Quick Protocol for Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) Version 3

New England Biolabs

Abstract

This is the "quick" version of Monarch® PCR & DNA Cleanup Kit (5 µg) 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 here.

There are two protocols available for this product:

DNA Cleanup and Concentration (below): 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.

Before start

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

All centrifugation steps should be carried out at 16,000 x 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 > 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).

NOTES
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A 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 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.

DURATION
00:01:00

Step 3.

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

DURATION
00:01:00

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Discarding flow-through is optional.

Step 4.

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

DURATION
00:01:00

Step 5.

Transfer column to a clean 1.5 ml microfuge tube.

NOTES
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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 ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute.

DURATION
00:02:00

Step 7.

Spin for 1 minute to elute DNA.

NOTES
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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.