Version 2

Nov 30, 2020

Quick Protocol for Oligonucleotide Cleanup Using the Monarch® PCR & DNA Cleanup Kit (5 ?g) (NEB #T1030) V.2

New England Biolabs[1]

[1]New England Biolabs

1 Works for me dx.doi.org/10.17504/protocols.io.bp9dmr26

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ABSTRACT

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

EXTERNAL LINK

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

ATTACHMENTS

ProtocolCard_T1030.pdf

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PROTOCOL CITATION

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ATTACHMENTS

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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. 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).

MATERIALS TEXT

MATERIALS

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

SAFETY WARNINGS

Please refer to SDS for safety warnings.

ABSTRACT

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

BEFORE STARTING

- All centrifugation steps should be carried out at 16,000 x *g* (~13,000 RPM).
- Add 4 volumes of ethanol (≥ 95%) to one volume of DNA Wash Buffer.
- Please review the important information under the “Guidelines" & "Warnings” tabs before beginning.

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

100 µl DNA Cleanup Binding Buffer

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

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

300 µl Ethanol (≥ 95%)

3 Insert column into collection tube and load sample onto column.

4 Spin for 1 minute, then discard flow-through.

00:01:00 Spinning

5 Re-insert column into collection tube.

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6 Add 500 µl DNA Wash Buffer and spin for 1 minute.

500 µl DNA Wash Buffer

00:01:00 Spinning

7 Discard flow-through.

8 **Repeat steps 5-7 (Optional).** Recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).

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 1 minute.

00:01:00 Re-spinning

10 Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix.

6 µl DNA Elution Buffer

11 Wait for 1 minute, then spin for 1 minute to elute DNA.

00:01:00 Waiting

00:01:00 Spinning

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.

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