Now includes modified bases and RNase R

RNA Technical Guide

TOOLS TO STREAMLINE RNA–RELATED WORKFLOWS

NEW ENGLAND Biolabs®

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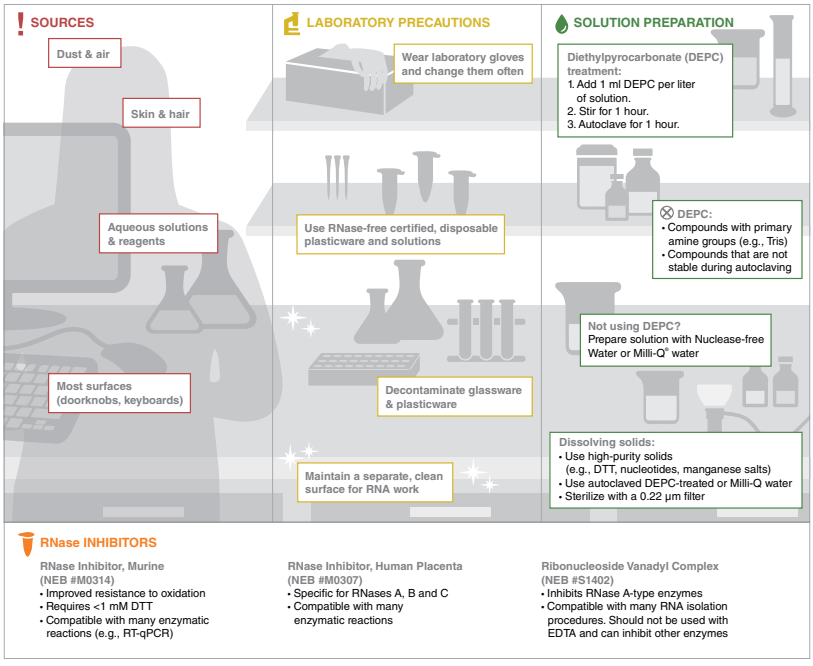
Let NEB Help Streamline Your RNA-related Workflows

Historically, our understanding of the function of RNA in the cell was limited to its role in translation of genetic information from DNA into protein. The major species of RNA described were; (A) messenger RNA (mRNA), which converts DNA into RNA, (B) transfer RNA (tRNA) that is charged with specific amino acids and, (C) ribosomal RNA (rRNA), a major component of the ribosome. More recently, RNA has been implicated in a diverse number of biological processes, including catalysis and transcriptional regulation. Technological advances and improvements in RNA analysis and detection have led to the discovery of many new classes of small and large non-coding RNAs with novel regulatory functions. Examples include, microRNA (miRNA), circular RNA, long non-coding RNA (lncRNA), small nucleolar RNA (snoRNA) and extracellular RNA (exRNA). In addition, RNA modifications have revealed added complexity to RNA. These biologically relevant modifications are an active area of exploration. These findings have helped usher in a renaissance of RNA-focused research in biology.

NEB offers a broad portfolio of reagents for the purification, quantitation, detection, synthesis and manipulation of RNA. These products are available from bench-scale to commercial-scale to enable both academic and industrial needs. Further, we provide these products at quality levels that support vaccine and diagnostic manufacturing. Experience improved performance and increased yields, enabled by our expertise in enzymology.

Getting Started: Avoiding RNase Contamination



Get started today at NEBrna.com

FEATURED PRODUCT

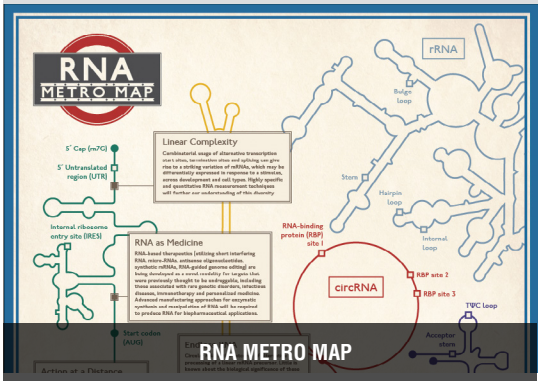
RNase Inhibitor, Murine (NEB #M0314)

- Improved resistance to oxidation, compared to human/porcine RNase inhibitor
- Ideal for reactions where low DTT concentrations are required (e.g., Real-time PCR)
- GMP-grade reagent also available (see page 16 for details)

FEATURED RESOURCES

Visit NEBrna.com to find additional resources, including:

- **RNA Synthesis Brochure**
  Learn more about NEB's products for synthesis of RNA, which range from template generation to poly(A) tailing
- **Monarch DNA & RNA Purification Brochures**
  It's time to transform your DNA and RNA purification experience! Learn about the advantages of choosing Monarch Nucleic Acid Purification Kits from NEB
- **Luna Universal qPCR and RT-qPCR Brochure**
  Make a simpler choice with Luna! Learn about the advantages of choosing Luna products for your qPCR & RT-qPCR, and see how Luna products compare to other commercially available reagents.
- **NEBNext for Illumina Brochure**
  Review NEB's extensive range of NGS sample prep products for RNA, Small RNA and DNA
- **Supporting COVID-19 Research**
  Find out how NEB is supporting customers developing vaccines and diagnostic tools for lab-based and point-of-care settings
- **Enzymes for Innovation**
  NEB offers novel enzymes with unique activities that can support RNA workflows
- **RNA Metro Map**
  Download our RNA poster to learn more about the various RNA structures and recent applications



RNA Purification

Isolating high-quality RNA is crucial to many downstream experiments, such as cloning, reverse transcription for cDNA synthesis, RT-PCR, RT-qPCR and RNA-seq. There are various approaches to RNA purification including phenol-chloroform extraction, spin column purification, and the use of magnetic beads.

Monarch Spin RNA Isolation Kit (Mini)

The Monarch Spin RNA Isolation Kit (Mini) is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from various biological samples, including cultured cells, mammalian tissues, microbes, plants, insects and blood. With just this single kit, users can purify up to 100 µg of high-quality total RNA from multiple sample types, from standard as well as tough-to-lyse samples, with included enzymes, reagents, and specialized gDNA removal columns. The kit uniquely enables binding capacities like RNA purification 'mini' kits, combined with the low elution volumes of 'micro' kits. Cleanup of enzymatic reactions and purification of RNA from TRIzol®-extracted samples is also possible using this kit. Purified RNA has metrics with $A_{260/280}$ and $A_{260/230}$ ratios typically > 2.0, high RNA integrity scores, and minimal residual gDNA. This kit captures RNA ranging in size from full-length rRNAs down to intact miRNAs. Purified RNA is suitable for downstream applications such as RT-qPCR, cDNA synthesis, RNA-seq, and RNA hybridization-based technologies.

ADVANTAGES

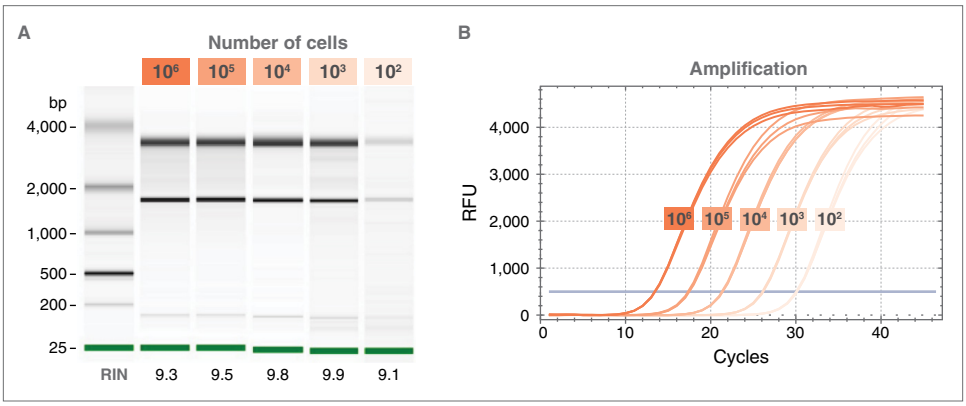
- Use with a wide variety of sample types, including cells, fibrous or lipid-rich tissues, bacteria, yeast, plants, insects and more
- Purify RNA of all sizes, including small RNAs < 200 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent, Monarch StabiLyse DNA/RNA Buffer (**NEB #T2111**)

SPECIFICATIONS

- **Binding Capacity:** 100 µg RNA
- **RNA Size:** > 20 nt
- **Purity:** $A_{260/280}$ and $A_{260/230}$ usually ≥ 1.8
- **Input Amount:** up to 10^6 cells or 10 mg tissue*
- **Elution Volume:** 30 – 100 µl
- **Yield:** varies depending on sample type
- **Compatible downstream applications:** cDNA synthesis, RT-qPCR, RNA-Seq, small RNA library prep, Northern blotting, hybridization-based workflows

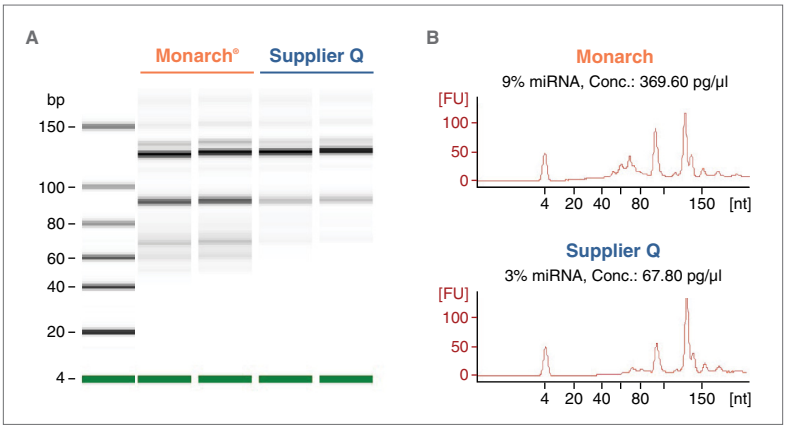
*See page 6 for more details and other sample types

Monarch Spin RNA Isolation Kit (Mini) enables extraction of high-quality RNA even from low input samples



RNA was extracted using the Monarch Spin RNA Isolation Kit (Mini) from varying amounts of HEK293 cells over 5 orders of magnitude starting from 1 million cells to 100. A. To assess RNA integrity, the extracted RNA was resolved using the Agilent® Pico Bioanalyzer chip on a Bioanalyzer 2100 with sample loading adjusted to the manufacturer's recommendation of assay input range. B. To demonstrate successful downstream application, the RNA was subjected to RT-qPCR using Luna® Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) in a 20 µl reaction targeting GAPDH mRNA. Expected amplification curves were observed showing a quantitative trendcorresponding to the varying input amounts.

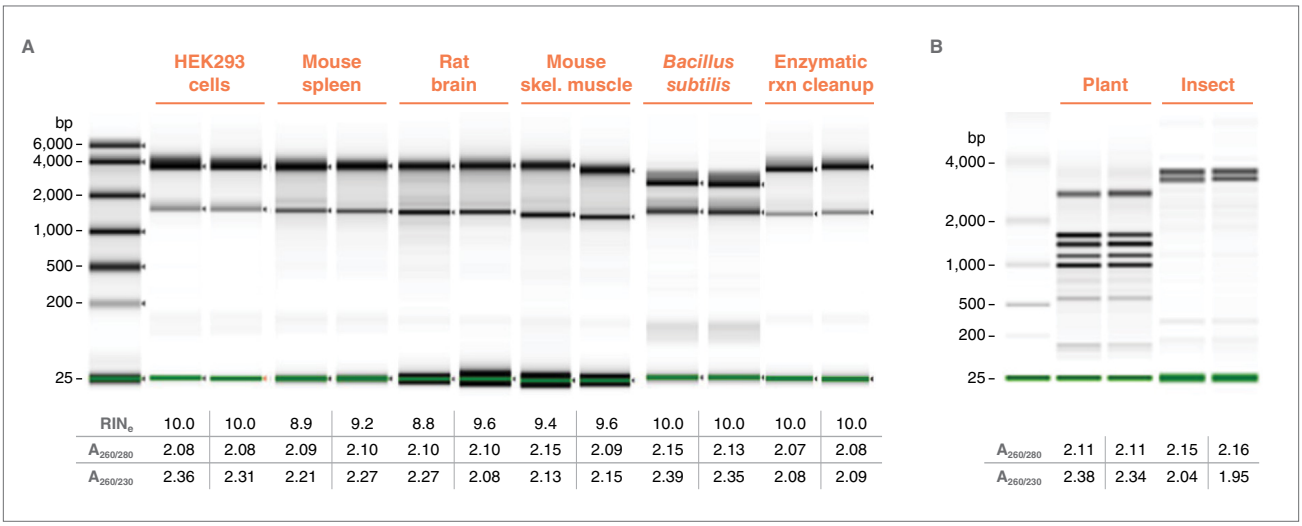
Monarch Spin RNA Isolation Kit (Mini) successfully purifies small RNAs below 200 nucleotides, enabling a more faithful representation of the total RNA pool compared to a leading supplier



RNA was extracted from 10 mg mouse brain tissues using Monarch Spin RNA Isolation Kit (Mini) and a comparable kit from Supplier Q. To visualize the isolation of small RNA species, the extracted RNA was resolved using the Agilent Small RNA chip on a Bioanalyzer 2100 according to manufacturer's instructions. Gel traces of RNA isolated from the two kits are shown (A), with representative electropherograms showing the small RNA size profiles (B).

Monarch-purified RNA is High-quality and Compatible with a Wide Variety of Downstream Applications

Monarch Spin RNA Isolation Kit (Mini) is a versatile solution for high-quality RNA extraction from a wide variety of sample types



	HEK293 cells		Mouse spleen		Rat brain		Mouse skel. muscle		*Bacillus subtilis*		Enzymatic rxn cleanup	
RIN_e	10.0	10.0	8.9	9.2	8.8	9.6	9.4	9.6	10.0	10.0	10.0	10.0
$A_{260/280}$	2.08	2.08	2.09	2.10	2.10	2.10	2.15	2.09	2.15	2.13	2.07	2.08
$A_{260/230}$	2.36	2.31	2.21	2.27	2.27	2.08	2.13	2.15	2.39	2.35	2.08	2.09

	Plant		Insect	
$A_{260/280}$	2.11	2.11	2.15	2.16
$A_{260/230}$	2.38	2.34	2.04	1.95

RNA was extracted in duplicate using the Monarch Spin RNA Isolation Kit (Mini) from various samples representing a range of biological properties in the starting material, including fibrous, fatty and nuclease-rich mammalian tissues, tough-to-lyse samples such as Gram-positive bacteria, plants and insects, as well as RNA cleanup reactions. RNA quality was assessed using A260/A280 and A260/A230 ratios measured using a microvolume spectrophotometer (Lunatic®, Unchained Labs®). RNA integrity was measured using Agilent® automated electrophoresis systems according to sample types. For samples with known typical RNA profiles, Agilent RNA ScreenTape® was used on the TapeStation® 4200 (A). For samples with known atypical RNA profiles such as plant green tissue with plastidal content, and insects with ribosomal gene breaks, Agilent Bioanalyzer® 2100 used with NanoChip (B).

TIPS FOR SUCCESSFUL RNA EXTRACTIONS

1. **Prevent RNase Activity:** Nucleases in your sample will degrade RNA, so inhibiting their activity is essential. Process samples quickly after harvest, use preservation reagents, and always ensure you are working in nuclease-free working environments.
2. **Inactivate RNases after harvesting your sample:** Nucleases in your sample will lead to degradation, so inactivating them is essential. Process samples quickly, or use preservation reagents, and always ensure nuclease-free working environments.
3. **Do not exceed recommended input amounts:** Buffer volumes are optimized for the recommended input amounts. Exceeding these can result in inefficient lysis and can also clog the column. See page 6.
4. **Ensure samples are properly homogenized/disrupted:** Samples should be disrupted and homogenized completely to release all RNA.
5. **For sensitive applications, ensure proper gDNA removal:** gDNA is removed by the gDNA removal column (Monarch Spin Column S2C) and subsequent on-column DNase I treatment. Off-column DNase I treatment can also be employed.

Choosing Sample Input Types for RNA Purification and Average Yields Expected

Below, we have provided some empirical yield, purity, and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110). It is important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

SAMPLE TYPE		RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	OBSERVED RIN or RINe	MAXIMUM INPUT AMOUNT
CULTURED CELLS					
HEK 293		1 x 10^6 cells	12–14	9–10	15 x 10^6 cells
NIH3T3		1 x 10^6 cells	8–12	9–10	1 x 10^7 cells
TISSUE					
Rat liver (frozen stabilized)		10 mg	40–60	8–9	20 mg
Rat spleen (frozen stabilized)		10 mg	40–50	9	20 mg
Rat kidney (frozen stabilized)		10 mg	7–10	9	50 mg
Rat brain (frozen stabilized)		10 mg	5–8	8–9	50 mg
Rat muscle (frozen stabilized)		10 mg	2–3	8–9	50 mg
Mouse heart (frozen stabilized)		10 mg	5–6	8–9	50 mg
BLOOD OR PLASMA					
Human	Fresh	200 µl	0.5–1.0	7–8	200 µl
	Frozen	200 µl	0.5–1.0	7–8	200 µl
Rat	Frozen	200 µl	5-6	9	200 µl
BLOOD CELLS					
PBMC (isolated from 5 ml whole blood)		5 ml	1–3	7	5 x 10^6 cells
YEAST					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	20–40	9–10	5 x 10^7 cells
	Frozen with Zymolyase®	1 x 10^7 cells	20–40	9	5 x 10^7 cells
BACTERIA					
E. coli	Frozen pellet with bead homogenizer	1 x 10^9 cells	10–15	10	1 x 10^9 cells
	Frozen pellet with lysozyme	1 x 10^9 cells	40–60	10	1 x 10^9 cells
B. subtilits	Frozen pellet with bead homogenizer	1 x 10^9 cells	15–20	9	1 x 10^9 cells
	Frozen pellet with lysozyme	1 x 10^9 cells	20–30	9–10	1 x 10^9 cells
PLANT					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Onion leaf (fresh with bead homogenizer)		50 mg	4–6	8*	50 mg
Root		50 mg	8–10	8*	50 mg
INSECTS					
Mosquito (preserved in ethanol dry ice bath)		10 mg	20–30	9*	20 mg
House fly (preserved in ethanol dry ice bath)		10 mg	10–20	9*	10 mg

RNA yields and RINs observed during evaluation are reported here. RNA yield and quality are influenced by several factors including sample growth stage and RNA content, storage condition, sample handling during processing as well as the chosen method of sample lysis and homogenization. It is crucial to consider these factors and use best practices to maximize RNA yield and quality. Please refer to Important Notes in the Product Manual before starting for recommendations on Working with RNA and Considerations for Sample Lysis and Homogenization.

**These samples contain RNA with an atypical ribosomal profile that is not applicable for standard RIN measurement. Users are encouraged to set their own threshold or assess RNA integrity by visually inspecting the bands generated by the automated gel electrophoresis platform.*

For updates, visit neb.com/MonarchRNAinputs

Troubleshooting Guide for Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110)

Our troubleshooting guide below outlines some of the most common pain points scientists encounter during RNA purification.

PROBLEM	COMMON CAUSE	SUGGESTIONS/SOLUTIONS
Column clogging	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Refer to recommendations under Choosing Sample Input Types.
	Insufficient Lysis	• Increase time of digestion or homogenization. • Centrifuge sample to pellet debris and use only supernatant for next steps. • Use larger volume of buffer for lysis and homogenization.
Low RNA yield	Insufficient Lysis	• Increase time of digestion or homogenization. • Centrifuge sample to pellet debris and use only supernatant for next steps. • Use larger volume of buffer for lysis and homogenization.
	Sample is degraded	• Use RNA preservation reagents to maintain RNA integrity during storage. • Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. • To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE. • See Product Manual under Important Notes Before Starting.
	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Overloading leads to inefficient purification, insufficient elution and reduced yield. See Choosing Input Amounts.
Low RNA quality	Sample is degraded	• Use RNA preservation reagents to maintain RNA integrity during storage. • Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. • To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE.
	Salt/Ethanol carryover	• Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution. Ensure wash steps are carried out prior to eluting sample. Do not skip any washes with Buffer BX and Buffer WZ. • Use care to ensure the column tip does not contact the flow-through. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a soft paper towel prior to reattachment to the column to remove any residual wash buffer. • Add additional wash step and/or extend spin time for final wash.
	Residual protein carryover	• Low $A_{260/280}$ values indicate residual protein in the purified sample. Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA purification column. Do not skip any washes with Buffer BX and Buffer WZ.
DNA contamination	DNA carryover	• Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample. • Perform in-tube/off-column DNase I treatment to remove gDNA. See Appendix in Product Manual.
	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the column tip does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the column for 2 minutes following the final wash with Monarch Buffer WZ. • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer. • Add additional wash step and/or extend spin time for final wash.
Unusual Spectrophotometric readings	RNA concentration is too low for spectrophotometric analysis	• For more concentrated RNA, elute with 10 µl of nuclease-free water. • Increase amount of starting material (within kit specifications). See Choosing Input Amounts or Product Manual.
	Silica fines in eluate	• Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the $A_{260/230}$ is unaffected by possible elution of silica particles.

RNA Cleanup and Concentration

The ability to quickly modify and manipulate RNA is in high demand and accordingly, the need for rapid and reliable RNA cleanup methods have become essential. For example, after RNA synthesis by *in vitro* transcription (IVT), unincorporated nucleotides, aborted transcripts, enzymes and buffer components should be removed before using the transcript for RNP formation or for microinjection. Removal of reactants is also beneficial following standard protocols such as RNA labeling, capping, Proteinase K treatment, and DNase I treatment. Sensitive workflows such as RNA-seq or RT-qPCR may also benefit from RNA cleanup prior to processing.

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 silica column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. These kits can also be used to extract RNA from cells, saliva, and buccal/nasopharyngeal swabs. The Monarch Spin RNA Cleanup Kits are available in 3 different binding capacities for flexibility in any application. Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA Following the standard protocol, RNA ≥ 25 nucleotides can be purified; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

FEATURED 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

- Isolate highly pure RNA ($A_{260/280}$ and $A_{260/230}$ ≥ 1.8) in minutes
- Clean up RNA with simple protocol utilizing a single wash buffer
- Elute in as little as 6 µl (NEB #T2030)
- Bind up to 500 µg of RNA (NEB #T2050)
- Adjust cutoff size down to 15 nt with a slight protocol modification

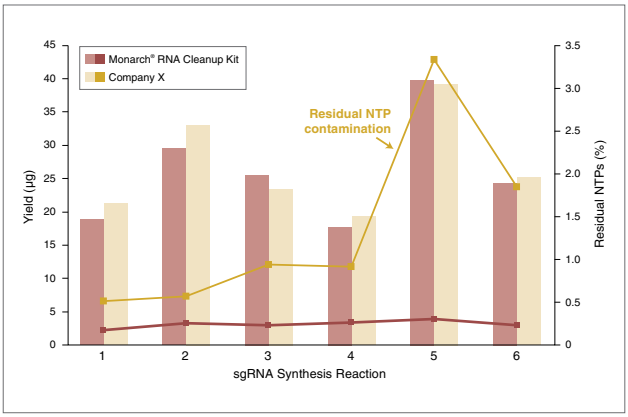
APPLICATIONS

- Cleanup & concentration after enzymatic reactions (e.g., DNase I and Proteinase K treatment)
- Cleanup after RNA synthesis (IVT and sgRNA synthesis)
- Cleanup & concentration of previously-purified RNA (e.g., after TRIzol extraction)
- RNA extraction from cells, saliva and swabs (buccal/NP)
- RNA Gel Extraction

Specifications:

MONARCH SPIN 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

The Monarch Spin RNA Cleanup Kit (50 µg) produces RNA 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.

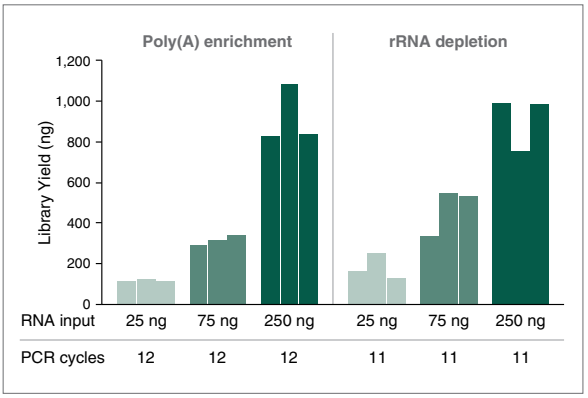
Following cleanup, residual nucleotides (NTPs) were measured by LC-MS and are reported as percent area NTPs (rATP+rCTP+rGTP+rUTP)/percent area sgRNA). The NEB Monarch Spin RNA Cleanup Kit consistently outperforms other commercially available RNA cleanup kits in the removal of residual NTPs from sgRNA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

RNA Sequencing

Next generation sequencing (NGS) can be used to determine the presence and quantity of RNA species in a sample, enabling sensitive and accurate gene expression analysis. For the Illumina sequencing platform, RNA libraries are prepared by either enrichment of mRNA or removal of ribosomal rRNA, then cDNA synthesis followed by DNA Library preparation steps: end repair, addition of a non-templated dA overhang, adaptor ligation, and PCR amplification.

NEBNext UltraExpress RNA Library Prep Kit

The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library prep, with a fast, streamlined workflow. The kit is compatible with mRNA isolation and rRNA depletion workflows and a wide range of sample types. With a 3-hour library prep protocol, the kit enables creation of high-quality RNA libraries in a single day, in conjunction with mRNA or rRNA depletion kits.

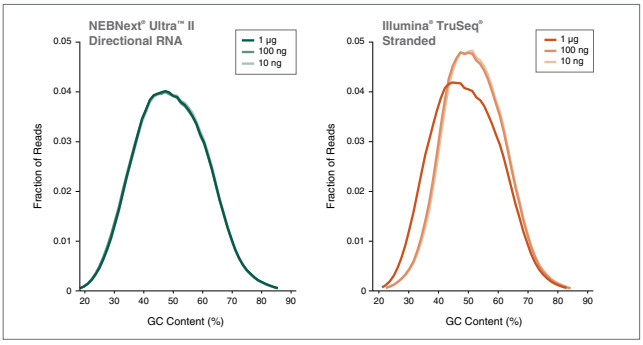


The NEBNext UltraExpress RNA Library Prep Kit produces high library yields for a range of inputs, in poly(A) enrichment and rRNA depletion workflows. Universal Human Reference RNA with the indicated input amounts was (A) enriched for poly(A) mRNA (NEB #E7490) or (B) depleted of ribosomal RNA (NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400), followed by creation of strand-specific libraries using the NEBNext UltraExpress RNA Library Preparation Kit. Library yields were assessed using TapeStation® 4200 and values shown are for three replicates for each input amount.

Get even more from less with NEBNext Ultra II

NEBNext Ultra II RNA Library Prep Kits for Illumina are available for both directional and non-directional (non-strand-specific) RNA library construction, and deliver significantly increased sensitivity and specificity from your RNA-seq experiments, from ever-decreasing amounts of input RNA. In conjunction with ribosomal RNA (rRNA) depletion or poly(A) mRNA enrichment, the kits enable the production of high quality libraries from 10 ng of Total RNA up to 1 µg.

NEBNext Ultra II Directional RNA libraries provide uniform GC content distribution, at a broad range of input amounts



Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (Agilent #740000), and libraries were made using the NEBNext Ultra II Directional RNA Kit (plus the NEBNext Poly(A) mRNA Magnetic Isolation Module) and Illumina TruSeq Stranded mRNA Kit. Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2 x 76 bp). Reads were mapped to the hg19 reference genome. GC content distribution for each library was calculated using mapped reads. Ultra II Directional RNA libraries had uniform GC content distribution across a range of input amounts, whereas for TruSeq Stranded the GC content distribution changed with different input amounts, indicating the introduction of input-dependent sequence bias.

FEATURED PRODUCTS

NEBNext UltraExpress RNA Library Prep Kit (NEB #E3330)

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760, #E7765)

NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770, #E7775)

NEBNext Immune Sequencing Kits:

- **Human (NEB #E6320)**
- **Mouse (NEB #E6330)**

NEBNext RNA Depletion Kits:

- **Human/Mouse/Rat rRNA (NEB #E7400, #E7405)**
- **Bacteria rRNA (NEB #E7850, #E7860)**
- **Globin mRNA & rRNA (NEB #E7750, #E7755)**
- **Customized Depletion (NEB #E7865, #E7870)**

NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)

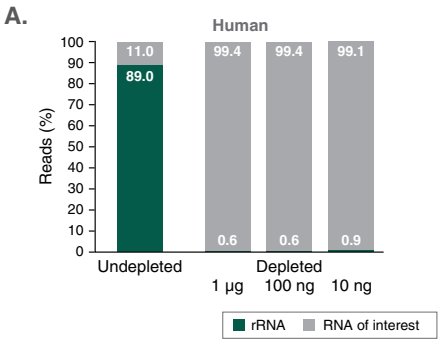
NEBNext Adaptors & Primers (neb.com/oligos)

ADVANTAGES

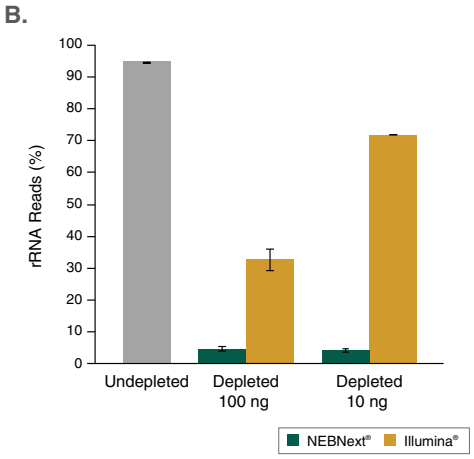
- Get more of what you need, with the **highest library yields**
- Generate **high quality libraries** even when you have only limited amounts of input RNA
  - 10 ng – 1 µg Total RNA (polyA mRNA workflow)
  - 10 ng – 1 µg Total RNA (v2 rRNA depletion workflow)
- **Minimize bias** with fewer PCR cycles required
- Increase the **complexity and transcript coverage** of your libraries
- **Save time** with streamlined workflows, reduced hands-on time, and automation compatibility
- Rely on **robust performance**, even with low quality RNA, including FFPE
- Compatible with NEBNext poly(A) mRNA isolation, rRNA depletion reagents and multiplexing adaptors and primers
- Use our NEBNext selector tool at **NEBNextSelector.neb.com** for help with selecting the right NEBNext product for your needs

Visit NEBNext.com to learn more and request a sample.

The NEBNext rRNA Depletion Kit v2 enriches for RNAs of interest across a wide range of total RNA inputs in human



The NEBNext rRNA Depletion Kit v2 efficiently depletes rRNA from degraded FFPE total RNA while preserving transcript abundances



Universal human reference total RNA (A) or human adult normal liver tissue FFPE Total RNA, RIN 2.3 (B) was depleted of rRNA using the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (A and B), or the TruSeq® Stranded Total RNA Gold kit (B). RNA-seq libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 10 Million reads (A) or 20 Million reads from depleted libraries and 200 million reads from undepleted libraries (B) reads were sampled (seqtk) and were identified as ribosomal using mirabait.

SEQUENCING SMALL RNAs?

The unique workflow of the NEBNext Small RNA library prep kits addresses the challenge of minimization of adaptor-dimers while achieving production of high-yield diverse multiplex libraries in a simple protocol.

- Minimized adaptor-dimer formation
- High yields
- Input can be total RNA
- Suitable for methylated small RNA's (e.g. RNAs as well as unmethylated small RNAs)
- 48 Indices available
  - **NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1) (NEB #E7770)**
  - **NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2) (NEB #E7580)**
  - **NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1–48) (NEB #E7560)**
  - **NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)**
- Visit NEBNext.com for more information

RNA SAMPLE INPUT GUIDELINES

Integrity of RNA

- We recommend determining the RNA sample input using the RNA Integrity Number (RIN) estimated by the Agilent TapeStation or similar instrumentation. Ideally the RNA sample will have a RIN value of 7 or higher but NEBNext RNA products are compatible for use with even samples with low RIN values.
- RNA should be completely free of DNA, and DNA digestion of the purified RNA using RNase-free DNase I (such as that provided with the Monarch Total RNA Miniprep Kit) is recommended.

Quantitation of RNA

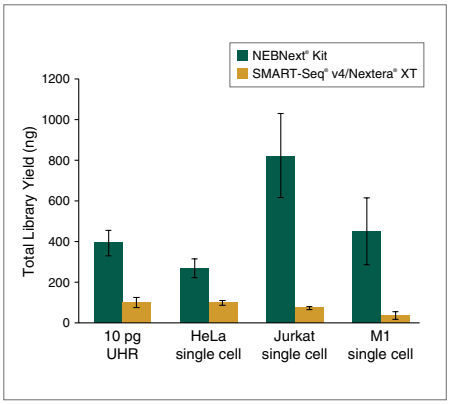
- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer or similar instrumentation, using pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. Note that free nucleotides or organic compounds used in some RNA extraction methods also absorb UV light near 260nm and will cause an over-estimation of RNA concentration.

Single Cell/Low Input RNA-Seq

NEBNext Single Cell/Low Input RNA Library Prep meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing libraries from single cells or as little as 2 pg–200 ng of total RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, using a unique protocol and suite of reagents, and even low-abundance transcripts are represented in the high yields of cDNA obtained. Subsequent library construction incorporates the Ultra II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.

Generate higher library yields with the NEBNext Single Cell/ Low Input RNA Library Prep Kit



Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent® #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech® #634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096) were used. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.

FEATURED PRODUCTS

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420)

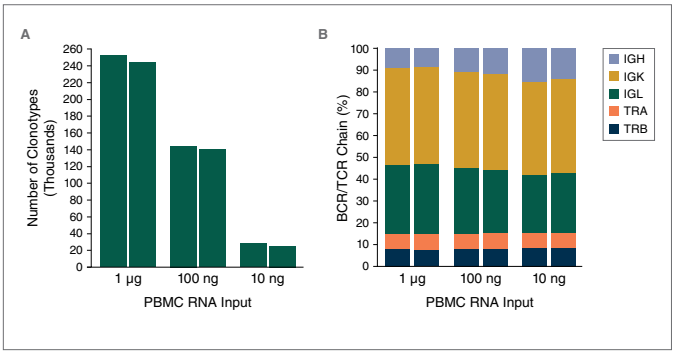
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module (NEB #E6421)

- Generate the **highest yields** of high-quality cDNA and sequencing libraries from single cells, or as little as 2 pg—200 ng total RNA
- **Experience unmatched detection** of low abundance transcripts
- Rely on **consistent and uniform transcript detection** for a wide range of input amounts and sample types
- **Use with a variety of RNA inputs**, including cultured or primary cells, or total RNA
- **Save time** with a fast, streamlined workflow, minimal hands-on time, and automation compatibility

Immune-cell Sequencing

The NEBNext Immune Sequencing Kits (Human) and (Mouse) enable exhaustive profiling of somatic mutations in the full-length immune gene repertoires of B cells and T cells, via the expression of complete antibody chains. This includes modular primer sets, providing information for complete V, D, and J segments and full isotype information analysis (IgM, IgD, IgG, IgA and IgE), and TCRα and TCRβ chain characterization.

The NEBNext Immune Sequencing Kit is able to generate both BCR and TCR libraries in one tube



Human BCR+TCR libraries were constructed from 1 µg, 100 ng and 10 ng human PBMC Total RNA (Takara Bio #636592) with replicates for each input. Libraries were downsampled to 950,000 reads for all the libraries. pRESTO tools were used for quality filtering of reads, sequence assemble, and generation of consensus sequence UMIs. V, D and J assignment was done using MiGMAP. (A) Number of clonotypes detected for each human PBMC Total RNA input. (B) B cell chains and T cell chains percentages in each library.

FEATURED PRODUCTS

NEBNext Immune Sequencing Kit (Human) (NEB #E6320)

NEBNext Immune Sequencing Kit (Mouse) (NEB #E6330)

- Unlock the immune system's complexity with a deeper analysis of receptor sequences
- Enrich for and sequence both B cell receptors (BCR) and T cell receptors (TCR)
- Generate full-length immune gene repertoires of B and T cells
- Accurately quantify transcripts with unique molecular identifiers (UMIs)
- Analyze data using a bioinformatic workflow based on the open-source pRESTO toolkit

qPCR and RT-qPCR

Quantitative PCR (qPCR) uses real-time fluorescence to measure the quantity of DNA present at each cycle during a PCR. A wide variety of approaches have been developed for generating a fluorescent signal, the most common of which use either hydrolysis probes (e.g., TaqMan®), or a double-stranded DNA binding dye, (e.g., SYBR® Green). qPCR can be modified to detect and quantitate RNA by adding a reverse transcriptase (RT) step upstream of the qPCR assay to generate cDNA (i.e., RT-qPCR). Reverse transcription can be performed separately from qPCR or directly in the qPCR mix (i.e., one-step RT-qPCR). One-step workflows are commonly favored in molecular diagnostic assays and where sample inputs may be limiting. Separate cDNA synthesis followed by qPCR (i.e., two-step RT-qPCR) is preferred when multiple interrogations will be made of the same starting material or where archiving of cDNA may be required.

Lighting the way with Luna

Luna products from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. All Luna products provide robust performance on diverse sample sources and target types.

Find the right Luna product for your application

1 Select your target

2 Select your detection method

1 Select your target	Dye-based	Probe-based
Genomic DNA or cDNA	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
Purified RNA — One-Step RT-qPCR	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006)* • 4X Mix with UDG (NEB #M3019)* • LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)
Purified RNA — Two-Step RT-qPCR	LunaScript® RT SuperMix (NEB #E3010/M3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix (NEB #E3010/M3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)

*No ROX Versions available.

Doing one-step RT-qPCR?

The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart® Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. The Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) is supplied at 4X concentration and enables higher amounts of sample input, which is relevant for applications where RNA present in low abundance is of interest, such as viral RNA or pathogen detection.

FEATURED PRODUCTS

- Luna Universal qPCR Master Mix (NEB #M3003)
- Luna Universal Probe qPCR Master Mix (NEB #M3004)
- Luna Universal One-Step RT-qPCR Kit (NEB #E3005)
- Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)
- Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007)
- Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019)
- Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX) (NEB #M3029)
- LyoPrime® Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)

Make a Simpler Choice

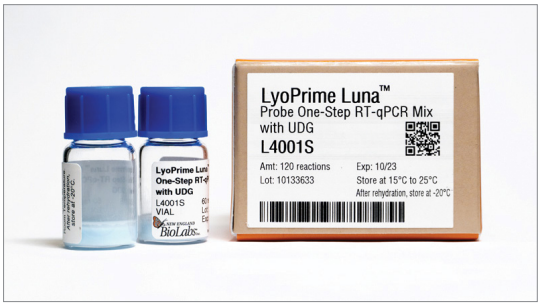
- Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors

Experience Best-in-class Performance

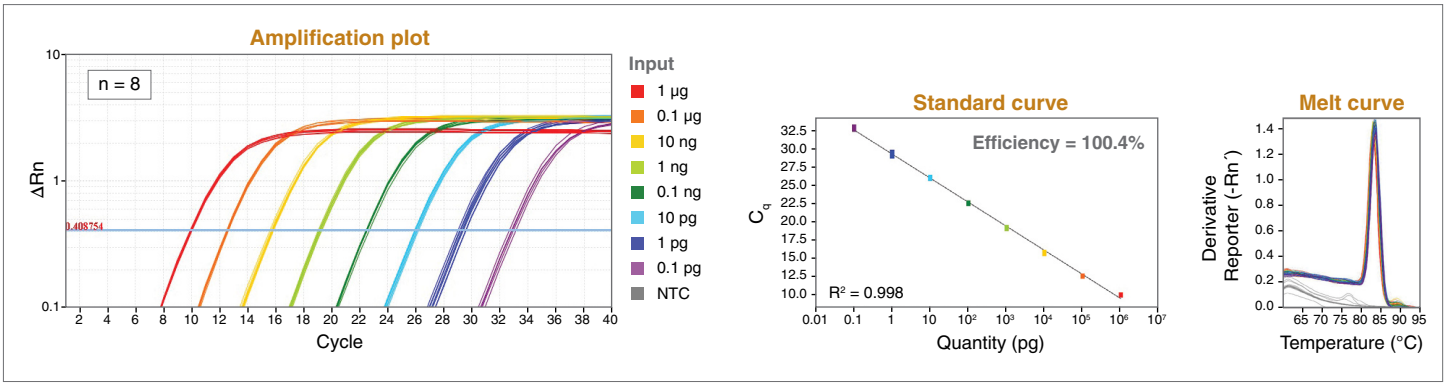
- Products perform consistently across a wide variety of sample sources
- Master mixes support carryover prevention

Optimize Your One-Step RT-qPCR with Luna WarmStart® Reverse Transcriptase

- Novel, thermostable reverse transcriptase (RT) improves performance
- WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness



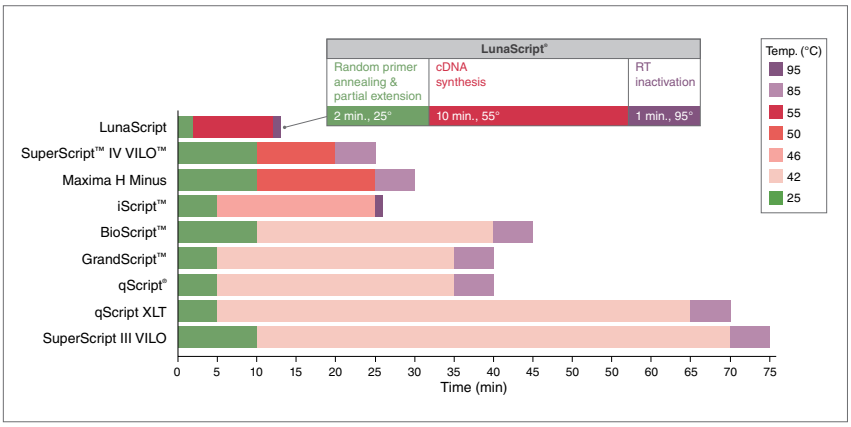
Luna WarmStart Reverse Transcriptase exhibits exceptional sensitivity, reproducibility and RT-qPCR performance



Doing two-step RT-qPCR?

LunaScript RT SuperMix is an optimized master mix for first strand cDNA synthesis up to 3 kb and can be used in amplicon sequencing or a two-step RT-qPCR workflow. LunaScript delivers best-in-class performance, user-friendly protocols, and includes a convenient blue dye to track your sample throughout the RT-qPCR workflow. cDNA products generated by LunaScript have been extensively evaluated in qPCR using the Luna Universal qPCR Master Mix (NEB #M3003) and Luna Universal Probe qPCR Master Mix (NEB #M3004). In combination, these products provide a two-step RT-qPCR workflow with excellent sensitivity and accurate, linear quantitations.

At just 13 minutes, the LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol



Comparison of recommended protocols for cDNA synthesis. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures, reducing complications from RNA secondary structure.

FEATURED PRODUCT

LunaScript RT SuperMix Kit (NEB #E3010)

LunaScript RT SuperMix (NEB #M3010)

LunaScript RT Master Mix Kit (Primer-free) (NEB #E3025)

Optimize your RT-qPCR

- Simplify reaction setup with **convenient supermix format** or incorporate your own primers using our primer-free mix (NEB #E3025)
- **Eliminate pipetting errors** with non-interfering, visible tracking dye
- Synthesize cDNA in **less than 15 minutes**
- LunaScript RT SuperMix (NEB #M3010) does not include a No-RT control mix or nuclease-free water
- Enjoy consistent linearity, sensitivity, and capacity for **reliable RNA quantification**

Visit LUNAqPCR.com to request a sample.

LOOKING FOR REVERSE TRANSCRIPTASES (RTs) FOR OTHER APPLICATIONS?

NEB offers several RTs, including ProtoScript® II Reverse Transcriptase (NEB #M0368) and Induro® Reverse Transcriptase (NEB #M0681), which supports long cDNA synthesis. See page 22 for the full list of RTs available and visit **www.neb.com/rt** for new product updates.

Optimization Tips for RT-qPCR with Luna

TIPS FOR OPTIMIZATION

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**.
The following tips can be used to help optimize your One-Step RT-qPCR.

TARGET SELECTION

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA TEMPLATE

- Use high quality, purified RNA templates whenever possible. Luna qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

PRIMERS

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's Tm calculator. (**Tmcalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

HYDROLYSIS PROBES

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

MULTIPLEXING

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

REVERSE TRANSCRIPTION

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

CYCLING CONDITIONS

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

REACTION SETUP

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

ASSAY PERFORMANCE

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Luna One-Step RT-qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Incorrect RT step temperature or RT step omitted	• For typical use, a 55°C RT step temperature is optimal for the Luna WarmStart Reverse Transcriptase
	Incorrect cycling protocol	• Refer to the proper RT-qPCR cycling protocol in product manual
	Reagent omitted from RT-qPCR assay Reagent added improperly to RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	RNA template or reagents are contaminated or degraded	• Prepare high quality RNA without RNase/DNase contamination • Confirm template input amount • Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in product manual • Rerun the RT-qPCR assay with fresh reagents
Inconsistent qPCR traces for triplicate data	Improper pipetting during RT-qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
Standard curve has a poor correlation coefficient/ efficiency or the standard curve falls outside the 90–110% range	Cycling protocol is incorrect	• Refer to the proper RT-qPCR cycling protocol in product manual • Use a 55°C RT step • For ABI instruments, use a 1 minute 60°C annealing/extension step
	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during RT-qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification/NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software
Amplification in No-RT control	RNA is contaminated with genomic DNA	• Treat sample with DNase I • Redesign amplicon to span exon-exon junction

RNA Synthesis

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 five *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.

For more information on products available for RNA synthesis, visit NEBrna.com and download our RNA Synthesis Brochure

* *"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
Q5® Hot Start High-Fidelity DNA Polymerase (GMP*)	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)			
phi29 DNA Polymerase (GMP*)	HiScribe T7 ARCA mRNA Synthesis Kit			Monarch Spin RNA Cleanup Kit (50 µg)
TelN Protelomerase (GMP*)	HiScribe T7 High Yield RNA Synthesis Components (GMP*)	Faustovirus Capping Enzyme (GMP*)		Monarch Spin RNA Cleanup Kit (500 µg)
dNTP solution mixes (GMP*)		Vaccinia Capping System (GMP*)		
BspQI* (GMP*)	HiScribe T7 Quick High Yield RNA Synthesis Kit	mRNA Cap 2´-O-Methyltranferase (GMP*)		Lithium Chloride
NEBuffer™ 4 (GMP*)	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	S-Adenosylmethionine (SAM) (GMP*)		
	T7 RNA Polymerase (GMP*)			
	Hi-T7 RNA Polymerase			
	Companion Products			**Companion Products**
	RNase inhibitor (Murine) (GMP*)			Monarch Buffer BX
	RNase Inhibitor (Human Placental)			Monarch Buffer WX
	Pyrophosphatase, Inorganic (*E. coli*) (GMP*)			Nuclease-free Water
	Pyrophosphatase, Inorganic (Yeast)			
	DNase I (RNase-free) (GMP*)			
	DNase I-XT			
	RNase R			
	NTPs (GMP*)			
	Modified NTPs			

GMP* = available in GMP-grade

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

Generate Microgram Quantities of RNA with HiScribe

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.

Application		T7 Kits					SP6 Kits
		HiScribe T7 High Yield RNA Synthesis Kit NEB #E2040	HiScribe T7 Quick High Yield RNA Synthesis Kit NEB #E2050	HiScribe T7 ARCA mRNA Kit NEB #E2065	HiScribe T7 ARCA mRNA Kit (with tailing) NEB #E2060	HiScribe T7 mRNA Kit with CleanCap Reagent AG NEB #E2080	HiScribe SP6 RNA Synthesis Kit NEB #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 high yield CleanCap Reagent AG capped RNA synthesis • Template encoded poly(A) tails • Non-polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation					●	
	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	●	●				●
	Post-transcriptional capping with Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	●	●				●
	Complete substitution of NTPs: 5-mC, pseudouridine, 5mCTP, Pseudo-UTP, N-Methyl-Pseudo-UTP, 5-Methyl-UTP, etc.	●				●	●
	Partial substitution of NTPs: 5-mC, pseudouridine, 5mCTP, Pseudo-UTP, N-Methyl-Pseudo-UTP, 5-Methyl-UTP, 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	●	●				●

Generating Guide RNA for CRISPR/Cas9 Experiments

Cas nucleases are central components of CRISPR-based immunity, a mechanism used to protect a bacterial or archaeal cell from invading viral and foreign DNA. CRISPRs (clustered regularly interspaced short palindromic repeats) are DNA loci that contain multiple, short, repeated sequences, separated by unique "spacer DNA". The CRISPR locus is transcribed and processed into short guide RNAs (gRNAs) that are incorporated into Cas nuclease. The RNA corresponding to the spacer DNA guides the Cas nuclease to its target by complementary base pairing; double-stranded DNA cleavage results.

Cas nucleases have been adapted for use in genome engineering because they can easily be programmed for target specificity by supplying gRNAs of any sequence. In cells and animals, genome targeting is performed by expressing nucleases and gRNA from DNA constructs (plasmid or virus), supplying RNA encoding Cas nuclease and gRNA, or by introducing RNA-programmed Cas nuclease directly.

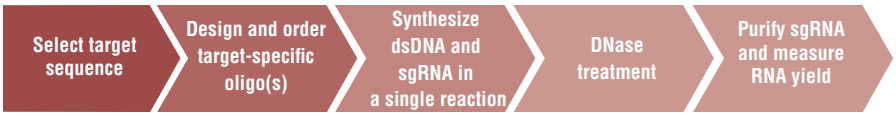
The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30-minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

FEATURED PRODUCT

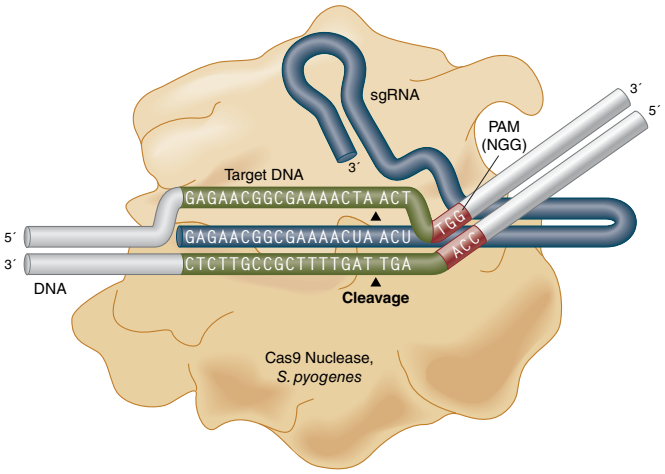
EnGen sgRNA Synthesis Kit (NEB #E3322)

- Reduce protocol time with single-reaction format
- Generate up to 25 µg of sgRNA
- Facilitate troubleshooting with included control oligo provided
- Save money with reduced cost per reaction

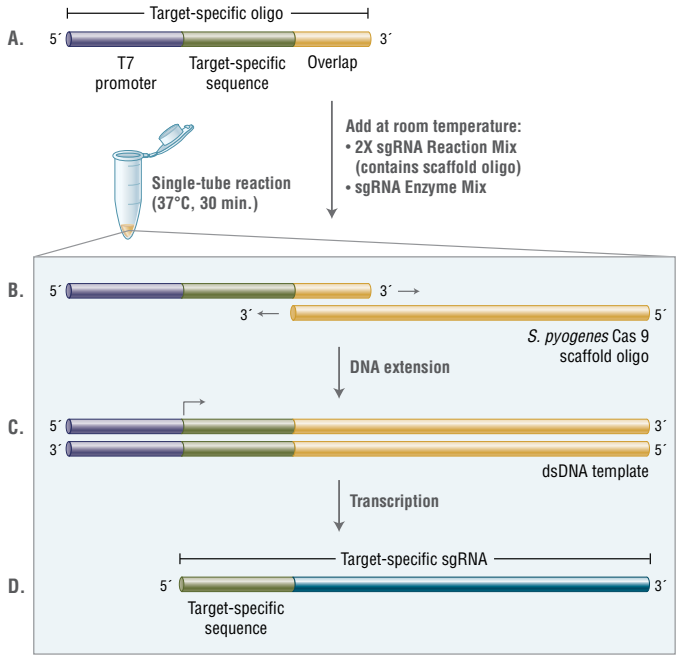
General workflow for the EnGen sgRNA Synthesis Kit, *S. pyogenes*.



Schematic representation of Cas9 Nuclease, *S. pyogenes* recognition and DNA cleavage



sgRNA Synthesis with EnGen is complete in less than one hour



Interested in Designing Your Own Workflows?

NEBs broad portfolio of enzyme specificities and reagents enables development of creative workflows for your RNA research. For example, RNA can be modified with enzymes and reagents that act selectively depending on the existence of particular structures, and hence are useful tools for characterizing RNA species. The selective properties of RNA modifying enzymes enable researchers to distinguish different RNA species. Some unique end modifications can be used to selectively degrade or isolate particular RNA species when treated sequentially with the appropriate series of enzymes. For instance, 5´-capped RNAs are not substrates for polynucleotide kinases, or RNA ligases, but are substrates for decapping enzymes. Similarly, ligation and poly-adenylation of RNA 3´ end requires a free 3´ -OH.

Featured product: RNA ligases

NEB offers a wide selection of ligases with varying activities to support a variety of applications. Use the selection chart to choose the best RNA ligase for your needs.

	RNA LIGASES							
	T4 RNA Ligase 1 (#M0204)	**T4 RNA Ligase 2** (#M0239)	**T4 RNA Ligase 2 Truncated** (#M0242)	**T4 RNA Ligase 2 Truncated K227Q** (#M0351)	**T4 RNA Ligase 2 Truncated KQ** (#M0373)	**ThermoStable 5´ App DNA/RNA Ligase** (#M0319)	**5´ Adenylation Kit** (#E2610)	**RtcB Ligase** (#M0458)
RNA APPLICATIONS								
Nicks in dsRNA		✔✔✔						
Labeling of 3´ Termini of RNA	✔✔✔		✔	✔	✔	✔		
Ligation of ssRNA to ssRNA	✔✔✔							
Ligation of Preadenylated Adaptors to RNA	✔✔		✔✔	✔✔	✔✔✔	✔✔		
5´ Adenylation							✔✔✔	
Ligation of 3´ P and 5´ OH of ssRNA								✔✔✔
DNA APPLICATIONS								
Ligation of Preadenylated Adaptors to ssDNA						✔✔✔		
DNA/RNA APPLICATIONS								
Joining of RNA and DNA in a ds-structure		✔✔						
Ligation of RNA and DNA with 3´ P and 5´ OH								✔✔
NGS APPLICATIONS								
NGS Library Prep dsDNA-dsDNA (Ligation)								
NGS Library Prep ssRNA-ssDNA (Ligation)	▲		▲	▲	▲	▲		
NGS Library Prep ssRNA-ds-Adaptor Splinted Ligation		▲						
FEATURES								
Thermostable						•	•	
Recombinant	•	•	•	•	•	•	•	•

KEY			
✔✔✔ Optimal, recommended ligase for selected application	✔✔ Works well for selected application	✔ Will perform selected application, but is not recommended	▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

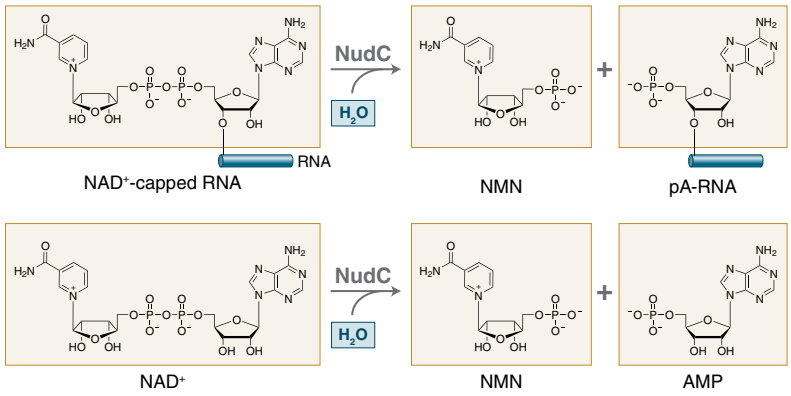
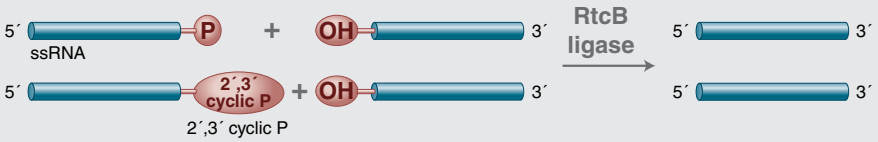
Enzymes for Innovation

The NEB catalog highlights a wide variety of enzyme functionalities found in nature or engineered for specific purposes. However, in molecular biology, new tools can often lead to new discoveries. Taking advantage of the enzymology expertise at NEB, we are offering novel enzymes with interesting and unique activities for manipulating DNA, RNA, proteins and glycans, even if specific applications for them have yet to be discovered. Our hope is that by engaging researchers' imaginations, our "Enzymes for Innovation" initiative will enable the development of new molecular techniques that so often lead to new discoveries..

FEATURED PRODUCTS

- NuDc Pyrophosphatase (NEB #M0607)
- RtcB Ligase (NEB #M0458)
- Sce PUS1 (NEB #M0526)

Visit www.neb.com/EnzymesForInnovation to view the full list of products available.

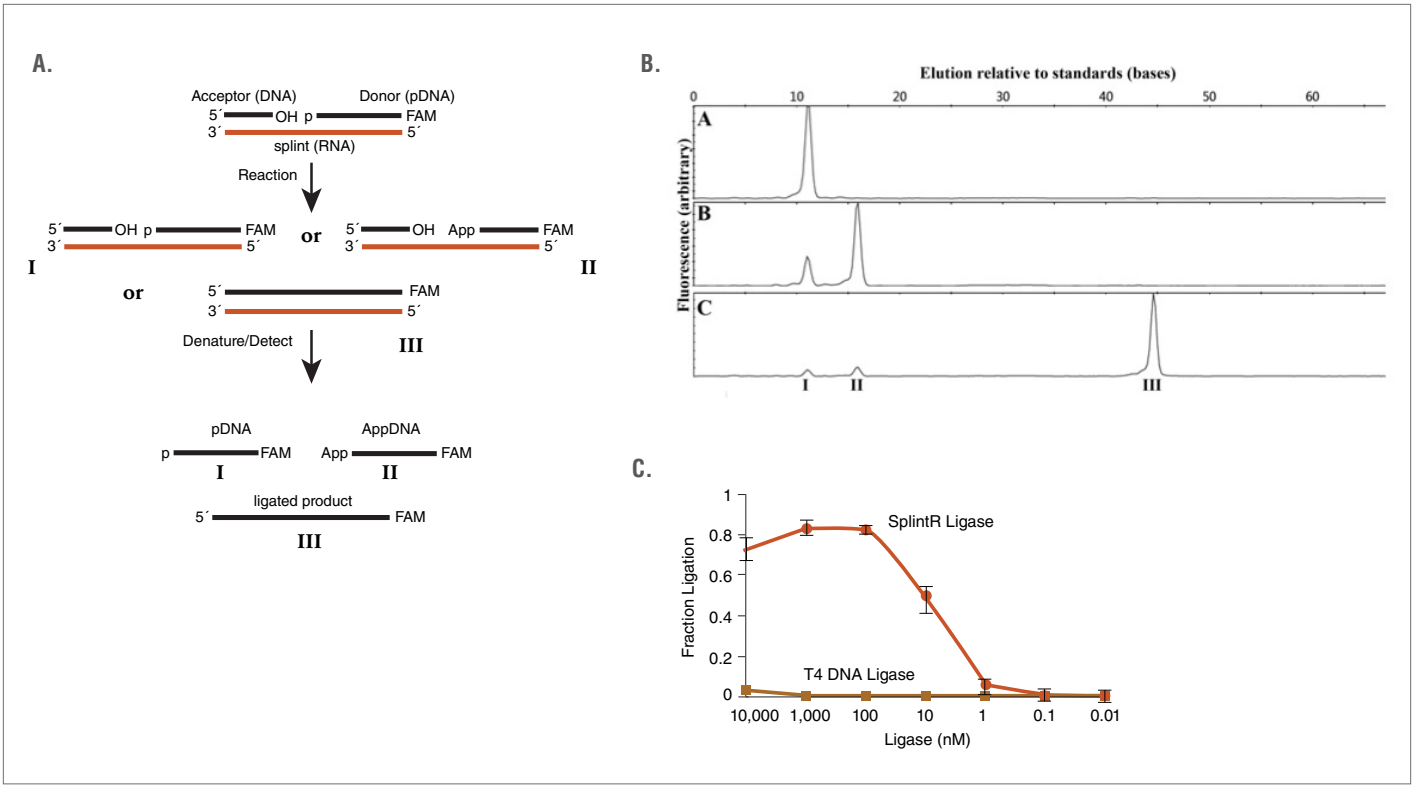
PRODUCT	NEB #	DESCRIPTION
NuDc Pyrophosphatase	M0607	 NudC is a NUDIX pyrophosphatase that efficiently hydrolyzes NAD+ - and NADH-capped RNA, generating a ligatible 5´ monophosphate on the RNA (NAD+ decapping or deNADding).
RtcB Ligase	M0458	 Joins single-stranded RNA with a 3´-phosphate or 2´,3´-cyclic phosphate to another RNA with a 5´ -hydroxyl.
Sce PUS1	M0526	 Sce Pseudouridine Synthase I (Sce PUS1) converts Uridine to Pseudouridine in single-stranded RNA, with a preference for Uridines in single-stranded RNA regions over Uridines in double-stranded RNA. The optimal substrate is an unstructured RNA that is 15 nt long or longer.

Featured product: SplintR Ligase

Our broad portfolio of RNA ligases includes unique specificities such as SplintR Ligase, which efficiently catalyzes the ligation of adjacent, single-stranded DNA splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Learn more about SplintR Ligase in our webinar at neb.com/NEBTVwebinars

Ligation of DNA splinted by RNA



(A) Outline of the ligation assay: a 5´-phosphorylated, 3´-FAM labeled DNA "donor" oligonucleotide and an unmodified DNA "acceptor" oligonucleotide are annealed to a complementary RNA splint. This substrate is reacted with a ligase to form a mixture of unreacted starting material (I), adenylylated DNA (II), and ligated product (III). These products are denatured, separated by capillary electrophoresis and detected by fluorescence. (B) Ligation of the RNA-splinted substrate in SplintR Ligase Reaction Buffer for 15 minutes at 25°C with (a) no enzyme, (b) 1 µM T4 DNA Ligase and (c) 100 nM SplintR Ligase. Indicated peaks correspond to starting pDNA (I), AppDNA (II) and ligated product (III) as determined by co-elution with synthetically prepared standards. (C) The fraction of ligated product catalyzed by either SplintR Ligase or T4 DNA Ligase was analyzed by performing sets of ligations with both ligases at concentrations between 10 pM and 10 µM for 15 minutes at 25°C. SplintR Ligase is clearly much more efficient at ligating RNA-splinted DNA than T4 DNA Ligase.

Ordering Information

RNA Synthesis

PRODUCT	NEB #	SIZE
HiScribe T7 mRNA Kit with CleanCap Reagent AG	E2080S/L	20/100 reactions
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S/L	50/250 reactions
HiScribe T7 High Yield RNA Synthesis Kit	E2040S/L	50/250 reactions
HiScribe SP6 RNA Synthesis Kit	E2070S	50 reactions
HiScribe T7 ARCA mRNA Kit	E2065S	20 reactions
HiScribe T7 ARCA mRNA Kit (with tailing)	E2060S	20 reactions
T3 RNA Polymerase	M0378S	5,000 units
T7 RNA Polymerase	M0251S/L	5,000/25,000 units
SP6 RNA Polymerase	M0207S/L	2,000/10,000 units
Hi-T7 RNA Polymerase	M0658S	5,000 units
E. coli Poly(A) Polymerase	M0276S/L	100/500 units
Poly(U) Polymerase	M0337S	60 units
E. coli RNA Polymerase, Core Enzyme	M0550S	100 units
E. coli RNA Polymerase, Holoenzyme	M0551S	50 units
Ribonucleotide Solution Set	N0450S/L	10/50 µmol of each
Ribonucleotide Solution Mix	N0466S/L	10/50 µmol of each
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
Pyrophosphatase, Inorganic (*E. coli*)	M0361S/L	10/50 units
Pyrophosphatase, Inorganic (yeast)	M2403S/L	10/50 units
Thermostable Inorganic Pyrophosphatase	M0296S/L	250/1,250 units
mRNA Decapping Enzyme	M0608S	2,000 units
Vaccinia Capping System	M2080S	400 units
Faustovirus Capping Enzyme	M2081S/L	500/2,500 units
Anti-Reverse Cap Analog 3´-O-Me-m^{7}G(5´)ppp(5´)G	S1411S/L	1/5 µmol
Standard Cap Analog m^{7}G(5´)ppp(5´)G	S1404S/L	1/5 µmol
Unmethylated Cap Analog G (5´) ppp(5´)G	S1407S/L	1/5 µmol
Methylated Cap Analog for A + 1 sites m^{7}G(5´)ppp(5´)A	S1405S/L	1/5 µmol
Unmethylated Cap Analog for A + 1 sites G(5´)ppp(5´)A	S1406S/L	1/5 µmol
mRNA Cap 2´-*O*-Methyltransferase	M0366S	2,000 units
3´-Desthiobiotin-GTP	N0761S	0.5 µmol

cDNA Synthesis

PRODUCT	NEB #	SIZE
ProtoScript II Reverse Transcriptase	M0368S/L/X	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase	M0253S/L	10,000/50,000 units
AMV Reverse Transcriptase	M0277S/L	200/1,000 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
ProtoScript II First Strand cDNA Synthesis Kit	E6560S/L	30/150 reactions
ProtoScript First Strand cDNA Synthesis Kit	E6300S/L	30/150 reactions

RNA Detection

PRODUCT	NEB #	SIZE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1,000/2,500 reactions
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1,000/2,500 reactions
Luna Probe One-Step RT-qPCR Kit (No ROX)	E3007E	2,500 reactions
LunaScript RT SuperMix Kit	E3010S/L	25/100 reactions
LunaScript RT SuperMix	M3010L/X/E	100/500/2,500 reactions
LunaScript RT Master Mix Kit (Primer-free)	E3025S/L	25/100 reactions
Luna Probe One-Step RT-qPCR 4X Mix with UDG	M3019S/L/X/E	200/500/1,000/2,500 reactions
Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)	M3029S/L/E	200/500/2,000 reactions
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG	L4001S	120 reactions

RNA Ligases & Modifying Enzymes

PRODUCT	NEB #	SIZE
T4 RNA Ligase 1 (ssRNA Ligase)	M0204S/L	1,000/5,000 units
T4 RNA Ligase 1 (ssRNA Ligase), high concentration	M0437M	5,000 units
T4 RNA Ligase 2 (dsRNA Ligase)	M0239S/L	150/750 units
T4 RNA Ligase 2, truncated	M0242S/L	2,000/10,000 units
T4 RNA Ligase 2, truncated K227Q	M0351S/L	2,000/10,000 units
T4 RNA Ligase 2, truncated KQ	M0373S/L	2,000/10,000 units
RtcB Ligase	M0458S/L	25 reactions
Thermostable 5´ AppDNA/RNA Ligase	M0319S/L	10/50 reactions
5´ DNA Adenylation Kit	E2610S/L	10/50 reactions
SplintR Ligase	M0375S/L	1,250/6,250 units
RNA 5´ Pyrophosphohydrolase (RppH)	M0356S	200 units
5´ Deadenylase	M0331S	1,000 units
RNase I$_f$	M0243S/L	5,000/25,000 units
RNase R	M0100S	400 units
RNase H	M0297S/L	250/1,250 units
RNase 4	M1284S/L	2,500/12,500 units
RNase 4 Digestion and 3´ End Repair Mix	M1288S/L	50/250 reactions
RNase HII	M0288S/L	250/1,250 units
Quick Dephosphorylation Kit	M0508S/L	100/500 units
Antarctic Phosphatase	M0289S/L	1,000/5,000 units
Alkaline Phosphatase Calf Intestinal (CIP)	M0290S/L	1,000/5,000 units
Shrimp Alkaline Phosphatase (rSAP)	M0371S/L	500/2,500 units
T4 Polynucleotide Kinase	M0201S/L	500/2,500 units
ShortCut RNase III	M0245S/L	200/1,000 units
XRN-1	M0338S/L	20/100 units
Exonuclease T	M0265S/L	250/1,250 units
NudC Pyrophosphatase	M0607	250 pmol
Sce PUS1	M0526	5,000 pmol

gRNA Synthesis

PRODUCT	NEB #	SIZE
EnGen sgRNA Synthesis Kit, *S. pyogenes*	E3322S	20 reactions

RNA Library Preparation for Next Generation Sequencing

PRODUCT	NEB #	SIZE
NEBNext UltraExpress RNA Library Prep Kit	E3330S/L	24/96 reactions
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 reactions
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module	E6421S/L	24/96 reactions
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 reactions
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 reactions
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 reactions
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 reactions
NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 reactions
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 reactions
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 reactions
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 reactions
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 reactions
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 reactions
NEBNext RNA Depletion Core Reagent Set	E7865S/L/X	6/24/96 reactions
NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	E7870S/L/X	6/24/96 reactions
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 reactions
NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 reactions

RNA Library Preparation for Next Generation Sequencing (cont.)

PRODUCT	NEB #	SIZE
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers 2)	E7780S	96 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 reactions
NEBNext Singleplex Oligos for Illumina	E7350S/L	12/60 reactions
DNase I	S1402S	10 ml (200 mM)

RNase Control

PRODUCT	NEB #	SIZE
RNase Inhibitor, Murine	M0314S/L	3,000/15,000 units
RNase Inhibitor, Human Placenta	M0307S/L	2,000/10,000 units
Ribonucleoside Vanadyl Complex	S1402S	10 ml (200 mM)

RNA Purification & Cleanup

PRODUCT	NEB #	SIZE
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch RNA Lysis Buffer	T2012L	100 ml
Monarch RNA Priming Buffer	T2013L	56 ml
Monarch RNA Wash Buffer	T2014L	50 ml
Monarch Collection Tubes II	T2018L	100 tubes
Monarch RNA Purification Columns	T2007L	100 columns
Monarch DNA/RNA Protection Reagent	T2011L	56 ml
Monarch Total RNA Miniprep Enzyme Pack (contains DNase I, Prot K, and associated buffers)	T2019L	1 pack
Monarch RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps
Monarch RNA Cleanup Kit (500 µg)	T2050S/L	10/100 preps
Monarch RNA Cleanup Binding Buffer	T2041L	80 ml
Monarch RNA Cleanup Wash Buffer	T2042L	40 ml
Monarch RNA Cleanup Columns (10 µg)	T2037L	100 columns and tubes
Monarch RNA Cleanup Columns (50 µg)	T2047L	100 columns and tubes
Monarch RNA Cleanup Columns (500 µg)	T2057L	100 columns and tubes
Magnetic mRNA Isolation Kit	S1550S	25 isolations
Epimark N6-Methyladenosine Enrichment Kit	E1610S	20 reactions
Oligo $d(T)_{25}$ Magnetic Beads	S1419S	25 mg
Oligo $d(T)_{25}$ Cellulose Beads	S1408S	250 mg
Streptavidin Magnetic Beads	S1420S	5 ml (20 mg)
Hydrophilic Streptavidin Magnetic Beads	S1421S	5 ml (20 mg)
polyA Spin mRNA Isolation Kit	S1560S	8 isolations
p19 siRNA Binding Protein	M0310S	1,000 units

RNA Markers & Ladders

PRODUCT	NEB #	SIZE
dsRNA Ladder	N0363S	25 gel lanes
microRNA Marker	N2102S	100 gel lanes
ssRNA Ladder	N0362S	25 gel lanes
Low Range ssRNA Ladder	N0364S	25 gel lanes
RNA Loading Dye (2X)	B0363S	4 ml
Universal miRNA Cloning Linker	S1315S	0.83 nmol

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