Quick Protocol for Monarch® PCR & DNA Cleanup Kit (5 ?g) (NEB #T1030) V.1

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Abstract

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

Guidelines

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

The full protocol is available **here.**

The video protocol is available **here**.

Materials

MATERIALS

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

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).

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

Note

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 for 1 minute at 16,000 x g, then discard flow-through.**

00:01:00

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.

00:01:00

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).

00:01:00

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.

Note

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.

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.**

00:02:00

Note

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.