Monarch® DNA Purification

HIGH PERFORMANCE, SUSTAINABILITY & VALUE

Make the better choice and migrate to Monarch

Monarch® Nucleic Acid Purification Kits are the perfect complement to many molecular biology workflows, offering exceptional value for a range of budgets. 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; kits and spin column components are made with significantly less plastic than leading suppliers, and are packaged with responsibly-sourced, recyclable packaging.

Available kits:

Monarch Spin Plasmid Miniprep Kit (NEB #T1110)

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

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

- Purify DNA in 5 minutes and elute in as little as 5 µl
- Prevent buffer retention and salt carry over with unique, optimized column design
- A modified protocol enables the purification of small DNA fragments and oligos

Monarch Spin DNA Gel Extraction Kit (NEB #T1120)

- Quickly extract highly-pure DNA from agarose gels with excellent yields
- Column design enables elution in as little as 5 µl and prevents buffer carryover

Monarch Spin gDNA Extraction Kit (NEB #T3010)

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

Monarch HMW DNA Extraction Kits (NEB #T3050 and #T3060)

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

Monarch Mag Viral DNA/RNA Extraction Kit (NEB #T4010)

- Hands-free extraction of viral DNA and/or RNA using a magnetic bead-based protocol
- Compatible with automated high-throughput workflows on a variety of platforms

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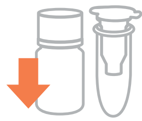
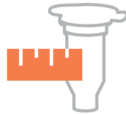
Visit **NEBMonarch.com** to learn more and request a sample.

Monarch kits are designed for sustainability and value

Reduced lab waste



Significantly less plastic as compared to leading supplier
Monarch kits still deliver high yields, purity and performance



Thinner-walled columns
Reduction in total plastic without affecting performance

Buffer bottles
Carefully designed to minimize plastic usage

Flexible purchasing options

Buffers and columns sold separately
Purchase only what you need and avoid wasted materials

Same performance, design and formulations
Standalone products are the same components that are included in complete kits

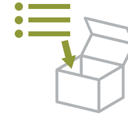
No excessive packaging

Sturdy, reusable boxes at just the right size
Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

Concise protocol cards replace printed manuals
Manuals are available online

Sustainable & recyclable packaging

Sourced for recyclability
All components are purposefully sourced for recyclability

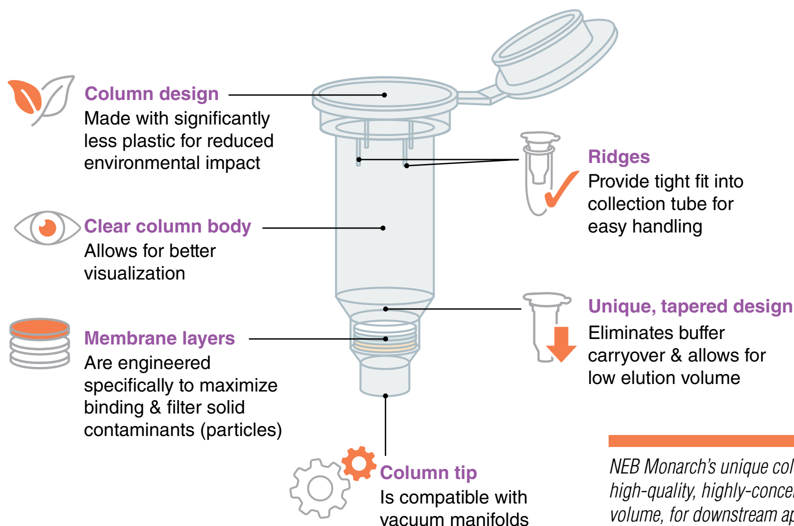


Instructions for recycling kit components
Can be found online

Recycled paper
Used to make the kit boxes, inserts and paper materials

Eco-friendly printing
Printing of boxes and packaging powered by green sustainable sources such as wind

Features of the Monarch Spin Column for Nucleic Acid Purification

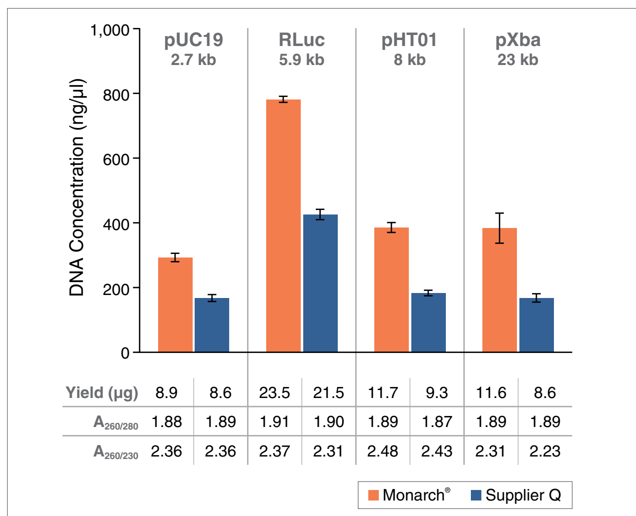


NEB Monarch's unique column design and membrane assembly allows high-quality, highly-concentrated nucleic acid purification with low elution volume, for downstream applications. The column is designed and made with significantly less plastic for a reduced environmental impact.

Monarch Spin Plasmid Miniprep Kit (NEB #T1110)

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.

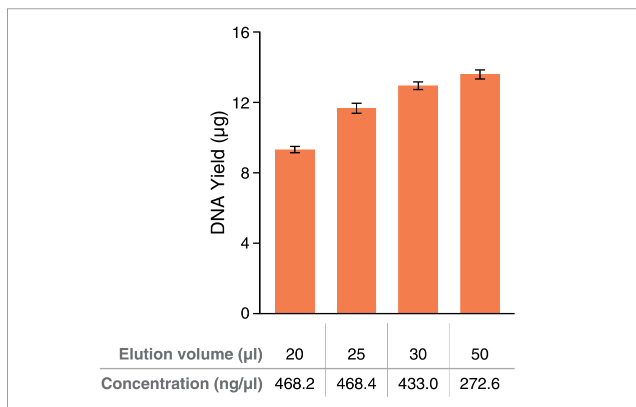
Monarch Spin Plasmid Miniprep Kit consistently produces more concentrated plasmid DNA with equivalent or better yield and purity compared to the leading supplier



	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

DNA concentration, yield and purity are higher with Monarch Spin Plasmid Miniprep Kit than leading supplier's kit, across different plasmids. Preps were performed according to recommended protocols using ~1 ml (OD600 = 3) aliquot for pUC19 and pHT01 and ~2 ml (OD600 = 6) aliquot for Rluc and pXba of the same overnight cultures. Concentrations of plasmid were measured using a Trinean DropSense 16.

Monarch Spin Plasmid Miniprep Kit can elute in as low as 30 µl for high yield



Elution volume (µl)	20	25	30	50
Concentration (ng/µl)	468.2	468.4	433.0	272.6

Various elution volumes used in Monarch Spin Plasmid Miniprep Kit can generate high yield, highly concentrated DNA. 2 ml of NEB 10-beta Competent E. coli (NEB #C3019) transformed with pUC19 plasmid (NEB #N3041) was used. Monarch Buffer EY was used to elute the plasmid with the volumes indicated. Concentrations of plasmid were measured using a Trinean DropSense 16.

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

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

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 DNA Wash Buffer provided helps ensure 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.

Monarch Spin DNA Gel Extraction Kit (NEB #T1120)

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.

> *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, Central Michigan University

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

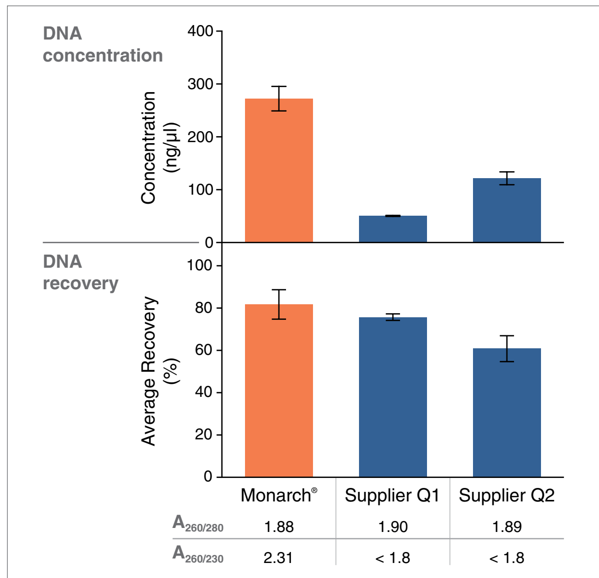
- **Binding Capacity:** up to 5 µg
- **DNA Size Range:** ~50 bp to 25 kb
  With protocol modification, oligos ≥ 12 bp (dsDNA) or ≥ 16 nt (ssDNA) can be purified with NEB #T1130
- **Typical Recovery:** up to 70–90%
- **Elution Volume:** ≥ 5 µl
- **Purity:** $A_{260/280}$ ≥ 1.8
- **Protocol Time:**
  Gel Extraction: 10–15 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.

DNA Cleanup & Gel Extraction (cont.)

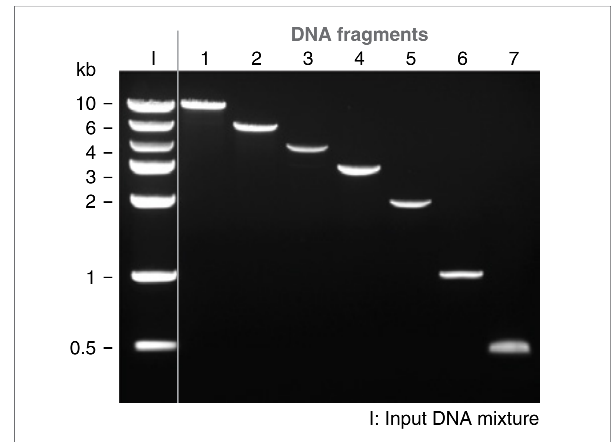
The Monarch Spin DNA Gel Extraction Kit consistently recovers DNA as well as or better than the leading supplier, and is more concentrated for greater downstream utility



	Monarch®	Supplier Q1	Supplier Q2
$A_{260/280}$	1.88	1.90	1.89
$A_{260/230}$	2.31	< 1.8	< 1.8

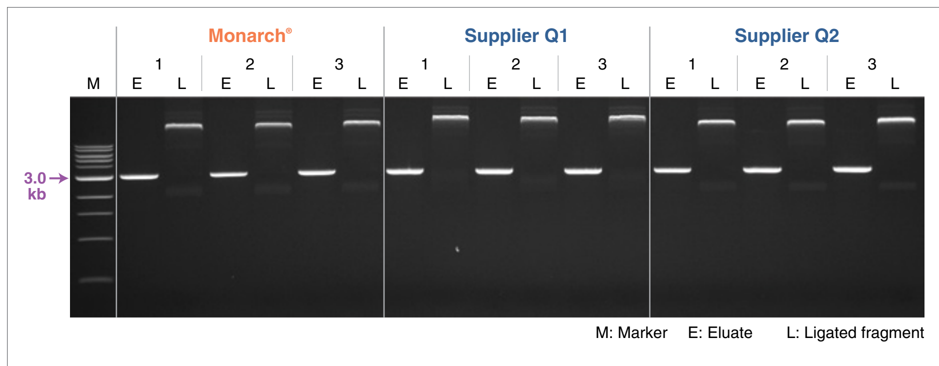
Using the Monarch Spin DNA Gel Extraction Kit, DNA concentrations and recovery are equal to or higher than a leading suppliers' kits. 2 µg of 3 kb fragment were resolved on a 1.2 % w/v TBE agarose gel, excised and processed with recommended protocols and elution volumes. Concentrations of DNA were measured using a Trinean DropSense 16 and percent recovery calculations are based on the eluted DNA concentration and elution volume used.

Monarch Spin DNA Gel Extraction Kit is effective for a wide range of DNA sizes



A mixture of 7 DNA fragments (lane I) ranging from 0.5 kb to 10 kb was prepared and resolved on a 1.2 % w/v TBE agarose gel. Each fragment was manually excised from the agarose gel and processed using the Monarch Spin DNA Gel Extraction Kit. The elution of each fragment was resolved on a new gel with the original mixture for comparison.

DNA purified from agarose gels using Monarch Spin DNA Gel Extraction Kit yields high-quality DNA, suitable for downstream applications



Use of the Monarch Spin DNA Gel Extraction Kit can effectively purify DNA from agarose and is suitable for downstream applications, performing as well as a leading supplier's kits. 2 µg of 3 kb fragments were resolved on a 1.2 % w/v TBE agarose gel, excised, and processed with recommended protocols. 1 µg of DNA was ligated using the Blunt/TA Ligase Master Mix (NEB #M0367). The equivalent amount of eluate and the ligated samples were resolved on a 1.2 % TBE agarose gel. M = 1 kb DNA Ladder (NEB #N3232).

Monarch Spin gDNA Extraction Kit (NEB #T3010)

The Monarch Spin gDNA Extraction 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).

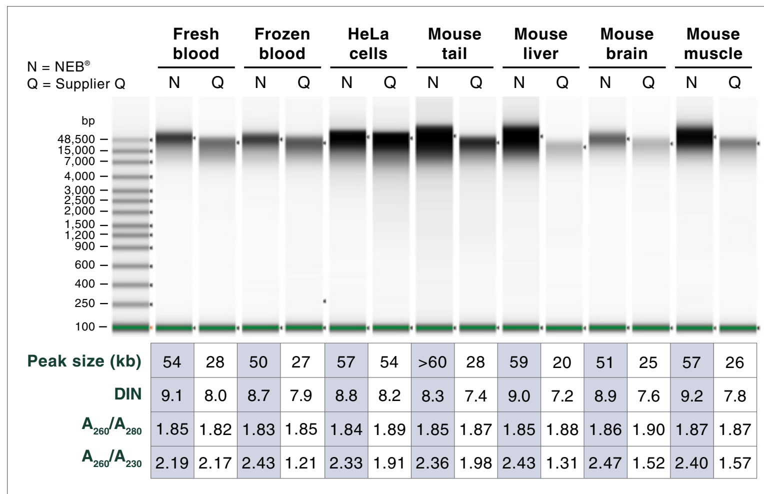
ADVANTAGES

- 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 10.
- **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:** $A_{260/280}$ ≥ 1.8, $A_{260/280}$ ≥ 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®)

The Monarch Spin gDNA Extraction Kit provides excellent yields of higher quality, higher molecular weight DNA than leading suppliers



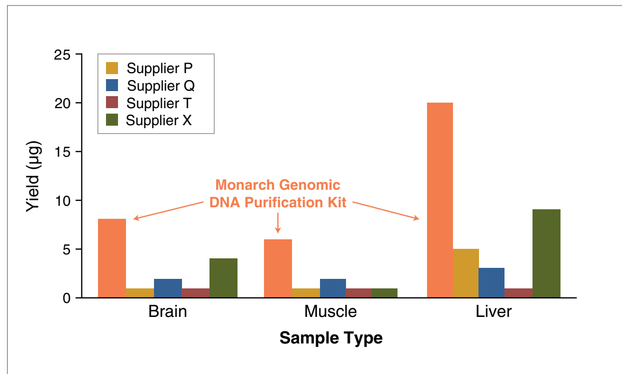
	Fresh blood N	Fresh blood Q	Frozen blood N	Frozen blood Q	HeLa cells N	HeLa cells Q	Mouse tail N	Mouse tail Q	Mouse liver N	Mouse liver Q	Mouse brain N	Mouse brain Q	Mouse muscle N	Mouse muscle Q
Peak size (kb)	54	28	50	27	57	54	>60	28	59	20	51	25	57	26
DIN	9.1	8.0	8.7	7.9	8.8	8.2	8.3	7.4	9.0	7.2	8.9	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 gDNA Extraction Kit and supplier Q's 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 10^6 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.

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

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

The Monarch Spin gDNA Extraction 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 gDNA Extraction Kit provides excellent yields for a wide range of tissues, which can be problematic for other commercial kits.

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

Residual RNA in Purified Nucleic Acid (%)

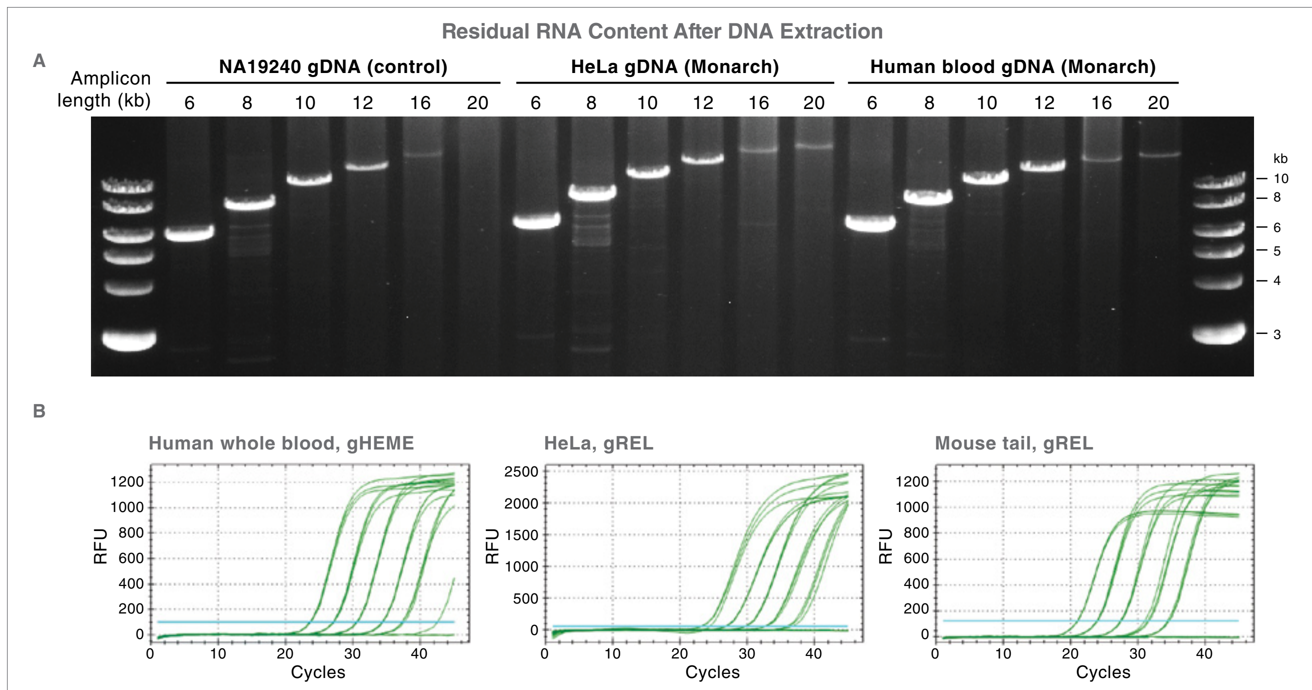
	Blood	HeLa	Tail	Liver	Brain	Muscle
Monarch®	0.1%	0.6%	0.3%	1.7%	0.7%	0.5%
Supplier P	33.9%	50.8%	32.1%	80.5%	91.3%	90.9%
Supplier Q	1.4%	10.1%	0.7%	5.9%	4.1%	2.7%
Supplier T	10.0%	2.2%	5.6%	39.0%	46.0%	7.1%
Supplier X	0.4%	5.1%	0.1%	0.3%	1.8%	3.1%

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 10^6 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 gDNA Extraction 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 gDNA Extraction Kit generates high quality genomic DNA suitable for sensitive applications like long range PCR and qPCR



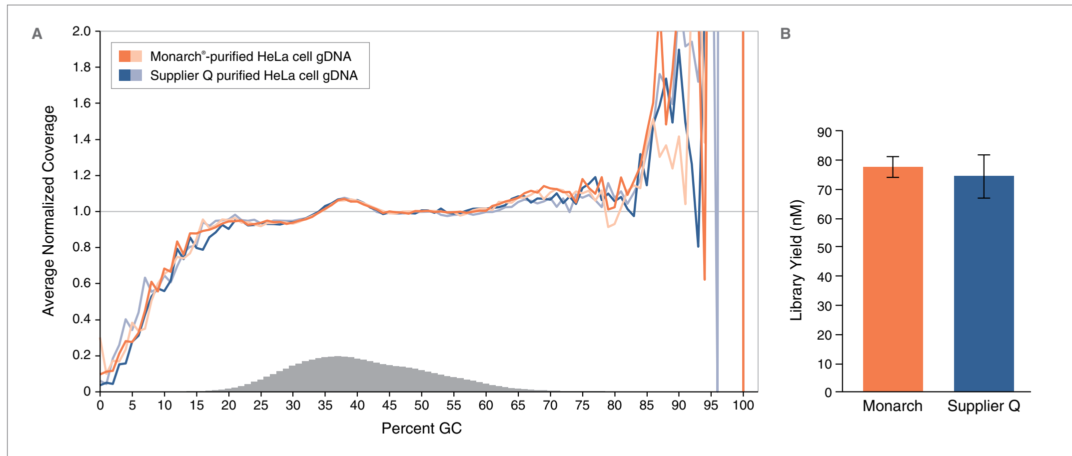
A. Amplification reactions were set up with primer pairs specific for 6, 8, 10, 12, 16, 20 kb amplicons from human DNA. LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) was used and 25 ng template DNA was added to each sample. PCR reactions were carried out on an Applied Biosystems 2720 Thermal Cycler. 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 gDNA Extraction 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.

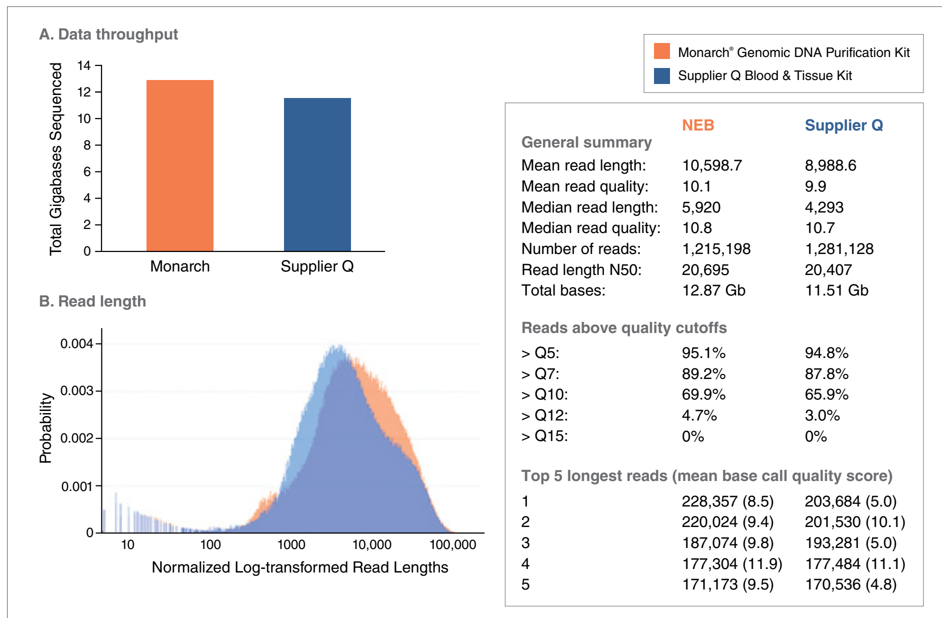
The Monarch Spin gDNA Extraction Kit generates excellent input material for NGS library preparation with NEBNext® kits for Illumina



A. Duplicate libraries were made from 100 ng HeLa cell gDNA purified with Monarch (orange) or another leading supplier (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 other supplier's 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 gDNA Extraction Kit generates high quality DNA for nanopore sequencing



	NEB	Supplier Q
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 gDNA Extraction Kit or another leading supplier's 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 Supplier Q 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 gDNA Extraction 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 gDNA Extraction 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 the product page 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.

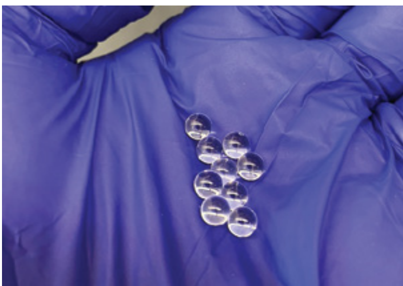
** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. 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 (NEB #T3050, #T3060)

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 HMW DNA Extraction



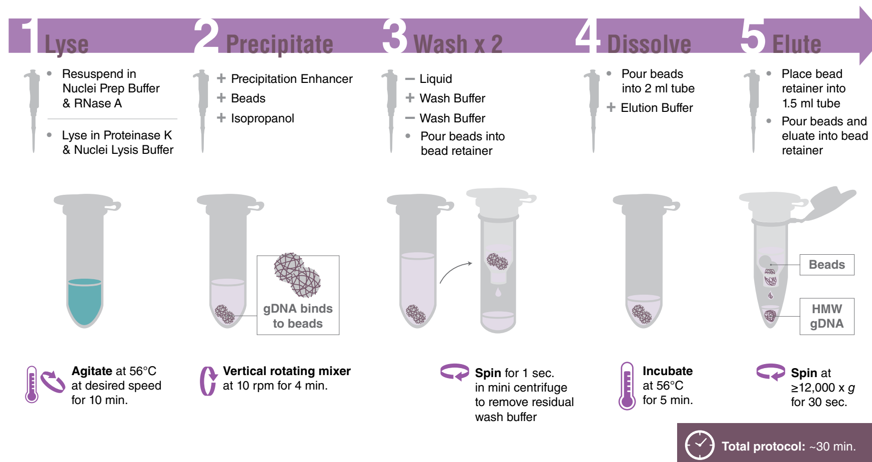
ADVANTAGES

- 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

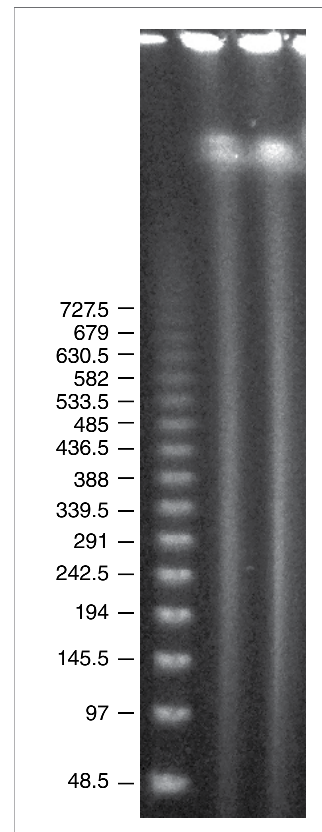
SPECIFICATIONS

- **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

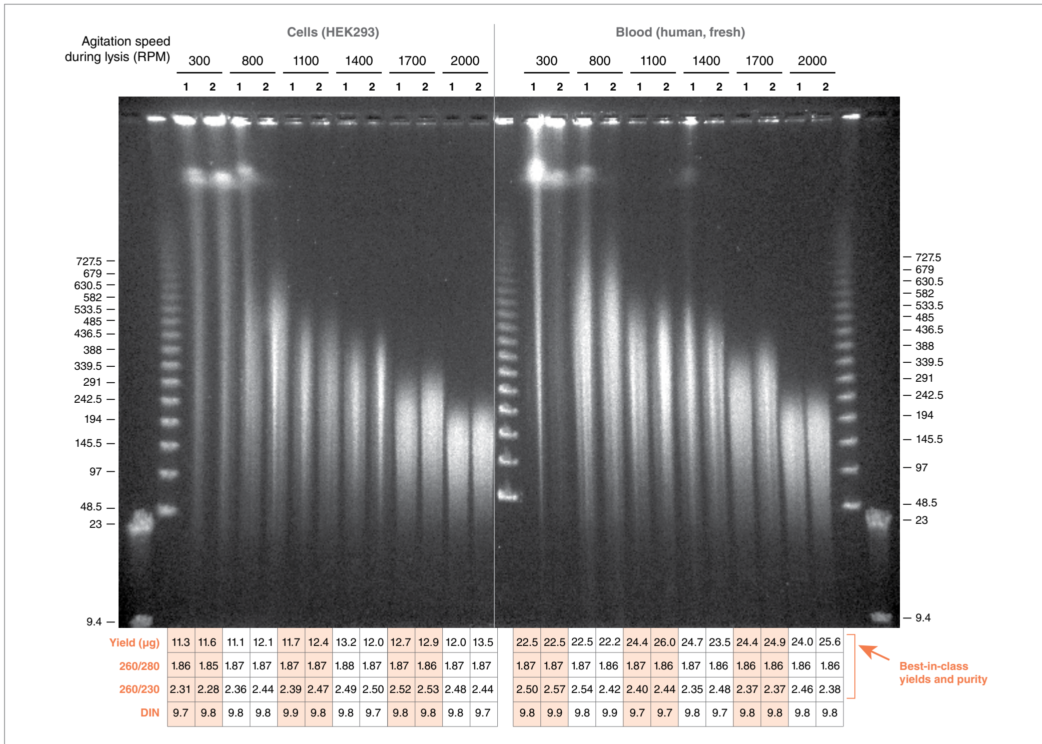


"We've had great success with obtaining HMW DNA for long read sequencing from a variety of cell types, using less input and obtaining a comparable yield...It is straightforward and easy to use."

– 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)

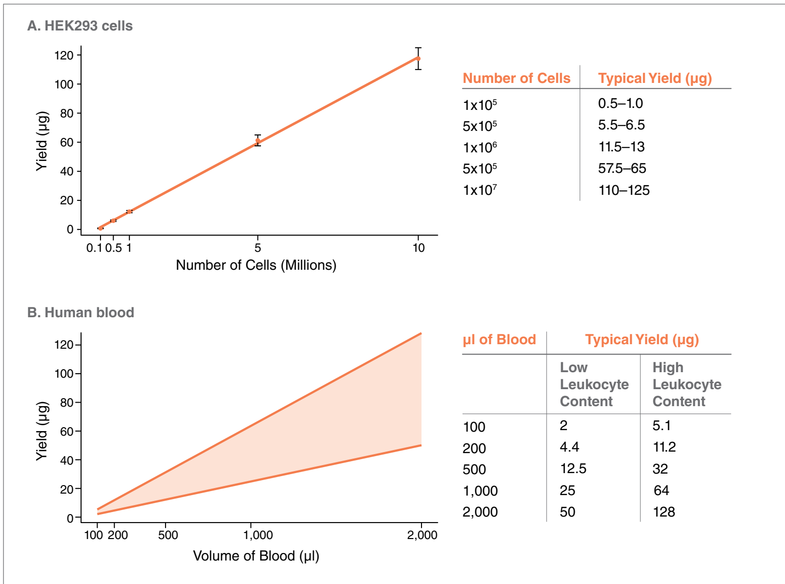
Agitation speed (RPM)	300		800		1100		1400		1700		2000	
	1	2	1	2	1	2	1	2	1	2	1	2
Yield (µg)	11.3	11.6	11.1	12.1	11.7	12.4	13.2	12.0	12.7	12.9	12.0	13.5
260/280	1.86	1.85	1.87	1.87	1.87	1.87	1.88	1.87	1.87	1.86	1.87	1.87
260/230	2.31	2.28	2.36	2.44	2.39	2.47	2.49	2.50	2.52	2.53	2.48	2.44
DIN	9.7	9.8	9.8	9.8	9.9	9.8	9.8	9.7	9.8	9.8	9.8	9.7

Blood (human, fresh)

Agitation speed (RPM)	300		800		1100		1400		1700		2000	
	1	2	1	2	1	2	1	2	1	2	1	2
Yield (µg)	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	1.87	1.87	1.87	1.86	1.87	1.86	1.87	1.86	1.86	1.86	1.86	1.86
260/230	2.50	2.57	2.54	2.42	2.40	2.44	2.35	2.48	2.37	2.37	2.46	2.38
DIN	9.8	9.9	9.8	9.9	9.7	9.7	9.8	9.7	9.8	9.8	9.8	9.8

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 anpurity 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



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

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.

Tested 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**

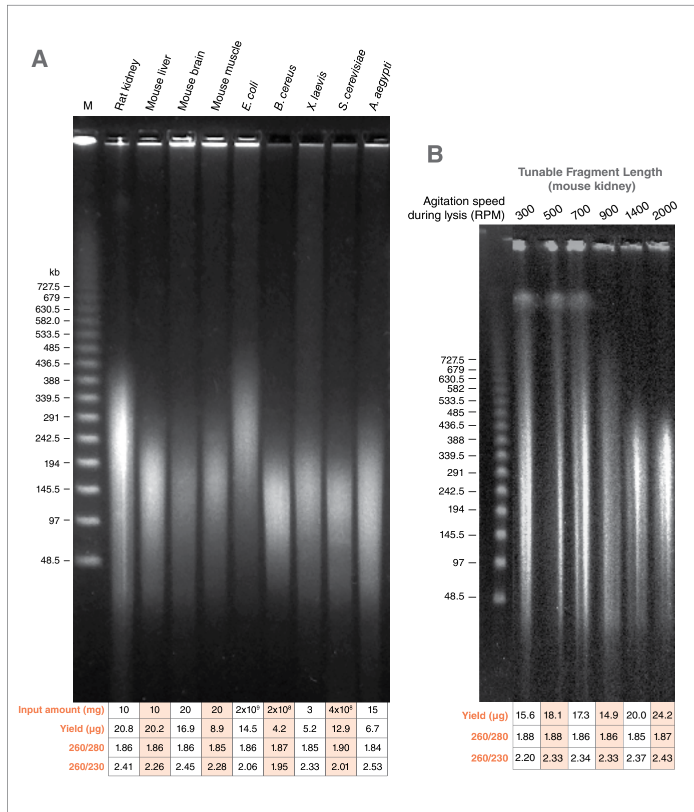
Chicken
Turkey

*Fresh and frozen samples have been validated
**Compatible with all common anticoagulants

For detailed guidance on input amounts and expected results, visit **NEB.com/MonarchHMWDNAinputs**

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



	Rat kidney	Mouse liver	Mouse brain	Mouse muscle	E. coli	B. cereus	X. laevis	S. cerevisiae	A. aegypti
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

Agitation speed during lysis (RPM)	300	500	700	900	1400	2000
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.*

Tissue workflow highlights

Following lysis and phase separation, use the same simple worflow 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.

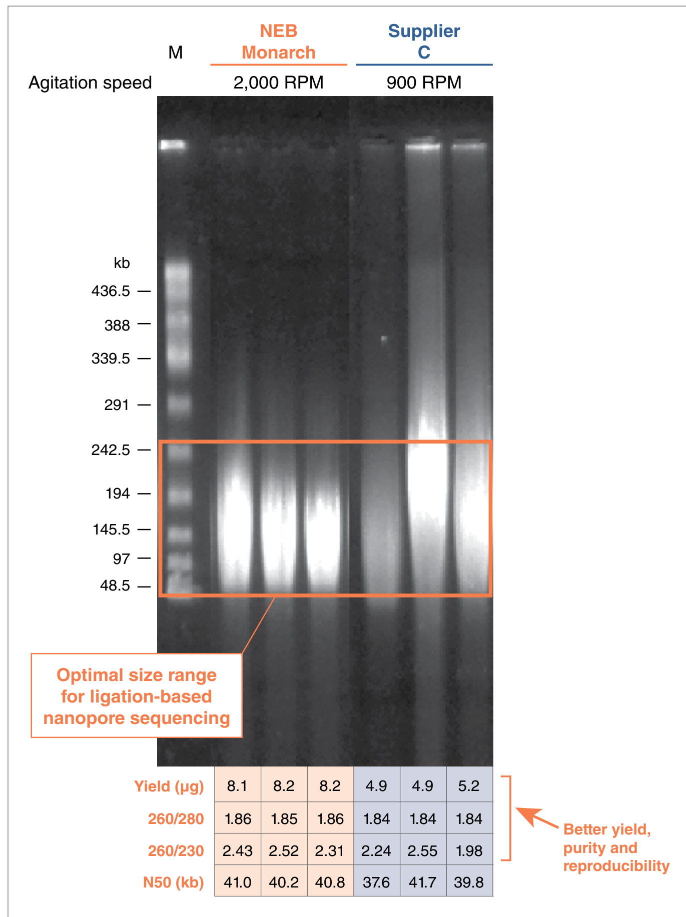
Use of beads simplifies washing and elution.

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.

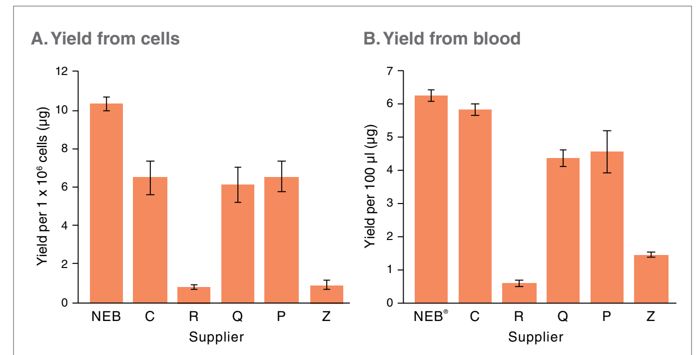
Reproducibly High Yields and Purity – Great for Long Read Sequencing

Monarch Kits extract HMW DNA with superior yields, purity and reproducibility when compared to another supplier



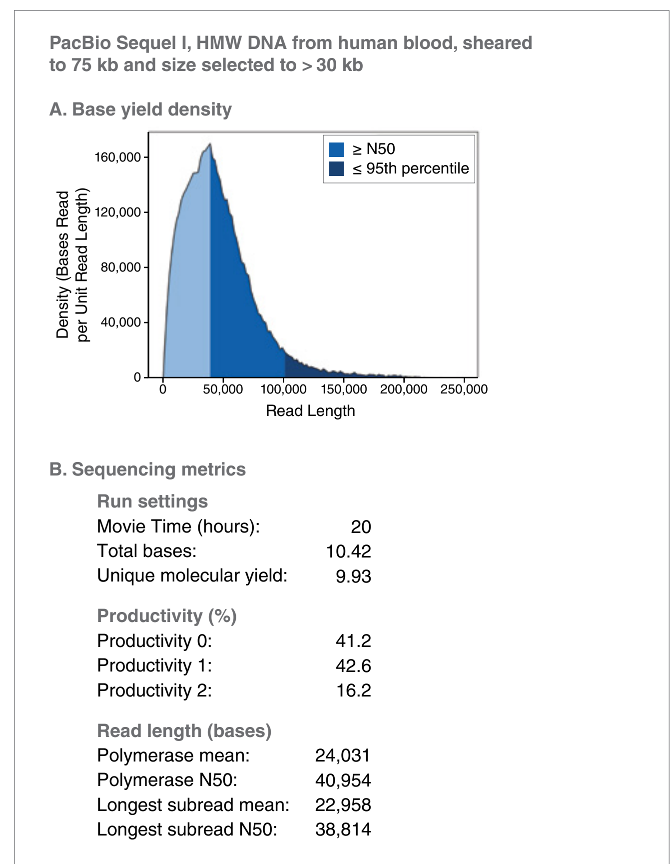
Genomic DNA was purified from 2 x 10⁶ HEK293 cells and 0.7 x 10⁶ K562 cells with the Monarch Kit and another supplier's kit, according to manufacturers' recommendations. 400 ng of HMW genomic DNA was separated on a 0.75% gel using a Pippin pulse gel system (Sage Science) at the 5–430 kb program. M = Lambda PFG Ladder (NEB #N0341). NEB Monarch and supplier C's samples were barcoded and analyzed on the same Oxford Nanopore Technologies flow cell.

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



HMW DNA was isolated from 1 x 10⁶ HEK293 cells (A) and fresh human blood (B) with kits from New England Biolabs (N) and other suppliers (C, R, Q, P, 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



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.

Excellent performance in Oxford Nanopore Technologies Sequencing

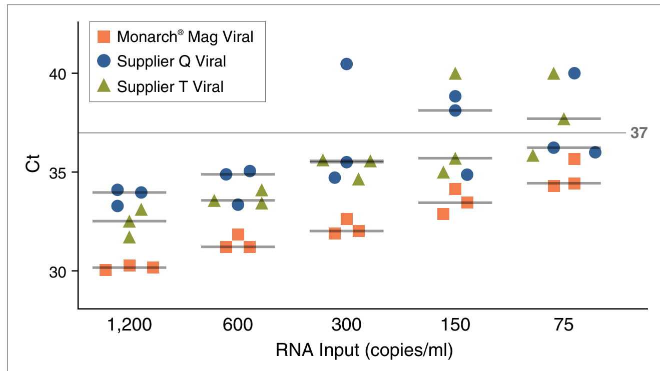
	HEK293	HUMAN BLOOD	MOUSE KIDNEY
Mean read length	21338.9	21522.6	27120.7
Mean read quality	12.8	13.4	13
Median read length	10388	10130	23150
Median read quality	13.2	13.9	13.5
Number of reads	377687	538090	164000
Read length N50	45432	46542	44631
Total bases	8059414490 (8.1 Gb)	11581090785 (11.6 Gb)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

DNA used for the sequencing libraries was extracted from HEK293 cells (1x10⁶ 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.

Monarch Mag Viral DNA/RNA Extraction Kit (NEB #T4010)

The Monarch Mag Viral DNA/RNA Extraction Kit provides a rapid and reliable magnetic bead-based process for extracting viral nucleic acids from saliva and respiratory swab samples. The kit combines the efficiency of silica-based nucleic acid purification with the ease of use of magnetic beads. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Kit sizes align to 96-well formats (100 preps, 600 preps, and 1800 preps), and the protocol is compatible with high throughput automation on a variety of platforms, including the KingFisher Flex magnetic particle processor and Agilent Bravo and MGISP liquid handler platforms.

Performance comparison of Monarch Mag Viral DNA/RNA Extraction Kit with other suppliers demonstrates high reproducibility and sensitivity of the Monarch kit



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 BioRad 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 extraction of viral DNA and/or RNA
- Utilizes magnetic bead-based methods. Compatible with manual and automated high-throughput workflows on a variety of instrument platforms, such as KingFisher® Flex, Agilent® Bravo®, MGISP® liquid handlers, and more
- 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

Products for Genomic DNA Extraction & Purification

PRODUCT	NEB #	SIZE
Monarch Spin gDNA Extraction 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
COLUMNS, PLASTICS & BEADS AVAILABLE SEPARATELY		
Monarch Spin Columns S2C and Tubes	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, Gel Extraction 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 & PLASTICS AVAILABLE SEPARATELY		
Monarch Spin Columns S1A and Tubes	T2037L	100 columns and tubes
Monarch Spin Columns S2D and Tubes	T1117L	100 columns and tubes
Monarch Spin Collection Tubes	T2118L	100 tubes
BUFFERS AVAILABLE SEPARATELY		
Monarch Buffer B1	T1111L	57 ml
Monarch Buffer B2	T1112L	54 ml
Monarch Buffer B3	T1113L	110 ml
Monarch Buffer BZ	T1114L	168 ml
Monarch Buffer WZ	T1115L	26 ml

Products for Viral Nucleic Acid Extraction

PRODUCT	NEB #	SIZE
Monarch Mag Viral DNA/RNA Extraction Kit	T4010S/L/X	100/600/1,800 preps

Time for change – try a Monarch sample

Try a sample of our Monarch Nucleic Acid Purification Kits by visiting

NEBMonarch.com

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