Monarch® DNA Gel Extraction Kit

NEB #T1020S/L

50/250 preps

Version 2.1_4/21

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Kit Components

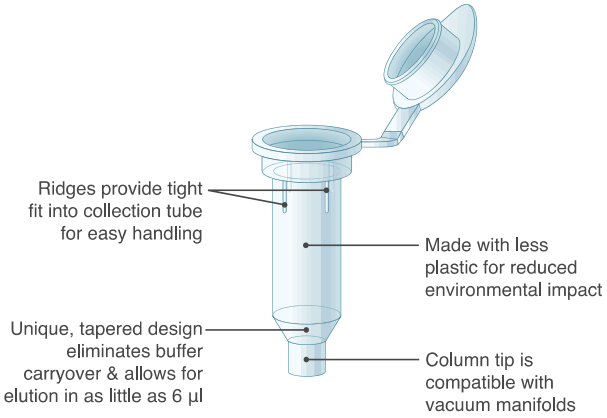
The kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. For information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com/T1020). Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.

	T1020S 50 preps	**T1020L 250 preps**	**STORAGE TEMPERATURE**
Monarch Gel Dissolving Buffer	47 ml	235 ml	Room temp.
Monarch DNA Wash Buffer	5 ml	25 ml	Room temp.
Monarch DNA Elution Buffer	3 ml	7 ml	Room temp.
Monarch DNA Cleanup Columns (5 µg)	50 columns	250 columns	Room temp.
Monarch Collection Tubes	50 tubes	250 tubes	Room temp.

Introduction

The Monarch DNA Gel Extraction Kit rapidly and reliably purifies up to 5 µg of concentrated high-quality, double-stranded DNA from agarose gels. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 15 minutes. The Monarch Gel Dissolving Buffer is used to dissolve the agarose gel slice and ensure the sample is compatible for loading the DNA onto the proprietary silica matrix under high salt conditions. The wash buffer ensures trace amounts of DNA binding dyes, electrophoresis buffer salts and gel loading buffer components 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 sample in volumes as low as 6 µl.

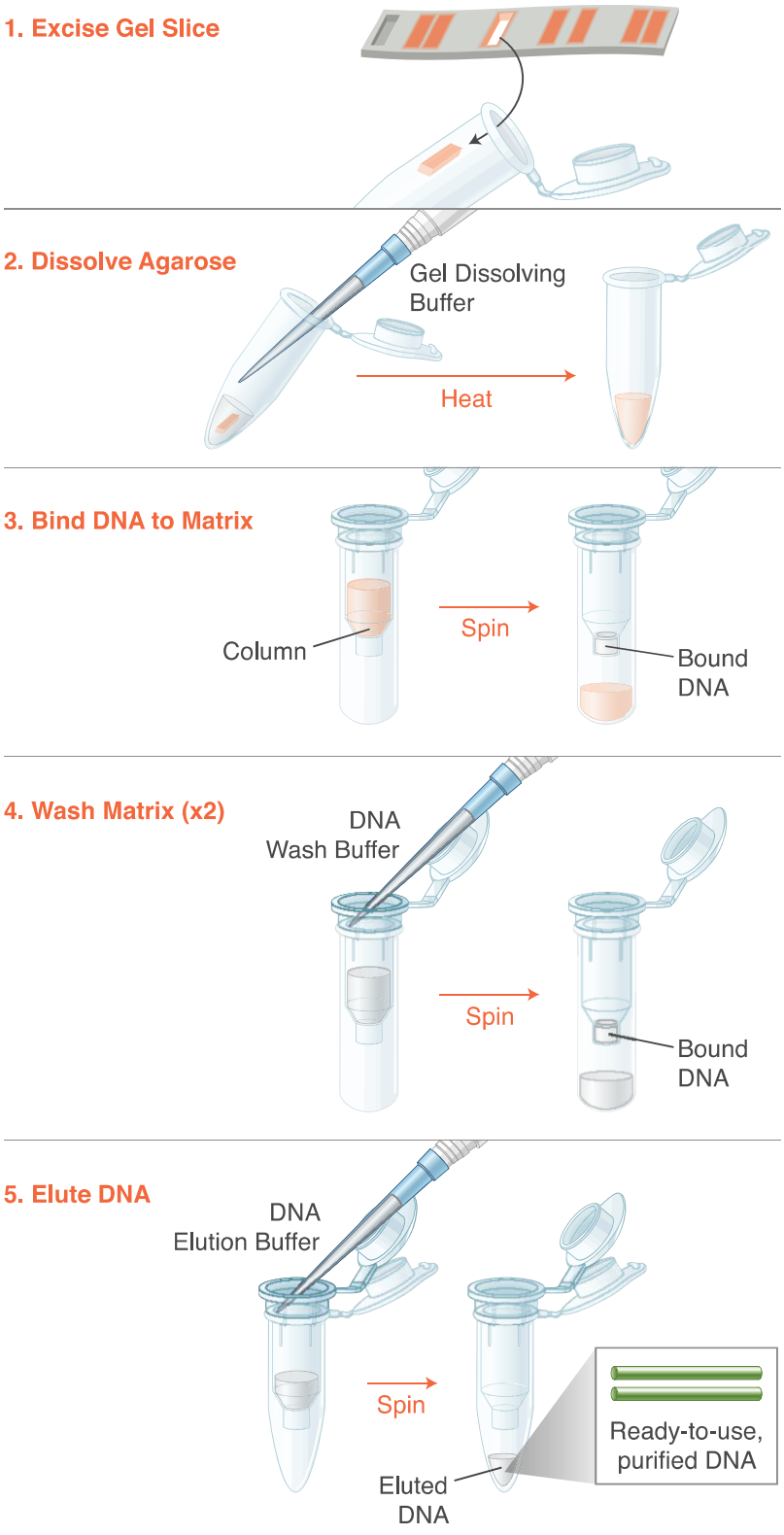
Figure 1: Column Design



Specifications

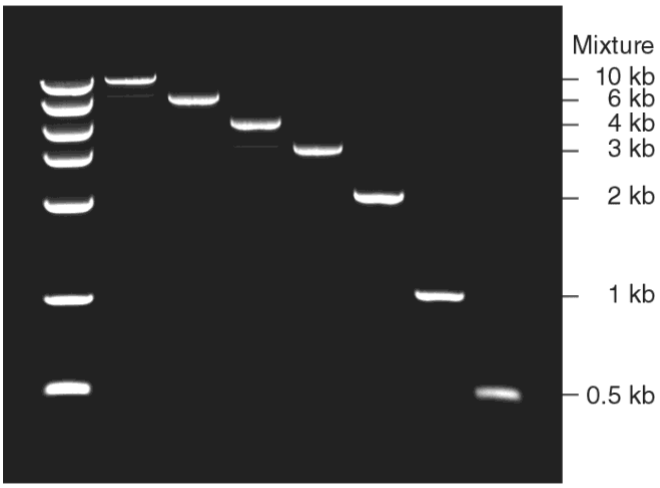
DNA Sample Type:	double-stranded DNA from agarose gels
Binding Capacity:	up to 5 µg
DNA Size Range:	~ 50 bp to 25 kb
Typical Recovery:	DNA (50 bp to 10 kb): 70–90% DNA (11–23 kb): 50–70%
Elution Volume:	≥ 6 µl
Purity:	$A_{260/280} \geq 1.8$
Protocol Time:	10 minutes of spin and incubation time
Compatible Downstream Applications:	ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing.

Figure 2: Workflow for gel extraction



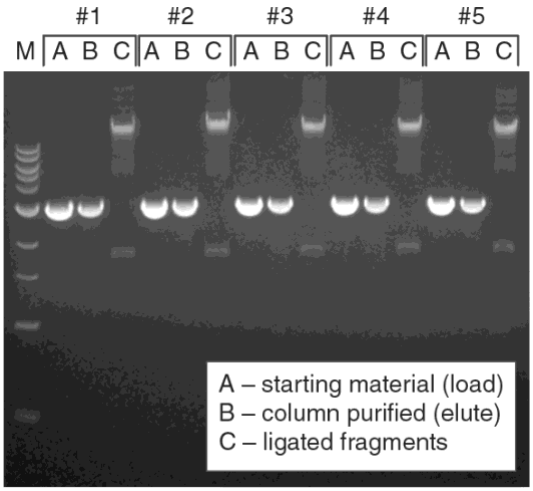
Performance Data

Figure 3: Monarch DNA Gel Extraction Kit reproducibly recovers DNA over a broad range of molecular weights.



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

Figure 4: DNA purified from agarose gels using the Monarch DNA Gel Extraction Kit can be reproducibly isolated and ligated.



Two micrograms of a 3 kb fragment with compatible ends was resolved on a 1% agarose gel, excised, and purified using the Monarch DNA Gel Extraction Kit. Samples were eluted in 20 µl and a fraction (1/4th of total) was ligated using the Blunt/TA Ligase Master Mix (NEB #M0367). Representative samples from 5 replicates were resolved on a second 1% agarose gel. M is the 1 kb DNA Ladder (NEB #N3232).

General Guidelines

The input amount of DNA to be purified should not exceed the binding capacity of the column (5 µg). DNA fragments are excised from an agarose gel and are diluted by addition of four volumes of Gel Dissolving Buffer. For a typical 100 mg (100 µl) gel slice, 400 µl of Gel Dissolving Buffer is added. Centrifugation should be carried out at 16,000 x *g* in a standard laboratory microcentrifuge at room temperature.

Important Notes Before You Begin

- Add ethanol to Monarch DNA Wash Buffer prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch DNA Wash Buffer).
    - For 50-prep kit, add 20 ml of ethanol to 5 ml of Monarch DNA Wash Buffer
    - For 250-prep kit, add 100 ml of ethanol to 25 ml of Monarch DNA Wash Buffer
- Please note that the column reservoir holds 800 µl and will need to be re-loaded if the volume of gel + dissolving buffer exceeds this volume.
- All centrifugation steps should be carried out at 16,000 x g (around 13K RPM in a typical microcentrifuge). This ensures all traces of buffer are removed at each step.
- Always keep all buffer bottles tightly closed when not actively in use.

Monarch DNA Gel Extraction Kit Protocol

1. Excise the DNA fragment to be purified from the agarose gel using a razor blade, scalpel, or other clean cutting tool. Use care to trim excess agarose. Transfer it to a 1.5 ml microcentrifuge tube and weigh the gel slice.
   Note: Using UV light to visualize the slice is common, but exposure time should be kept as short as possible to minimize damage to the DNA. Use long-wave UV when possible, as shorter wavelengths induce greater damage. Also, trim off excess agarose from the perimeter of the band to minimize the amount of dissolving buffer needed, and to reduce the time necessary to extract the DNA.

2. Add 4 volumes of Monarch Gel Dissolving Buffer to the tube with the slice (e.g., add 400 µl Gel Dissolving Buffer per 100 mg agarose). If the gel slice is > 150 mg, consider reducing the amount of Gel Dissolving Buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow.
   Note: If the volume of the dissolved sample exceeds 800 µl, the loading of the sample onto the column should be performed in multiple rounds to not exceed the volume constraints of the spin column.

3. Incubate the sample between 37–55°C (typically 50°C), inverting periodically until the gel slice is completely dissolved (generally 5–10 minutes).
   Note: For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 mg gel slice: 400 µl Gel Dissolving Buffer: 150 µl water). Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the DNA and potential clogging of the column by particles of agarose.

4. Insert the column into collection tube and load sample onto the column. Spin for 1 minute, then discard flow-through.

   To save time, spin for 30 seconds, instead of 1 minute.

   If using a vacuum manifold* instead of centrifugation, insert the column into the manifold and switch the vacuum on. 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. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.

   If using a vacuum manifold, add 200 µl of DNA Wash Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

6. Repeat wash (Step 5).

7. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, re-spin for 1 minute before placing into clean microfuge tube.

   If using a vacuum manifold: Since vacuum set-ups can vary, a 1-minute centrifugation is recommended prior to elution to ensure that no traces of salt and ethanol are carried over to the next step.

8. Add ≥ 6 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.
   Note: Typical elution volumes are 6–20 µl. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

   To save time, spin for 30 seconds, instead of 1 minute.

Troubleshooting

Low DNA Yield

- Reagents added incorrectly. Check protocol to ensure correct buffer reconstitution, order of addition for buffers and proper handling of column flow-through and eluents.
- 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 Gel Dissolving Buffer for the specified time and within the proper temperature range. Mix the sample and inspect periodically to monitor 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 DNA Elution Buffer 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 DNA off the column at the cost of dilution of the sample and increased processing times. For typical fragments below 10 kb, the recommended elution volumes and incubation times should be sufficient, unless the maximal yield is desired. For the purification of larger fragments, heating the DNA Elution Buffer to 50°C prior to eluting and ex- tending the incubation time after buffer addition to 5 minutes can improve yield. Additionally, multiple rounds of elution can be employed to increase the amount of DNA eluted, at the expense of dilution of the sample.

Low DNA Performance

- Gel slice not fully dissolved. Undissolved agarose may leach salts into the eluted DNA. Be sure to incubate the gel slice and the Monarch Gel Dissolving Buffer mixture for the specified time and temperature. Mix the sample and inspect periodically to monitor dissolving of the agarose.
- Ethanol has been carried over. Ensure final wash spin time is 1 minute to ensure complete removal of the wash buffer from the column and be careful when transferring the column to a new tube for elution step to ensure column tip does not contact column flow-through.
- Trace amounts of salts that produce low $OD_{260/230}$ ratios can also be carried over during the elution step. Be careful when transferring column to new tube for elution step to ensure the column tip does not contact column flow-through.

High 260/230 Ratio or Peak at 230-250 nm

- A low 260/230 ratio and a peak at 230-250 nm is caused by the presence of guanidine salt in the eluted DNA. Guanidine salt is a components of the Gel Dissolving Buffer and when this buffer is used in large quantities, the salt can be carried over and will absorb strongly in this range on the spectrophotometer. If working with large agarose plugs (> 150 mg), consider using 3 to 3.5 volumes of Gel Dissolving Buffer instead of the 4 volumes recommended in the protocol; this will help decrease the amount of guanidine salt present in the workflow. Excess guanidine salt cannot be removed from the column with additional washes, so it is best to start with the smallest gel slice possible for optimal results. 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 PCR & DNA Cleanup Kit (NEB #T1030), which is a 5-minute spin-column based protocol.

Ordering Information

PRODUCT	NEB #	SIZE
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps
COLUMNS SOLD SEPARATELY		
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns
BUFFERS SOLD SEPARATELY		
Monarch Gel Dissolving Buffer	T1021L	235 ml
Monarch DNA Wash Buffer	T1032L	25 ml
Monarch DNA Elution Buffer	T1016L	25 ml

COMPANION PRODUCTS

PRODUCT	NEB #	SIZE
Quick-Load® Purple 1 kb DNA Ladder	N0552S	125 lanes
Quick-Load Purple 100 bp DNA Ladder	N0551S	125 lanes
Quick-Load Purple 1 kb Plus DNA Ladder (0.1 – 10.0 kb)	N0550S/L	250/750 lanes
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
Exo-CIP Rapid PCR Cleaning Kit	E1050S/L	100/400 reactions
Monarch Genomic DNA Purification Kit	T3010S/L	50/150 preps
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps

Revision History

REVISION #	DESCRIPTION	DATE
1.0		11/15
1.1		4/16
1.2		3/17
2.0	New format applied. Replaced “vortexing periodically” with “inverting periodically” in step 3. Corrected kit component table and buffer preparation typo.	1/21
2.1	Kit components correction for T1020L, Monarch collection tubes is 250 tubes.	4/21

How to Recycle Monarch Kit Components*

Component	Recycling Notes**
Kit Box (paper)	For the greatest environmental benefit, please reuse this box. It is fully recyclable in paper recycling. The small magnets do not prohibit recycling.
Columns and Collection Tubes (hard plastic)	Columns and collection tubes are made from polypropylene and are recyclable. After use, please refer to your institutional policies for proper disposal, especially when working with biohazardous materials.
Plastic Bottles (hard plastic)	Bottles are made from high-density polyethylene, and caps are polypropylene. Please rinse before recycling.
Plastic Bags (plastic film)	Bags are made from low-density polyethylene and can be recycled with other plastic bags and films.
Protocol Card (paper)	Recycle with mixed paper, or keep in your lab notebook for reference. The finish on this card does not prohibit recycling.

* Information as of November 2015. Please visit NEBMonarchPackaging.com for updates.
** Please defer to your institutional policies for proper disposal of this kit and its components.
Consult with your local and institutional authorities to learn how to maximize your landfill diversion and materials recovery.

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