INSTRUCTION MANUAL

Monarch® Spin DNA Gel Extraction Kit

NEB #T1120S/L

50/250 preps

Version 1.0 06.24

Table of Contents

Kit Contents and Storage

Component	NEB #	Application/Usage	T1120S 50 preps	T1120L 250 preps	Storage Temperature
Monarch Buffer BY	T1121	Gel dissolving buffer	45 ml	230 ml	15-25°C
Monarch Buffer WZ	T1115	Wash buffer concentrate (5X)	5 ml	26 ml	15-25°C
Monarch Buffer EY	T1116	Elution buffer	3 ml	7 ml	15-25°C
Monarch Spin Columns S1A	T2037	Spin column for nucleic acid purification	50 columns	250 columns	15-25°C
Monarch Spin Collection Tubes	T2118	Collection tube	50 tubes	250 tubes	15-25°C

Storage Recommendations

- 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 DNA Gel Extraction Kit 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 BY contains guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. Do not add bleach or acidic solution directly to the buffers or to the sample preparation waste.
- For more information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com/T1120).
- 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 DNA Gel Extraction Kit is a rapid and reliable method for the extraction, purification, and concentration of up to 5 µg of high-quality, double-stranded DNA from agarose gels. 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 5 µg) and high purity in the extraction and purification of DNA from agarose gels, with the capability to remove contaminants and residual salts.
- **High Concentration**: Elute in very small volumes, in as little as 5 µl for elution, allowing for highly concentrated DNA.
- **Time Savings:** A short protocol of only 10 minutes of spin and incubation time is needed to complete the workflow.
- **Unique Column Design:** Spin column features a unique design that enables elution in low volumes and minimizes buffer retention and contaminant carryover.
- **Optimized Buffers:** Buffer system is optimized, without the need to adjust pH or add isopropanol.
- **Application Compatibility**: Purified DNA is ready for downstream molecular applications, such as 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.

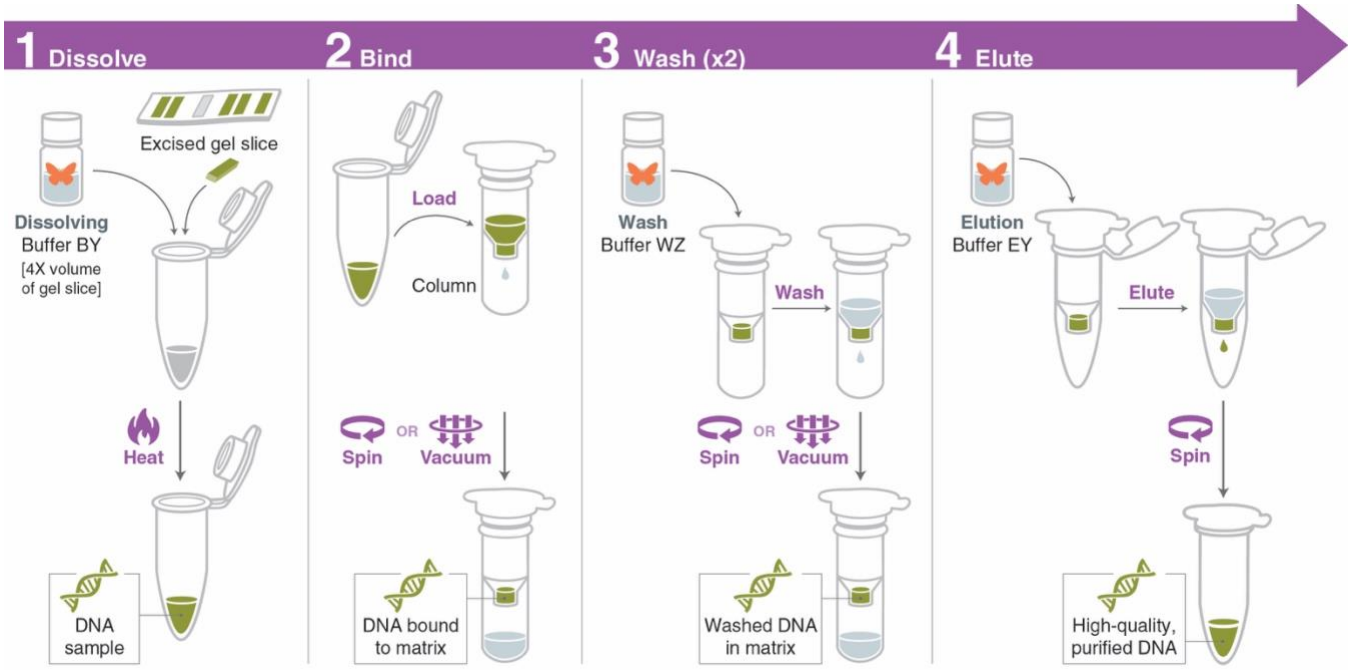
- **Sustainable performance**: Significantly less plastic is used in spin columns, bottles and other plastic parts, compared to similar kits from other leading suppliers. Monarch kits still deliver high yields, purity, and performance.
- **Thinner-walled columns:** Reduction in total plastic without affecting performance.
- **Flexible purchasing options:** Columns 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 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 DNA Gel Extraction Kit

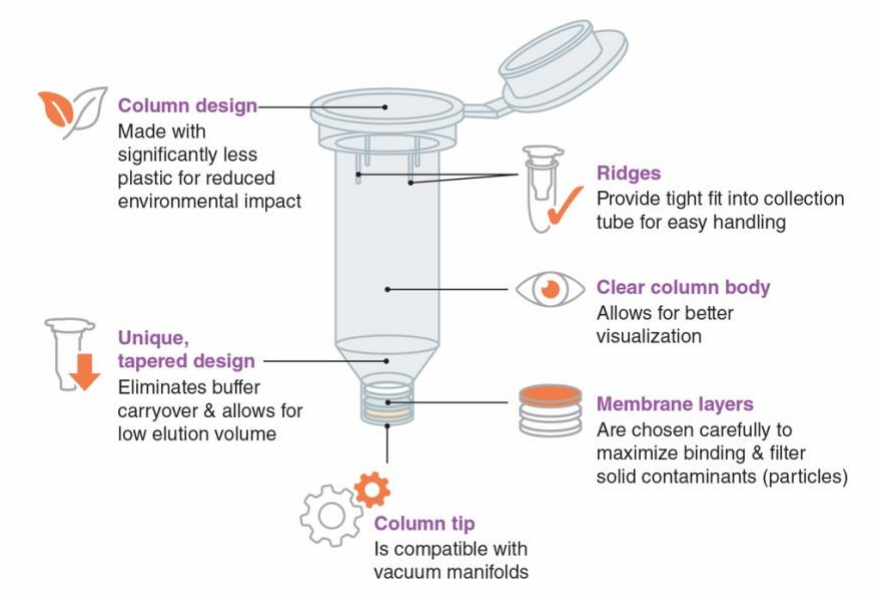
The Monarch Spin DNA Gel Extraction Kit employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in the purification of DNA in only 10-15 minutes. The Monarch Buffer BY is used to dissolve the agarose gel slice and ensure the sample is compatible with loading the DNA onto a proprietary silica matrix under high salt conditions. The wash buffer ensures trace amounts of DNA binding dyes, electrophoresis buffer salts, gel-loading buffer components and any residual gel dissolving buffer are removed. Low-volume elution produces concentrated, high-purity DNA ready for use in restriction digests, DNA sequencing, ligation, and other enzymatic manipulations. The unique column design ensures zero buffer retention and no carryover of contaminants, allowing elution of the sample in volumes as low as 5 µl.

Figure 1. Gel Extraction Workflow



The Monarch Spin DNA Gel Extraction Kit uses a dissolve/bind/wash/elution method using a spin column.

Figure 2: Monarch Column Design



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 a reduced environmental impact.

Properties

To view functional performance data, please visit the product webpage.

Purification format	Spin-column for nucleic acid purification
Compatible methods	Centrifugation or vacuum manifold
DNA Sample	dsDNA from agarose gels
Typical Recovery	70-90% for < 10 kb and 50-70% for ≥ 10 kb
DNA Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8
Binding Capacity	Up to 5 µg
Elution Volume	5-20 µl
DNA Size Range	Standard protocol: 100 bp – 25 kb
Protocol Time	10-15 minutes of spin and incubation time
Compatible Downstream Applications	Ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing

Important Notes Before Starting

The Monarch Spin DNA Gel Extraction Kit is designed to ensure optimal DNA yield and quality, accounting for influencing factors such as agarose concentration, gel electrophoresis buffers and DNA size. Although the kit is optimized for a broad range of conditions, it is crucial to carefully consider these factors to ensure high quality and maximize DNA recovery.

Agarose Concentration

Agarose gel electrophoresis is a fundamental technique for the separation of DNA fragments based on their size. The choice of agarose concentration depends on the required resolution and efficiency of DNA separation on the gel. Lower percentages of agarose are preferable for separating larger DNA fragments, whereas higher percentages enhance separation and resolution for smaller fragments. Typically, agarose concentrations ranging from 0.5% to 2% w/v are utilized in most conditions, but higher concentrations can be used based on experimental needs.

NEB's Monarch Spin DNA Gel Extraction Kit is designed to offer flexibility in gel extraction procedures, accommodating agarose concentrations up to 4% (w/v). However, if agarose concentration > 2% is used, it is recommended to use low melting and low molecular weight agarose. This enhances the resolution of DNA during gel electrophoresis and facilitates the efficient dissolving of the gel slice during the gel extraction process.

Gel Electrophoresis Buffers

Various buffers are used in agarose gel electrophoresis for DNA analysis, with Tris-acetate EDTA (TAE) and Tris-borate EDTA (TBE) as common choices. TAE facilitates faster migration and enhanced resolution of DNA larger DNA (> 1-2 kb), albeit with a weaker buffer capacity. Conversely, the TBE buffer supports a slower DNA migration but allows enhanced resolution of smaller DNA (< 1-2 kb) with stronger buffer capacity compared to TAE. In gel extraction application, both TAE and TBE buffer can be used with TAE buffer yielding slightly higher DNA recovery.

DNA Size

Typically, longer DNA exhibits a stronger affinity to silica in the presence of chaotropic salt, resulting in tight binding. For Monarch Spin DNA Gel Extraction Kit, DNA size exceeding 10 kb may tightly bind to the silica column which can be difficult to elute. If working with a DNA longer than 10 kb, adding less Monarch Buffer BY (gel dissolving buffer) and 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.

Exposure to UV light

While using UV light to visualize the DNA in agarose gel is common, exposure time should be minimized to avoid damage to the DNA. For the Monarch Spin DNA Gel Extraction Kit, long-wave UV is recommended as damage to the DNA is reduced compared to short-wave.

General Guidelines for Monarch Spin DNA Gel Extraction Kit

- The input amount of DNA to be purified should not exceed the binding capacity of the columns (5 µg).
- 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 buffer are eluted at each step.
- If using a vacuum manifold, read and follow the manufacturer's instructions before starting the prep.
- The column holds a maximum volume of 800 µl. If your agarose gel plug and gel dissolving buffer exceeds this volume, load first 800 µl, spin and reload the remaining volume.
- If precipitate has formed in Monarch Buffer BY, incubate at 30-37°C, inverting periodically to dissolve.
- Always keep columns tightly sealed in the provided bag.

Equipment and Reagents Required & Supplied by the User

Equipment

- Benchtop microcentrifuge
- Heat block or water bath that can heat up to 55°C
- Vacuum manifold (for the vacuum manifold protocol)
- Vacuum pump (for the vacuum manifold protocol)

Reagents/supplies

- 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

Add ethanol to the Monarch DNA Buffer WZ prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch Buffer WZ).

- For T1120S (50-prep) kit, add 20 ml of ethanol to Monarch Buffer WZ.
- For T1120L (250-prep) kit, add 104 ml of ethanol to Monarch Buffer WZ.

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

Monarch Spin DNA Gel Extraction Kit Protocols:

1. Standard Gel Extraction Protocol using centrifugation
2. Standard Gel Extraction Protocol using a vacuum manifold

Gel Extraction Protocol using Centrifugation

1. **Excise the DNA fragment from the agarose gel, taking care to trim excess agarose. Transfer to a 1.5 ml microfuge tube and weigh the gel slice.** Minimize exposure to UV light to avoid damage to the DNA. Trimming excess agarose from the perimeter of the band reduces the required amount of gel-dissolving buffer and dissolving time to extract the DNA.
2. **Add 4 volumes of Monarch Buffer BY to 1 volume of gel slice** (e.g., 400 µl buffer per 100 µl (≅100 mg agarose). If the DNA is > 10 kb add 3 volumes of Monarch Buffer BY instead of 4 volumes.
3. **Incubate the sample between 37-55°C (typically 50°C), vortexing periodically until the gel slice is completely dissolved (generally 5-10 minutes).** If using > 2% agarose concentration and/or lower gel dissolving temperature (37-45°C), the dissolving time may increase by 2-5 minutes. For DNA fragments > 10 kb, an additional 1.5 volume of water should be added after the slice is fully dissolved to mitigate the tighter binding of larger pieces of DNA to the silica matrix (e.g., 100 mg gel slice: 300 µl Gel Dissolving Buffer: 150 µl water).
4. **Insert the Monarch Spin Column S1A into the Monarch Spin Collection Tube and load the sample onto the column. Spin for 1 minute, then discard the flow-through.** If the total volume is > 800 µl, load 800 µl first and spin. Reload the rest of the sample and spin. Repeat as needed.
5. **Re-insert the column into the collection tube. Wash by adding 200 µl of Monarch Buffer WZ and spin for 1 minute.** Discarding flow-through is optional.
6. **Repeat wash (step 5).**
7. **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.
8. **Add 5-20 µl of Monarch Buffer EY to the center of the matrix to elute DNA. Wait for 1 minute, and spin for 1 minute.** Typical elution volumes are 5-20 µl. Nuclease-free water can also be used to elute the DNA. Yield may slightly increase if a larger volume of Monarch Buffer EY is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), incubate the column with elution buffer at room temperature for 5 minutes to maximize the yield. Alternatively, heating the elution buffer to 50°C prior to use can be used.

Gel Extraction Protocol using a Vacuum Manifold

1. **Excise the DNA fragment from the agarose gel, taking care to trim excess agarose. Transfer to a 1.5 ml microfuge tube and weigh the gel slice.** Minimize exposure to UV light to avoid damage to the DNA. Trimming excess agarose from the perimeter of the band reduces the required amount of gel-dissolving buffer and dissolving time to extract the DNA.
2. **Add 4 volumes of Monarch Buffer BY to 1 volume of gel slice** (e.g., 400 µl buffer per 100 µl (≅100 mg agarose). If the DNA is > 10 kb add 3 volumes of Monarch Buffer BY instead of 4 volumes.
3. **Incubate the sample between 37-55°C (typically 50°C), vortexing periodically until the gel slice is completely dissolved (generally 5-10 minutes).** If using > 2%, agarose concentration and/or a lower gel dissolving temperature (37-45°C), the dissolving time may increase by 2-5 minutes. For DNA fragments > 10 kb, an additional 1.5 volume of water should be added after the slice is fully dissolved to mitigate the tighter binding of larger pieces of DNA to the silica matrix (e.g., 100 mg gel slice: 300 µl Gel Dissolving Buffer: 150 µl water).
4. **Insert the Monarch Spin Column S1A 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 manifold manufacturer's instructions to set up the manifold and connect it properly to a vacuum source.
5. **Wash by adding 200 µl of Monarch Buffer WZ and switch the vacuum on. Allow the solution to pass through the columns, then switch the vacuum source off.**
6. **Repeat wash (step 5).**
7. **(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.
8. **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.
9. **Add 5-20 µl of Monarch Buffer EY to the center of the matrix to elute DNA. Wait for 1 minute, and spin for 1 minute.** Typical elution volumes are 5-20 µl. Nuclease-free water can also be used to elute the DNA. Yield may slightly increase if a larger volume of Monarch Buffer EY is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), incubate the column with elution buffer at room temperature for 5 minutes to maximize the yield. Alternatively, heating the elution buffer to 50°C prior to use can be used.

Troubleshooting

Problem	Common Cause	Suggestions/Solutions
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.
	Gel slice not fully dissolved	Small clumps of agarose may clog the column or interfere with DNA binding. Be sure to incubate the gel slice in the Monarch Buffer BY (gel dissolving buffer) for the specified time and within the proper temperature range. Mix the sample and inspect periodically to monitor the dissolution of the agarose.
	Gel dissolved above 60°C	The DNA may become denatured if incubated at higher temperatures than the specified range of 37–55°C.
	Incomplete elution during prep	Ensure the Monarch Buffer EY (elution buffer) is added correctly to the center of the matrix. Larger elution volumes and longer incubation times 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	Gel slice is not fully dissolved	Undissolved agarose may leach salts into the eluted DNA. Be sure to incubate the gel slice and the Monarch Buffer BY (gel dissolving buffer) mixture for the specified time and temperature. Mix the sample and inspect periodically to monitor the dissolving of the agarose.
	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.
Low 260/230 ratio or Peak at 230-250 nm	Presence of guanidine salts in the eluted DNA	If working with large agarose plugs (>150 mg), consider using 3 volumes of Monarch Buffer BY instead of 4 volumes as recommended in the protocol. Additionally, an extra wash step can remove residual salt carry-over (total of 3 wash steps). These will help decrease the amount of guanidine salt present in the workflow. The presence of guanidine salt does not affect performance in downstream applications but will result in over-estimating the concentration of DNA. If removal of guanidine salt is necessary after elution, the DNA can be cleaned up using the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130), which is a 5-minute spin-column based protocol.

Ordering Information

View the entire Monarch DNA& RNA Purification portfolio at www.nebmonarch.com

Monarch Spin DNA Gel Extraction Kit

PRODUCT	NEB #
Monarch Spin DNA Gel Extraction Kit	T1120
Columns and collection tubes also offered separately	
Monarch Spin Columns S1A and Tubes	T2037
Monarch Spin Collection Tubes	T2118

NEB Companion Products

PRODUCT	NEB #
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	M0379
β-Agarase I	M0392

Revision History

REVISION	DESCRIPTION	DATE
1.0		06/24