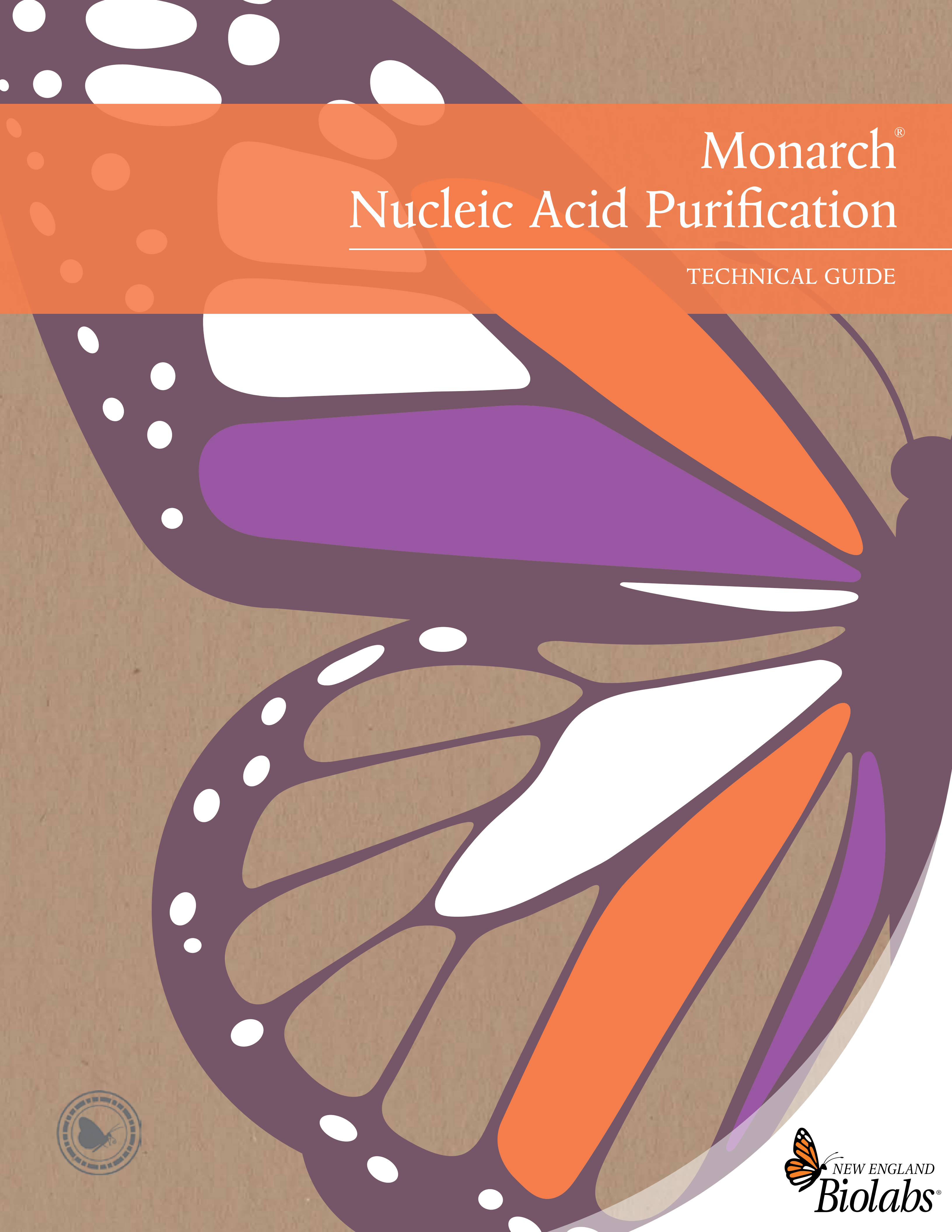
Monarch®
Nucleic Acid Purification

TECHNICAL GUIDE



NEW ENGLAND
Biolabs®

Make the right choice and migrate to Monarch

Monarch® Nucleic Acid Purification Kits are the perfect complement to many molecular biology workflows. 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. The Monarch nucleic acid purification portfolio can serve your needs, whether you are isolating nucleic acids from biological samples, cleaning up DNA and RNA from enzymatic reactions, extracting DNA fragments from gels, or purifying plasmids.

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 Mag Viral DNA/RNA Extraction Kit NEW

- Designed for hands-free extraction of viral DNA and/or RNA
- Utilizes magnetic beads. Compatible with manual and automated high-throughput workflows on a variety of instrument platforms
- Suitable for saliva, respiratory swab and wastewater samples

Monarch Spin Genomic DNA Purification Kit

- Purify high-quality, genomic DNA from several sample types
- Achieve excellent DNA yields with fast, user friendly protocols

Monarch HMW DNA Extraction Kits

- Quickly and easily extract ultra-high molecular weight DNA
- Available for cells & blood as well as tissues, bacteria and other samples

Monarch Total RNA Miniprep Kit

- Extract total RNA from a variety of sample types with this all-in-one kit
- Validated with cells, blood, saliva, swabs, tissues, bacterial, plant and more

Monarch Spin RNA Cleanup Kits

- Purify and concentrate RNA after enzymatic reactions and RNA synthesis
- Three convenient binding capacities (10 µg, 50 µg and 500 µg)

Monarch Spin Plasmid Miniprep Kit

- Easily purify plasmids from bacterial cultures
- Monitor your progress with our convenient colored-buffer system

Monarch Spin PCR & DNA Cleanup Kit

- Purify DNA in 5 minutes and elute in as little as 5 µl
- Unique column design eliminates buffer carryover
- Purify small DNA fragments and oligos with modified protocol

Monarch Spin DNA Gel Extraction Kit

- Quickly extract highly-pure DNA with excellent yields
- Column design enables elution in as little as 5 µl

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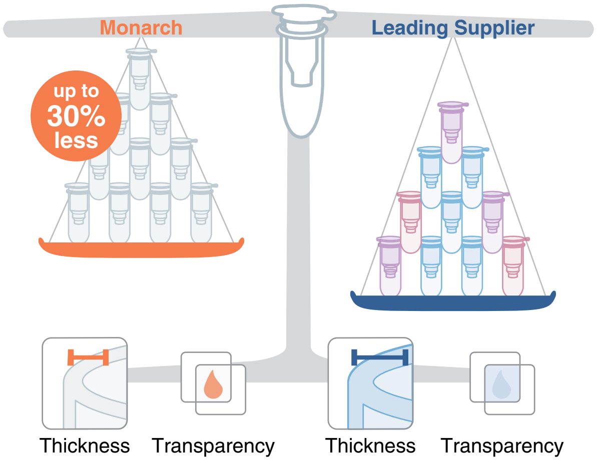
COLUMNS & COLLECTION TUBES
Weight in plastic

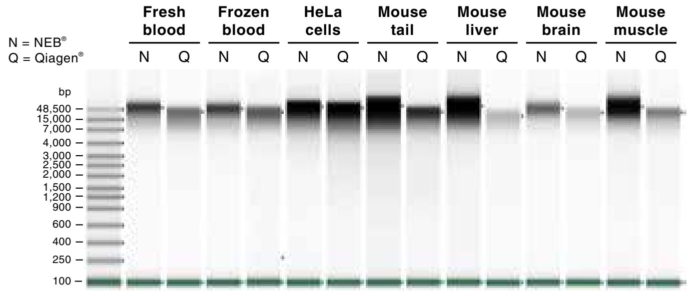


Visit **NEBMonarch.com** to learn more and request a sample.

Monarch Spin Genomic DNA Purification Kit

The Monarch Spin Genomic DNA Purification Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood and mammalian tissues. Excellent results are achieved even with challenging samples like fatty (e.g., brain) and fibrous (e.g., muscle, mouse tail) tissues. Additionally, bacteria, yeast and insects can be processed with minor protocol modifications to enhance lysis in these tough-to-lyse samples. Protocols are also included to enable purification from clinically-relevant samples, such as saliva and cheek swabs, as well as rapid cleanup of previously extracted gDNA. Purified gDNA has high quality metrics and minimal residual RNA. The purified gDNA is suitable for downstream applications, such as endpoint PCR, qPCR and library prep for next generation sequencing (NGS).

The Monarch Spin Genomic DNA Purification Kit provides excellent yields of higher quality, higher molecular weight DNA than the Qiagen® DNeasy® Blood & Tissue Kit



	Fresh blood		Frozen blood		HeLa cells		Mouse tail		Mouse liver		Mouse brain		Mouse muscle	
	N	Q	N	Q	N	Q	N	Q	N	Q	N	Q	N	Q
Peak size (kb)	54	28	50	27	57	54	>60	28	59	20	51	25	57	26
DIN	9.1	8.0	8.7	7.9	8.8	8.2	8.3	7.4	9.0	7.2	8.9	7.6	9.2	7.8
A_{260}/A_{280}	1.85	1.82	1.83	1.85	1.84	1.89	1.85	1.87	1.85	1.88	1.86	1.90	1.87	1.87
A_{260}/A_{230}	2.19	2.17	2.43	1.21	2.33	1.91	2.36	1.98	2.43	1.31	2.47	1.52	2.40	1.57

Agilent Technologies® 4200 TapeStation® Genomic DNA ScreenTape was used for analysis of gDNA purified from blood, cultured cells and tissue samples using the relevant protocols of the Monarch Spin Genomic DNA Purification Kit and the Qiagen DNeasy Blood & Tissue Kit. gDNA was eluted in 100 µl and 1/100 of the eluates (~1 µl) was loaded on a Genomic DNA ScreenTape. Starting materials used: 100 µl fresh human whole blood, 100 µl frozen pig blood, 1 x 10⁶ HeLa cells and 10 mg frozen tissue powder. Monarch-purified gDNA samples typically show peak sizes 50–70 kb and DINs of ~9. DNeasy-purified gDNA peak sizes are typically < 30 kb with DINs ~7–8. DNeasy kits produce lower yields and low $A_{260/230}$ ratios for liver, brain, muscle and frozen blood.

ADVANTAGES

- Use with a wide variety of sample types
- Achieve higher yields, especially with difficult tissue samples (e.g., brain and muscle)
- Effectively remove RNA (< 1% residual RNA) with optimized buffer chemistry and included RNase A
- Isolate longer DNA (peak size > 50 kb), which is suitable for long read sequencing platforms
- Save time with fast protocols, efficient lysis steps and minimal hands on time
- Can also be used to clean up genomic DNA

SPECIFICATIONS

- **Recommended Input Amount:** Varies by sample type. See page 6.
- **Binding Capacity:** 30 µg genomic DNA
- **Genomic DNA Size:** Peak size > 50 kb for most sample types; may be lower for saliva and buccal swabs
- **Elution Volume:** ≥ 35 µl (100 µl is recommended)
- **Purity:** $A_{260/280} \geq 1.8$, $A_{260/280} \geq 2.0$
- **RNA Content:** < 1% (with included RNase A treatment)
- **Compatible Downstream Applications:** endpoint PCR, qPCR, library preparation for NGS (including Oxford Nanopore Technologies® and PacBio®)

> *This kit yielded the highest purity DNA I have ever seen from a commercial spin column kit.*
>
> – Stephen, Lake Superior State University

1 Lyse

+ 200 µl sample & Lysis Buffer
+ 1–10 µl Proteinase K
+ 3 µl RNase A*†

Blood/cells: 5 min. (56°C)
Tissue: 60 min. (56°C)

2 Bind

+ 400 µl gDNA Binding Buffer

3 min. (1,000 xg)
1 min. (max. speed)

No ethanol required

3 Wash (2X)

+ 500 µl gDNA Wash Buffer

1 min. (max. speed)

4 Elute

+ 35–100 µl gDNA Elution Buffer (60°C)

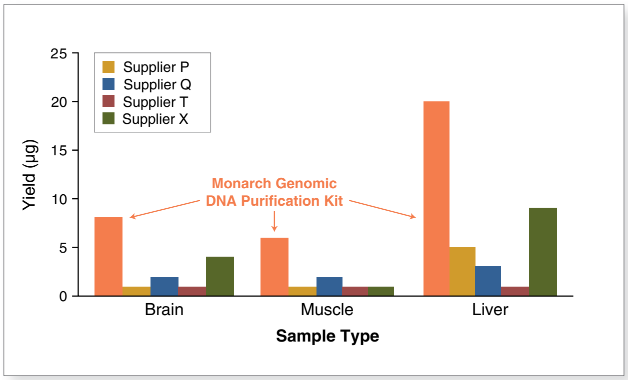
1 min. (max. speed)

One elution step

* Add separately for tissue samples
† Include debris removal spin for fibrous tissues

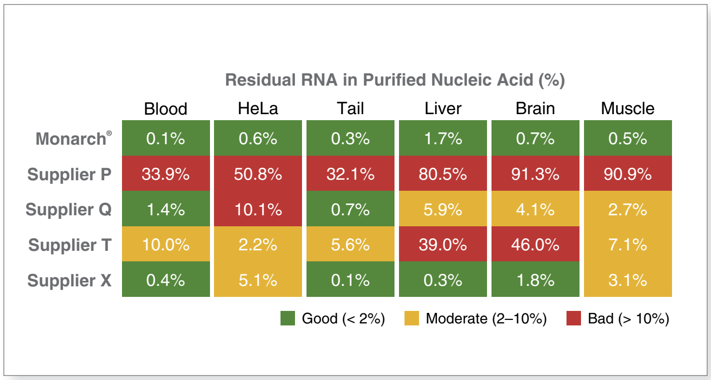
Purified DNA is High Yield, Highly Pure, Free from RNA and Ready for use in Downstream Applications

The Monarch Spin Genomic DNA Purification Kit provides excellent yields for difficult tissue types



Duplicate 10 mg samples of RNAlater®-stabilized rat tissue were cut to small pieces and subsequently lysed and purified according to the protocols provided for each kit. Optional RNase A steps were included. Elution was carried out with 100 µl elution buffer provided in the respective kits. Yields displayed are averages of the duplicate samples, and represent the genomic DNA yield after correcting for the RNA content as determined by LC-MS. Results indicate that the Monarch Spin Genomic DNA Purification Kit provides excellent yields for a wide range of tissues, which can be problematic for other commercial kits.

DNA purified with the Monarch Spin Genomic DNA Purification Kit has significantly lower residual RNA across all sample types



Residual RNA in Purified Nucleic Acid (%)

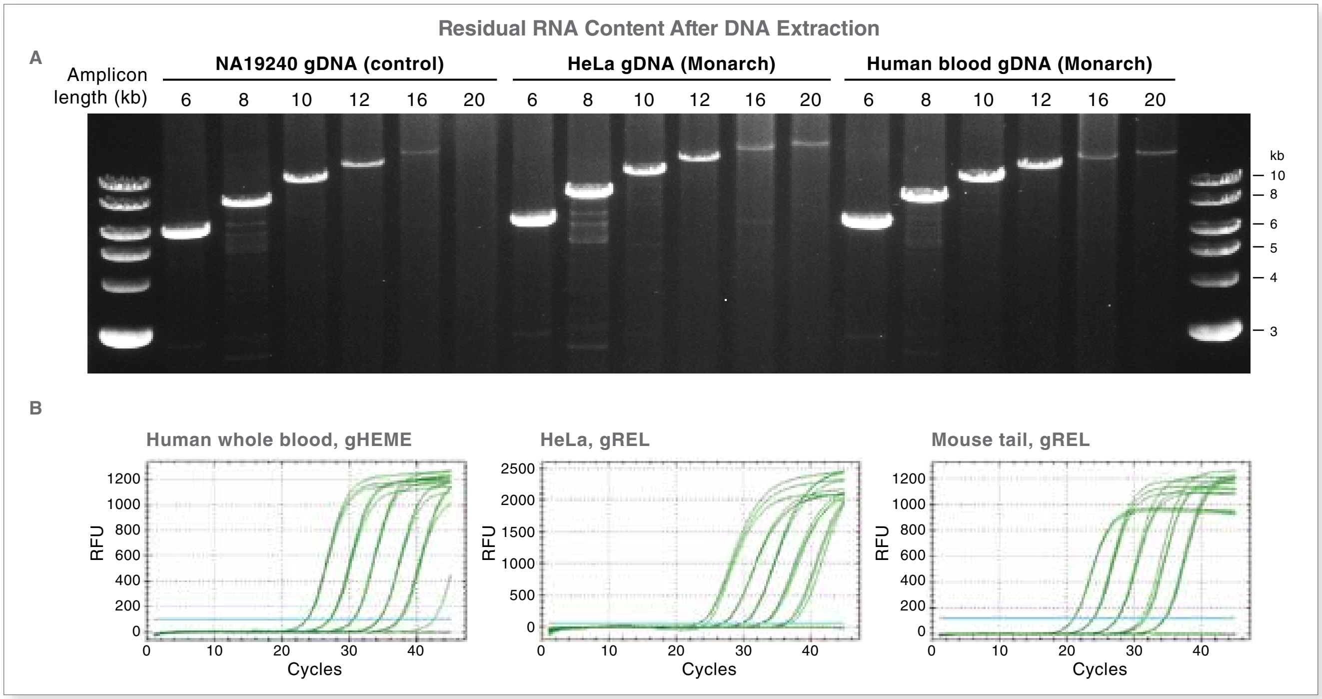
	Blood	HeLa	Tail	Liver	Brain	Muscle
Monarch®	🟩 0.1%	🟩 0.6%	🟩 0.3%	🟩 1.7%	🟩 0.7%	🟩 0.5%
Supplier P	🟥 33.9%	🟥 50.8%	🟥 32.1%	🟥 80.5%	🟥 91.3%	🟥 90.9%
Supplier Q	🟩 1.4%	🟥 10.1%	🟩 0.7%	🟨 5.9%	🟨 4.1%	🟨 2.7%
Supplier T	🟨 10.0%	🟨 2.2%	🟨 5.6%	🟥 39.0%	🟥 46.0%	🟨 7.1%
Supplier X	🟩 0.4%	🟨 5.1%	🟩 0.1%	🟩 0.3%	🟩 1.8%	🟨 3.1%

🟩 Good (< 2%) 🟨 Moderate (2–10%) 🟥 Bad (> 10%)

RNA content present in genomic DNA eluates from various kits was evaluated by LC-MS. All samples were processed in duplicate according to manufacturers' recommendations and were eluted in 100 µl. Starting materials used: 100 µl human blood, 1 x 10⁶ HeLa cells, 10 mg of RNAlater-stabilized mouse (tail) and rat tissue samples (others). 1 µg of each sample was treated with the Nucleoside Digestion Mix (NEB #M0649) and subjected to LC-MS.

Values displayed are averages of duplicate measures and indicate the percentage of riboguanoside (rG) versus the total amount of ribo- and deoxyriboguanoside in the samples. Actual RNA content may be lower for all samples, since rG is more abundantly co-purified in silica preps than other RNA bases. The Monarch Spin Genomic DNA Purification Kit consistently delivers residual RNA below 1%–2% levels, which is usually undetectable with most analysis methods and lower than what is seen for other commercial kits.

The Monarch Spin Genomic DNA Purification Kit generates high quality genomic DNA suitable for sensitive applications like long range PCR and qPCR



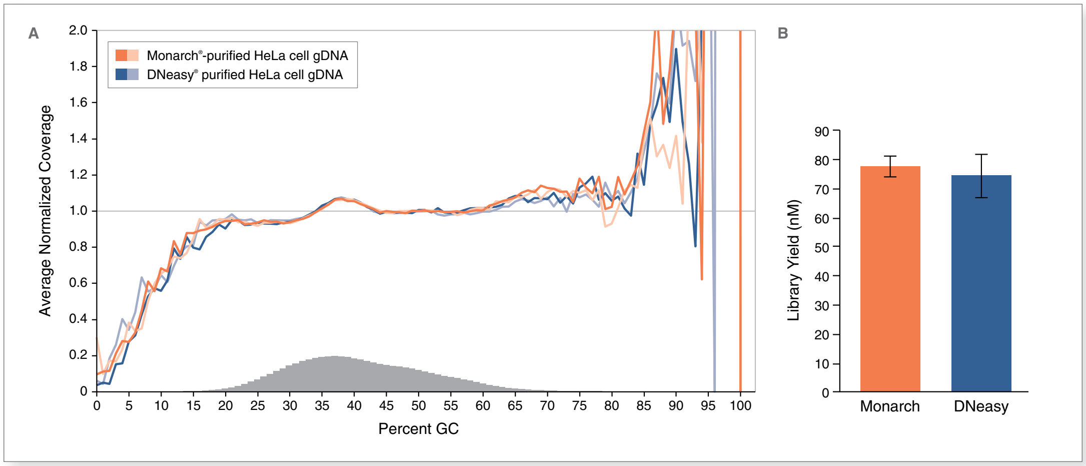
A. *Amplification reactions were set up with primer pairs specific for 6, 8, 10, 12, 16, 20 kb amplicons from human DNA. LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) was used and 25 ng template DNA was added to each sample. PCR reactions were carried out on an Applied Biosystems 2720 Thermal Cycler. Monarch-purified genomic DNA isolated from HeLa cells and human blood were compared to commercially available reference DNA from the human cell line NA19240 F11. 10 µl was loaded on a 1.5% agarose gel, using the 1 kb DNA Ladder (NEB #N3232) as a marker. Results indicated DNA was of high-integrity and suitable for long range PCR.*

B. *Monarch-purified genomic DNA from human whole blood, HeLa cells and mouse tail was diluted to produce a five log range of input template concentrations. The results were generated using primers targeting gHEME (human whole blood) and gREL (HeLa, mouse tail) for qPCR assays with the Luna® Universal qPCR Master Mix (NEB #M3003) and cycled on a BioRad® CFX Touch qPCR thermal cycler. Results indicated that DNA is highly pure and free from inhibitors, optimal for qPCR.*

An Outstanding Choice for Illumina® and Long Read Sequencing

The Monarch Spin Genomic DNA Purification Kit is an excellent choice for DNA extraction upstream of library preparation for next generation sequencing. DNA purified with this kit is high quality and of high molecular weight (peak size > 50 kb), making it an outstanding choice for preparation of libraries for nanopore sequencing and other long read platforms. The kit is also optimized to selectively bind DNA, not RNA, and is also supplied with RNase A for (optional) removal of any residual RNA, allowing for purification of DNA with extremely low RNA contamination.

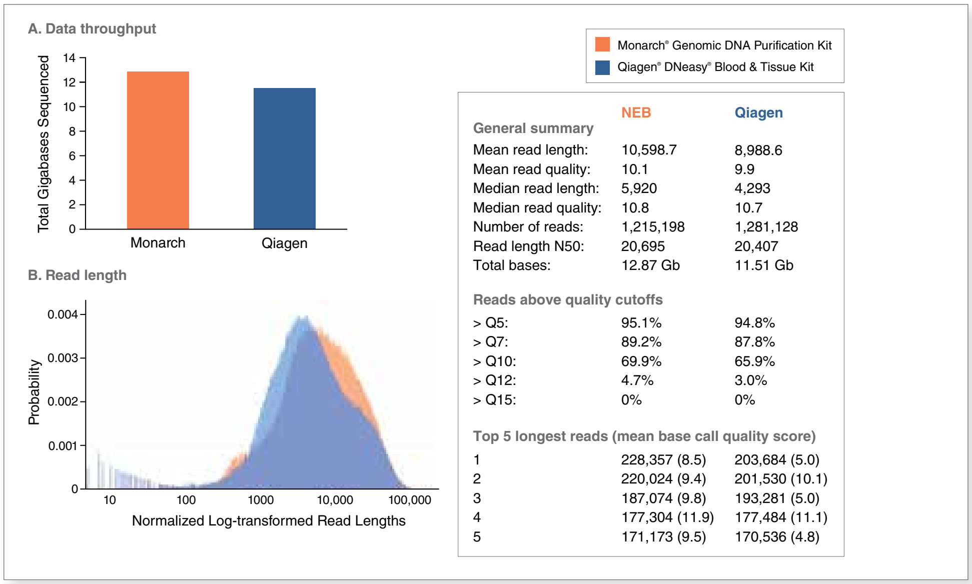
The Monarch Spin Genomic DNA Purification Kit generates excellent input material for NGS library preparation with NEBNext® kits for Illumina



A. Duplicate libraries were made from 100 ng HeLa cell gDNA purified with Monarch (orange) or Qiagen DNeasy Blood & Tissue Kit (blue) using the NEBNext Ultra™ II FS DNA Library Prep Kit for Illumina (NEB #E7805). Libraries were sequenced on an Illumina MiSeq®. Reads were mapped using Bowtie 2.2.4 and GC coverage was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each %GC is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. Monarch GC coverage matched Qiagen DNeasy results.

B. High yield libraries are achieved from Monarch-purified gDNA. Library yields of the samples described above were assessed on an Agilent Technologies 2100 BioAnalyzer® using a High Sensitivity DNA Kit.

The Monarch Spin Genomic DNA Purification Kit generates high quality DNA for nanopore sequencing



	NEB	Qiagen
General summary		
Mean read length:	10,598.7	8,988.6
Mean read quality:	10.1	9.9
Median read length:	5,920	4,293
Median read quality:	10.8	10.7
Number of reads:	1,215,198	1,281,128
Read length N50:	20,695	20,407
Total bases:	12.87 Gb	11.51 Gb
Reads above quality cutoffs		
> Q5:	95.1%	94.8%
> Q7:	89.2%	87.8%
> Q10:	69.9%	65.9%
> Q12:	4.7%	3.0%
> Q15:	0%	0%
Top 5 longest reads (mean base call quality score)		
1	228,357 (8.5)	203,684 (5.0)
2	220,024 (9.4)	201,530 (10.1)
3	187,074 (9.8)	193,281 (5.0)
4	177,304 (11.9)	177,484 (11.1)
5	171,173 (9.5)	170,536 (4.8)

HeLa cell genomic DNA was extracted using either the Monarch Spin Genomic DNA Purification Kit or the Qiagen DNeasy Blood & Tissue Kit. One microgram of purified DNA was used to prepare Oxford Nanopore Technologies sequencing libraries following the 1D Ligation Sequencing Kit (SQK-LSK109) protocol without DNA fragmentation. Libraries were loaded on a GridION® (Flow cell R9.4.1) and the data was collected for 48 hrs. Libraries produced using the Monarch gDNA exceeded the Qiagen libraries on common sequencing metrics including: A. total sequencing data collected, B. read length. Data was generated using NanoComp (Bioinformatics, Volume 34, Issue 15, 1 August 2018, Pages 2666–2669).

At **www.NEBNext.com,** learn how NEBNext Library Prep can support your short or long read NGS workflows.

Guidelines for Choosing Sample Input Amounts When Using the Monarch Spin Genomic DNA Purification Kit

Genomic DNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield, purity, and DIN 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 Genomic DNA Purification Kit. It is very important not to overload the column and the buffer system when extracting and purifying gDNA, as DNA yields, purity, integrity, and length may suffer. Inputs that will result in ~100 ng of gDNA should be considered the minimum input amount for this kit (5 µl whole blood, 1 x 10^4 cultured cells or 0.2 mg tissue). If using smaller amounts, the use of carrier RNA is recommended (see product manual for more details). Visit **www.neb.com/MonarchgDNAinputs** for updates and additional validated samples.

SAMPLE TYPE	RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
TISSUE*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
Spleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
BLOOD**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
CELLS				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
BACTERIA				
E. coli (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (Gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
ARCHAEA				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
YEAST				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
SALIVA/BUCCAL CELLS***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

*** Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Monarch HMW DNA Extraction Kits

The Monarch HMW DNA Extraction Kits provide a rapid and reliable process for extracting high molecular weight, intact genomic DNA from cultured cells, whole blood, various tissues, bacteria and other sample types (e.g., amphibian, insect). Utilizing a novel and optimized process that combines lysis with tunable fragment length generation, followed by precipitation of the extracted DNA onto the surface of large glass beads, the prep proceeds rapidly and efficiently. DNA size ranges from 50 kb into the Mb range, depending on the agitation speed used during lysis. Purified DNA is recovered in high yield with excellent purity, including nearly complete removal of RNA. For cells, the process time is only 30 minutes, while blood samples require erythrocyte lysis and are processed in approximately 60 minutes. Tissues and bacteria are processed in about 90 minutes, leading the market in speed. Purified HMW DNA is suitable for a variety of downstream applications including long read sequencing (Oxford Nanopore Technologies and Pacific Biosciences®), optical mapping, and linked-read genome assembly.

Glass beads used for HWM DNA Extraction

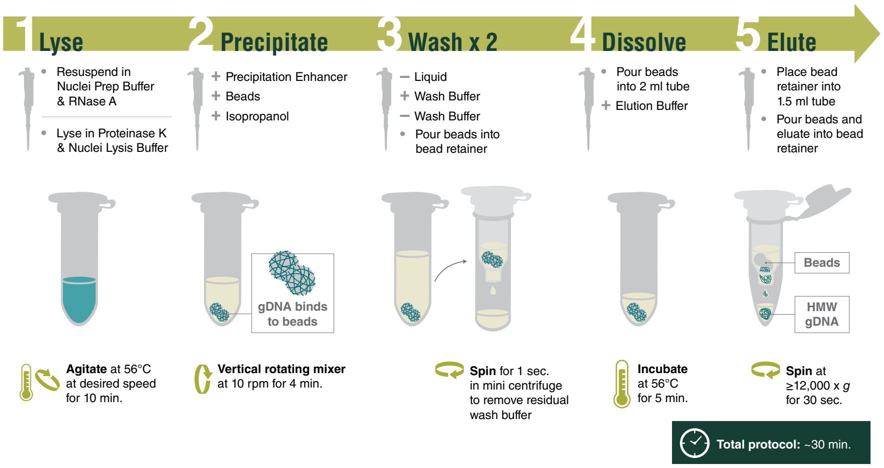
ADVANTAGES

- Fast workflow (cells: 30 min, blood: 60 min, tissue/bacteria: 90 min)
- Extract DNA into the megabase (Mb) range with cells, blood, soft organ tissues, and bacteria
- Tune DNA size based on agitation speed during lysis
- Achieve best-in-class yields and purity
- Consistently achieve reproducible results
- Effectively remove RNA
- Elute DNA easily and completely

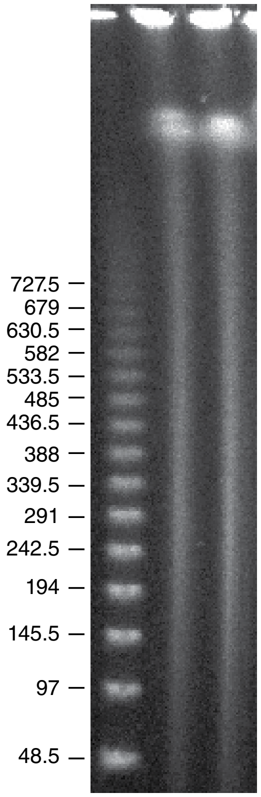
SPECIFICATIONS

- **Binding Mechanism:** precipitation on glass beads
- **Input Amount:** Cells: 1 x 10^5 – 1 x 10^7 cells
  Blood: 100 µl – 2 ml
  Tissue: 2 – 25 mg
  Bacteria: 5 x 10^8 – 5 x 10^9 cells
- **Genomic DNA Size:** 50 kb up to several MB; dependant on agitation speed and sample quality/type
- **Purity:** $OD_{260/280}$ typically 1.8 – 1.9
  $OD_{260/230}$ typically 2.1 – 2.5
- **RNA Content:** < 2%

Workflow for cell samples



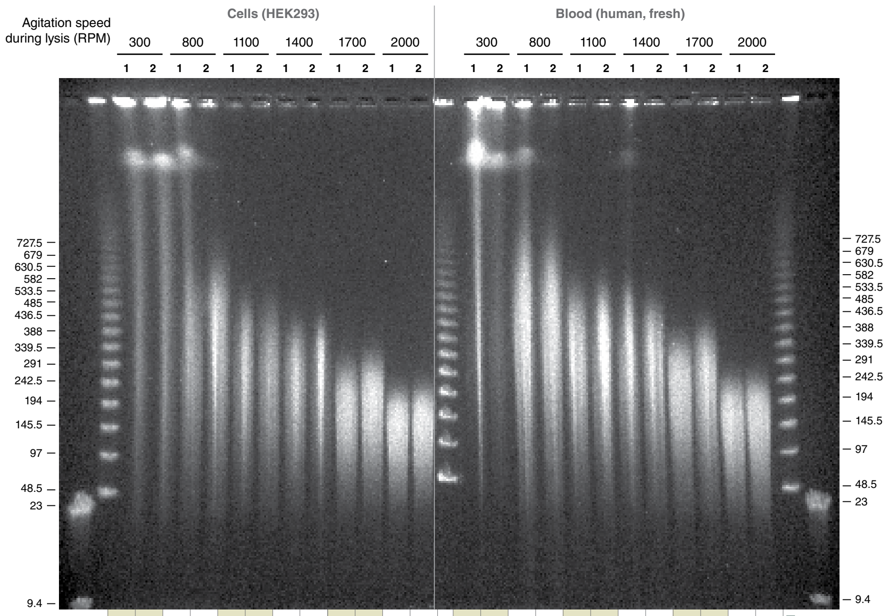
HMW DNA from HEK293 cells



> *We've had great success with obtaining HMW DNA for long read sequencing from a variety of cell types, using less input and obtaining a comparable yield...It is straightforward and easy to use."*
>
> – Inswasti Cahyani & Matt Loose, DeepSeq,
> University of Nottingham

Easily Extract Megabase-sized DNA from Cells and Blood with High Yields and Purity

DNA fragment size is tunable based on agitation speed during lysis



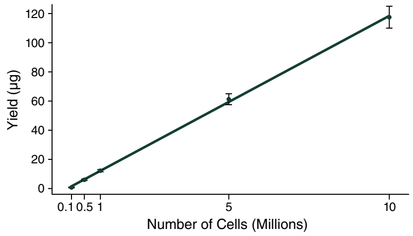
	Cells (HEK293)												Blood (human, fresh)											
Agitation speed during lysis (RPM)	300		800		1100		1400		1700		2000		300		800		1100		1400		1700		2000	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Yield (µg)	11.3	11.6	11.1	12.1	11.7	12.4	13.2	12.0	12.7	12.9	12.0	13.5	22.5	22.5	22.5	22.2	24.4	26.0	24.7	23.5	24.4	24.9	24.0	25.6
260/280	1.86	1.85	1.87	1.87	1.87	1.87	1.88	1.87	1.87	1.86	1.87	1.87	1.87	1.87	1.87	1.86	1.87	1.86	1.87	1.86	1.86	1.86	1.86	1.86
260/230	2.31	2.28	2.36	2.44	2.39	2.47	2.49	2.50	2.52	2.53	2.48	2.44	2.50	2.57	2.54	2.42	2.40	2.44	2.35	2.48	2.37	2.37	2.46	2.38
DIN	9.7	9.8	9.8	9.8	9.9	9.8	9.8	9.7	9.8	9.8	9.8	9.7	9.8	9.9	9.8	9.9	9.7	9.7	9.8	9.7	9.8	9.8	9.8	9.8

Best-in-class yields and purity

Preps were performed on duplicate aliquots of 1 x 10^6 HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad CHEF-DR III System). Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #T3041 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.

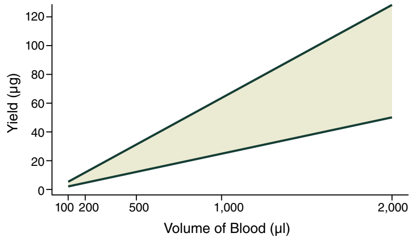
Linear correlation between DNA yield and input for cell and blood samples

A. HEK293 cells



Number of Cells	Typical Yield (µg)
1x10^5	0.5–1.0
5x10^5	5.5–6.5
1x10^6	11.5–13
5x10^5	57.5–65
1x10^7	110–125

B. Human blood



µl of Blood	Typical Yield (µg)	
	Low Leukocyte Content	High Leukocyte Content
100	2	5.1
200	4.4	11.2
500	12.5	32
1,000	25	64
2,000	50	128

Summarized yield data for HMW DNA preps are shown carried out at 2,000 rpm during lysis, using HEK293 cultured cells and fresh human blood samples from different donors as input material in the corresponding protocols. The starting materials were diluted to 5 different concentrations to cover the entire recommended input range. Cell samples ≤ 5 x 10^5 cells and blood samples < 500 µl were purified using the recommended volumes for low input samples. Obtained yields show a high degree of linearity over the displayed input range.

Validated Sample Types*

Cells & Blood Kit

Cells

- HEK293
- HeLa
- NIH3T3
- Jurkat
- K562 (suspension cells)
- HCT116
- A549
- U5Os
- HepG2
- NCI-460
- SK-N-SH
- Aa23

Mammalian Blood**

- Human
- Mouse
- Rat (fresh only)
- Rabbit
- Pig
- Horse
- Cow
- Rhesus monkey
- Goat

Nucleated Blood**

- Chicken
- Turkey

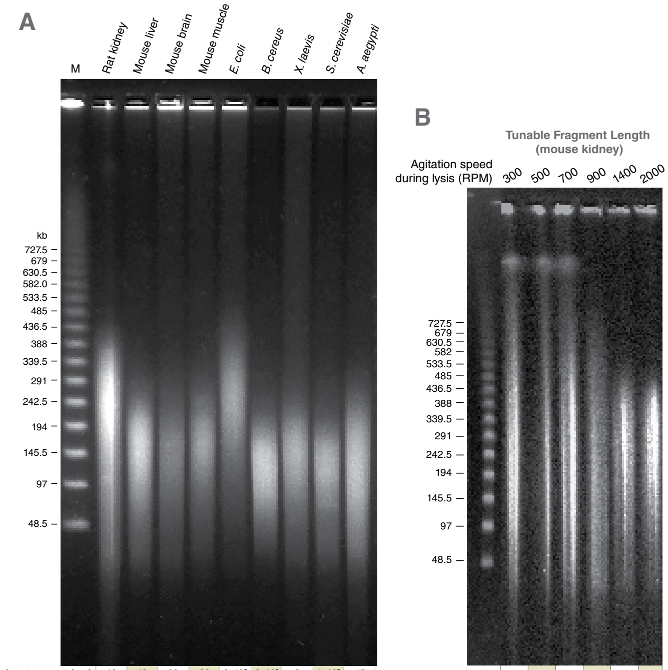
*Fresh and frozen samples have been validated

**Compatible with all common anticoagulants

Visit **NEB.com/MonarchHMWDNAinputs** for detailed guidance on input amounts and expected results.

Simply and Effectively Isolate HMW DNA from a Variety of Tissue Types, with Tunable Fragment Length

Successful extraction of HMW DNA from various tissue samples with tunable DNA size for soft organ tissues



Input amount (mg)	10	10	20	20	2x10^9	2x10^8	3	4x10^8	15
Yield (µg)	20.8	20.2	16.9	8.9	14.5	4.2	5.2	12.9	6.7
260/280	1.86	1.86	1.86	1.85	1.86	1.87	1.85	1.90	1.84
260/230	2.41	2.26	2.45	2.28	2.06	1.95	2.33	2.01	2.53

Yield (µg)	15.6	18.1	17.3	14.9	20.0	24.2
260/280	1.88	1.88	1.86	1.86	1.85	1.87
260/230	2.20	2.33	2.34	2.33	2.37	2.43

A. *HMW genomic DNA extracted from various samples using the Monarch HMW DNA Extraction Kit for Tissue (species and input amounts indicated in the figure). Preps were performed according to the kit instructions, with sample agitation at 2000 rpm during lysis. A modified workflow was used to process S. cerevisiae samples.* ***B.*** *HMW genomic DNA from mouse kidney (10 mg) was purified using the Monarch HMW DNA Extraction Kit for Tissue. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. 500 ng (**A**) or 300 ng (**B**) of purified DNA was resolved by PFGE. Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder (NEB #N0341) was used as a molecular weight standard.*

TIPS FOR SUCCESSFUL HMW DNA EXTRACTION

1. Pay close attention to input amounts and follow the protocol guidance for your recommended inputs. Lysis volumes may need to be reduced for optimal binding, especially when working with low input tissue samples.
2. For optimal results in the blood protocol, resuspend leukocyte pellets carefully and completely at each resuspension step.
3. When working with low-input tissue samples, stop agitation in the thermal mixer after 15 minutes, and continue the incubation without shaking.
4. Carry out the inversions exactly as directed to ensure the DNA binds completely to the beads. If inversions are done manually, do them slowly and gently, each inversion taking about 5-6 seconds.
5. If using low agitation speeds for the highest molecular weight DNA, additional inversions during DNA binding will maximize yields.

Tissue workflow highlights

Following lysis and phase separation, use the same simple workflow for binding and elution as in the cell workflow

Microtube pestle and grinding tube included for optimal sample homogenization.

Phase separation step optimizes protein removal.

Use of beads simplifies washing and elution.

Tissue input recommendations

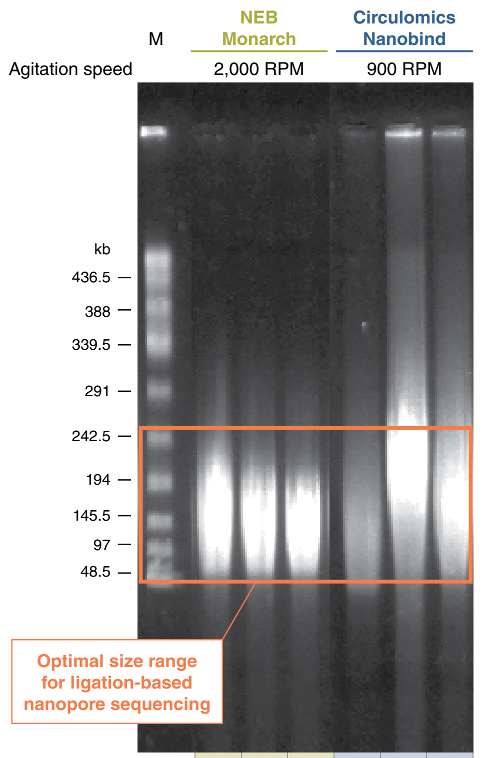
		RECOMMENDED INPUT (INPUT RANGE), mg	YIELD (µg) FOR RECOMMENDED INPUT (YIELD PER mg)
Mammalian Tissue			
Mouse brain	Fresh	15 (2**–20)	12–21
	Frozen	15 (2**–20)	15–21 (1–1.5)
Mouse liver	Fresh (w/NaCl)	10 (2–15)	7
	Frozen (w/NaCl)	10 (2–15)	17–19 (1.7–1.9)
	Fresh*	10 (2–15)	20
	Frozen*	10 (2–15)	27–31 (2.7–3.1)
Mouse muscle	Fresh	20 (2–25)	8–9
	Frozen	20 (2–25)	12–16 (0.6–0.8)
Mouse kidney	Fresh	10 (2–15)	23–34
	Frozen	10 (2–15)	32–41 (3.2–4.1)
Mouse tail	Frozen	20 (2–25)	20 (1.8–2.1)
Mouse ear punch	Fresh	10 (2–15)	15–16 (1.5–1.6)
Rat kidney	Frozen	10 (2–15)	20–25
Bacteria			
E. coli (Gram-negative)	Frozen	1 x 10^9 cells (5 x 10^8 – 5 x 10^9 cells)	8–9
B. cereus (Gram-positive)	Frozen	2 x 10^8 cells (2 – 4 x 10^8 cells)	4–5
M. luteus (gram-positive)	Frozen	1 x 10^8 cells	2.0
Amphibian			
X. laevis	Fresh	3–4	5
Yeast			
S. cerevisiae	Fresh	3.8 x 10^8 cells	15
Insect			
A. aegypti	Frozen	15	6

*Standard protocol without recommended NaCl treatment.

**If working with input amounts < 5 mg, refer to the product manual for guidance on reducing buffer volumes.

Reproducibly High Yields and Purity – Great for Long Read Sequencing

Monarch Kits extract HMW DNA with superior yields, purity and reproducibility when compared to Circulomics® Nanobind®



Yield (µg)	8.1	8.2	8.2	4.9	4.9	5.2
260/280	1.86	1.85	1.86	1.84	1.84	1.84
260/230	2.43	2.52	2.31	2.24	2.55	1.98
N50 (kb)	41.0	40.2	40.8	37.6	41.7	39.8

Better yield, purity and reproducibility

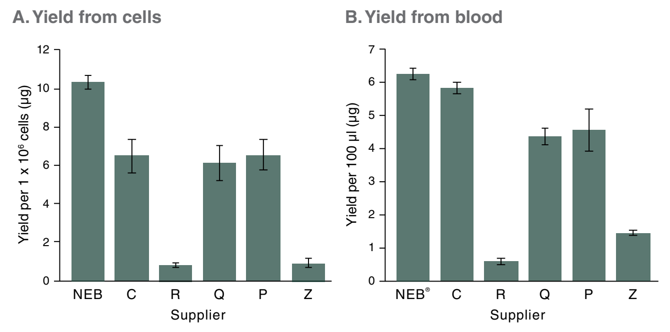
Genomic DNA was purified from 2 x 10^6 HEK293 cells and 0.7 x 10^6 K562 cells with the Monarch Kit and the Circulomics Nanobind CBB Big DNA Kit, according to manufacturers' recommendations. 400 ng of HMW genomic DNA was separated on a 0.75% gel using a Pippin pulse gel system (Sage Science) at the 5–430 kb program. M = Lambda PFG Ladder (NEB #N0341). NEB Monarch and Circulomics Nanobind samples were barcoded and analyzed on the same Oxford Nanopore Technologies flow cell.

Excellent performance in Oxford Nanopore Technologies Sequencing

	HEK293	HUMAN BLOOD	MOUSE KIDNEY
Mean read length	21338.9	21522.6	27120.7
Mean read quality	12.8	13.4	13
Median read length	10388	10130	23150
Median read quality	13.2	13.9	13.5
Number of reads	377687	538090	164000
Read length N50	45432	46542	44631
Total bases	8059414490 (8.1 Gb)	11581090785 (11.6 Gb)	4447789727 (4.4 Gb)

DNA used for the sequencing libraries was extracted from HEK293 cells (1x10^6 cells), human blood (500 µl) and mouse kidney (10 mg fresh, homogenized with rotor stator) using the Monarch kit, without further size selection. Libraries were prepared using the NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing (NEB #E7180) and sequencing was performed on a GridION Mk1 (LSK109 kit, FLO-MIN106D flow cell) for up to 48 hours, or shorter if no more data was generated by the flow cell. No additional treatment of the flow cell (e.g., flushing) was employed. Read lengths are indicated in bases.

DNA yields from cell and blood preps using various commercially available kits

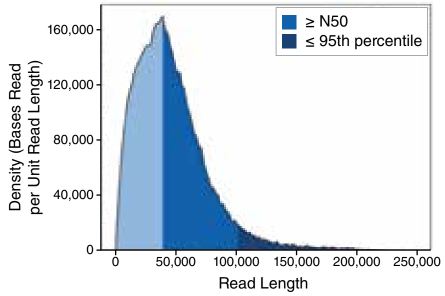


HMW DNA was isolated from 1 x 10^6 HEK293 cells (A) and fresh human blood (B) with kits from New England Biolabs (N), Circulomics [Nanobind CBB Big DNA Kit (C)], RevoluGen® [Fire Monkey (R)], QIAGEN [MagAttract HMW DNA Kit (Q)], Promega® [Wizard HMW DNA Extraction Kit (P)] and Zymo® Research [Quick-DNA HMW MagBead Kit (Z)]. Blood input volumes were used as specified in manufacturers' protocols (N: 500 µl, C: 200 µl, R: 500 µl, Q: 200 µl, P: 300 µl, Z: 200 µl). Yields for the blood samples were normalized for 100 µl blood.

Excellent performance in PacBio sequencing

PacBio Sequel I, HMW DNA from human blood, sheared to 75 kb and size selected to > 30 kb

A. Base yield density



B. Sequencing metrics

Run settings

Movie Time (hours):	20
Total bases:	10.42
Unique molecular yield:	9.93

Productivity (%)

Productivity 0:	41.2
Productivity 1:	42.6
Productivity 2:	16.2

Read length (bases)

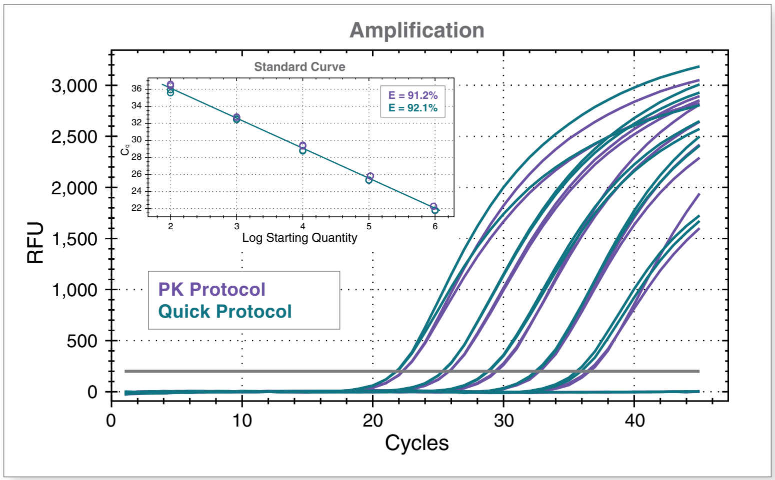
Polymerase mean:	24,031
Polymerase N50:	40,954
Longest subread mean:	22,958
Longest subread N50:	38,814

HMW DNA was extracted from human blood with the Monarch HMW DNA Extraction Kit for Cells & Blood with agitation speed of 2000 rpm during lysis. DNA was sheared to 75 kb with a Megaruptor, and SMRTbells were constructed with the SMRTbell Express Template Prep Kit 2.0 and size-selected to a minimum of 30 kb with a Blue Pippin. CLR reads were obtained on a PacBio Sequel I using 12 pM on-plate loading concentration and a 20 hour movie with no pre-extension.

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. Total RNA, including viral RNA, can also be extracted from clinically-relevant samples like saliva, buccal swabs and nasopharyngeal swabs. 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.

The Monarch Total RNA Miniprep Kit successfully purifies synthetic SARS-CoV-2 viral RNA from saliva samples



The Monarch Total RNA Miniprep Kit Proteinase K and Quick Protocols were used to isolate total RNA from saliva samples containing 10-fold serial dilutions of synthetic SARS-CoV-2 N-gene RNA. Purified RNA was eluted in 100 µl nuclease-free water to yield 50 to 500,000 copies of viral RNA/µl. Using the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006), titers as low as 50 copies (the lowest input tested) were detected and linear, quantitative recovery of the SARS-CoV-2 N-gene was observed over a 5-Log range.

ADVANTAGES

- Use with a wide variety of sample types, including clinically-relevant ones like swabs (buccal/NP) and saliva
- Purify RNA of all sizes, including miRNA & small RNAs ≥ 20 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent
- RNA Extraction from some samples is automatable on the QIAcube® and KingFisher Flex platforms
- Save money with value pricing for an all-in-one kit

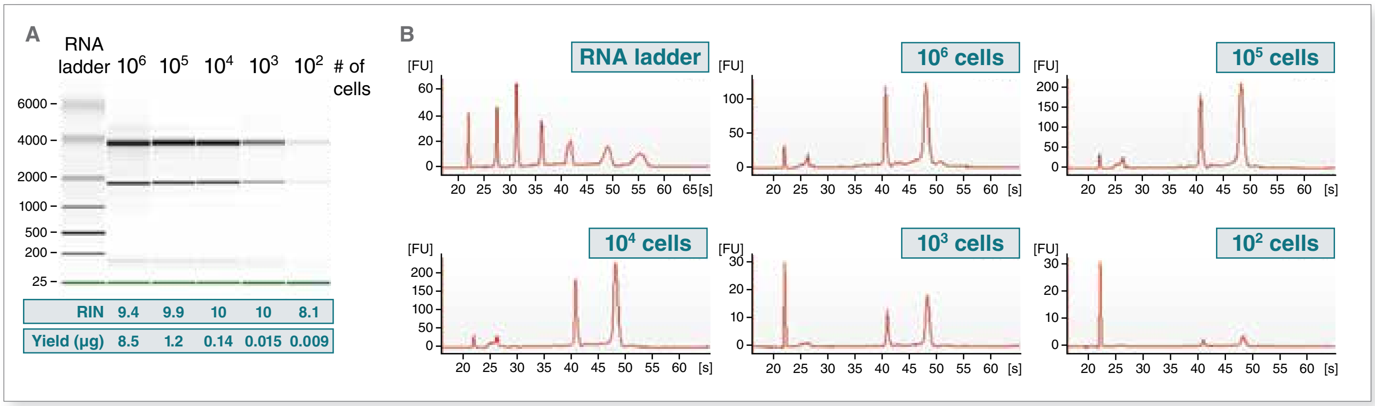
SPECIFICATIONS

- **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 cells or 50 mg tissue*
- **Elution Volume:** 30–100 µl
- **Yield:** varies depending on sample type
- **Compatible downstream applications:** RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

**See page 13 for more details and other sample types*

> *The Monarch Total RNA Miniprep offers better yield and quality; easy protocol and good and consistent results among samples.*
>
> – Gisele, Mississippi State University

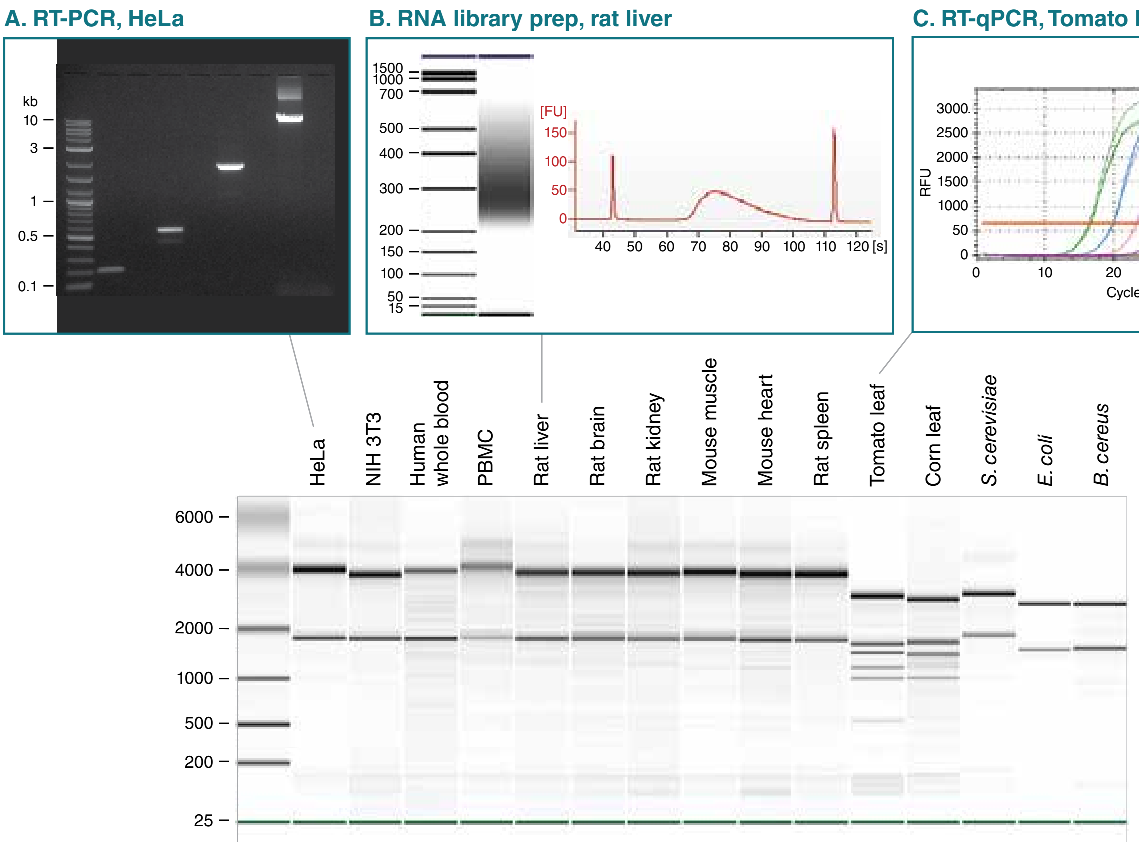
The Monarch Total RNA Miniprep Kit can generate high quality RNA from as few as 100 HeLa cells



Total RNA was isolated using the Monarch Total RNA Miniprep Kit (NEB #T2010) from varying amounts of HeLa cells over 5 orders of magnitude and eluted in 100 µl of nuclease-free water. Samples were analyzed on a Bioanalyzer Pico chip, with RIN values and total yields shown below the lanes (A). Electropherograms are included as a reference (B).

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

RNA extracted from a wide variety of sample types is high quality and ready for downstream use



	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 Directional RNA Library Prep Kit (NEB #E7760) and RT-qPCR (C) using Luna One-Step RT-qPCR Reagents (NEB #E3005).

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 13.
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 and subsequent on-column DNase I treatment. Off-column DNase I treatment can also be employed.

Doing qPCR or RT-qPCR?

Learn more about Luna and request a sample at **LUNAqPCR.com**

Sample Inputs & Expected Recovery for the Monarch Total RNA Miniprep Kit

RNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Empirical yield and RIN data from a wide variety of sample types are provided below, as well as guidance on the maximum input amounts. It is important NOT to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer. Visit **www.neb.com/MonarchRNAinputs** for updates and additional validated sample types.

SAMPLE TYPE (1)		INPUT	AVERAGE YIELD (µg)	OBSERVED RIN	MAXIMUM STARTING MATERIAL
CULTURED MAMMALIAN CELLS					
HeLa		1 x 10^6 cells	12–15	9–10	1 x 10^7 cells
HEK 293		1 x 10^6 cells	12–14	9–10	1 x 10^7 cells
NIH3T3		1 x 10^6 cells	8–12	9–10	1 x 10^7 cells
MAMMALIAN BLOOD (2)					
Human	Fresh	200 µl	0.5–1.0	7–8	3 ml
	Frozen	200 µl	0.5–1.0	7–8	3 ml
	Stabilized	200 µl	0.5–1.0	7–8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml*
BLOOD CELLS					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 x 10^7 cells
TISSUE					
Rat liver	Frozen pulverized	10 mg	25	8–9	20 mg
	Stabilized solid	10 mg	50–60	8–9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40–50	9	20 mg
Rat kidney (frozen pulverized)		10 mg	7–10	9	50 mg
Rat brain	Frozen pulverized	10 mg	2–3	8–9	50 mg
	Stabilized solid	10 mg	0.5–1.5	8–9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5–8	8–9	50 mg
Rat muscle (frozen pulverized)		10 mg	2–3	8–9	50 mg
Mouse muscle	Frozen pulverized	10 mg	3	8–9	50 mg
	Powder with bead homogenizer	10 mg	5	7–8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8–10	9	50 mg
Mouse heart (stabilized solid with bead homogenizer)		10 mg	5–6	8–9	50 mg
YEAST					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	50	9–10**	5 x 10^7 cells
	Fresh with Zymolyase	1 x 10^7 cells	60	9**	5 x 10^7 cells
BACTERIA					
E. coli	Frozen	1 x 10^9 cells	5	10	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^9 cells	10	10	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	70	10	1 x 10^9 cells
B. cereus	Frozen with lysozyme	1 x 10^8 cells	20–30	9	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^8 cells	8	9–10	1 x 10^9 cells
PLANT					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg

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(1) RNA for other samples including drosophila, zebrafish embryos/larvae, plasma, serum, saliva, buccal swabs and nucleated blood have been successfully purified with this kit; protocols are available in the product manual online.

(2) Protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Mouse blood also has a maximum input of 1 ml.

** *S. cerevisiae* total RNA was run on an Agilent Nano 6000 Chip using plant assay.

Monarch Spin Kits for RNA Cleanup

The Monarch Spin RNA Cleanup Kits provide a fast and simple silica column-based solution for cleanup and concentration of RNA after enzymatic reactions (including *in vitro* transcription (IVT), DNase I and Proteinase K treatment, capping, tailing and labeling) as well as after RNA isolation (e.g., TRIzol extraction). These kits can also be used to extract RNA from cells, saliva and buccal/nasopharyngeal swabs. Kits are available in three different binding capacities: 10 µg, 50 µg and 500 µg, each containing unique columns designed to prevent buffer retention and ensure no carryover of contaminants.

Monarch Kit 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

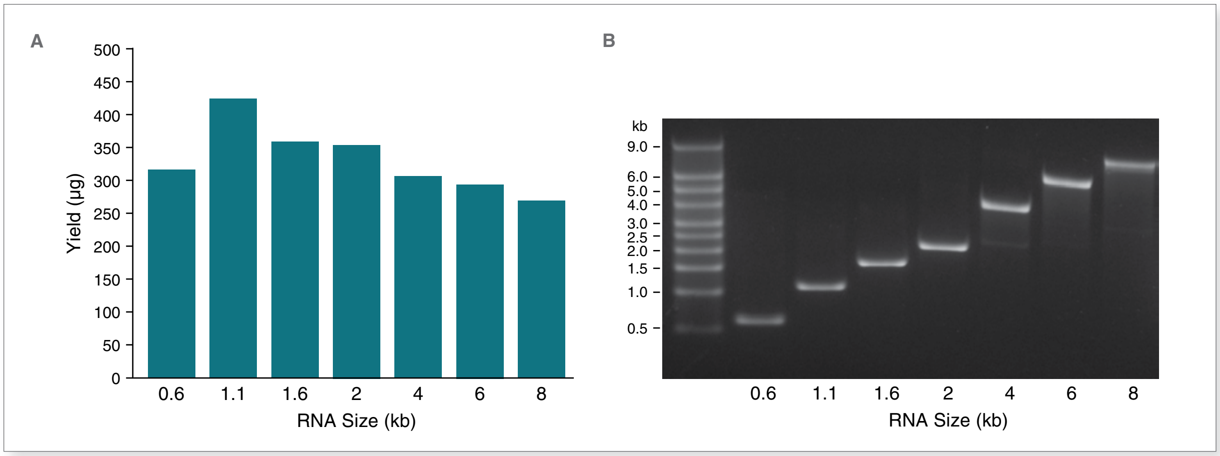
ADVANTAGES

- 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) or 20 µl (NEB #T2040)
- Bind up to 500 µg of RNA (NEB #T2050)
- Adjust cutoff size down to 15 nt with a slight protocol modification
- Can be used for RNA extraction from some samples; extraction from saliva can be automated on the QIAcube and KingFisher Flex platforms

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

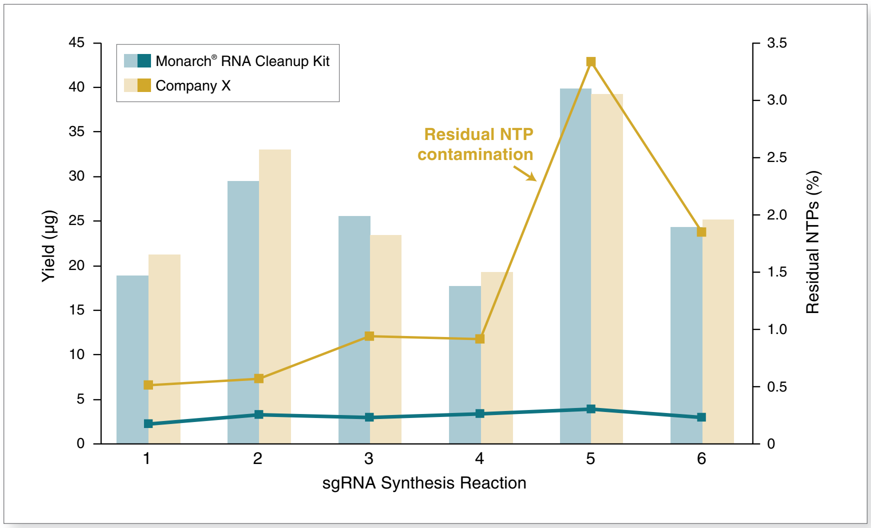
The Monarch Spin RNA Cleanup Kit (500 µg) is suitable for cleaning up large quantities (> 250 µg) of RNA from *in vitro* transcription reactions



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

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

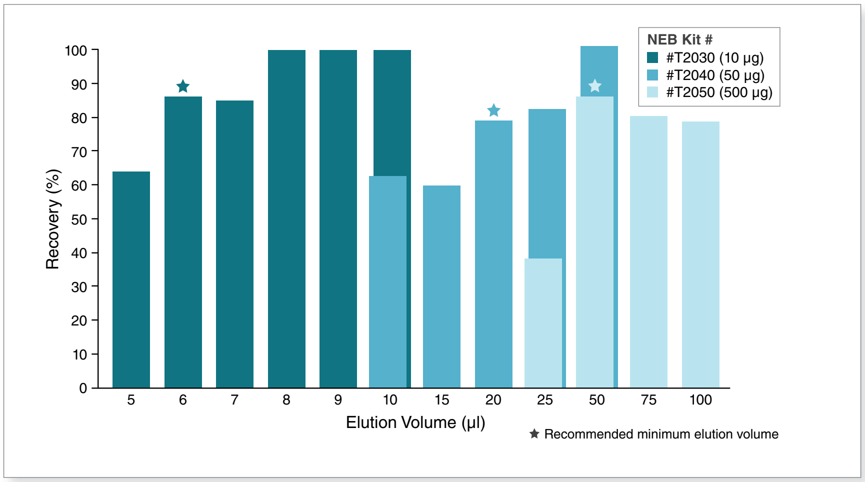
The Monarch Spin RNA Cleanup Kit (50 µg) produces sgRNA yields consistent with other competitor RNA cleanup kits and with lower residual NTP contamination



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

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

Recovery of RNA from Monarch Spin RNA Cleanup Kits with varying elution volumes



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

Learn more at **NEB.com/MonarchRNACleanup**

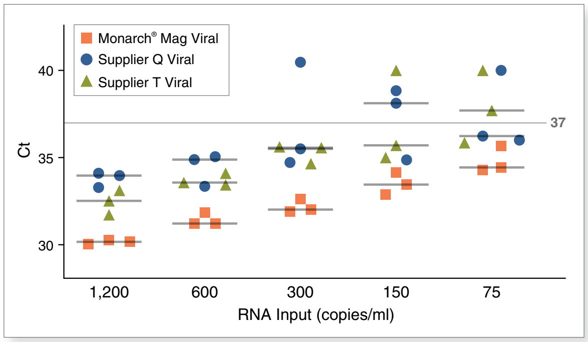
Learn more about NEB's reagents for synthesis of high-quality RNA – from template generation and transcription, capping, tailing, and cleanup after synthesis. **Visit neb.com/IVT.**

> “ *We recently switched to NEB for our spin column kits, and I just wanted to say that I am so impressed with the thoughtfulness of the packaging—how the protocol is stored, the tiny bags that the columns are in, and the quality of the box itself, which we will keep to store items after we finish the kit. We also like that you can customize the elution volume. Cheers to your team for doing your part and designing a great product!*
>
> – Jaclyn, George Washington University

Monarch Mag Viral DNA/RNA Extraction Kit

The Monarch Mag Viral DNA/RNA Extraction Kit offers reliable hands-free and high-throughput isolation of viral DNA and RNA from saliva and respiratory swab samples, and is compatible with wastewater samples. This kit features a magnetic bead-based method for extraction and purification of viral nucleic acid and is compatible with various automated instruments, as well as manual workflows. The kit enables highly sensitive detection and amplification in various downstream applications.

Monarch Mag Viral DNA/RNA Extraction Kit demonstrates high reproducibility and sensitivity



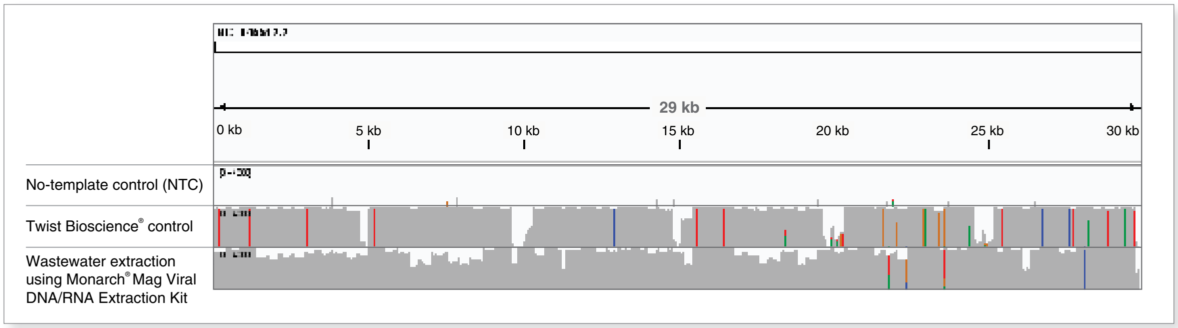
Mock samples representing decreasing viral loads were prepared using heat-inactivated SARS-CoV-2 (ATCC®) in VTM (Hardy Diagnostics®). Extraction was performed using Monarch Mag Viral DNA/RNA Extraction Kit and similar kits from two other suppliers. RT-qPCR was performed using NEB #E3019 and Bio-Rad® CFX96 Touch™ Real-Time PCR Detection System. Monarch Mag Viral DNA/RNA Extraction Kit showed consistently low Cts and reproducible data, even at low viral loads, compared to the competitor kits tested.

ADVANTAGES

- Designed for hands-free, highly-concentrated viral DNA and/or RNA extraction in low elution volumes
- Compatible with manual and automated workflows on instrument platforms, such as KingFisher® Flex, Agilent® Bravo®, MGISP® liquid handlers
- Tested for saliva and respiratory swab sample types. Compatible with wastewater samples, after enrichment steps (not supplied)
- Suitable for qPCR/RT-qPCR, ddPCR, library prep for sequencing/NGS and other downstream applications
- Includes carrier RNA for sensitive detection in RNA-based amplification workflows

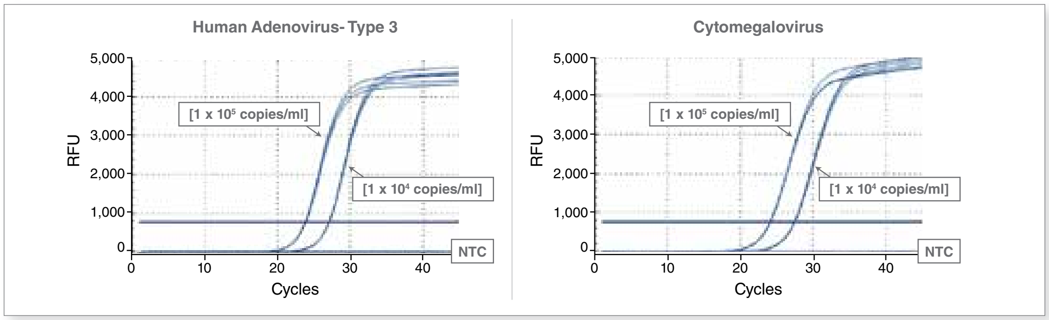
For detailed guidance and more information on kit components, visit **NEB.com/t4010**

Complete SARS-CoV-2 genome coverage from wastewater samples using the Monarch Mag Viral DNA/RNA Extraction Kit



Integrative Genome Viewer visualization of read coverage across the SARS-CoV-2 genome (log scale). RNA was extracted from 750,000 copies of inactivated SARS-CoV-2 viral particles (ATCC 1986HK) spiked into wastewater samples, using the Monarch Mag Viral DNA/RNA Extraction Kit. Viral particle enrichment was applied to the contrived wastewater samples with Nanotrap® Microbiome A Particles prior to RNA extraction. 20,000 copies of Twist Bioscience® Synthetic SARS-CoV-2 RNA Control 23 template served as a positive control for amplicon generation and library prep. Amplicons and libraries were prepared with NEBNext® VarSkip Short v2 SARS-CoV-2 primer pools and the NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®). Libraries were sequenced on a NextSeq® 500/550 instrument (2x75 bp). Coverage depth per base was determined, reads were down-sampled with seqtk and aligned to SARS-CoV-2 reference genome (NCBI, NC_045512) with Bowtie2.

qRT-PCR on DNA extracted using the Monarch Mag Viral DNA/RNA Extraction Kit demonstrates accurate detection and quantification of target DNA viruses

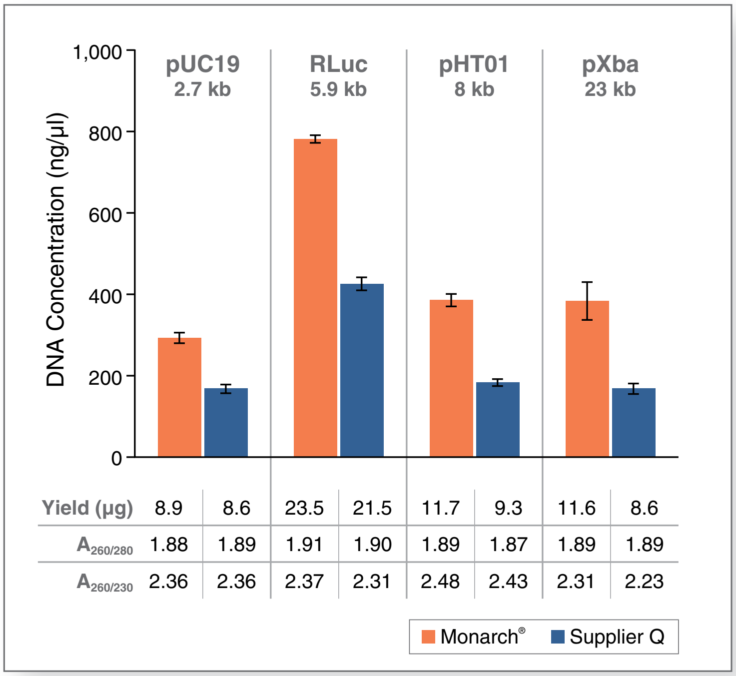


Monarch Mag Viral DNA/RNA Extraction Kit was used to extract DNA from Human Adenovirus-Type 3 (ZeptoMetrix®) and Cytomegalovirus (ZeptoMetrix) at two different viral loads using the automated KingFisher protocol. The eluted DNA was subjected to qRT-PCR on a BioRad CFX96 Touch Real-Time PCR Detection System. Amplification curves demonstrate successful extraction of viral DNA with expected signal corresponding to the respective viral loads.

Monarch Spin Plasmid Miniprep Kit

The Monarch Spin 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.

Monarch Spin Plasmid Miniprep Kits provide high purity and yields



	pUC19		RLuc		pHT01		pXba	
Yield (µg)	8.9	8.6	23.5	21.5	11.7	9.3	11.6	8.6
$A_{260/280}$	1.88	1.89	1.91	1.90	1.89	1.87	1.89	1.89
$A_{260/230}$	2.36	2.36	2.37	2.31	2.48	2.43	2.31	2.23

This kit provides a rapid and reliable method for the extraction and purification of high-quality plasmid DNA from various bacterial strains. Quickly and easily purify up to 20 µg in as low as 30 µl, without genomic DNA or RNA contamination. This new and improved kit provides higher purity and yield when compared to the leading supplier.

Monarch nucleic acid purification kits are evolving!
For more information, visit **NEB.com/MonarchEvolves**

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:** 9½–12½ 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.

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 and Gel Extraction Needs

Monarch Spin 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 5 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.

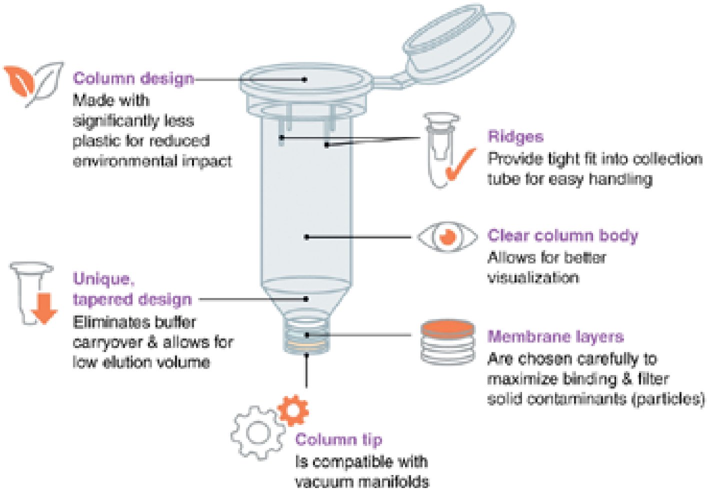
Monarch Spin DNA Gel Extraction Kit

The Monarch Spin 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 Spin PCR & DNA Cleanup Kit (5 µg)

The Monarch Spin 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 Monarch Buffer WZ provided ensures enzymes, short primers, 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.

Features of the Monarch Spin Column S1A



NEB Monarch's unique column design and membrane assembly allows high-quality DNA purification with low-elution volume, for highly-concentrated DNA for downstream applications. The column is designed and made with significantly less plastic for reduced environmental impact..

ADVANTAGES

- Elute in as little as 5 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
- With protocol modification, DNA ≥ 12 bp (dsDNA) or ≥ 16 nt (ssDNA) can be purified with NEB #T1130

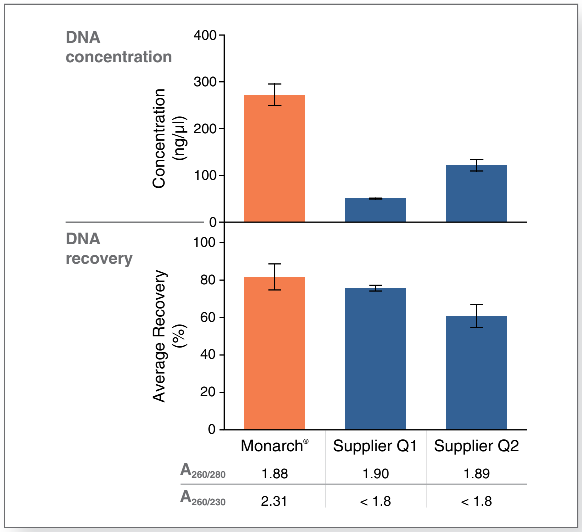
SPECIFICATIONS

- **Binding Capacity:** up to 5 µg
- **DNA Size Range:** ~50 bp to 25 kb
  With protocol modification, oligos ≥ 12 bp (dsDNA) or ≥ 16 nt (ssDNA) can be purified with NEB #T1130
- **Typical Recovery:** DNA (50 bp to 10 kb): 70–90%
  DNA (11–25 kb): 50–70%
  dsDNA ≥ 12 bp and ssDNA ≥ 16 nt: 70–85%
  (NEB #T1130 only)
- **Elution Volume:** ≥ 5 µl
- **Purity:** $A_{260/280} \geq 1.8$
- **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

TIPS FOR SUCCESSFUL GEL EXTRACTIONS

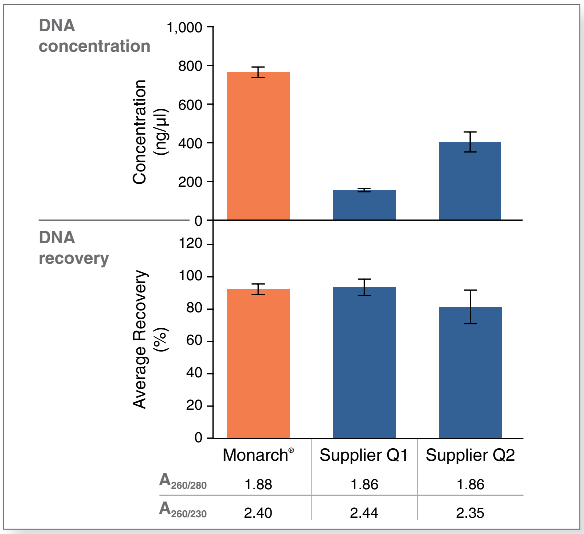
1. **Use the smallest possible agarose plug:** The less agarose in solution, the more efficient the extraction will be. More agarose means more melting time and more buffer needed to dissolve it (introducing more salts which can co-elute with your sample). If the plug is greater than 160 mg, the volume 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.

Monarch Spin DNA Gel Extraction Kit extracts high quality dsDNA from agarose gels



For the extraction, purification, and concentration of up to 5 µg of high-quality, double-stranded DNA from agarose gels. This new kit is effective for a wide range of DNA sizes and features a rapid 10 min protocol and the ability to elute in as little as 5 µl. It recovers DNA better than the leading supplier and is more highly concentrated for greater downstream utility.

Monarch Spin PCR & DNA Cleanup Kit purifies and concentates both dsDNA and ssDNA



This new kit provides a rapid method for the purification and concentration of up to 5 ug of high-quality, double-stranded and single-stranded DNA from enzymatic reactions such as PCR, restriction digestion, and reverse transcription. Purify small DNA fragments, including oligonucleotides, with this single kit, elute in as little as 5 µl, and complete the entire workflow in only 5 minutes. The kit produces more concentrated DNA with better DNA recovery and purity compared to the leading supplier.

Savings for the planet. Savings for you.

Our Monarch nucleic acid purification kits are evolving, with new updates that deliver significant improvements to performance, sustainability and value.

Redesigned column delivers higher purity and yield

Packaging is streamlined even further, with recycled materials throughout and concise protocols cards provided

Spin columns, bottles and other parts are still manufactured with significantly less plastic

Cost savings from these improvements have been passed on to you

Migrate to the new Monarch

Use the table below to migrate to the newest version of each Monarch kit.

ORIGINAL PRODUCT NAME	ORIGINAL NEB #	NEW PRODUCT NAME	NEW NEB #
Monarch Plasmid Miniprep Kit	T1010S T1010L	Monarch Spin Plasmid Miniprep Kit	T1110S T1110L
Monarch DNA Gel Extraction Kit	T1020S T1020L	Monarch Spin DNA Gel Extraction Kit	T1120S T1120L
Monarch PCR & DNA Cleanup Kit (5 ug)	T1030S T1030L	Monarch Spin PCR & DNA Cleanup Kit (5 µg)	T1130S T1130L

Troubleshooting Guide for Genomic DNA Purification

PROBLEM	CAUSE	SOLUTION
LOW YIELD		
Cells	Frozen cell pellet was thawed and/or resuspended too abruptly	• Thaw cell pellets slowly on ice and flick tube several times to release the pellet from bottom of tube. Use cold PBS, and resuspend gently by pipetting up and down 5–10 times until pellet is dissolved
	Cell Lysis Buffer was added concurrently with enzymes	• Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer
Blood	Blood was thawed, allowing for DNase activity	• Keep blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples
	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Formation of hemoglobin precipitates	• In blood from species with high hemoglobin content, (e.g., guinea pig) insoluble hemoglobin complexes may accumulate and clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
Tissue	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
	Sample was not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Genomic DNA was degraded (common in DNase-rich tissues)	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
	Column is overloaded with DNA	• Some organ tissues (e.g., spleen, kidney, liver) are extremely rich in genomic DNA. Using inputs larger than recommended will result in the formation of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material.
	Incorrect amount of Proteinase K added	• Most samples are digested with 10 μl Proteinase K, but for brain, kidney and ear clips, use 3 μl.
DNA DEGRADATION		
Tissue	Tissue samples were not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before Proteinase K can lyse the tissue.
	High DNase content of soft organ tissue	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
Blood	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Blood was thawed, allowing for DNase activity	• Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples
SALT CONTAMINATION		
	Guanidine thiocyanate salt from the binding buffer was carried over into the eluate	• When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip and always pipet carefully onto the silica membrane. • Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the spin column. • Close the caps gently to avoid splashing the mixture into the upper column area and move the samples with care in and out of the centrifuge. • If salt contamination is a concern, invert the columns a few times (or vortex briefly) with gDNA Wash Buffer as indicated in the protocol.
PROTEIN CONTAMINATION		
Tissue	Incomplete digestion	• Cut samples to the smallest possible pieces. Incubate sample in the lysis buffer for an extra 30 minutes to 3 hours to degrade any remaining protein complexes.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small, indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Blood	High hemoglobin content	• Some blood samples (e.g., horse) are rich in hemoglobin, evidenced by their dark red color. Extend lysis time by 3–5 minutes for best purity results.
	Formation of hemoglobin precipitates	• In blood from species with high hemoglobin content, (e.g., guinea pig) insoluble hemoglobin complexes may accumulate and clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
RNA CONTAMINATION		
Tissue	Too much input material	• DNA-rich tissues (e.g., spleen, liver and kidney) will become very viscous during lysis and may inhibit RNase A activity. Do not use more than the recommended input amount.
	Lysis time was insufficient	• Extend lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved
TISSUE DIGESTION TAKES TOO LONG		
	Tissue pieces too large	• Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis
	Tissue pieces are stuck to bottom of tube	• Vortex to release pieces from the tube bottom, and immediately after adding Proteinase K and Tissue Lysis Buffer
	Too much starting material	• Use recommended input amount
TISSUE LYSATE APPEARS TURBID		
	Formation of indigestible fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
RATIO A_{260}/A_{230} > 2.5		
	Slight variations in EDTA concentration in eluates	• EDTA in elution buffer may complex with cations like Mg^{2+} and Ca^{2+} samples present in genomic DNA, which may lead to higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Troubleshooting Guide for Total RNA Extraction & Purification

PROBLEM	CAUSE	SOLUTION
Clogged column	Insufficient sample disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Protection Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample-specific protocols in the product manual or online at www.neb.com/T2010.
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 13.
Low RNA yield	Incomplete elution	• After addition of Nuclease-free Water (NEB #B1500) to column matrix, incubate 5-10 min at room temperature and then centrifuge to elute • Perform a second elution (note: this will dilute sample)
	Sample is degraded	• Store input sample at -80°C prior to use • Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage
	Insufficient disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample specific protocol in the product manual or online at www.neb.com/T2010. • For Proteinase K treated samples, doubling Proteinase K (from 5% to 10%) may lead to an increase in RNA yield
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 13.
RNA degradation	Starting material not handled/stored properly	• Store input sample at -80°C prior to use. Degradation of RNA may occur if sample is not flash frozen or protected by a preservation reagent. Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage.
	Deviation from the stated protocol may expose RNA to unwanted RNase activities	• Refer to the General Guidelines for Working with RNA in the product manual
	RNase contamination of eluted materials or kit buffers may have occurred	• See General Guidelines for Working with RNA in the product manual for advice on reducing risks of contamination
Low OD ratios	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.
	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. Use care to ensure the tip of the column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
DNA contamination	Genomic DNA not removed by column	• 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
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts on page 13.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the tip of the RNA Purification Column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the RNA Purification Column for 2 minutes following the final wash with RNA Wash Buffer • 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 30 µl of nuclease-free water • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Sample Input Amounts on page 13.
	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

Troubleshooting Guide for RNA Cleanup

PROBLEM	CAUSE	SOLUTION
Low RNA yield	Reagents added incorrectly	• Check protocol to ensure correct buffer reconstitution, order of addition for buffers and ethanol, and proper handling of column flow-through and eluents
	Insufficient mixing of reagents	• Ensure the ethanol is thoroughly mixed with mixture of RNA sample and Monarch Buffer BX before applying sample to the purification column
	Incomplete elution during prep	• Ensure the nuclease-free water used for elution is delivered directly to the center of the column so that the matrix is completely covered and elution is efficient. Larger elution volumes and longer incubation times can increase yield of RNA off the column at the cost of dilution of the sample and increased processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient. Additionally, multiple rounds of elution can be employed to increase the amount of RNA eluted, at the expense of dilution of the sample. The first elution can be used to elute a second time to maximize recovery and minimize sample dilution.
	Complex secondary structure affects binding and elution of smaller RNAs (< 45 nt)	• Diluting your sample with 2 volumes of ethanol instead of one volume in Step 2.
	Poor extraction from Agarose gel	• Be sure to incubate the sample between 37–55°C after addition of both the Monarch Buffer BX and the ethanol. Additionally, incubate the column with nuclease-free water at 65°C for 5 minutes prior to spinning to elute the RNA.
Purified RNA is degraded	RNase contamination	• Wear gloves and use disposable RNase-free tips (not provided) and collection tubes during the procedure • Keep all kit components tightly sealed when not in use
	Improper storage of RNA	• Use RNA immediately in downstream applications or store at -70°C
Low OD ratios	Residual guanidine salts have been carried over during elution	• Ensure wash steps are carried out prior to eluting sample • Use care to ensure the tip of the column does not contact the flow-through. If unsure, please repeat centrifugation • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer
Low performance of RNA in downstream steps	Salt and/or ethanol carry-over	• Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over in the eluted RNA
DNA contamination	DNA present in sample	• Incubate RNA sample with DNase I (NEB #M0303) and clean up RNA using the Monarch Spin RNA Cleanup Protocol

Products for Genomic DNA Extraction & Purification

PRODUCT	NEB #	SIZE
Monarch Spin Genomic DNA Purification Kit	T3010S/L	50/150 preps
Monarch HMW DNA Extraction Kit for Cells & Blood	T3050S/L	5/50 preps
Monarch HMW DNA Extraction Kit for Tissue	T3060S/L	5/50 preps
Monarch Mag Viral DNA/RNA Extraction Kit	T4010S/L/X	100/600/1,800 preps
COLUMNS, PLASTICS AND BEADS AVAILABLE SEPARATELY		
Monarch Spin Columns S2C	T3017L	100 columns and tubes
Monarch Spin Collection Tubes	T2118L	100 tubes
Monarch DNA Capture Beads	T3005L	200 beads
Monarch Bead Retainers	T3004L	100 retainers
Monarch Pestle Set	T3000S	100 sets
Monarch 2 ml Tubes	T3003L	100 tubes
BUFFERS & REAGENTS AVAILABLE SEPARATELY		
Monarch gDNA Tissue Lysis Buffer	T3011L	34 ml
Monarch gDNA Cell Lysis Buffer	T3012L	20 ml
Monarch gDNA Blood Lysis Buffer	T3013L	20 ml
Monarch gDNA Binding Buffer	T3014L	65 ml
Monarch gDNA Wash Buffer	T3015L	60 ml
Monarch gDNA Elution Buffer	T3016L	34 ml
Monarch RNase A	T3018L	1 ml
Proteinase K, Molecular Biology Grade	P8107S	2 ml
Monarch gDNA Nuclei Prep & Lysis Buffer Pack	T3054L	1 pack
Monarch RBC Lysis Buffer	T3051L	160 ml
Monarch HMW gDNA Tissue Lysis Buffer	T3061L	62 ml
Monarch Protein Separation Solution	T3062L	36 ml
Monarch Precipitation Enhancer	T3055L	10 ml
Monarch gDNA Elution Buffer II	T3056L	24 ml

Products for DNA Cleanup and Plasmid Purification

PRODUCT	NEB #	SIZE
Monarch Spin DNA Gel Extraction Kit	T1120S/L	50/250 preps
Monarch Spin PCR & DNA Cleanup Kit (5 µg)	T1130S/L	50/250 preps
Monarch Spin Plasmid Miniprep Kit	T1110S/L	50/250 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch Spin Columns S1A	T2037L	100 columns and tubes
Monarch Spin Columns S2D	T1117L	100 columns and tubes
Monarch Spin Collection Tubes	T2118L	100 tubes

Accessories

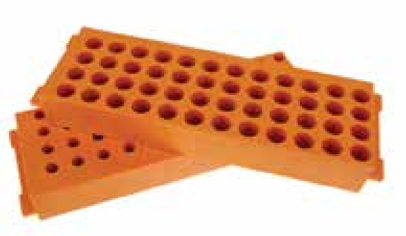
PRODUCT	NEB #	SIZE
Monarch Microfuge Tube EcoRack	T5020S	2 racks
Monarch Spin Collection Tubes	T2118L	100 tubes
Monarch Pestle Set	T3000S	100 sets
Monarch Bead Retainers	T3004L	100 retainers
Monarch 2 ml Tubes	T3003L	100 tubes

Products for RNA Purification

PRODUCT	NEB #	SIZE
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch Mag Viral DNA/RNA Extraction Kit	T4010S/L/X	100/600/1,800 preps
Monarch Spin RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch Spin RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps
Monarch Spin RNA Cleanup Kit (500 µg)	T2050S/L	10/100 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch RNA Purification Columns	T2007L	100 columns and tubes
Monarch gDNA Removal Columns	T2017L	100 columns and tubes
Monarch Spin Collection Tubes	T2118L	100 tubes
Monarch Spin Columns S1A	T2037L	100 columns and tubes
Monarch Spin Columns S2A	T2047L	100 columns and tubes
Monarch Spin Columns S2B	T2057L	100 columns and 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
Monarch Buffer BX	T2041L	80 ml
Monarch Buffer WX	T2042L	40 ml
Nuclease-free Water	B1500S/L	25 ml/100 ml

Monarch Microfuge Tube EcoRack

The Monarch Microfuge Tube EcoRack (NEB #T5020) is a bench-top tube rack made from plastic recovered during the manufacture of Monarch nucleic acid purification columns. Plastic that would otherwise be discarded during the injection molding process is recovered and re-molded into this useful lab accessory that can hold up to 48 tubes each side. One side can accommodate tubes 1.5-2 mls and the other can accommodate 0.5 ml tubes.



NEW ENGLAND
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