INSTRUCTION MANUAL

Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg)

NEB #T1135V/S/L

10/50/200 preps

Version 1.0_05/25

Table of Contents

Kit Contents

Component	NEB #	Application/Usage	T1135V 10 preps	T1135S 50 preps	T1135L 200 preps	Storage Temperature
Monarch Buffer BZ	T1114	Binding buffer concentrate (1.42X)	8.4 ml	42 ml	168 ml	15-25°C
Monarch Buffer WZ	T1115	Wash buffer concentrate (5X)	5 ml	12 ml	50 ml	15-25°C
Monarch Buffer EY	T1116	Elution buffer	3 ml	25 ml	45 ml	15-25°C
Monarch Spin Columns S3A	T1157	Spin column for nucleic acid purification	10 columns	50 columns	200 columns	15-25°C
Monarch Spin Collection Tubes	T2118	Collection tube	10 tubes	50 tubes	200 tubes	15-25°C

Buffers may require reconstitution prior to use. See Buffer Preparation section for guidance.

Storage Recommendation

- All kit components should be stored at room temperature.
- Always keep reagent bottles tightly closed.
- Keep columns sealed in the enclosed bag.
- See individual component labels for specific storage guidance.

Intended Use

The Monarch Spin High-Capacity DNA Cleanup Kit (100 µg) is developed for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Safety Information

- Monarch Buffer BZ contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. Do not add bleach or acidic solutions directly to the buffers or sample preparation waste.
- For more information regarding the composition of buffers, please consult the Safety Data Sheets available on our website www.neb.com/T1135.
- Proper laboratory safety practices should be employed using this kit, including the use of lab coats, gloves, and eye protection.

Quality Control

To help ensure consistent quality and performance, each lot of this kit is tested for predetermined quality control and functional specifications.

Introduction

The Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg) is a rapid and reliable method for the purification and concentration of up to 100 µg of high-quality, double-stranded and single-stranded DNA from enzymatic reactions such as restriction digestion, ligation, and reverse transcription, PCR, RCA, etc. Designed with sustainability in mind, these kits use significantly less plastic than other kits on the market.

Features of this kit include:

- **High Performance:** Achieve high yields (up to 100 µg) and high purity in the purification, cleanup, and concentration of DNA, with the capability to remove short primers, detergents, and other low-molecular-weight reaction components (e.g., nucleotides, DMSO, betaine).
- **High Concentration:** Elute in very small volumes, in as little as 50 µl for elution, allowing for highly concentrated DNA.
- **High Compatibility:** Designed for maximum compatibility with microcentrifuges, centrifuges and vacuum manifolds using a spin column format. No extra accessories or plastics required.
- **Powerful Flexibility:** Purify large (> 25 kb) or small DNA fragments (< 40 bp), including oligonucleotides, with this single kit, using the modified protocols provided.
- **Unique Design:** Spin column features a unique design that enables elution in low volumes and minimizes buffer retention and contaminant carryover.
- **Optimized**: Buffer system is optimized without the need to adjust pH.
- **Application Compatibility:** Purified DNA is ready for downstream molecular applications, such as *in vitro* transcription template, restriction digests, DNA sequencing, ligation, amplification, and other enzymatic reactions.

Sustainability and Recycling Information

Monarch DNA and RNA Purification Kits are designed for sustainability and developed for performance. Learn more about Monarch sustainability at www.neb.com/monarchsustainability.

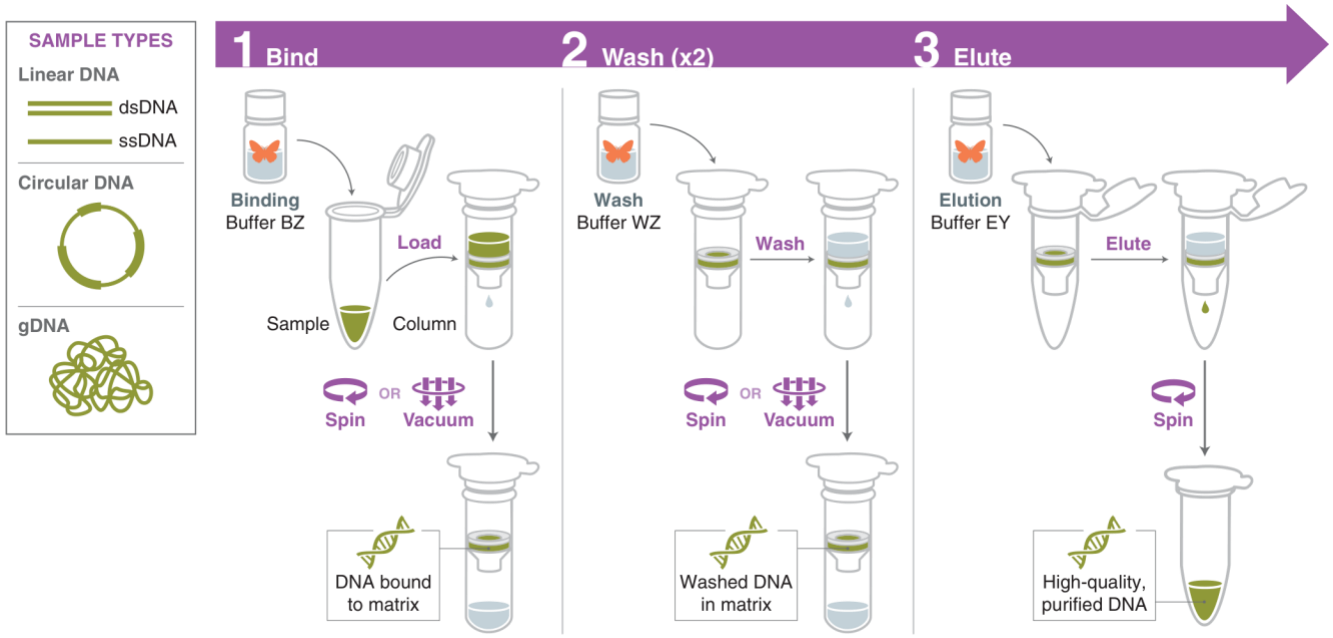
- **Sustainable performance**: Significantly less plastic is used in spin columns, bottles, and other plastic parts than in similar kits from leading suppliers.
- **Thinner-walled columns:** Reduction in total plastic without affecting performance.
- **Flexible purchasing options:** Columns and buffers also available separately. Purchase only what you need and avoid wasted materials.
- **Same performance, design, and formulations:** Standalone products are the same components and formulations that are included in complete kits.
- **Streamlined packaging:** Sturdy, reusable boxes at just the right size with concise protocol cards that replace printed manuals.
- **Sustainable and recyclable packaging**: Packaging printed with less ink using eco-friendly practices and powered by sustainable sources such as wind, where possible. Packaging is sourced for recyclability and recycled paper is used where possible to make the kit boxes, inserts, and paper materials.

Help keep Monarch sustainable by recycling after using. Learn more on how to recycle Monarch boxes and kit components at www.neb.com/monarchrecycling.

Overview of Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg)

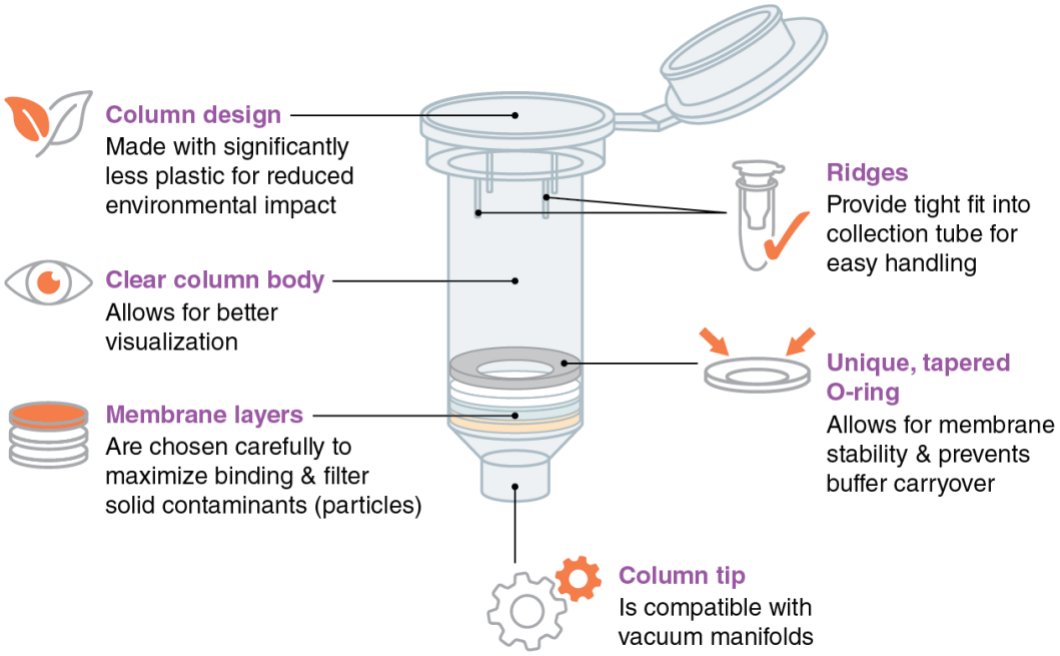
The Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg) employs an advanced bind/wash/elute workflow combined with silica-membrane technology. Monarch Buffer BZ (DNA binding buffer) is designed with high salt concentrations, enabling the optimization of the binding of the sample DNA to our unique spin column. Subsequently, the Monarch Buffer WZ (wash buffer) effectively removes enzymes, salts, detergents, and reaction components. Elution under low-salt conditions yields highly pure DNA, suitable for diverse downstream applications, including *in vitro* transcription templates, restriction digests, DNA sequencing, ligation, and other enzymatic manipulations. The innovative column design, with precision-engineered silica matrices and layers, enables minimal elution volumes as low as 50 µl with reduced buffer retention and contaminants. Combined with our optimized buffer system, the kit offers rapid and reliable purification and concentration of up to 100 µg of high-quality DNA.

Figure 1: DNA Cleanup Workflow



The Monarch Spin High-Capacity DNA Cleanup Kit (100 µg) uses the bind/wash/elute method and a unique spin column.

Figure 2. Column Design



NEB's unique column design for high-capacity DNA purification enables high-quality DNA purification with low elution volume. The column is designed with significantly less plastic for a reduced environmental impact.

Properties

Purification format	Spin column
DNA Sample*	DNA from various enzymatic reactions (restriction digestion, ligation, and reverse transcription, PCR, RCA, etc.)
	ssDNA or dsDNA oligonucleotides from enzymatic reactions can also be purified using the Oligonucleotide Cleanup Protocol
Typical Recovery	70-90 %
DNA Purity	$A_{260/280} > 1.8$ and $A_{260/230} > 1.8$
Nucleic Acid Binding Capacity	Up to 100 µg DNA
Elution Volume	50 - 200 µl
DNA Size Range	Standard protocol: 40 bp – 25 kb
	Oligonucleotide Cleanup protocol: ssDNA ≥ 12 nt and dsDNA ≥ 10 bp
Protocol Time	9 minutes of spin and incubation time
Compatible Downstream Applications	*in vitro* transcription template, ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing

*See next table for the extended list of DNA samples that can be used with this kit

Applications & Usage

Listed are selected examples of applications and usage. To see an updated list, refer to the product webpage.

PCR cleanup	DNA from PCR reactions can be purified after amplification to remove polymerases, primers, detergents, dNTPs, etc.
Enzymatic reaction cleanup	Restriction enzymes and modifying enzymes such as ligases, kinases, nucleases, phosphatases are efficiently removed, allowing for effective desalting and concentration of the DNA sample.
cDNA cleanup	DNA/RNA complexes can be purified post-reverse transcription/amplification to enable removal of the RT and polymerase as well as nucleotides.
Labeling cleanup	Unincorporated radiolabeled or fluorescently labeled nucleotides can be removed from the DNA substrate.
Plasmid cleanup	Plasmid preps from unknown sources may contain inhibitors and unwanted contaminants. Purification and concentration can be easily achieved using this kit.
Oligonucleotide and ssDNA purification	ssDNA oligonucleotides (≥ 12 nt) and dsDNA fragments (≥ 10 bp) can be purified using the Oligonucleotide Cleanup Protocol (page 9).
gDNA, RCA cleanup	gDNA (DNA size >25 kb) or RCA product can be purified using supplemental protocols provided on the product webpage.

Important notes before starting

The Monarch Spin High-Capacity DNA Cleanup Kit (100 µg) is designed for optimal DNA yield and quality, accommodating various types of DNA and sizes. Although the kit is optimized for a broad range of conditions, it is crucial to carefully consider these influencing factors to ensure high quality and maximize DNA recovery.

DNA Size

Typically, longer DNA exhibits a stronger affinity to silica in the presence of chaotropic salt, resulting in tight binding. For the Monarch Spin High-Capacity DNA Cleanup Kit (100 µg), DNA exceeding 15 kb may bind tightly to the silica column and become difficult to elute. If working with DNA longer than 15 kb, a modified elution method can be employed to increase elution efficiency. For a more detailed procedural guide, we recommend reading the full protocol provided in this manual.

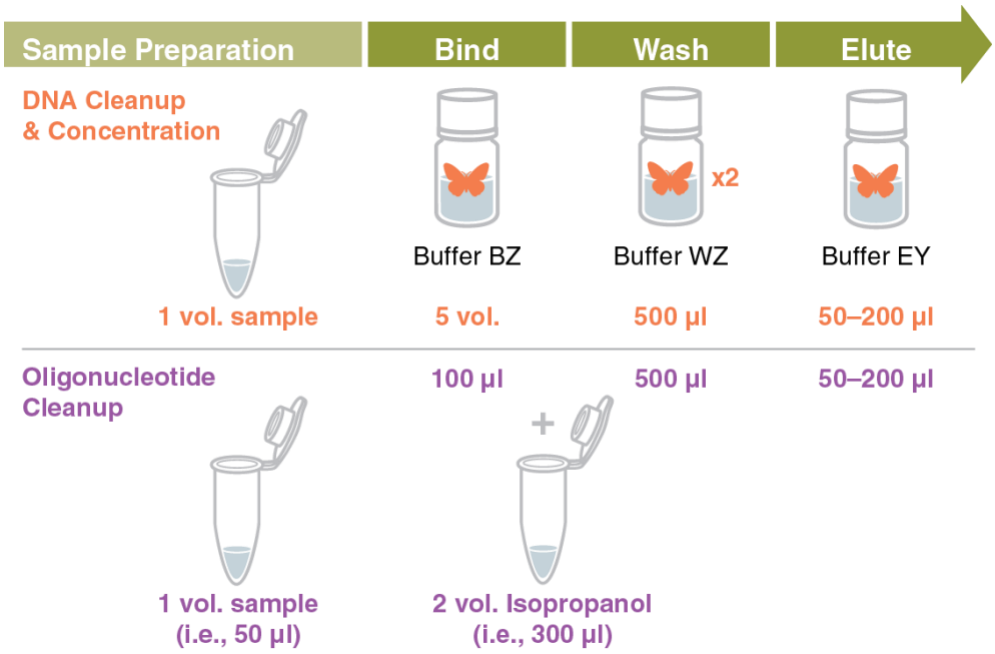
For DNA < 50 bp or oligonucleotide purification, we provide a separate protocol to allow optimal recovery. Please read the next section describing two different protocols in detail to choose the best protocol for your samples.

Monarch Protocol Overview and Comparison

The Monarch Spin High-Capacity DNA Cleanup Kit (100 µg) provides two different protocol types for the different applications of 1) DNA Cleanup and 2) Oligonucleotide Cleanup. For gDNA (DNA > 25 kb) or RCA reaction cleanup, the DNA Cleanup protocol can be used with lower yield (< 70%). To maximize the yield, please refer to the optimized protocol available for both gDNA (DNA > 25 kb) and RCA reaction cleanup on our product webpage.

	DNA Cleanup	**Oligonucleotide Cleanup**
Effective size range	dsDNA > 40 bp and ssDNA > 200 nt	dsDNA ≥ 10 bp or ssDNA ≥ 12 nt
Typical usage	• Purification from PCR and other enzymatic reactions • Removes primers, nucleotides, enzymes and other enzyme reaction components	• Purification and recovery of oligonucleotide and ssDNA • This protocol will not remove primers from PCR reactions as DNA size cutoff is 10 bp/12 nt • This protocol can be used for a total DNA cleanup

Workflow diagram comparing DNA Cleanup and Oligonucleotide Cleanup



See detailed protocol for specific instructions.

General Guidelines for Monarch® Spin High-Capacity DNA Cleanup Kit (100 µg)

- The input amount of DNA to be purified should not exceed the binding capacity of the columns (100 µg).
- A starting sample volume of 50-200 µl is recommended. For smaller samples, nuclease-free water or TE buffer can be used to adjust the volume to the recommended volume range. Sample volumes larger than 200 µl can be used; however, additional Monarch Buffer BZ may be required. If extra Monarch Buffer BZ is needed, it is available for purchase separately.
- Centrifugation should be carried out at 16,000 x *g* (~13,000 RPM) in a standard laboratory microcentrifuge at room temperature. This ensures all traces of the buffer are removed at each step.
- The column holds a maximum volume of 800 µl.
- Always keep columns tightly sealed in the provided bag.

Equipment and Reagents Required & Supplied by the User

Equipment

- Benchtop microcentrifuge
- Vacuum manifold (for the vacuum manifold protocol)
- Vacuum pump (for the vacuum manifold protocol)

Reagents/supplies

- Isopropanol (100%)
- Ethanol (≥ 95%)
- 1.5 ml or 2 ml microfuge tubes
- Optional: Nuclease-free water for elution, if provided elution buffer will not be used

Buffer Preparation

- Monarch Buffer BZ and Monarch Buffer WZ are provided as a concentrate. Please prepare the buffers according to the table below:

	T1135V (10 prep)	**T1135S (50 prep)**	**T1135L (200 prep)**
Monarch Buffer BZ Add 0.43 volume of isopropanol per volume of Buffer BZ	Add 3.6 ml of isopropanol	Add 18 ml of isopropanol	Add 72 ml of isopropanol
Monarch Buffer WZ Add 4 volumes of ethanol (≥ 95%) per volume of Buffer WZ	Add 20 ml of ethanol	Add 48 ml of ethanol	Add 200 ml of ethanol

- Always keep all buffer bottles tightly closed when not actively in use.

Protocols

1. DNA Cleanup Protocol using centrifugation
2. DNA Cleanup Protocol using a vacuum manifold
3. Oligonucleotide Cleanup Protocol

DNA Cleanup Protocol Using Centrifugation

1. **Add 5 volumes of Monarch Buffer BZ to 1 volume of sample. Mix well by pipetting up and down or flicking the tube. Do not vortex.** Using a sample volume of 50-200 µl is recommended. For samples less than 50 µl, adjust the volume with TE or nuclease-free water to exceed 50 µl. The provided Buffer BZ is sufficient for sample volumes up to 200 µl. If the sample volume exceeds 200 µl, additional Monarch Buffer BZ may be required and is available separately.

2. **Insert the Monarch Spin Column S3A into the collection tube and load the sample onto the column. Spin for 1 minute, then discard the flow-through.** The column holds a maximum volume of 800 µl. If the sample diluted in Monarch Buffer BZ volume exceeds 800 µl, load 800 µl first and spin. Reload the rest of the sample and spin. Repeat as needed.

3. **Re-insert the column into the collection tube. Wash by adding 500 µl of Monarch Buffer WZ and spin for 1 minute. Discard the flow-through.**

4. **Repeat wash (step 3).**

5. **Transfer the column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not touch the flow-through. If in doubt, re-spin for 1 minute.

6. **Add 50-200 µl of Monarch Buffer EY to the center of the matrix to elute DNA. Wait for 5 minutes, and spin for 1 minute.** A 100 µl elution volume is typically recommended. Using a lower elution volume may slightly decrease the yield. To increase yield with minimal elution volume, incubate the columns at room temperature for a few extra minutes or reload the eluate onto the column and spin again. For larger DNA fragments (≥ 15 kb), incubate the column with elution buffer at 50°C for 5 minutes to maximize yield.

DNA Cleanup Protocol Using a Vacuum Manifold

1. **Add 5 volumes of Monarch Buffer BZ to the sample. Mix well by pipetting up and down or flicking the tube. Do not vortex.** Using a sample volume of 50-200 µl is recommended. For samples less than 50 µl, adjust the volume with TE or nuclease-free water to exceed 50 µl. The provided Buffer BZ is sufficient for sample volumes up to 200 µl. If the sample volume exceeds 200 µl, additional Monarch Buffer BZ may be required and is available separately.

2. **Insert the Monarch Spin Column S3A into the vacuum adapter or manifold directly, switch the vacuum on, and load the sample onto the column. Allow the solution to pass through the column, then switch the vacuum source off.** Make sure to follow the manufacturer's instructions to set up the manifold and connect it properly to a vacuum source.

3. **Wash by adding 500 µl of Monarch Buffer WZ and switch the vacuum on. Allow the solution to pass through the columns, then switch the vacuum source off.**

4. **Repeat wash (step 3).**

5. **(Recommended) Insert the column into the collection tube and centrifuge for 1 minute.** Since vacuum set-ups can vary, centrifugation is recommended before the elution step to ensure no traces of buffer and ethanol are carried over.

6. **Transfer the column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not touch the flow-through. If in doubt, re-spin for 1 minute.

7. **Add 50-200 µl of Monarch Buffer EY to the center of the matrix to elute DNA. Wait for 5 minutes, and spin for 1 minute.** A 100 µl elution volume is typically recommended. Using a lower elution volume may slightly decrease the yield. To increase yield with minimal elution volume, incubate the columns at room temperature for a few extra minutes or reload the eluate onto the column and spin again. For larger DNA fragments (≥ 15 kb), incubate the column with elution buffer at 50°C for 5 minutes to maximize yield.

Oligonucleotide Cleanup Protocol

1. **Add 2 volumes (e.g., 100 µl) Monarch Buffer BZ (ensure that isopropanol has been added, as indicated on the bottle label) to 1 volume (e.g., 50 µl) of sample.** Using a sample volume of 50-200 µl is recommended. For samples less than 50 µl, adjust the volume with TE or nuclease-free water to exceed 50 µl..
2. **Add 2 volumes of isopropanol. Mix well by pipetting up and down or flicking the tube. Do not vortex.** E.g., If the sample volume is 50 µl, and 100 µl of Buffer BZ was added in step 1 (making a total volume of 150 µl), then add 300 µl of isopropanol (which is 2 volumes of the combined sample and buffer).
3. **Insert the Monarch Spin Column S3A into the collection tube and load the sample onto the column. Spin for 1 minute, then discard the flow-through.** The column holds a maximum volume of 800 µl. If the total volume of the sample exceeds 800 µl, load 800 µl first and spin. Reload the rest of the sample and spin. Repeat as needed.
4. **Re-insert the column into the collection tube. Wash by adding 500 µl Monarch Buffer WZ and spin for 1 minute. Discard the flow-through.**
5. **Repeat wash (Step 4).**
6. **Transfer the column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not touch the flow-through. If in doubt, re-spin for 1 minute.
7. **Add 50-200 µl of Monarch Buffer EY to the center of the matrix to elute DNA. Wait for 5 minutes, and spin for 1 minute.** Typically, a 100 µl elution volume is recommended. Lower elution volume may result in a slight decrease in yield. If minimal elution volume is used, incubating the columns at room temperature for a few minutes longer or reloading the eluate on the column and spinning again can increase the yield. For larger-size DNA (≥ 15 kb), incubate the column with elution buffer at 50°C for 5 minutes to maximize the yield.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
No DNA purified	Ethanol not added to wash buffer	Ensure proper amount of ethanol was added to the wash buffer.
Low DNA yield	Reagent added incorrectly	Check protocol to ensure correct buffer reconstitution, order of addition of buffers and proper handling of column flow-through and eluates.
	Incomplete elution	Ensure the Monarch Buffer EY (elution buffer) is added correctly to the center of the matrix. Larger elution volumes and longer incubation time can increase the yield of DNA, especially when the amount of DNA is close to maximum binding capacity and the DNA size is large (>10 kb). Alternatively, heating the elution buffer to 50°C prior to elution step may also increase the yield.
Low DNA purity and performance	Ethanol is carried over	Ensure the final wash spin time is 1 minute for the complete removal of the wash buffer. Carefully transfer the column to a microfuge tube ensuring the tip of the column does not touch the flow-through.
	Trace amounts of salt carried over	Carried-over salts will be indicated by a low $A_{260/230}$ ratio. Ensure the column tip does not touch the flow through.

For more troubleshooting and FAQs, please visit product webpage or reach out to our technical support team at info@neb.com

Ordering Information

Monarch Spin High-Capacity DNA Cleanup Kit (100 µg)

PRODUCT	NEB#
Monarch Spin High-Capacity DNA Cleanup Kit (100 µg)	T1135
Kit components sold separately	
Monarch Spin Columns S3A and Tubes	T1157
Monarch Spin Collection Tubes	T2118
Monarch Buffer BZ	T1114
Monarch Buffer WZ	T1115

NEB Companion Products

PRODUCT	NEB#
Monarch Spin PCR & DNA Cleanup Kit (5 µg)	T1130
Exo-CIP Rapid PCR Cleanup Kit	E1050
Gel Loading Dye, Purple (6x)	B7024
Gel Loading Dye, Purple (6x), no SDS	B7025
Quick-Load Purple® 1 kb DNA Ladder	N0552
Quick-Load Purple 100 bp DNA Ladder	N0551
Quick-Load Purple 1kb Plus DNA Ladder	N0550
T4 DNA Ligase	M0202
Blunt/TA Ligase Master Mix	M0367
Instant Sticky-end Ligase Master Mix	M0370

Revision History

REVISION #	DESCRIPTION	DATE