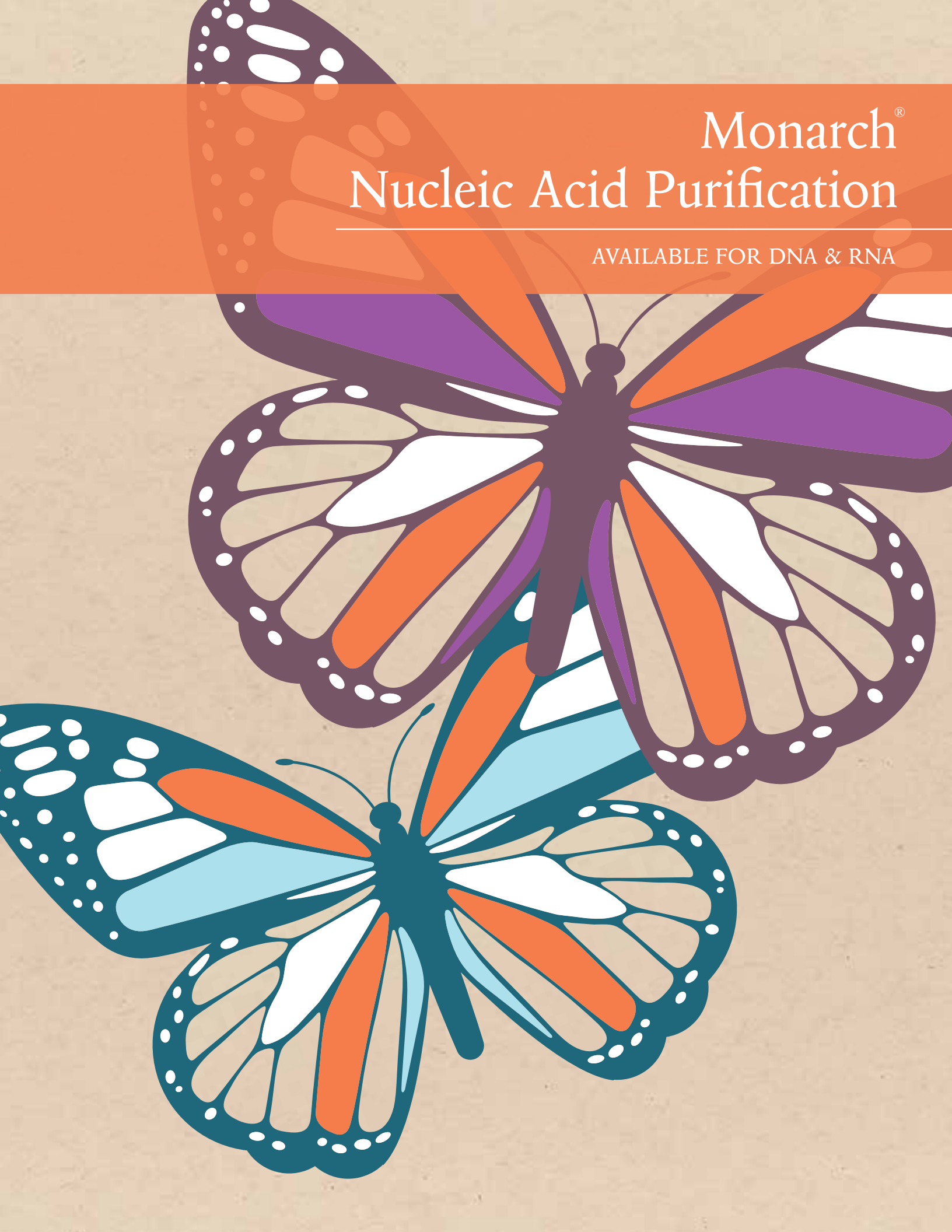
Monarch®
Nucleic Acid Purification

AVAILABLE FOR DNA & RNA



Make the right choice and migrate to Monarch

Monarch® Nucleic Acid Purification Kits are the perfect complement to many molecular biology workflows, including enzyme digests, transformation, electrophoresis, RT-PCR and library preparation. Purify high-quality nucleic acids suitable for direct use in a variety of downstream applications. Recover pure, intact DNA and RNA in minutes with our fast, user-friendly protocols and optimized buffer systems, and focus your time on the experiments that will drive your research forward. Monarch DNA columns are designed without a retaining ring, eliminating buffer carryover and allowing for low volume elution. The Monarch Total RNA Miniprep Kit is optimized for use with a wide variety of sample types, so that one convenient kit can serve your RNA purification needs.

Monarch kits are all designed with sustainability in mind; whenever possible, kits and components are made with significantly less plastic and are packaged with responsibly-sourced, recyclable packaging. Furthermore, plastic recovered during the manufacture of Monarch columns is used to manufacture other plastic-based NEB products.

AVAILABLE KITS:

Monarch Plasmid Miniprep Kit

- Easily purify plasmids from bacterial cultures
- Monitor your progress with our convenient colored-buffer system
- Eliminate buffer carryover with columns that do not contain retaining rings
- Utilize convenient labeling tabs

Monarch DNA Gel Extraction Kit

- Recover DNA from agarose gels quickly
- Elute in ≥ 6 µl for highly concentrated DNA
- Eliminate buffer carryover with columns that do not contain retaining rings

Monarch PCR & DNA Cleanup Kit

- Purify your DNA from enzymatic reactions in 5 minutes
- Elute in ≥ 6 µl for highly-concentrated DNA
- Eliminate buffer carryover with columns that do not contain retaining rings
- Use the same kit to purify small DNA fragments and oligos

NEW Monarch Total RNA Miniprep Kit

- Choose one convenient, all-in-one kit for multiple sample types
- Efficiently remove DNA with included DNase I and gDNA removal columns
- Process blood and tissue using included Proteinase K

*Visit NEBMonarchPackaging.com for details.

PURCHASE COLUMNS AND BUFFERS SEPARATELY FOR ADDED CONVENIENCE

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

Monarch Plasmid Miniprep Kit

The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis, and neutralization steps, with the additional benefit of color indicators at certain steps to easily monitor completion. Unique wash buffers ensure salts, proteins, RNA and other cellular components are removed, allowing low-volume elution of concentrated, highly pure DNA. Protocols are fast and user-friendly. Elution in as little as 30 µl provides concentrated DNA for use in downstream applications, such as restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

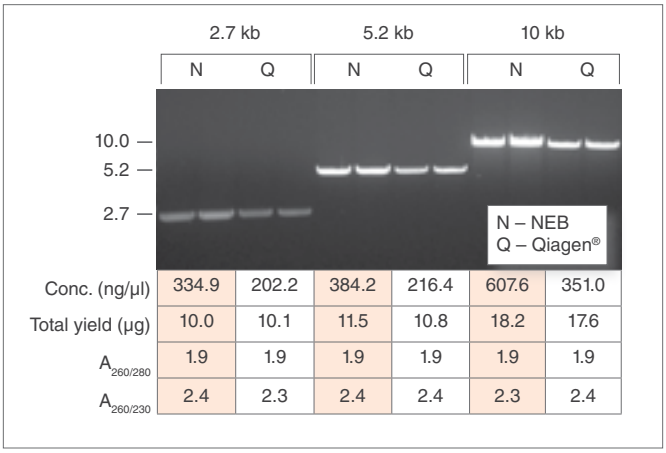
ADVANTAGES

- Elute in low volumes
- Prevent buffer retention and salt carryover with optimized column design
- Reduce hands on time with faster protocols and less spin time
- Monitor completion of certain steps using colored buffer system
- No need to add RNase before starting
- Easily label columns using tab and frosted surfaces

SPECIFICATIONS

- **Culture Volume:** 1–5 ml, not to exceed 15 O.D. units
- **Binding Capacity:** up to 20 µg
- **Plasmid Size:** up to 25 kb
- **Typical Recovery:** up to 20 µg, yield depends on plasmid copy number, host strain, culture volume, and growth conditions
- **Elution Volume:** ≥ 30 µl
- **Purity:** $A_{260/280}$ and $A_{260/230}$ ≥ 1.8
- **Protocol Time:** 10½ minutes of spin and incubation time
- **Compatible Downstream Applications:** restriction digestion and other enzymatic manipulations, transformation, transfection of robust cells, DNA sequencing, PCR, labeling, cell-free protein synthesis, etc.

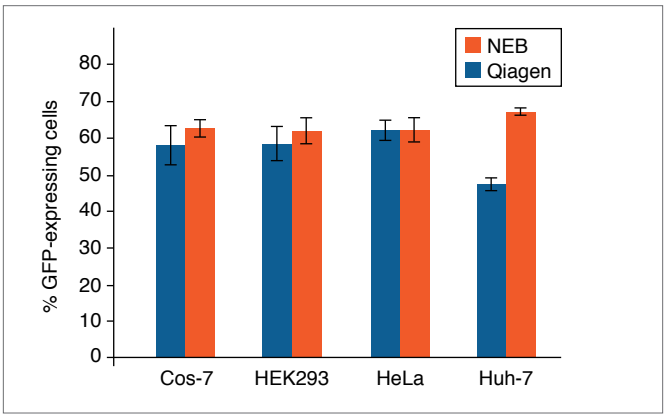
Monarch Plasmid Miniprep Kits consistently produce more concentrated plasmid DNA with equivalent yield, purity and functionality as compared to the leading supplier



	2.7 kb		5.2 kb		10 kb	
	N	Q	N	Q	N	Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

Preps were performed according to recommended protocols using 1.5 ml aliquots of the same overnight culture. One microliter of each prep was digested with HindIII-HF® (NEB #R3104) to linearize the vector and the digests were resolved on a 1% w/v agarose gel.

Plasmid DNA purified using the Monarch Plasmid Miniprep Kit produces transfection efficiencies equivalent to or better than plasmid DNA purified using the Qiagen® QIAprep® Spin Miniprep Kit



Plasmid DNA encoding constitutively expressed GFP (pEGFP-C2) was prepared using either Monarch Plasmid Miniprep Kit or Qiagen QIAprep Spin Miniprep Kit. Four different cell lines (Cos-7, HEK293, HeLa, and Huh-7) were grown to 80-90% confluence and transfected with 100 ng of each plasmid, in complex with 0.3 µl Lipofectamine 2000, and 10 µl Opti-MEM. Five replicates for each cell type were performed using both DNA preps. GFP expressing cells were counted by flow cytometry 48 hrs post-transfection with a minimum of 2000 events collected per well. Average percentage of cells expressing GFP from all replicates is graphed and used as a measure of transfection efficiency.

TIPS FOR SUCCESSFUL MINIPREPS

1. **Don't use too many cells (culture should not exceed 15 O.D. units):** Using the optimal amount of cells increases lysis efficiency and ensures that excess cell debris does not clog the column.
2. **Lyse cells completely:** In order to release all plasmid DNA, ALL of the cells need to be lysed. Resuspend cells completely, and incubate for the recommended time.
3. **Don't vortex cells after lysis:** Vortexing can cause shearing of host chromosomal DNA, resulting in gDNA contamination.
4. **Allow the RNase to do its job:** Do not skip or reduce the incubation with RNase (which is included in the neutralization buffer), otherwise you may observe RNA contamination.
5. **Don't skip any washes:** Proper washes ensure the removal of cell debris, endotoxins and salts.
6. **Heat the elution buffer for large plasmids:** Large DNA binds more tightly; heating the elution buffer helps to more efficiently release the DNA from the column matrix.

Monarch Kits for your DNA cleanup

Monarch DNA cleanup kits rapidly and reliably purify up to 5 µg of concentrated, high-quality DNA. These kits utilize a bind/wash/elute workflow with minimal incubation and spin times. The columns provided with each kit ensure zero buffer retention and no carryover of contaminants, enabling elution of sample in volumes as low as 6 µl. Monarch Buffers have been optimized, and do not require monitoring of pH. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations.

Monarch DNA Gel Extraction Kit

The Monarch DNA Gel Extraction Kit can be used to quickly purify DNA from agarose gels. Unlike other kits, there is no need to add isopropanol to the melted agarose prior to loading on the column, saving you a step. Enjoy high yields and minimal hands on time.

Monarch PCR & DNA Cleanup Kit (5 µg)

The Monarch PCR & DNA Cleanup Kit (5 µg) can be used to purify DNA from a variety of enzymatic reactions, such as PCR, restriction digestion, ligation and reverse transcription. The DNA Wash Buffer provided ensures enzymes, short primers (≤ 25 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed. A simple protocol modification also enables purification of small DNA and oligonucleotides.

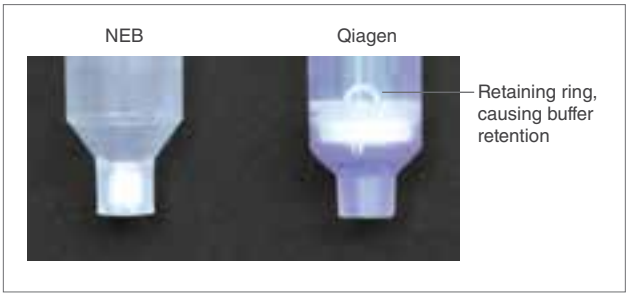
ADVANTAGES

- Elute in as little as 6 µl
- Prevent buffer retention and salt carryover with optimized column design
- Purify oligos and other small DNA fragments with simple protocol modification
- Save time with fast, user-friendly protocols
- Designed with sustainability in mind

SPECIFICATIONS

- **Binding Capacity:** up to 5 µg
- **DNA Size Range:** ~50 bp to 25 kb
  With protocol modification, oligos ≥ 15 bp (dsDNA) or ≥18 nt (ssDNA) can be purified with NEB #T1030
- **Typical Recovery:** DNA (50 bp to 10 kb): 70–90%
  DNA (11–25 kb): 50–70%
  ssDNA ≥18 nt and dsDNA ≥ 15 bp: 70-85% (NEB #T1030 only)
- **Elution Volume:** ≥ 6 µl
- **Purity:** $A_{260/280}$ ≥ 1.8
- **Protocol Time:**
  Gel Extraction: 10 min of spin and incubation time
  PCR & DNA Cleanup: 5 min of spin and incubation time
- **Compatible Downstream Applications:** ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing

Optimized Monarch column design



Many purification columns are built with a retaining ring to hold the membrane in place, but this can trap buffer. Monarch columns' silica matrix is held in place without the use of a retaining ring, eliminating buffer retention and ensuring worry-free purification.

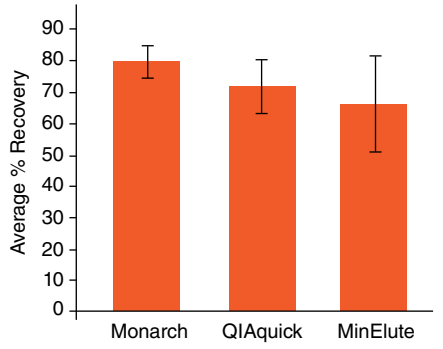
> *This is a great, easy-to-use, small footprint kit... it was great to elute in such a small volume while feeling confident that the elution buffer managed to get to all of the surface area of the membrane.*
>
> – Michelle, Principal Investigator, Central Michigan University

TIPS FOR SUCCESSFUL GEL EXTRACTIONS

1. **Use the smallest possible agarose plug:** The less agarose in solution, the more efficient the extraction will be. More agarose means more melting time and more buffer needed to dissolve it (introducing more salts which can co-elute with your sample). If the plug is greater than 160 mg, the volume of agarose plus buffer will exceed that of the column reservoir (800 µl), and will require that your sample be loaded onto the column in two steps.
2. **Minimize exposure to UV light:** Excise the gel slice as quickly as possible, as exposure to UV light damages DNA. As long as the excision is done quickly, damage will be negligible.
3. **Melt the agarose completely:** If the agarose is not completely melted, DNA remains trapped inside and cannot be extracted properly.
4. **Heat the elution buffer for large DNA fragments:** Large DNA binds more tightly; heating the elution buffer helps to more efficiently release the DNA from the column matrix.

and gel extraction needs

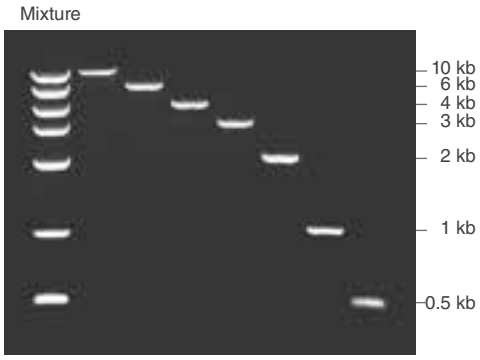
DNA purified from the Monarch DNA Gel Extraction Kit is recovered with similar efficiency and purity as the leading supplier, but is more highly concentrated, facilitating its use in downstream applications



	Monarch	QIAquick®	MinElute®
$A_{260/280}$	1.9+/-0.08	1.9+/-0.08	1.9+/-0.04
Yield (µg)	0.80+/-0.05	0.72+/-0.09	0.66+/-0.13
Conc. (ng/µl)	144.9+/-9.6	25.7+/-3.1	73.8+/-14.2

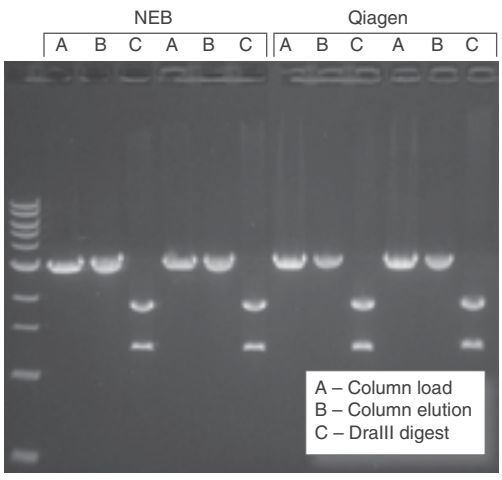
One microgram aliquots of a 3 kb fragment were resolved on a 1% w/v agarose gel, excised, and processed with different kits using manufacturer-specified minimum elution volumes. Values reported are the concentration and purity data determined by Nanodrop™ readings, as well as recovery calculations based on the eluted DNA concentration and recovered volume.

Monarch DNA Gel Extraction Kit reproducibly recovers DNA over a broad range of molecular weights



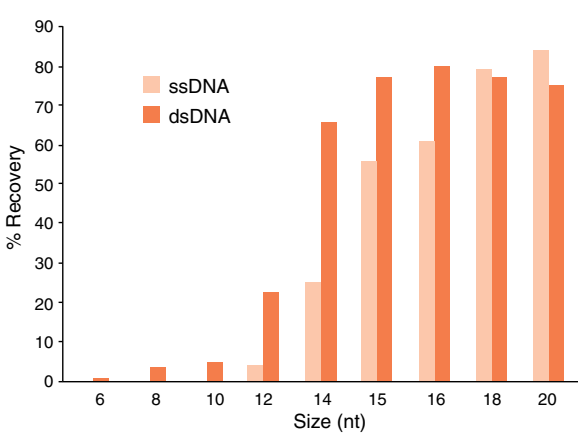
A mixture of 7 DNA fragments ranging from 10 kb to 0.5 kb was prepared and one-half of the mixture was resolved on a 1% w/v agarose gel. Each fragment was manually excised from the agarose gel and processed using the Monarch DNA Gel Extraction Kit. The entire elution of each fragment was resolved on a new gel with the remainder of the original mixture for comparison.

Monarch PCR & DNA Cleanup Kit (5 µg) performs equivalently to the leading supplier



Preps were performed according to recommended protocols. 1 µg of a 3 kb DNA fragment was incubated with 1 µM primers and OneTaq® Quick-Load® 2X Master Mix (NEB #M0486). DNA was eluted in 20 µl (NEB) and 40 µl (Qiagen) Elution Buffer. Half of the total elution volume was digested with 5 units of DraIII-HF (NEB #R3510). The digest and the unused portion of the elution were resolved on a 1% w/v agarose gel along with a representative sample of the starting material.

With simple protocol modification, the Monarch PCR & DNA Cleanup Kit can efficiently recover ssDNA and dsDNA oligonucleotides (1 µg), expanding the utility of the kit



Synthesized ssDNA and dsDNA oligonucleotides (1 µg in 50 µl H_2O) of varying lengths (6-20 nt) were purified using the oligonucleotide cleanup protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030), and were eluted in 50 µl water. The average percent recovery (n=3) of the oligonucleotides was calculated from the resulting A_{260} as measured using a Trinean® DropSense™ 16. Use of the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) results in the efficient removal of small oligonucleotides (6-12 nt) and > 70% recovery and cleanup of oligonucleotides ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA).

Monarch Total RNA Miniprep Kit

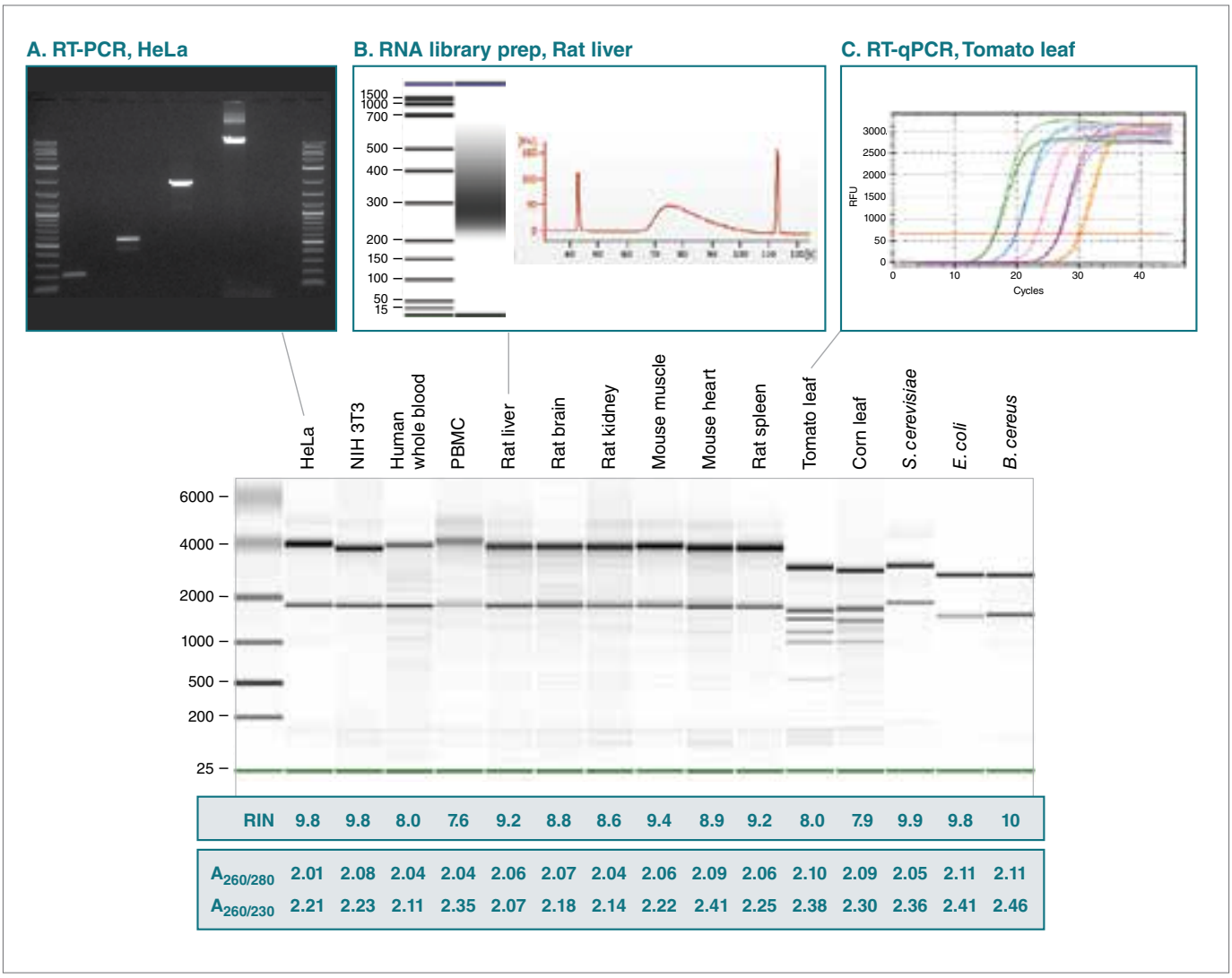
The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios ≥1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

Monarch has been validated for the following sample types:

- HeLa Cells
- HEK 293 Cells
- NIH 3T3 Cells
- Human Blood
- Rat Blood
- PBMC's
- Rat Liver
- Rat Spleen
- Rat Kidney
- Rat Brain
- Rat Muscle
- Mouse Muscle
- Mouse Heart
- Mouse Kidney
- *S. cervisiae*
- *E. coli*
- *B. cereus*
- Corn Leaf
- Tomato Leaf

For information on input amounts, yield, RIN values, please refer to the product manual at **neb.com/T2010**.

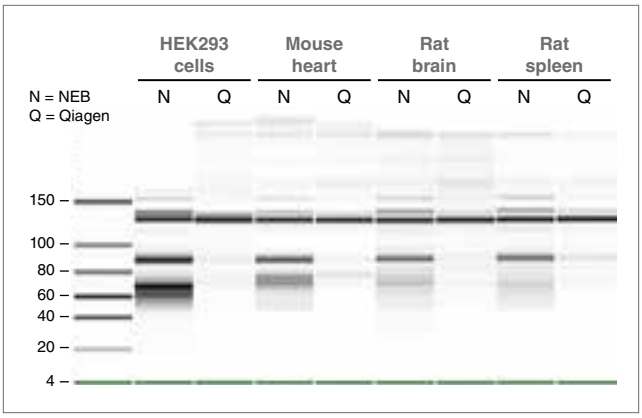
Monarch-purified RNA is high-quality and compatible with a wide variety of downstream applications



	HeLa	NIH 3T3	Human whole blood	PBMC	Rat liver	Rat brain	Rat kidney	Mouse muscle	Mouse heart	Rat spleen	Tomato leaf	Corn leaf	*S. cerevisiae*	*E. coli*	*B. cereus*
RIN	9.8	9.8	8.0	7.6	9.2	8.8	8.6	9.4	8.9	9.2	8.0	7.9	9.9	9.8	10
$A_{260/280}$	2.01	2.08	2.04	2.04	2.06	2.07	2.04	2.06	2.09	2.06	2.10	2.09	2.05	2.11	2.11
$A_{260/230}$	2.21	2.23	2.11	2.35	2.07	2.18	2.14	2.22	2.41	2.25	2.38	2.30	2.36	2.41	2.46

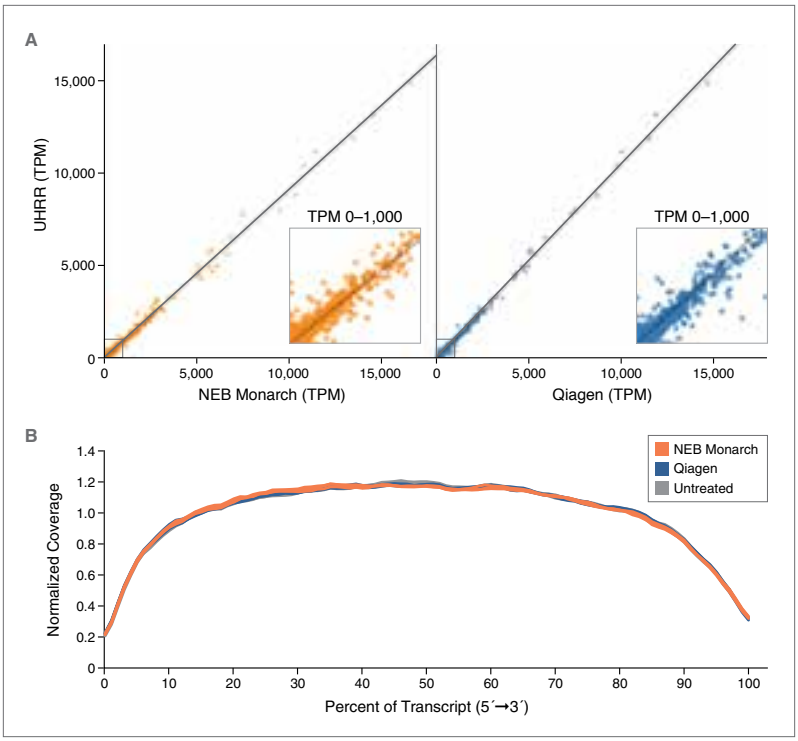
Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit (NEB #T2010). Aliquots were run on an Agilent® Bioanalyzer® 2100 using the Nano 6000 RNA chip (S. cerevisiae RNA was run using a plant Nano assay). RIN values and O.D. ratios confirm the overall integrity and purity of the RNA. To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/– RT) (A) for detection of 4 different RNA species using Protoscript® II Reverse Transcriptase (NEB #M0368)/LongAmp® Taq DNA Polymerase (NEB #M0323), NGS library prep (B) using NEBNext® Ultra™ II RNA Library Prep Kit (NEB #E7760) and RT-qPCR (C) using Luna® One-Step RT-qPCR Reagents (NEB #E3005).

The Monarch Total RNA Miniprep Kit successfully purifies small RNAs below 200 nucleotides, enabling a more accurate representation of the total RNA pool



RNA preps were performed on HEK293 cells, mouse heart, rat brain, or rat spleen using the Monarch Total RNA Miniprep Kit (N) (NEB #T2010) and the RNeasy® Mini Kit from Qiagen (Q). Equivalent amounts were resolved on a Bioanalyzer 2100 using the Small RNA chip. Monarch-purified RNA contains significantly more RNA in the sub-200 nucleotide pool.

Monarch-purified RNA can be used to prepare high quality RNA-seq libraries for gene expression analysis



Transcript levels in Universal Human Reference RNA (UHRR, Agilent) are compared before and after re-purification using either Qiagen RNeasy® or the Monarch Total RNA Miniprep Kit. Strong correlation with untreated UHRR is observed for both methods (Pearson R > 0.99 for both samples). All samples display consistent end-to-end coverage of transcripts indicating an absence of detectable degradation during purification. Poly-A selected RNA was selected from 100 ng of untreated, Qiagen and Monarch samples using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). RNA-seq libraries were then prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina® (NEB #E7760) before sequencing on a Miseq® instrument (2 x150). 1.6M reads were randomly sampled from each library and adapter trimmed (Seqprep v1.1). Levels of all Gencode v26 transcripts were assessed using Salmon (0.4) and plotted above (panel A). Average 5'-3' Coverage of Gencode v26 transcripts (assessed by Picard's CollectRnaSeqMetrics 1.56 after mapping to the GRCh38 reference genome with Hisat v2.0.3 and marking duplicates with Picard's MarkDuplicates 1.56) is shown below (panel B).

ADVANTAGES

- Use with a wide variety of sample types
- Purify RNA of all sizes, including miRNA & small RNAs >20 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent
- Protocols available for RNA fractionation and RNA cleanup
- Save money with value pricing for an all-in-one kit

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 10^7 or 50 mg tissue*
- **Elution Volume:** 50 – 100 µl
- **Yield:** varies depending on sample type
- **Compatible downsteam applications:** RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

**View manual for other sample types*

Kit Component	Monarch RNA Purification Kit	Qiagen RNeasy Kits		
		Mini	Protect Mini	Plus Mini
gDNA Removal Columns	✓	×*	×*	✓
DNase I	✓	×	×	×
Proteinase K	✓	×	×	×
RNA Protection Reagent	✓	×	✓	×

✓ = *Included* **Not included and not sold separately*

TIPS FOR SUCCESSFUL RNA EXTRACTIONS

1. **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.
2. **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.
3. **Ensure samples are properly homogenized/ disrupted:** Samples should be disrupted and homogenized completely to release all RNA.
4. **For sensitive applications, ensure proper gDNA removal:** gDNA removal is removed by the gDNA removal column and subsequent on-column DNase I treatment. Off-column DNase I treatment can also be employed.

Ordering Information

DNA PURIFICATION KITS	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns and tubes
Monarch Plasmid Miniprep Columns	T1017L	100 columns and tubes
BUFFERS AVAILABLE SEPARATELY		
Monarch DNA Cleanup Binding Buffer	T1031L	235 ml
Monarch DNA Wash Buffer	T1032L	25 ml
Monarch DNA Elution Buffer	T1016L	25 ml
Monarch Gel Dissolving Buffer	T1021L	235 ml
Monarch Plasmid Lysis Buffer (B2)	T1012L	2 x 27 ml
Monarch Plasmid Neutralization Buffer (B3)	T1013L	110 ml
Monarch Plasmid Resuspension Buffer (B1)	T1011L	55 ml
Monarch Plasmid Wash Buffer 1	T1014L	2 x 27 ml
Monarch Plasmid Wash Buffer 2	T1015L	30 ml

RNA PURIFICATION KIT	NEB #	SIZE
Monarch Total RNA Miniprep Kit	T2010S	50 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch RNA Purification Columns	T2007L	100 columns and tubes
Monarch gDNA Removal Columns	T2017L	100 columns and tubes
Monarch Collection Tubes II	T2018L	100 tubes
BUFFERS & REAGENTS AVAILABLE SEPARATELY		
Monarch DNA/RNA Protection Reagent	T2011L	56 ml
Monarch RNA Lysis Buffer	T2012L	100 ml
Monarch Total RNA Miniprep Enzyme Pack (contains DNase I, Prot K, and associated buffers)	T2019L	1 pack
Monarch RNA Priming Buffer	T2013L	56 ml
Monarch RNA Wash Buffer	T2014L	50 ml
Nuclease-free Water	B1500S/L	25 ml/100 ml

Time for change – try Monarch for free.

For a limited time, try one of our Monarch Nucleic Acid Purification Kits by visiting NEBMonarch.de (in Germany & Austria) or NEBMonarch.fr (in France)

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nqa ISO 14001 Registered Environmental Management
nqa ISO 13485 Registered Medical Devices

Monarch – Version 2.0 – 11/17