Version 5

May 14, 2021

Oligonucleotide Cleanup Using Monarch® PCR & DNA Cleanup Kit (5 µg) Protocol (NEB #T1030) V.5

New England Biolabs[1]

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1 Works for me Share dx.doi.org/10.17504/protocols.io.btd4ni8w

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ABSTRACT

The Monarch PCR & DNA Cleanup Kit protocol can be modified to enable the purification of ssDNA, oligonucleotides, and other small DNA fragments. 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.

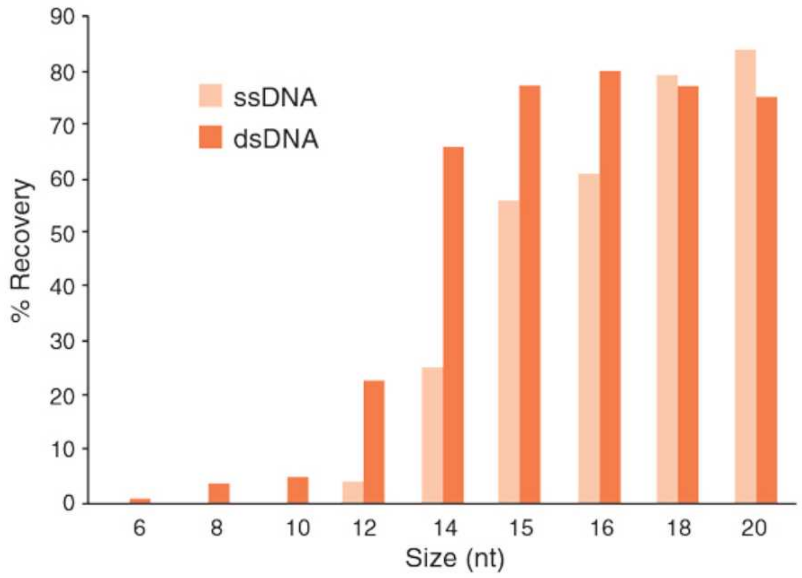

Figure 1: Recovery of ssDNA and dsDNA oligonucleotides (1 µg) using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit.

Synthesized ssDNA and dsDNA oligonucleotides (1 µg in 50 µl H2O) 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 A260 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).

You can also download the Quick Protocol Card here.

EXTERNAL LINK

https://www.neb.com/protocols/2017/04/25/oligonucleotide-cleanup-using-monarch-pcr-dna-cleanup-kit-5-g-protocol-neb-t1030

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LICENSE

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OWNERSHIP HISTORY

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May 13, 2021	Isabel Gautreau	New England Biolabs
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PROTOCOL INTEGER ID

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GUIDELINES

DNA Cleanup and Concentration: for the purification of up to 5 µg of DNA (ssDNA > 200 nt and dsDNA > 50 bp) from PCR and other enzymatic reactions.

Oligonucleotide Cleanup: 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). A detailed protocol and quick protocol are available for your convenience.

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 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

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.

Oligonucleotide Cleanup 5m

2

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.

3 A starting sample volume of 50 µl is recommended. For smaller samples, nuclease-free water can be used to adjust the volume.

4 

Add **100 µl DNA Cleanup Binding Buffer** to the 50 µl sample.

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5

Add **300 µl ethanol (≥ 95%)** . Mix well by pipetting up and down or flicking the tube. Do not vortex.

6 1m

Insert column into collection tube, 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.*

7 1m

Re-insert column into collection tube. Add **500 µl DNA Wash Buffer** and spin for **00:01:00** at **16000 x g** . 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.

8 1m

(Optional): Repeat previous step: Re-insert column into collection tube. Add **500 µl DNA Wash Buffer** and spin for **00:01:00** at **16000 x g** . 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.

This step is recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).

9 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 the next step.

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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.

10 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 the DNA.

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.

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