

Quick Protocol for Monarch® PCR & DNA Cleanup Kit (5 μg) (NEB #T1030) Version 2

New England Biolabs

Abstract

This is the "quick" version of Monarch® PCR & DNA Cleanup Kit (5 μ g) Protocol (NEB #T1030). For the full protocol, please click here.

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Guidelines

For detailed protocol and more information, visit www.neb.com/T1030

The full protocol is available here.

The video protocol is available <u>here</u>.

There are two protocols available for this product:

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

Oligonucleotide Cleanup: for the purification of up to 5 μ g of DNA fragments \geq 15 bp (dsDNA) or \geq 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 <u>detailed protocol</u> and <u>quick protocol</u> are available for your convenience.

Before start

Add 4 volumes of ethanol (\geq 95%) to one volume of DNA Wash Buffer.

All centrifugation steps should be carried out at $16,000 \times g$ (~13,000 RPM).

Materials

Monarch® PCR & DNA Cleanup Kit (5 μg) T1030 by New England Biolabs

Protocol

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.

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

NOTES

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A sample volume of 20–100 μ l is recommended. For smaller samples, TE can be used to adjust the volume. For diluted samples larger than 800 μ l, load a portion of the sample, proceed with step 2, and then repeat as necessary.

Step 2.

Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.

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00:01:00

Step 3.

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

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Discarding flow-through is optional.

Step 4.

Repeat Step 3: re-insert column into collection tube. Add 200 μ l DNA Wash Buffer and spin for 1 minute.

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00:01:00

Step 5.

Transfer column to a clean 1.5 ml microfuge tube.

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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.

Step 6.

Add \geq 6 μ l of DNA Elution Buffer to the center of the matrix. Wait for 1 minute.

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00:02:00

Step 7.

Spin for 1 minute to elute DNA.

NOTES

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Typical elution volumes are 6–20 μ l. 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 (\geq 10 kb), heating the elution buffer to 50°C prior to use can improve yield.