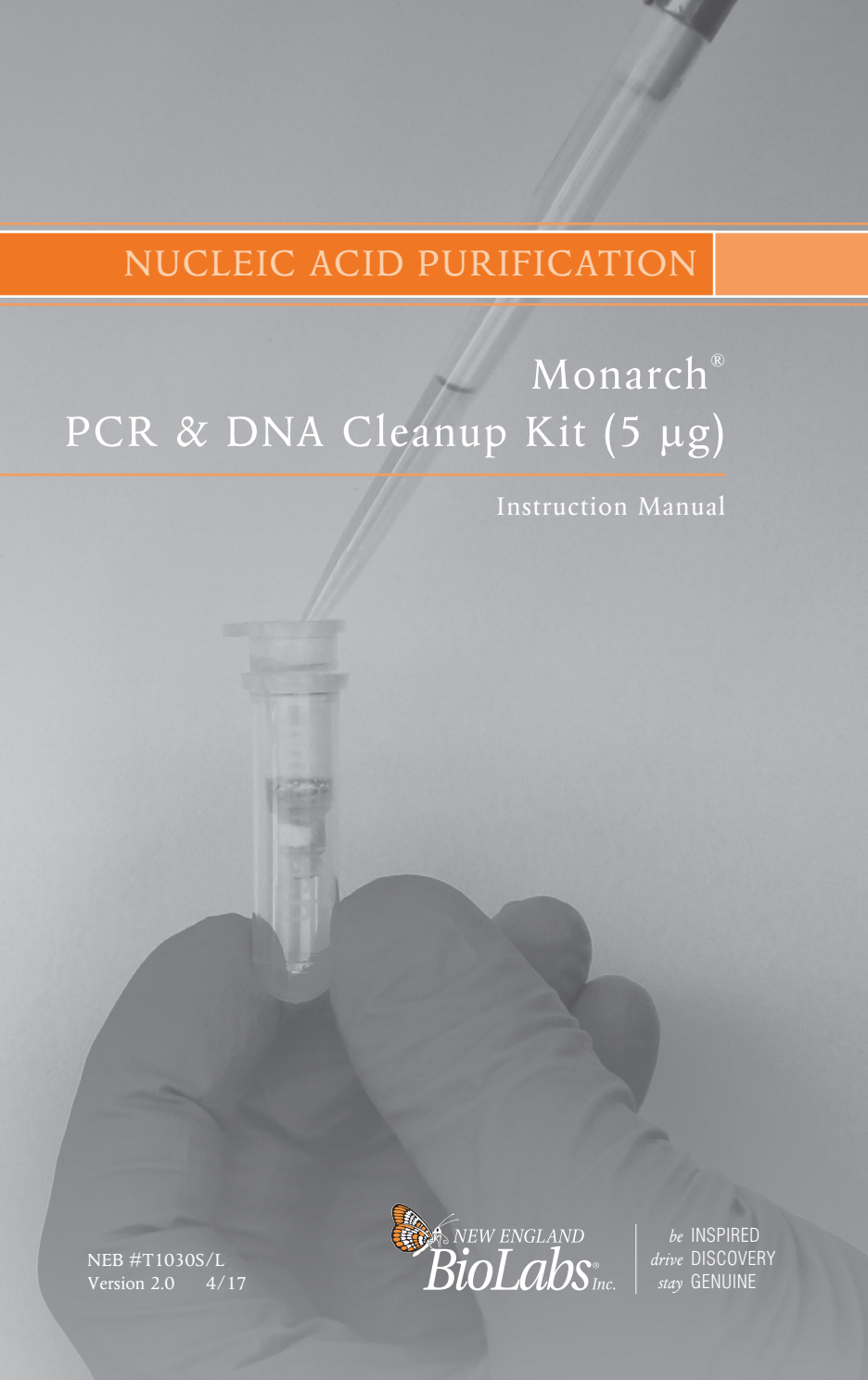


NUCLEIC ACID PURIFICATION

Monarch®
PCR & DNA Cleanup Kit (5 µg)

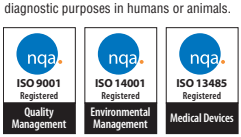
Instruction Manual

NEB #T1030S/L
Version 2.0 4/17

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 PCR & DNA Cleanup Kit (5 µg)

Table of Contents:

Kit Components:

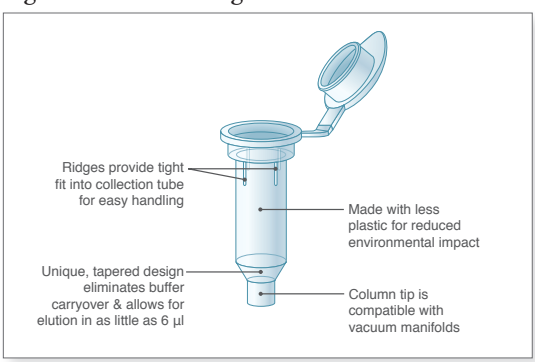
The kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. For information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (www.neb.com/T1030). Proper laboratory safety practices should be employed, including the use of lab coats, gloves and eye protection.

	T1030S 50 preps	T1030L 250 preps	STORAGE TEMP.
Monarch DNA Cleanup Binding 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 PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is 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. The protocol can also be modified to enable the purification of smaller DNA fragments, including oligonucleotides.

Figure 1: Column design



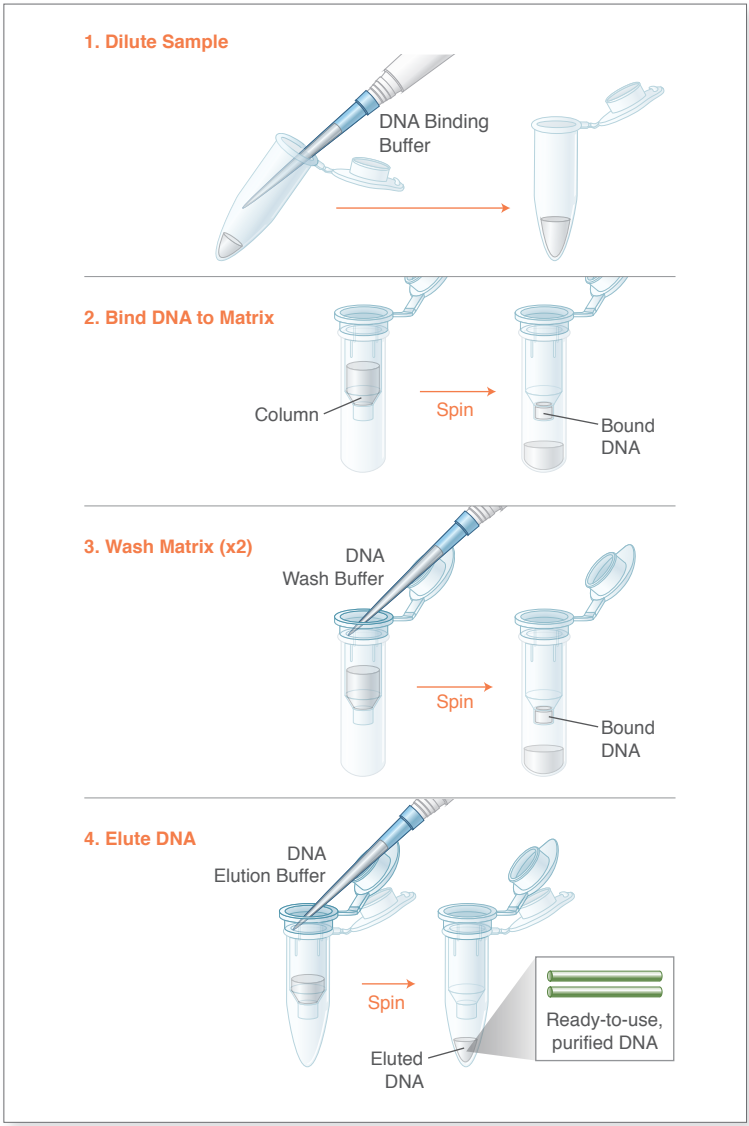
Specifications:

DNA Sample Type:	DNA from PCR and other enzymatic reactions (e.g., restriction digests, kinase reactions, ligations).*
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 and $A_{260/230}$ > 1.8
Protocol Time:	5 minutes of spin and incubation time
Compatible Downstream Applications:	ligation, restriction digestion, labeling and other enzymatic manipulations, library construction and DNA sequencing.

* ssDNA or dsDNA oligonucleotides from enzymatic reactions can also be purified using the Oligonucleotide Cleanup Protocol on page 7.

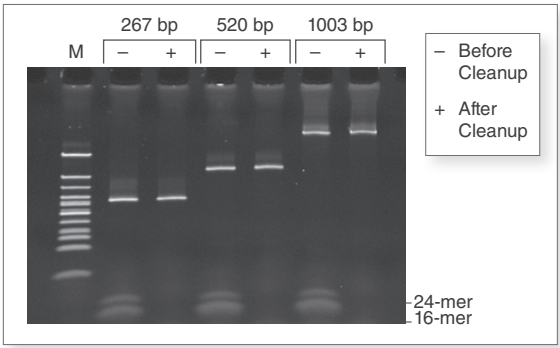
** DNA ≥ 15 bp to 25 kb (dsDNA) and DNA ≥ 18 nt to 10 kb (ssDNA) can also be purified using the Oligonucleotide Cleanup Protocol on page 7.

Figure 2: Workflow for DNA Cleanup



Performance Data

Monarch PCR & DNA Cleanup Kit (5 µg) removes low molecular weight primers from dsDNA samples.



Three independent amplicons (267 bp, 520 bp, 1003 bp) were spiked with two oligonucleotides (16-mer, 24-mer) to a final concentration of 1 µM. Half of each mix was purified with the Monarch PCR & DNA Cleanup Kit (5 µg) following the included protocol. Equivalent fractions of the original mixture and the eluted material were resolved on a 20% TBE acrylamide gel at 100V for one hour and stained with SYBR Green II.

General Guidelines:

Input amount of DNA to be purified should not exceed the binding capacity of the column (5 µg). A starting sample volume of 20–100 µl is recommended. For smaller samples, TE can be used to adjust the volume to the recommended volume range. Centrifugation should be carried out at 16,000 x g in a standard laboratory microcentrifuge at room temperature.

APPLICATIONS	
PCR cleanup	DNA from PCR reactions can be purified after amplification to remove polymerases, primers, detergents, dNTPs, etc.
Enzymatic reaction cleanup	Modifying enzymes such as ligases, kinases, nucleases, phosphatases are efficiently removed allowing efficient 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 cleanup	ssDNA oligonucleotides (≥ 18 nt) and dsDNA fragments (≥ 15 bp) can be purified using the Oligonucleotide Cleanup Protocol.

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

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

Monarch PCR & DNA Cleanup Kit (5 µg) Protocol

All centrifugation steps should be carried out at 16,000 x g. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1. Dilute sample with DNA Cleanup Binding Buffer according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex. A starting sample volume of 20–100 µl is recommended. For smaller samples, TE can be used to adjust the volume. For diluted samples larger than 800 µl, load a portion of the sample, proceed with Step 2, and then repeat as necessary.

SAMPLE TYPE	RATIO OF BINDING BUFFER: SAMPLE	EXAMPLE
dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl:100 µl
dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl:100 µl
ssDNA (cDNA, M13)	7:1	700 µl:100 µl

2. Insert column into collection tube and load sample onto 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.*

3. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discard flow-through.

   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.

4. Repeat wash (Step 3).

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

5. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

   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 or ethanol are carried over to the next step.

6. Add ≥ 6 µl of 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.

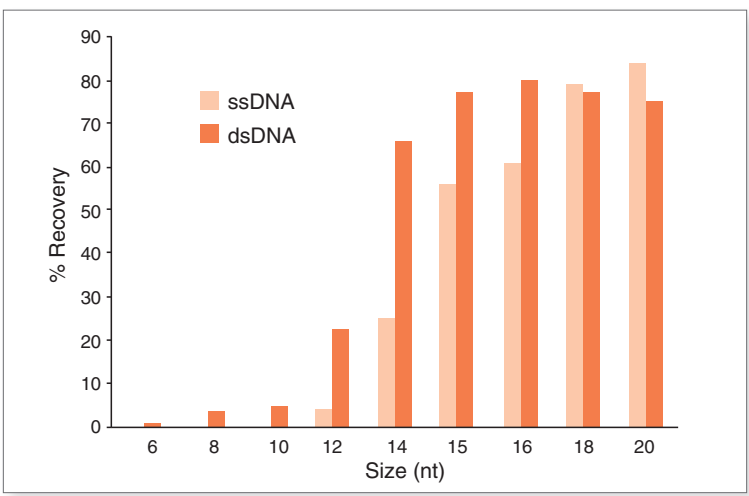
   

   To save time, spin for 30 seconds, instead of 1 minute.

Oligonucleotide Cleanup Protocol (NEB #T1030)

The Monarch PCR & DNA Cleanup Kit protocol can be modified to purify shorter DNA fragments and single-stranded oligonucleotides. The following modified protocol utilizes the same columns and bind/wash/elute workflow of the Monarch PCR & DNA Cleanup Kit with > 70% recovery and cleanup of oligonucleotides ≥ 15 bp (dsDNA) or ≥18 nt (ssDNA). The Oligonucleotide Cleanup protocol efficiently removes unincorporated nucleotides, short oligos, dyes, enzymes, and salts from labeling and other enzymatic reactions.

Figure 1: Recovery of ssDNA and dsDNA oligonucleotides (1 µg) using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit.



Synthesized ssDNA and dsDNA oligonucleotides (1 µg in 50 µl H_2O) of varying lengths (6-20 nt) were purified using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) and were eluted in 50 µl water. The average percent recovery (n=3) of the oligonucleotides was calculated from the resulting A_{260} as measured using a Trinean DropSense™ 16. Use of the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) results in the efficient removal of small oligonucleotides (6-12 nt) and > 70% recovery and cleanup of oligonucleotides ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA).

General Guidelines:

Input amount of DNA to be purified should not exceed the binding capacity of the column (5 µg). A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume to the recommended volume range. 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

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

All centrifugation steps should be carried out at 16,000 x g. (~13K RPM in a typical microcentrifuge). This ensures all traces of buffer are eluted at each step.

1. A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume.
2. Add 100 µl DNA Cleanup Binding Buffer to the 50 µl sample.
3. Add 300 µl ethanol (≥ 95%). Mix well by pipetting up and down or flicking the tube. Do not vortex.
4. Insert column into collection tube and load sample onto column and close the cap. 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.*
5. Re-insert column into collection tube. Add 500 µl DNA Wash Buffer and spin for 1 minute. Discard flow-through.

   If using a vacuum manifold, add 500 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 Step 5 (Optional). This step is recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).*

7. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.

   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 or ethanol are carried over to the next step.

8. Add ≥ 6 µl of 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.

   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.

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

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.
- 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

- 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 OD260/230 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 PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
COLUMNS SOLD SEPARATELY		
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns
BUFFERS SOLD SEPARATELY		
Monarch DNA Cleanup Binding Buffer	T1031L	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® Purple1 kb DNA Ladder	N0552S	125 lanes
Quick-Load Purple 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 DNA Gel Extraction Kit	T1020S/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  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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