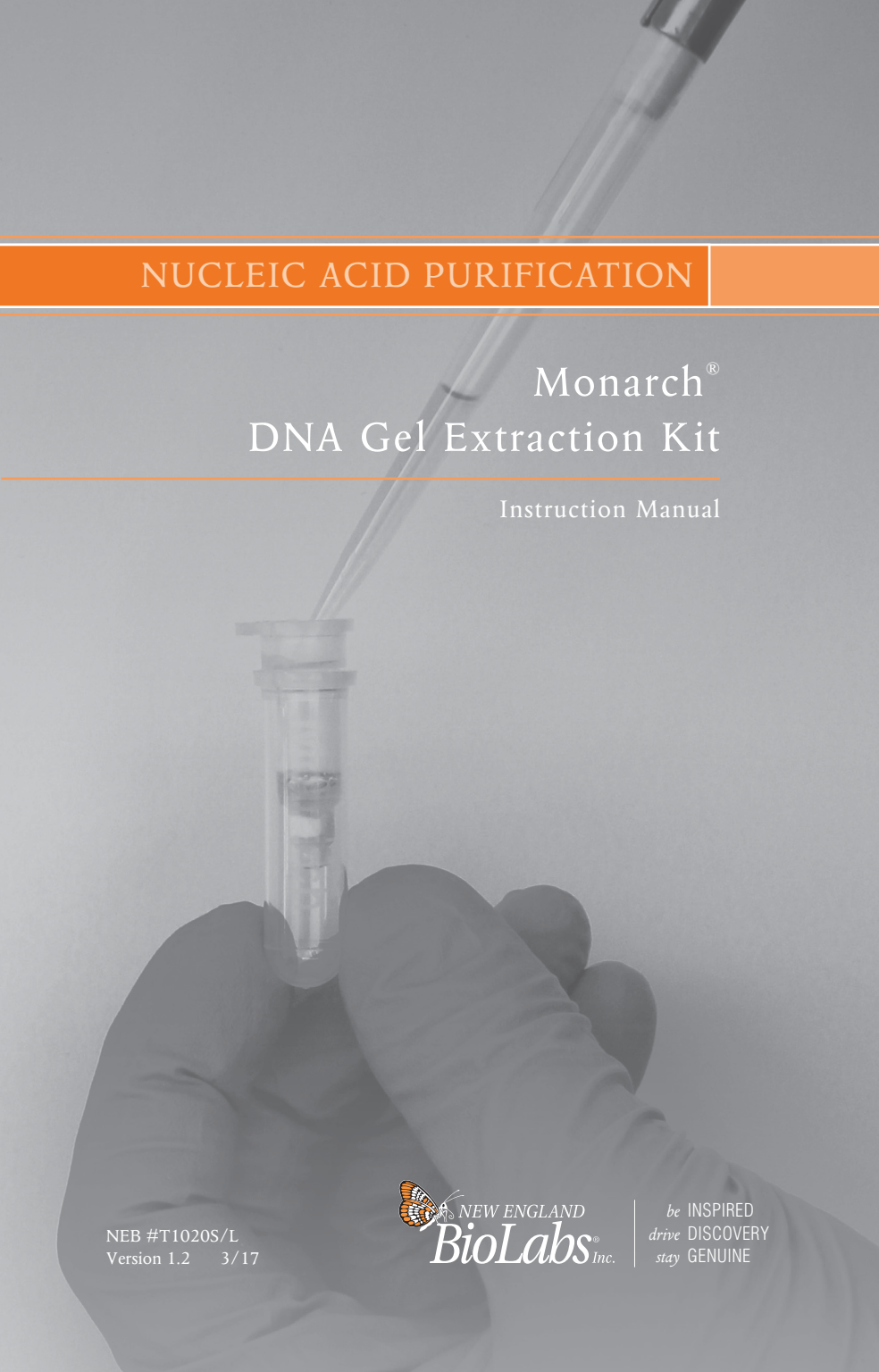


NUCLEIC ACID PURIFICATION

Monarch®
DNA Gel Extraction Kit

Instruction Manual

NEB #T1020S/L
Version 1.2 3/17

NEW ENGLAND BioLabs® Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Monarch DNA Gel Extraction Kit

Table of Contents:

Kit Components:

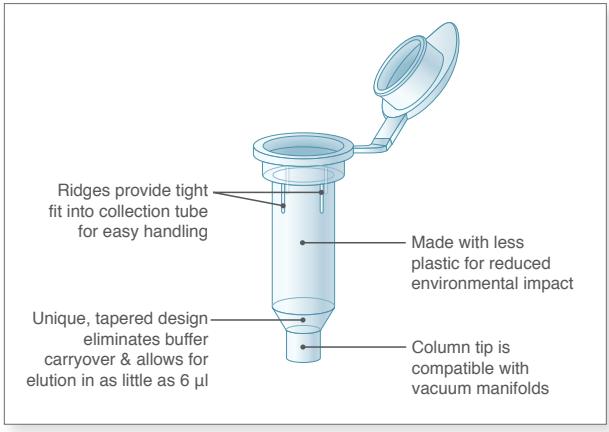
The kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. For information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com/T1020). Proper laboratory safety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

	T1020S 50 preps	T1020L 250 preps	STORAGE TEMP.
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	250	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.

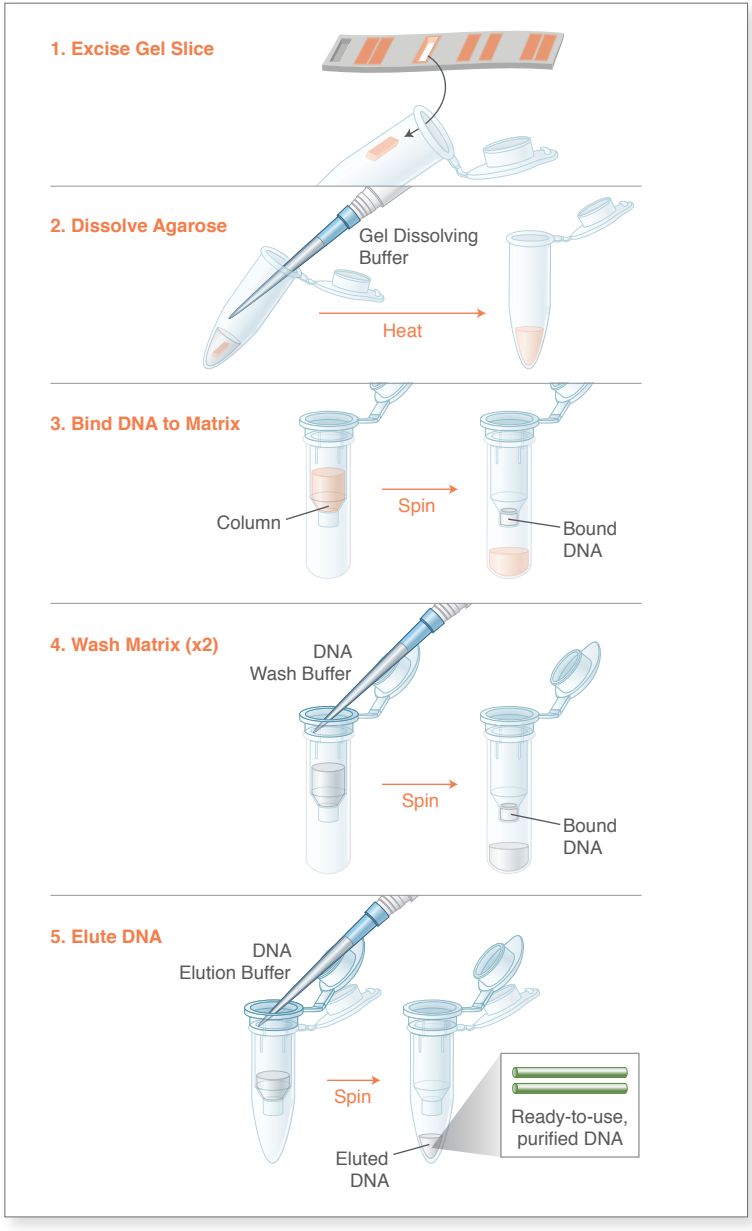
Figure 1: Column design



Specifications:

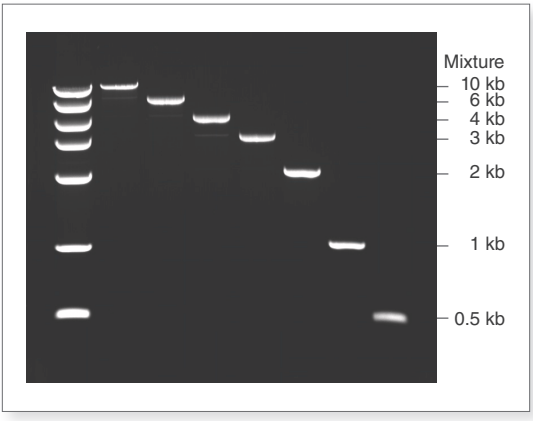
DNA Sample Type:	double-stranded DNA from agarose gels
Binding Capacity:	up to 5 µg
DNA Size Range:	~50 bp to 25 kb
Typical Recovery:	DNA (50 bp to 10 kb): 70–90% DNA (11–23 kb): 50–70%
Elution Volume:	≥ 6 µl
Purity:	$A_{260/280}$ > 1.8
Protocol Time:	10 minutes of spin and incubation time
Compatible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

Figure 2: Workflow for gel extraction



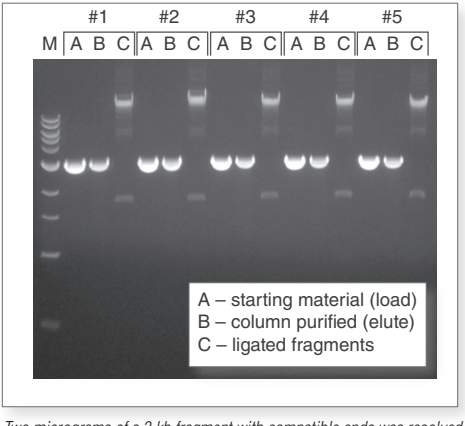
Performance Data

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 manually 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.

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 (1/4 th 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.

Buffer Preparation:

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: column holds 800 µl.
Always keep all buffer bottles tightly closed when not in use.

Monarch DNA Gel Extraction Kit Protocol

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 eluted at each step.

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.

   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), vortexing 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 µl 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 can be reduced to 30 seconds.

   If using a vacuume 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.*

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 column 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.

** Make sure to follow the manifold manufacturer's instructions to set-up the manifold and connect it properly to a vacuum source.*

8. Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, and 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 can be reduced to 30 seconds.

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 extending 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.

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		
Gel Loading Dye, Purple (6X)	B7024S	4 ml
Gel Loading Dye, Purple (6X), no SDS	B7025S	4 ml
Quick-Load® Purple 1 kb DNA Ladder	N0552S	125 lanes
Quick-Load 100 bp DNA Ladder	N0551S	125 lanes
Quick-Load Purple 2-Log DNA Ladder (0.1 - 10.0 kb)	N0550S	250 lanes
T4 DNA Ligase	M0202S/T/L/M	20,000/100,000 units
Blunt/TA Ligase Master Mix	M0367S/L	50/250 rxns
Instant Sticky-end Ligase Master Mix	M0370S/L	50/250 rxns
RELATED PRODUCTS		
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps

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 5 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 2, and caps are polypropylene 5. Please rinse before recycling.
Plastic Bags (plastic film)	Bags are made from low-density polyethylene 4 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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