Version 3

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DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) V.3

New England Biolabs[1]

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ABSTRACT

Protocol for DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). You can also download the Quick Protocol Card here.

EXTERNAL LINK

https://www.neb.com/protocols/2015/11/23/monarch-pcr-and-dna-cleanup-kit-protocol

DOI

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PROTOCOL CITATION

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OWNERSHIP HISTORY

Mar 16, 2021	Liz Brydon	Protocols.io
May 13, 2021	Isabel Gautreau	New England Biolabs
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May 14, 2021	Isabel Gautreau	New England Biolabs

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GUIDELINES

There are two protocols available for this product:

DNA Cleanup and Concentration (steps): for the purification of up to 5 µg of DNA (ssDNA > 200 nt and dsDNA > 50 bp) from PCR and other enzymatic reactions. A detailed protocol and a quick protocol are available for your convenience.

Oligonucleotide Cleanup protocol: for the purification of up to 5 µg of DNA fragments ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA). Expected recovery is > 70%. When purifying ssDNA of any size, recovery can be increased by using this protocol; however, it is important to note that this protocol shifts the cutoff for smaller fragments to 18 nt (rather than 50 nt for the DNA Cleanup and Concentration Protocol).

MATERIALS TEXT

MATERIALS

Monarch® PCR & DNA Cleanup Kit (5 µg) **New England Biolabs Catalog #T1030**

KIT COMPONENTS

A	B	C	D
	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.

SAFETY WARNINGS

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.

BEFORE STARTING

Please review the important information under the “Guidelines" tab before beginning.

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.

Buffer Preparation

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1 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).

DNA Cleanup and Concentration 5m

2 

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 the next step, and then repeat as necessary.

A	B	C
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 > 200 nt*	7:1	700 µl:100 µl

**Please note that recovery of ssDNA < 200 nts can be increased by using the Oligonucleotide Cleanup Protocol, but doing so will shift the cutoff size for DNA binding to 18 nt (versus 50 nt).*

3  1m

Insert column into collection tube and load sample onto column and close the cap. Spin for **00:01:00** at **16000 x g** , 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.

4  1m

Re-insert column into collection tube. Add **200 µl DNA Wash Buffer** and spin for **00:01:00** at **16000 x g** . 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

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through the column, then switch the vacuum source off.

5  1m

Repeat wash: Re-insert column into collection tube. Add **200 µl DNA Wash Buffer** and spin for **00:01:00** at **16000 x g** . 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 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.

7  2m

Add ≥ **6 µl DNA Elution Buffer** to the center of the matrix. Wait for **00:01:00** , then spin for **00:01:00** at **16000 x g** to elute DNA.

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

> *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.*

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