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

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.

Before start

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

- For 50-prep kit, add 20 ml of ethanol to 5 ml of Monarch DNA Wash Buffer
- For 250-prep, kit add 100 ml of ethanol to 25 ml of Monarch 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. 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.

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 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 at 16,000 x g, then discard flow-through.

DURATION

00:01:00

Step 3.

Re-insert column into collection tube. Add 200 µl DNA Wash Buffer (with ethanol added) and spin for 1 minute at 16,000 x g. Discarding flow-through is optional.

DURATION

00:01:00

Step 4.

Repeat Step 3. (Step 3: Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute at 16,000 x g. Discarding flow-through is optional).

DURATION

00:01:00

Step 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 1 minute.

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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, then spin for 1 minute at 16,000 x g to elute the DNA.

DURATION

00:02:00

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.