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Feb 25, 2022

Quick Protocol for DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) V.6

New England Biolabs[1]

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

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https://www.neb.com/protocols/2015/12/08/quick-protocol-for-monarch-pcr-dna-cleanup-kit-5-g-t1030

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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.
A step-by-step protocol is available here.

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).

Add isopropanol to Monarch DNA Cleanup Binding Buffer prior to use*:

- For the 50-prep kit, add **14 mL** of isopropanol to the DNA Cleanup Binding Buffer.
- For the 250-prep kit, add **63.5 mL** of isopropanol to the DNA Cleanup Binding Buffer.
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Add ethanol to Monarch DNA Wash Buffer prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch DNA Wash Buffer)

- For the 50-prep kit, add **20 mL** of ethanol to the Monarch DNA Wash Buffer
- For the 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.

All centrifugation steps should be carried out at **16.000 x g** (~ **13.000 rpm**).

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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.

A	B	C
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).

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.

2

Insert column into collection tube and load sample onto column. Spin at **16000 x g** for **00:01:00** , then discard flow-through.

3

Re-insert column into collection tube. Add **200 µL DNA Wash Buffer** and spin at **16000 x g** for **00:01:00** .

Discarding flow-through is optional.

4

Repeat Step 3: Re-insert column into collection tube. Add **200 µL DNA Wash Buffer**

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and spin at **16000 x g** for **00:01:00** .

Discarding flow-through is optional.

5 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** .

6 

Add ≥ **6 µL DNA Elution Buffer** to the center of the matrix. Wait for **00:01:00** .

7 Spin 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. For larger size DNA (≥ 10 kb), heating the elution buffer to **50 °C** prior to use can improve yield. 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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