual nucleotides (NTPs) were measured by LC-MS and are reported as percent area NTPs (rATP+rCTP+rGTP+rUTP)/percent area sgRNA). The NEB Monarch Spin RNA Cleanup Kit consistently outperforms other commercially available RNA cleanup kits in the removal of residual NTPs from sgRNA synthesis reactions.

Figure 22. Recovery of RNA from Monarch Spin RNA Cleanup Kits with Varying Elution Volumes

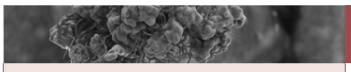


10, 50 or 500 µg of RNA (6S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch Spin RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040)(500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A_{260} as measured using a Trinean DropSense 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch Spin RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch Spin RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch Spin RNA Cleanup Kit (500 µg, NEB #T2050).

RNA Cleanup Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
Low RNA Yield	Reagents added incorrectly	Check protocol to ensure correct buffer reconstitution, order of addition of buffers and ethanol, and proper handling of column flow-through and eluents.
	Insufficient mixing of reagents	Ensure the ethanol is thoroughly mixed with RNA sample and RNA cleanup binding buffer (Buffer BX) before applying the sample to the RNA cleanup column.
	Incomplete elution during prep	Ensure the nuclease-free water used for elution is delivered directly to the center of the column so that the matrix is completely saturated. Larger elution volumes, multiple elutions, and longer incubation times can increase yield of RNA but will dilute the sample and may increase processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient.
	High degree of RNA secondary structure	Binding and elution of smaller RNAs (< 45 nt) can be affected by secondary structure of the RNA molecules. If poor yield of a small RNA is observed, we recommend diluting your sample with 2 volumes of ethanol instead of one volume in Step 2 of the protocol.
Purified RNA is Degraded	RNase contamination	In order to avoid RNase contamination during RNA cleanup, make sure to work on a clean lab bench, wear gloves and use disposable RNase-free pipet tips and microfuge tubes (not provided). Keep all kit components tightly sealed when not in use.
	Improper storage of RNA	Purified RNA should be used immediately in downstream applications or stored at -70°C.
Low $A_{260/230}$ Ratios	Residual guanidine salt carry-over	Ensure wash steps are carried out prior to eluting sample. Use care to ensure the tip of the column does not contact the flow-through. If unsure, repeat centrifugation. When reusing collection tubes, blot the rim of the tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
Low Performance of RNA in Downstream Steps	Salt and/or ethanol carry-over	Ethanol and salt remaining after the washes may inhibit downstream applications. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-centrifuge for 1 minute to ensure traces of salt and ethanol are not carried over in the eluted RNA.
	DNA contamination	DNA removal may be necessary for certain applications. Incubate RNA sample with DNase I (NEB #M0303) and cleanup RNA using the Monarch Spin RNA Cleanup Protocol.

Ordering Information



PRODUCT	NEB #	SIZE
Template generation		
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 units
Q5 Hot Start High-Fidelity DNA Polymerase	M0493S/L	100/500 units
Q5 Hot Start High-Fidelity 2X Master Mix	M0494S/L	100/500 reactions
Deoxynucleotide (dNTP) Solution Set	N0446S	25 µmol each
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol each
BspQI	R0712S/L	500/2,500 units
SapI	R0569S/L	250/1,250 units
***In vitro* transcription**		
T3 RNA Polymerase	M0378S	5,000 units
T7 RNA Polymerase	M0251S/L	5,000/25,000 units
Hi-T7® RNA Polymerase	M0658S	50,000 units
SP6 RNA Polymerase	M0207S/L	5,000/25,000 units
Pyrophosphatase, Inorganic (yeast)	M2403S/L	10/50 units
Pyrophosphatase, Inorganic (*E. coli*)	M0361S/L	10/50 units
Ribonucleotide Solution Set	N0450S/L	10/50 µmol each
Ribonucleotide Solution Mix	N0466S/L	10/50 µmol each
RNase Inhibitor, Murine	M0314S/L	3,000/15,000 units
RNase Inhibitor, Human Placenta	M0307S/L	2,000/10,000 units
Circular RNA		
RNase R	M0100S	20,000 units
T4 RNA Ligase 1	M0204S/L	1,000/5,000 units

Ordering Information Cont'd

PRODUCT	NEB #	SIZE
In vitro transcription & mRNA synthesis kits		
EnGen sgRNA Synthesis Kit, *S. pyogenes*	E3322S	20 reactions
HiScribe T7 mRNA Kit with CleanCap Reagent AG	E2080S/L	20/100 reactions
HiScribe T7 ARCA mRNA Kit (with tailing)	E2060S	20 reactions
HiScribe T7 ARCA mRNA Kit	E2065S	20 reactions
HiScribe T7 High Yield RNA Synthesis Kit	E2040S/L	50/250 reactions
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S/L	50/250 reactions
HiScribe SP6 RNA Synthesis Kit	E2070S	50 reactions
N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP)	N0431S	0.1 ml
5-Methyl-Cytidine-5'-Triphosphate (5-Methyl-CTP)	N0432S	0.1 ml
Pseudouridine-5'-Triphosphate (Pseudo-UTP)	N0433S	0.1 ml
5-Methoxy-Uridine-5'-Triphosphate (5-Methoxy-UTP)	N0434S	0.1 ml
Capping & tailing		
Faustovirus Capping Enzyme	M2081S/L	500/2,500 units
Vaccinia Capping System	M2080S	400 units
mRNA Cap 2´-O-Methyltransferase	M0366S	2,000 units
m^7G(5´)ppp(5´)G RNA Cap Structure Analog	S1404S/L	1.0/5.0 µmol
3´-O-Me-m^7G(5´)ppp(5´)G Cap Structure Analog (ARCA)	S1411S/L	1.0/5.0 µmol
m^7G(5´)ppp(5´)A Cap Structure Analog	S1405S/L	1.0/5.0 µmol
G(5´)ppp(5´)A Cap Structure Analog	S1406S/L	1.0/5.0 µmol
G(5´)ppp(5´)G Cap Structure Analog	S1407S/L	1.0/5.0 µmol
E. coli Poly(A) Polymerase	M0276S/L	100/500 units
Adenosine-5´ Triphosphate (ATP)	P0756S/L	1.0/5.0 µmol
RNA Purification		
Monarch Spin RNA Cleanup Kit (10 µg)	T2030S/L	10 preps / 100 preps
Monarch Spin RNA Cleanup Kit (50 µg)	T2040S/L	10 preps / 100 preps
Monarch SpinRNA Cleanup Kit (500 µg)	T2050S/L	10 preps / 100 preps
Monarch Spin Columns S1A (10 µg)	T2037L	100 columns + tubes
Monarch Spin Columns S2A (50 µg)	T2047L	100 columns + tubes
Monarch Spin Columns S2B (500 µg)	T2057L	100 columns + tubes
Monarch Buffer BX	T2041L	80 ml
Monarch Buffer BZ	T1114L	40 ml
RNA Analysis		
RNase 4 Digestion and 3´ End Repair Mix	M1288S/L	50/250 reactions
RNase 4	M1284S/L	2,500/12,500 units
RNase H	M0297S/L	250/1,250 units
Nucleoside Digestion Mix	M0649S	50 reactions
Template Removal		
DNase I (RNase-free)	M0303S/L	1,000/5,000 units
DNase I-XT	M0570S/L	1,000/5,000 units
Other RNA reagents		
RNA Loading Dye (2X)	B0363S	4 x 1.0 ml
RNA markers		
ssRNA Ladder	N0362S	0.05 ml
Low Range ssRNA Ladder	N0364S	0.05 ml

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