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Feb 28, 2022

Quick Protocol for Oligonucleotide Cleanup Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) V.4

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dx.doi.org/10.17504/protocols.io.bg9sjz6e

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ProtocolCard_T1030.pdf

DOI

dx.doi.org/10.17504/protocols.io.bg9sjz6e

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

New England Biolabs 2022. Quick Protocol for Oligonucleotide Cleanup Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bg9sjz6e Isabel Gautreau

Oligonucleotides, DNA, Cleanup

protocol ,

Jun 07, 2020

Feb 28, 2022

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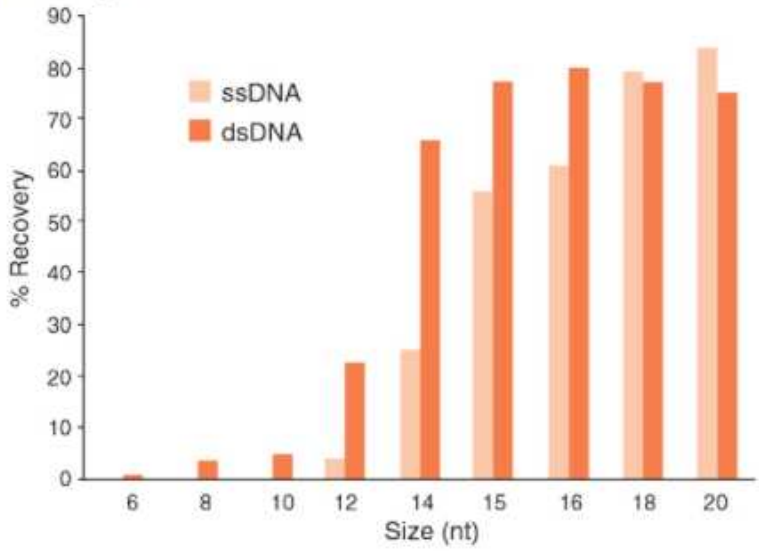
DNA Cleanup and Concentration: for the purification of **up to 5 µg** of **DNA** (ssDNA > 200 nt and dsDNA > 50 bp) from PCR and other enzymatic reactions.
A detailed protocol and a quick protocol are available for your convenience.

Oligonucleotide Cleanup (steps): 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 step-by-step protocol is available here.

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).

General Guidelines:

Input amount of DNA to be purified should not exceed the binding capacity of the

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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 16000 x *g* in a standard laboratory microcentrifuge at room temperature.

MATERIALS

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

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

- All centrifugation steps should be carried out at **16000 x g** (~ **13000 rpm**).
- Add isopropanol to Monarch DNA Cleanup Binding Buffer prior to use*:

1. For the 50-prep kit, add **14 mL** of isopropanol to the DNA Cleanup Binding Buffer.
2. For the 250-prep kit, add **63.5 mL** of isopropanol to the DNA Cleanup Binding Buffer.

- Add ethanol to Monarch DNA Wash Buffer prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch DNA Wash Buffer)

1. For 50-prep kit add **20 mL** of ethanol to the Monarch DNA Wash Buffer.
2. For 250-prep kit add **100 mL** of ethanol to the Monarch DNA Wash Buffer.

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

**Beginning in April 2021, the DNA Cleanup Binding Buffer will be changed to a concentrated format which requires the addition of isopropanol by the user. Please refer to the instructions inside of the product that you receive.*

1

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

We recommend a sample volume of 50 µl. For smaller samples, adjust the volume with

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nuclease-free water.

2 

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

3 Insert column into collection tube and load sample onto column and close the cap.

4 

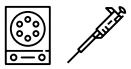
Spin at **16000 x g** for **00:01:00** , then discard flow-through.

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.

6 

Add **500 µL DNA Wash Buffer** and spin at **16000 x g** for **00:01:00** .

7 Discard flow-through.

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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 **Repeat steps 5-7 (Optional).**

Recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).

go to step #5 Repeating steps 5-7

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 **00:01:00** .

10 

Add ≥ **6 µL DNA Elution Buffer** to the center of the matrix.

11 

Wait for **00:01:00** , then spin at **16000 x g** for **00:01:00** to elute DNA.

Typical elution volumes are **6 µL** – **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.

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

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